“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

Brad Power
March 4, 2024

“A network view of cancer provides a revolutionary, modern view of what cancer is. Beyond identifying oncogenic drivers, if also find synthetic lethal vulnerabilities and the master regulator constellation that underlies the malignant phenotype. The challenge is to develop a functional understanding of the impact of all the genomic aberrations in a cancer - and there may be dozens or hundreds of abnormalities. To achieve ‘network consciousness’ in molecular diagnosis provides an understanding of the functional organization of malignant behavior in mechanistic terms - that forms the requisite foundation for curing advanced complex malignancies. Without that, treatment will always just be a shot in the dark. For most of the history of cancer treatment, there was no hope of understanding the disease at this level, but the ready availability of whole exome NGS and the tools to make sense of it using biosimulation has brought us to an inflection point in the history of medicine where we can finally see what we should be targeting. Somehow, we need to evolve patient-centric combination therapies that target the cancer network where it matters most. The goal is to take out the key resources of the disease network.” – Michael Castro, MD

“When I get the results of the genomic analysis, I'm looking for how many things I can treat, and then I try to target as many as possible.” – Michael Castro, MD

“Synthetic lethality can kill cancer cells without causing toxicity to normal tissues, thus bypassing the traditional oncologic requirement to give maximum tolerated dose and the necessity for toxicity. I'm focused on the concept of targeting multiple synthetic lethal nodes simultaneously as a way of taking down the cancer network… where you can stack multiple therapies simultaneously without impacting normal tissues.” – Michael Castro, MD

“The forefront of cancer research is decades ahead of the clinic. Unfortunately, most of what is being learned by the great public investment in cancer will never be translated into therapy, because that translation is driven by one thing, and that's return on investment… Without profit, the translation of science into practice is usually deemed “uninvestable.. But the issue is that it’s not a matter of whether we can treat the disease better, but a matter of whether anyone can get rich in the process. Hence there is a chasm between the forefront of scientific understanding about cancer and how oncology is practiced in the clinic.” - Michael Castro, MD

Meeting Summary

The “one-size fits all” approach to treating cancer (the “standard of care”) derived through evidence from randomized clinical trials has drawbacks. In some situations, approved therapies may have no disease impact for patients. We can do a lot better by personalizing treatment. For example, in prostate cancer a study showed that the chemotherapy docetaxel was superior to another chemotherapy mitoxantrone plus the steroid prednisone. We suppose that all patients would be better off with docetaxel. However, **for some patients the combination of**

mitoxantrone plus prednisone is four-fold more effective, and docetaxel has no impact, the very opposite of what the randomized trial concluded.

Ideally, one would get a comprehensive molecular diagnosis of cancer (Whole exome NGS and transcriptome). This is seldom done and most oncologists shun the approach because they don’t know what to do with the results. The oncogene-addiction paradigm is relevant fo0r 9-10% of patients only. The single drug approach to block a single pathway in cancer, such as targeting EGFR in lung cancer or the androgen receptor (AR) in prostate cancer, doesn't get very far. In the other 90% of cancers, there are numerous genomic aberrations causing disruption of dozens of signaling pathways. The idea is to measure all of this so that the master regulator network and synthetic lethal vulnerabilities in cancer can be determined. Once achieved, it is possible to understand the disease as a functional network that seeks robust perfect adaptation under stress, that is, therapeutic resistance. By achieving an engineering level understanding of a cancer’s mechanistic determinants, the dynamics, dependencies, and vulnerabilities of the cancer can be discerned, what I call “network consciousness.”
Using computational biosimulation at Cellworks, it is possible to simulate how drugs and drug combinations interact with the network. If you attack as many of the disease-specific nodes in the network as possible with combination therapy, that is “Network-targeting Combination Therapy (NTCT)”, the cancer will collapse. Because the Cellworks model is based on mathematics (differential equations), you get a quantitative measure of how one treatment compares to other treatments and the signaling pathways that show you why.

Michael Castro, MD, and Chief Medical Officer of Cellworks, is uniquely qualified as a clinician who is also an expert in molecular pathology, to look at molecular pathways and personalize treatments for cancer patients. Cellworks provides a report on the signaling pathways and the convergence of particular biochemical enzymes which he uses to understand master regulators, network nodes, DNA repair, and transcriptional (RNA) drivers. Identifying the dysregulated proteins that contribute to your cancer is crucial for developing personalized treatment plans.

The Cellworks model is an investigational tool and not yet sold.

How does this biosimulation approach to personalized treatment work?

1. Conduct detailed and extensive genomic testing (whole exome, RNA sequencing, proteomics, liquid biopsy) to uncover your unique molecular profile.
2. Identify as many susceptible nodes (synthetic lethal nodes, oncogenic nodes, or master regulator nodes) that can be treated as possible in your cancer network. One approach, VIPER (Virtual Inference of Protein-activity by Enriched Regulon) analyzes protein activity from gene expression data (RNA sequencing) to depict your unique cancer network. Synthetic lethal nodes in a cancer network are extremely important because normal tissue doesn't have them.
3. Treat with a combination of as many therapies as possible that target your personalized susceptible nodes, with an emphasis on synthetic lethal targeting, where you can stack multiple therapies simultaneously without impacting normal tissues much.

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For example, using this approach, a patient with an aggressive brain cancer (glioblastoma) got a comprehensive molecular diagnosis that led to the identification of a disease network with about a dozen synthetic lethal nodes. She was treated with a five-drug combination (lomustine, olaparib, digoxin, metformin, and high dose intravenous ascorbate) targeting the synthetic lethal nodes, with intra-patient dose escalation to safely deliver the treatment. There was no toxicity. She got a **complete remission, and she is now 54 months from original diagnosis, 2.5 years from relapse, without a trace of disease.**

What are the challenges to building a personalized network model of your disease?

- **Complexity**: There are hundreds of pathways >1500 transcription factors, 2500 microRNAs that modulate translation of mRNA into protein. Each of these pathways is complex. DNA repair has about 497 genes in it. Transcription factors are at the end of signaling pathways that are also complexly dysregulated in the tumor. They compete against each other to upregulate and downregulate gene expression.
- **Interpretation**: There's no one alive who could sit down and tell you all the protein interactions in the body. That is why a model of signaling pathway behavior and the consequences of genomic, epigenetic, transcriptional, posttranslational impacts is desperately needed.
- **Statistical validation**: Statistical validation requires data sets of patients, preferably with controls. Such data sets are nearly impossible to come by. However, Cewllworks has such a data set coming for NSCLC addressing the issue of which patients benefit from the addition of chemotherapy to immunotherapy.
- **Data**: You need the right data for the question you’re trying to answer. For a model that tries to predict clinical response to combination therapies using genomic signatures, the good news is that we have (arguably) lots of cancer genomics data, but the problem is that a limited amount of that data has comprehensive clinical outcome data, and that data is presumably biased to represent the standard of care. As such, we’re probably able to build a decent model to predict whether a patient will respond better to medicine A vs. medicine B.
- **Model validation**: The current Cellworks model is informed by scientific work. Though it is unvalidated, the decision for patients is whether to prefer a blind evidence based approach where everyone is considered the same for the sake of treatment, or whether to allow scientific discoveries to inform clinical decision making. I prefe th4e lights on approach,
- **Synthetic lethal nodes**: Synthetic lethal nodes are a “holy grail” for cancer because if you can find a drug that is very effective only when a certain mutation is present, the selectivity impact against cancer compared to normal could be enormous. We have started to0 understand that when chemotherapy really works well, it is often because there is a synthetic lethal vulnerability that confers responsiveness. But without that synthetic lethal susceptibility, many types of chemotherapy may indeed do little.

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What are the concerns and countermeasures to drug combinations and other non-standard (“off label”) drug recommendations?

- **Toxicity**: Conventional wisdom in cancer treatment strategy, largely derived from chemotherapy, argues that the similarities between normal human cells and cancer cells means there is no way to avoid toxicity for normal tissue when you treat cancer cells with drug combinations. With combinations of five or more toxic treatments, eventually the human body can only take so much. However, if you kill cancer by attacking synthetic lethal nodes, you won’t harm normal tissue. (Synthetic lethality is where mutations in two genes together result in cell death, but a mutation in either gene alone does not.) This could be the key for how we're going to go forward in combination therapy for cancer. You can stack therapies, one on top of the other, without really ever getting anywhere near the maximum tolerated dose.
- **Lack of evidence from clinical trials**: No one has done clinical trials on many of the recommendations that derive from molecular biology, so they are viewed as speculative by treating physicians. However, molecular biology researchers have taken these pathway models and drug relationships as facts. The breakdown is in translational medicine, taking molecular biology insights from laboratories into clinical practice. Jason Sicklick and Razelle Kurzrock have done observational clinical trials (I-PREDICT) which show drug combinations are effective and safe. Statistician Drew Watson is taking a given parameter, weighting that with exponents according to its relationship with survival, and then in the end, and creating an equation which will tell you whether or not immunotherapy is going to work, and what the patient's survival will be.

How viable is the biosimulation approach for targeting disease?

- **Likely Pharma interest**: Biosimulation tools are of very high interest to a variety of pharma teams that plan/design trials, especially for identifying hypotheses for medications that work synergistically in combination.
- **Computationally possible**: Modeling genome-wide networks has been a tractable computational problem for a number of years now. There were a number of problems that were challenging to tackle about a decade ago, but they’ve been largely solved through the combination of cloud computing and more efficient numerical algorithms. You can always think up a more elaborate model, but as long as you’ve got the money, then you can find a computer to build/run it.

Should you pursue this method of personalized network modeling and drug combinations?

It comes down to your philosophy about how you want to be treated, and where your comfort level is. You can either go with the “standard of care" (the NCCN guidelines, consensus medicine), or you can ask, “Is there anything else that's been learned about cancer that could help me?” “Is there anything that anyone could learn about my tumor that I can leverage as a therapy?”

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You should pursue this if you believe in following cutting edge scientific research and are willing to go outside the box and be in an uncomfortable relationship with your doctors who may say, “There's no evidence for this. Therefore, I wouldn't do it.” There's an old saying that, “All models are wrong, but some of them are useful.” While the models are incomplete, they are often further ahead than any doctors can do on their own.

What can we do to accelerate the exploration and adoption of this new approach?

- **Stop doing things that don't work**. If one adopts this approach in the clinic, it leads to radical re-thinking because the standard of care is predicated on giving drugs that don’t work for most patients based on the randomized trial method.
- **Gather evidence**: We're going to need to do a prospective randomized trial with these biosimulation tools to change medicine. This will need to be funded.
- **Educate**: Increase understanding of molecular biology among clinicians and patients.
- **Expand the data inputs**: We can get the entire genome and transcriptome. We need to increase experience with the transcriptome and look at the proteome as an input.
- **Certify molecular oncologists**: There is a chasm between molecular biology and clinical medicine. We need a hybrid person who has both skills and has AI to unravel this complex molecular biologic system. There are a few molecular pathologists, and there is no certification for molecular oncologists.

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Meeting Notes

KEYWORDS

patients, work, cancer, pathways, question, docetaxel, drug, proteomics, mutation, targeting, oncogenes, cell, disease, gene, network, therapy, nodes, synthetic, people, treatment

SPEAKERS

Michael Castro (78%), Allen Morris (8%), Frank Nothaft (4%), Brian McCloskey (3%), Brad Power (3%), Ian Lewington (2%), Robert Gurmankin (1%), Vanessa Hugo (1%)

OUTLINE

1. Cancer treatment strategies using computational biology. (0:00)
2. Using AI to analyze cancer genomics and identify potential treatments. (6:17)
3. Personalized cancer treatment based on audio transcript. (12:39)
4. Personalized cancer treatment using liquid biopsies and bio simulations. (19:22)
5. Targeting cancer cells with synthetic lethality. (26:40)
6. Personalized cancer treatment using liquid biopsy. (35:35)
7. Personalized cancer treatment using AI and synthetic lethal targeting. (43:23)
8. Personalized cancer treatment using multi-omics data. (52:51)
9. Using proteomics and transcriptomics to understand cancer. (58:48)
10. Personalized cancer treatment and the limitations of current methods. (1:05:13)
11. Molecular biology and immunology in cancer treatment. (1:12:04)
12. Cancer biology and treatment strategies. (1:18:57)

SUMMARY

- **Cancer treatment strategies using computational biology. 0:00**
    - Michael Castro reveals breakthrough in cancer treatment using computational biostimulation.
    - In 1996, chemotherapy became a standard of care for metastatic prostate cancer, despite lack of survival benefit.
    - Docetaxel is a new standard of care for hormone refractory cancer, with 10-year follow-up supporting its use.
- **Using AI to analyze cancer genomics and identify potential treatments. 6:17**
    - Michael Castro explains the concept of explainable AI in cancer research, highlighting the importance of targeting “synthetic lethal nodes” for effective therapy.
    - He shows a case with 1092 genes affected by mutations and copy number aberrations, but low tumor mutation burden.
    - He highlights the "elephant in the room" of cancer research: the missing and extra chromosomes causing disease.
- **Personalized cancer treatment based on audio transcript. 12:39**
    - Michael Castro identifies potential treatment options for patients, including PARP inhibitors.

    - Platinum drugs and P16 inhibition show additive effect on olaparib sensitivity, while radiation is inferior due to non-homologous end joining pathway deficits.
    - Investigator notes that many cancer therapies are marginal and over-celebrated, with only 30% of patients benefiting from a particular treatment.
- **Personalized cancer treatment using liquid biopsies and bio simulations.** 19:22
    - Michael Castro believes individualized medicine can improve patient outcomes, seeks to raise awareness and conduct prospective trials with CellWorks biostimulation.
    - He discusses challenges of treating cancer with heterogeneous tumors, using allele frequency to prioritize treatment.
    - He suggests liquid biopsy and bone biopsy for personalized cancer treatment, emphasizing complementary nature of both methods.
- **Targeting cancer cells with synthetic lethality.** 26:40
    - Inventor of "synthetic lethal nodes" for cancer treatment, discusses successful cases of complete remission in patients with relapsed glioblastoma.
    - Roger Royce explains the concept of synthetic lethality in cancer treatment, highlighting the potential for targeted therapies to overcome resistance by attacking multiple vulnerabilities in the cancer network.
    - Allen Morris guesses that synthetic lethal nodes include DNA repair pathways, but Michael Castro corrects him, highlighting the importance of P 53 for nucleotide excision repair and energy production.
    - [AM: Ask Dr. Castro again - I believe Castro would categorize p53 in his “semantic” oncogenic node category even though it is the prototype of a tumor suppressor vs. a true synthetic lethal node. Indeed, see above, Dr. Castro broadens his semantic of “susceptible” nodes to: not only synthetic lethal nodes, but also, oncogenic nodes, master regulator, and transcription factor nodes)].
    - Frank Nothaft notes that targeting ATP synthesis and oxidative stress may be a promising approach to cancer treatment, despite lack of clinical evidence.
- **Personalized cancer treatment using liquid biopsy.** 35:35
    - Researchers are developing a machine learning model to predict cancer immunotherapy response based on genomic data, with potential to improve patient outcomes.
    - Lung cancer treatment strategy involves addressing clinical conundrum with chemotherapy and targeting specific molecular pathways.
    - Ian Lewington discusses his experience with advanced prostate cancer, including resistance to treatment and the use of liquid biopsies to monitor mutation levels.
- **Personalized cancer treatment using AI and synthetic lethal targeting.** 43:23
    - Ian Lewington discusses challenges in biopsying prostate cancer, including difficulty accessing tumors in bone.
    - Neurooncologist aims to improve diagnosis for GBM patients, focusing on early detection and personalized treatment.
    - Michael Castro emphasizes the importance of molecular diagnosis and AI-powered tools in understanding complex diseases like cancer.
    - He discusses potential for synthetic lethal targeting in cancer treatment, highlighting the need to stop doing things that don't work and harm patients.
- **Personalized cancer treatment using multi-omics data.** 52:51
    - Brian McCloskey discusses incorporating immune profiling and other datasets into existing models to improve drug sensitivity predictions.
    - Michael Castro discusses the use of Losartan in pancreatic cancer, highlighting its potential role in blocking TGF beta and improving outcomes for patients.

    - He also shares their insights on the limitations of genomics and the importance of proteomics in understanding cancer, citing examples from breast cancer research.
- **Using proteomics and transcriptomics to understand cancer. 58:48**
    - Michael Castro highlights the importance of proteomics in developing cancer therapies, particularly for identifying antigens on the surface of cancer cells.
    - Brian McCloskey raises a point of validation, noting that there may not be a direct correlation between DNA/RNA and proteomics, and that understanding this relationship is crucial for cancer diagnosis and treatment.
    - Michael Castro discusses the complexity of modeling biology and the need for a new approach, calling it "Vanguard medicine."
    - He believes their work is outside the box and aims to find solutions to problems, rather than continuing to do things that don't work.
- **Personalized cancer treatment and the limitations of current methods. 1:05:13**
    - Michael Castro argues that the profit-driven medical system hinders cancer research progress.
    - He emphasizes the importance of empathy in drug development, citing personal experience with cancer.
    - Clinicians should be shrewd about using Cellworks bio simulation tool for personalized medicine, as it provides valuable insights but is not perfect.
- **Molecular biology and immunology in cancer treatment. 1:12:04**
    - Allen Morris and Michael Castro discuss the rare combination of molecular biology and clinical medicine expertise, with less than 300 (estimated by AM) board-certified molecular pathologists in the US.
    - Allen Morris, having been seeded in real time with the new concept “synthetic lethal node” proposes the phrase and concept of a "primordial osteo-immunologic node" which bridges the adaptive immune system and bone, and evolved in jawed chordates. And notes there is already a field termed osteoimmunology, but wonders about the history and state of that field?
    - Allen Morris lists the above as one of his prostate cancer curiosities.
- **Cancer biology and treatment strategies. 1:18:57**
    - Pseudo progression vs. Hyperprogression in cancer are a recent common conundrum in radiology, and serial scans presumably over many months are needed to differentiate.
    - He discusses the complexity of cancer research, mentioning the need for a network view of cancer as a complex proteomic network.
    - He highlights the challenges of integrating and predicting the behavior of multiple pathways in cancer, citing the example of transcription factor biology.
    - He argues that understanding the complex network of transcription factors and signaling pathways in cancer cells is key to developing effective treatments.

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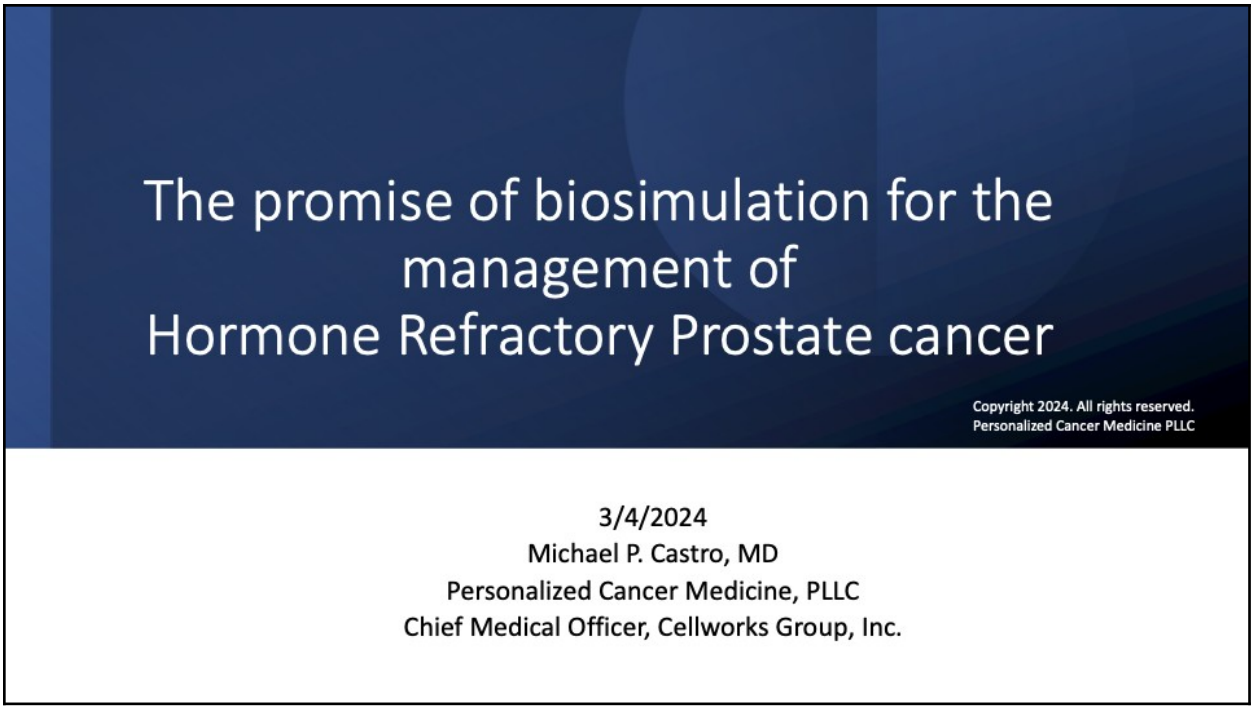
Meeting Transcript

Brad Power
This is the Cancer Patient Lab. We're excited to have Michael Castro with us again today. He's spoken to our group of patients before. He's a pioneer in looking at pathways and figuring out how to block those. He's an unusual combination of a researcher with an MD. He's treating patients in Southern California, and he's based in the Southern California area.

Michael, you were starting to say something really interesting about what brought you here today?

Michael Castro 1:01
In the course of my ordinary patient care, I came up with a rather spectacular realization, and I wanted to share it with you guys. I re-invited myself.



I work for this company Cellworks, a computational biosimulation company that takes complex genomic information, and then does signaling pathway analysis.

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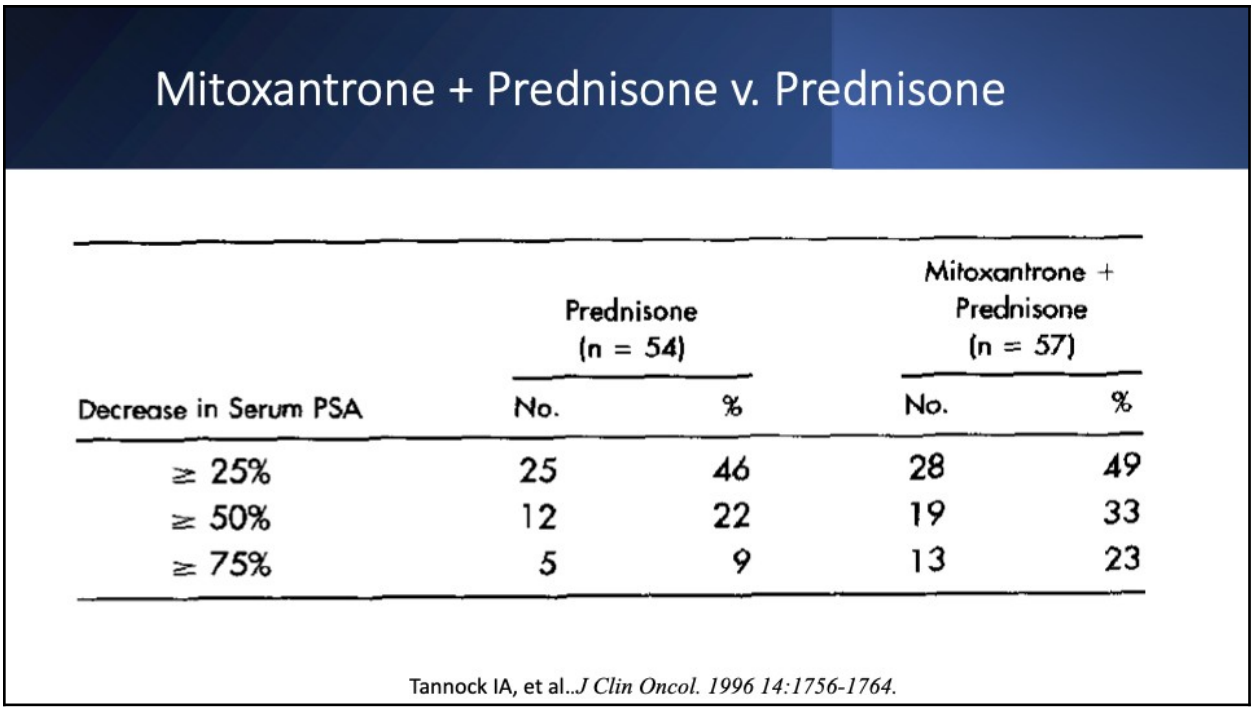

I want to go all the way back to 1996, where the medical oncologist’s role in managing prostate cancer really began. At that time, we were giving prednisone (a steroid which decreases inflammation, slows an overactive immune system, or replaces cortisol - a hormone that helps your body respond to stress, illness and injury) as a palliation (reliever of symptoms and suffering). There was interestingly some improvement in PSA (prostate-specific antigen, a measure of prostate cancer progression) that could be achieved from that, probably because prednisone inhibits NF-κB (NF-kappa B, a proinflammatory signaling pathway).
[AM editorial: Note - The curious nexus of Prednisone, prostate cancer, and NF-kB]
AM: Would you ask Dr. Castro for a more magnified view of the above report?

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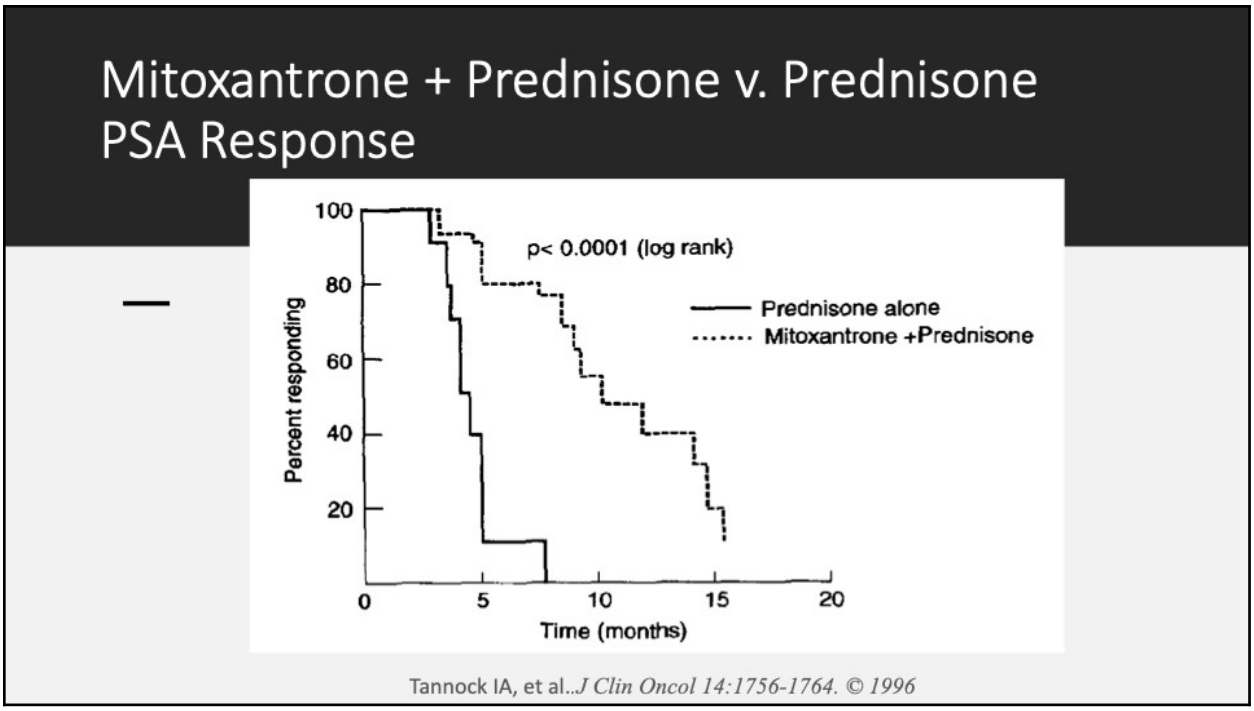
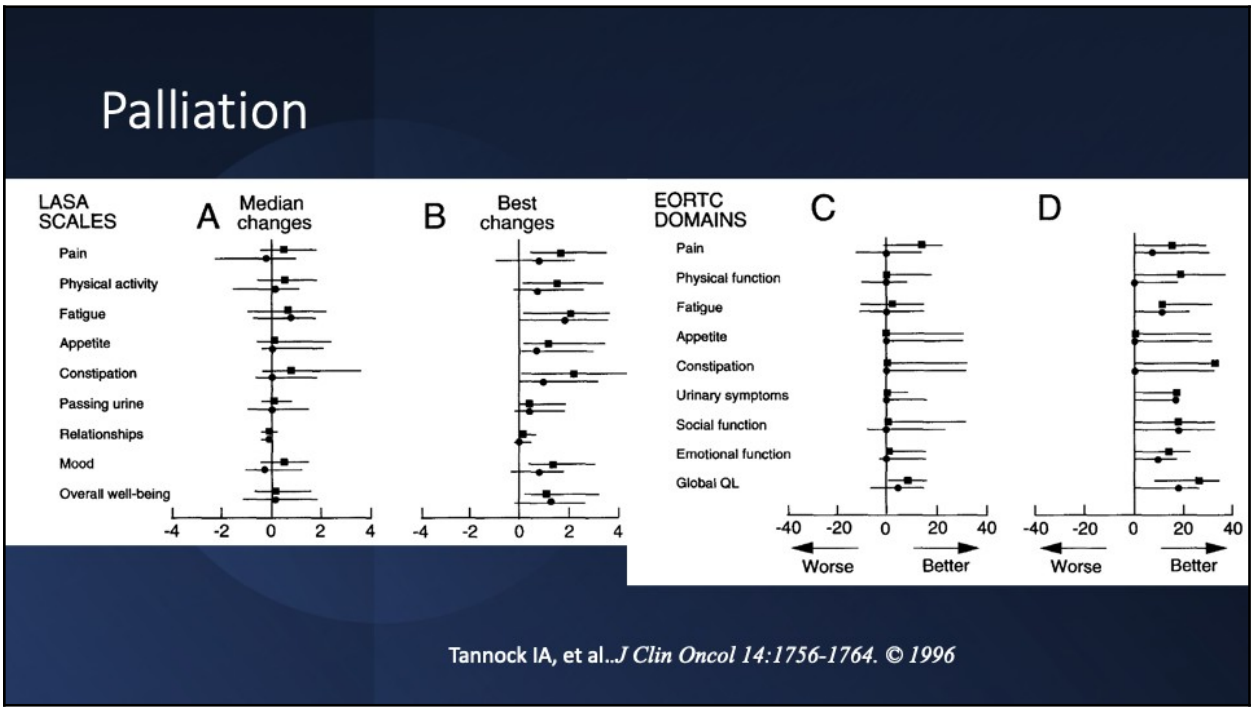


In any case, Mitoxantrone (a chemotherapy) was the first drug to come along. It shows an improvement in PSA response, as you can see, and the PSA response curves look in favor of chemotherapy. Thus began the era of giving chemotherapy to metastatic prostate cancer patients.



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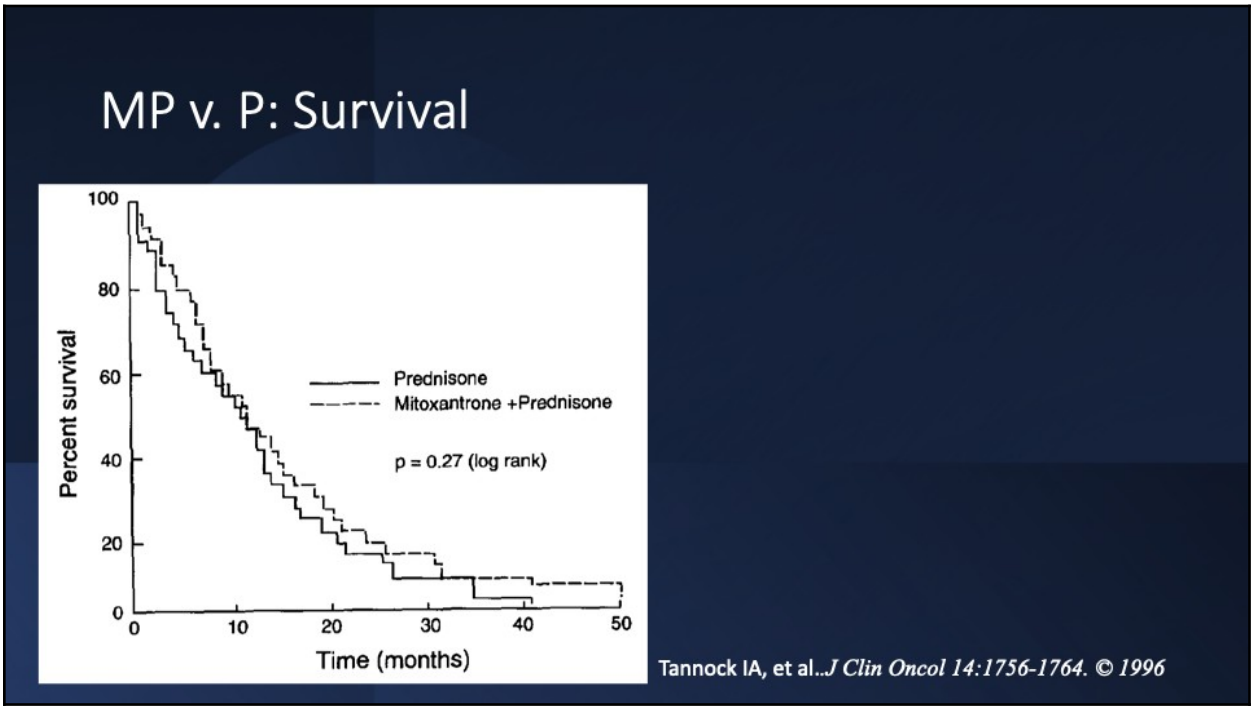
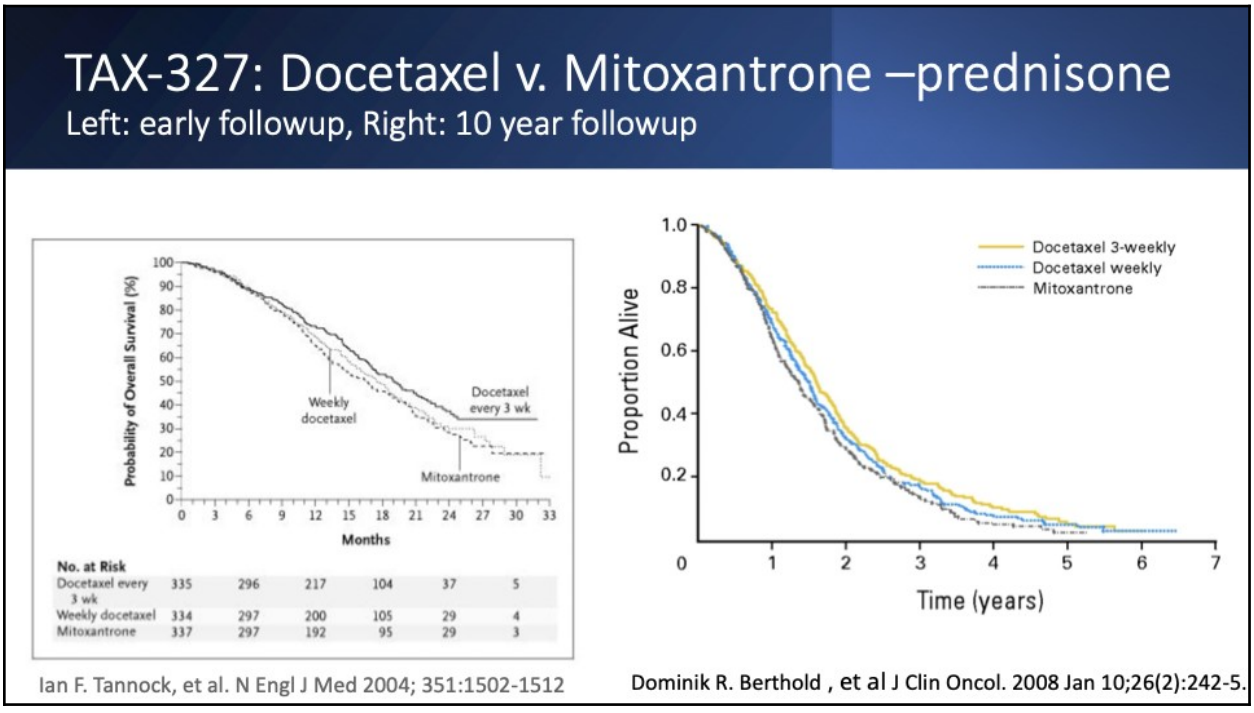
In this trial, they did a very admirable job of looking at quality of life. In spite of giving a cytotoxic drug, the argument was that patients actually felt better, and therefore, it was a reasonable thing to do. There was an absence of people feeling worse, for example.



On the other hand, there was no improvement in survival by adding Mitoxantrone. Nevertheless, this was the standard of care for nearly a decade until …



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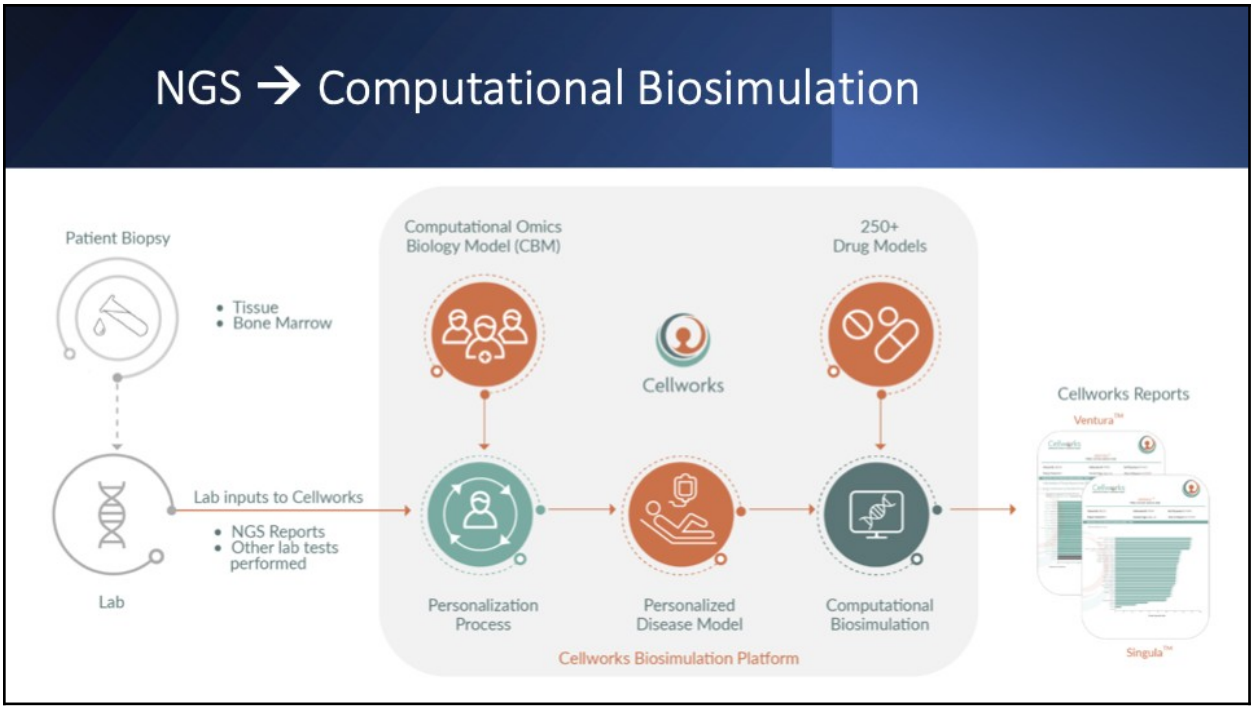
Michael Castro 3:37
… this famous landmark study of docetaxel versus Mitoxantrone and prednisone. They had about 1000 patients who were getting docetaxel either in a weekly or every three weeks schedule. You can see that the every three weekly schedule surpasses this course. You had to live about eight months in order for there to be any difference at all. But after that there was a clear benefit in favor of docetaxel. As we do in evidence-based medicine, we declared that docetaxel is the new standard of care, and Mitoxantrone has pretty much been in the waste bin of medical oncology ever since.

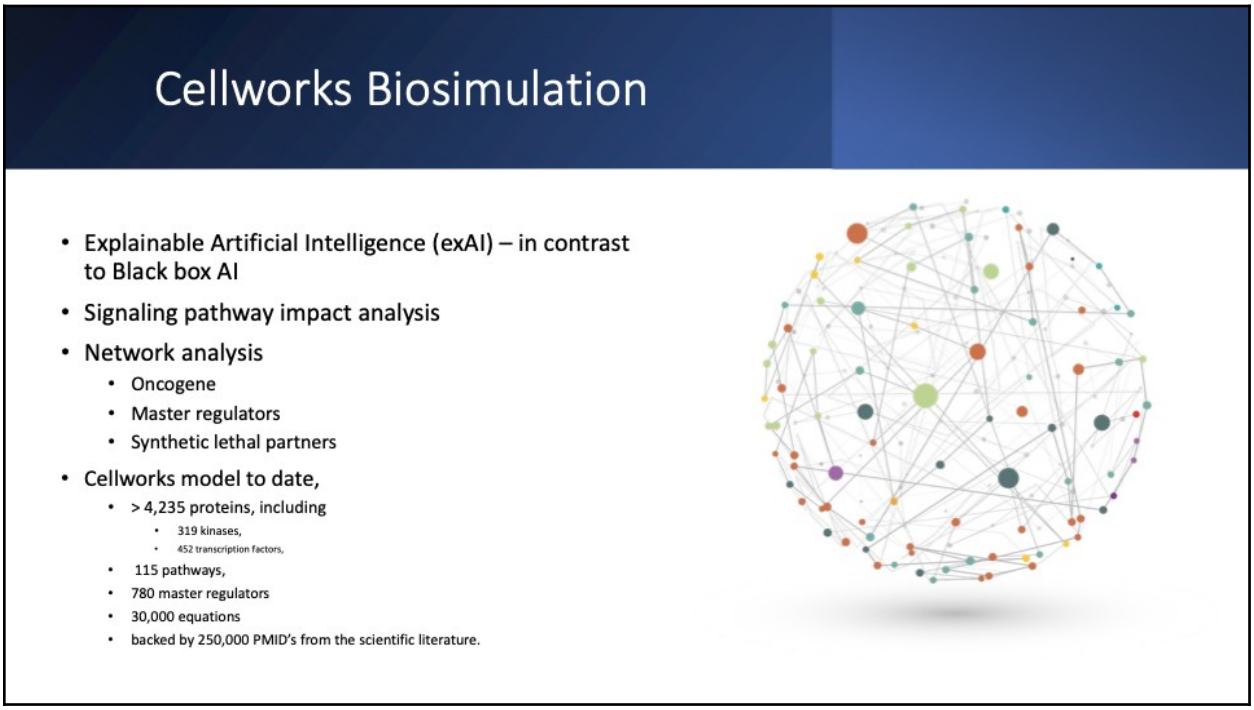
The 10 year follow-up supported this conclusion and enforced this as the standard of care, and today for all patients who show up with hormone-refractory disease, docetaxel is the first line of treatment. In fact, now it's given in the upfront setting with hormone deprivation simultaneously because you can get an even bigger increment.



Michael Castro 5:02
At Cellworks, we take the results of NGS and input it into a computational model of signaling pathways, which can give us a picture of how the disease creates the malignant phenotype, by which I'm talking about the hallmarks of cancer. This is a sophisticated group of people I'm talking to, and you know the hallmarks are uncontrolled proliferation, blockade of apoptosis, angiogenesis, replication, immortality, angiogenesis, immune evasion, and so on, and so forth. There are four new ones, by the way, as of last year.

Once we create this network of disease biology, then we can simulate how drugs and drug combinations interact with the network. And because the whole thing is based on arithmetic, or

actually differential equations, we get a quantitative output about how we think one treatment will be compared to another treatment. Then we get the signaling pathways that are going to show us why.



This is what I call “explainable artificial intelligence”, in contrast to the normal concept of “black box AI”, where you give something 100,000 data points, and then you let the computer find the associations. In the end, you get a model, and you're not quite sure why it says what it says. This, on the other hand, is much more satisfying, particularly if you're giving patients drugs based on it, because you can see what you're doing, and why you're doing it.

Essentially, it's a signaling pathway impact analysis tool.

It shows you the network, the complex, proteogenetic or genomic network that cancer is. This network is characterized by specific nodes, which are oncogenes, master regulators. Master regulators, of course, are involved in determining cells’ fate. All the cells in the body have the same DNA, but they have different specializations based on their transcriptional organization.

Then we have synthetic lethal nodes, which are extremely important because normal tissue doesn't have those. You can kill a cancer by attacking synthetic lethal nodes without harming normal tissue, which is the opposite of how most of us were taught to think about cancer therapy, where the concept is because of the overlap between normal human biology and cancer, there was no alternative except to cause normal tissue toxicity and go to maximum tolerated dose in order to get benefit. If you think about it, synthetic lethality targeting is the opposite. You can kill a cancer without affecting tissue.

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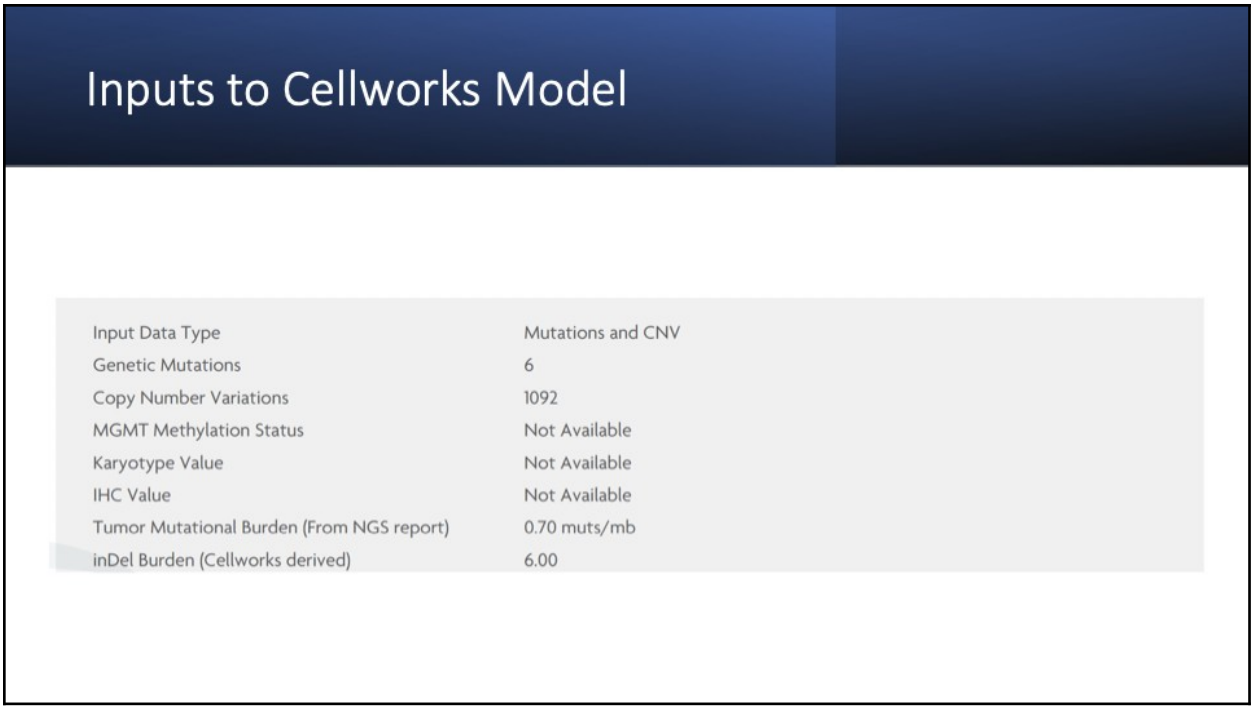
I'm specializing these days in the concept of targeting multiple synthetic lethal nodes simultaneously as a way of taking down the cancer network.



Michael Castro 8:42
I want to show you this specific case that came my way at Cellworks where we have a patient who had six mutations and quite a disorderly copy number aberrations. There were 1092 genes which were either deleted or amplified, low tumor mutational burden (a predictive biomarker of response to immune checkpoint inhibitors, like pembrolizumab/Keytruda), and low inDel burden (insertion-deletion, a highly immunogenic mutational class and thus a potentially superior biomarker). So not an immunogenic disease.

The Cellworks report is long. When you have 1092 pathways, this is what happens. We have a little summary of the mutations. They were all loss-of-function mutations. None of them would be considered particularly actionable. Some of these things, I would have to confess that I would have to look them up, even though I specialize in this. There's a lot of obscurity. Which is why we need a tool like this because there's no oncologist alive, who could sit down and tell you what all these things do. I would challenge anybody to do that.

Here's what the chromosomal aberrations look like (see below). By the way, this is what I call the elephant in the room, because even though the reports that we get from our companies – I'm happy to see that we have somebody from BostonGene here today – focus on mutations. The reason we do that is because the paradigm of treating oncogenes with tyrosine kinase inhibitors has been practice-changing, and transformed the entire field of oncology in the last 20 years. The truth is, it's just the low hanging fruit. Because what's going on in cancer is this:

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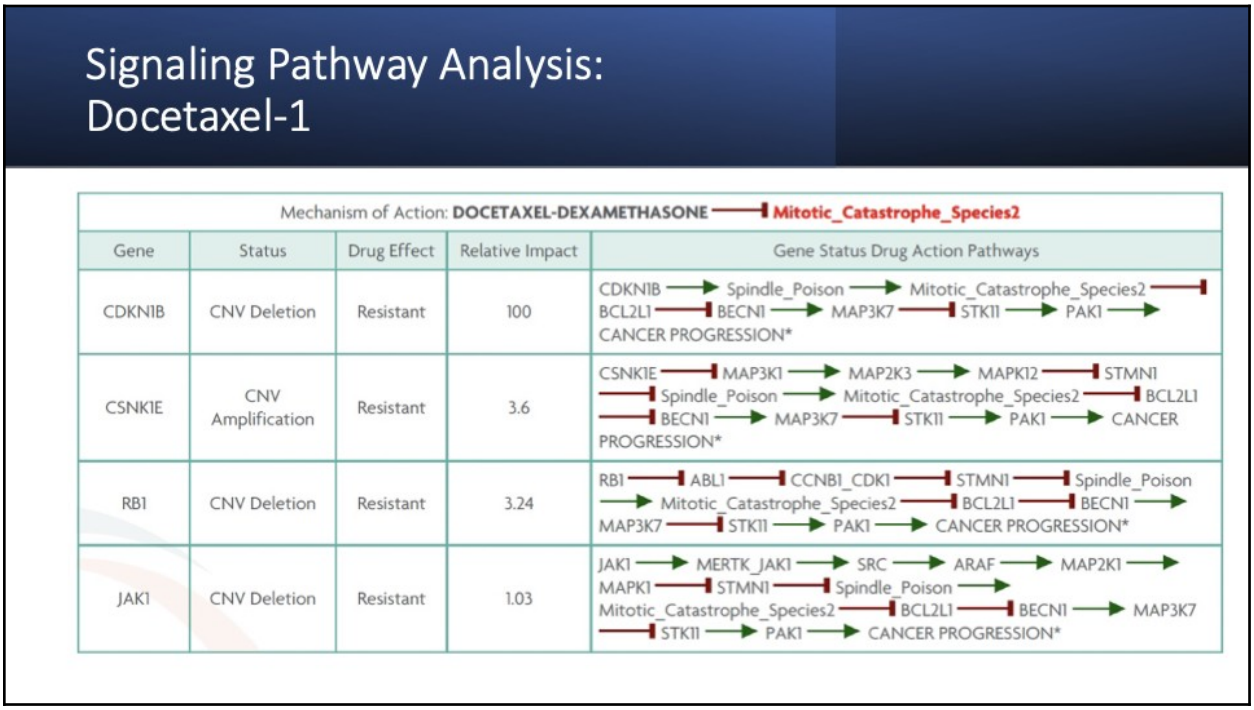
Michael Castro 11:26
We have all of these pieces of chromosomes that are missing and extra copies. This is really what happened. This is really where the disease is coming from.

At Cellworks we are trying to incorporate all of this information in the model to come up with predicting how this makes the cancer be the unique thing it is, and what impact that has on the treatments that are available to us.



Michael Castro 12:18
When we look at this patient's response to docetaxel, you're seeing the gene aberration on the left, the effect that that aberration has on the tumor, and then the signaling pathway that explains what's going on.

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SPIA: Docetaxel-2

Mechanism of Action: **DOCETAXEL-DEXAMETHASONE → SPINDLE POISON ⊣ ANAPC1_CDC26_CDC20 ⊣ CCNB1_CDK1 → MITOTIC_CATASTROPHE ⊣ MITOTIC_SLIPPAGE ⊣ APOPTOSIS**

Gene	Status	Drug Effect	Relative Impact	Gene Status Drug Action Pathways
AURKAIP1	CNV Amplification	Sensitive	NA	AURKAIP1 ⊣ AURKA → PLK1 ⊣ MITOTIC_CATASTROPHE
BIRC5	CNV Amplification	Resistant	NA	BIRC5 → MITOTIC_SLIPPAGE
BRCA2	CNV Deletion	Resistant	NA	BRCA2 → BUB1B → CCNB1_CDK1 → MITOTIC_CATASTROPHE ⊣ MITOTIC_SLIPPAGE ⊣ APOPTOSIS
ENO1	CNV Amplification	Resistant	NA	ENO1 ⊣ SPINDLE POISON
ERBB2	CNV Amplification	Resistant	NA	ERBB2 → PIK3CA → PI345P3 → PDPK1 → AKT ⊣ AKT1S1 ⊣ MTOR → HIF1A → TUBB3 ⊣ DOCETAXEL-DEXAMETHASONE
HSP90AA1	CNV Deletion	Resistant	NA	HSP90AA1 → NR3C1 → NFKBIA ⊣ NFKB1 → CANCER PROGRESSION
NOTCH1	CNV Amplification	Resistant	NA	NOTCH1 → HES1 ⊣ NR3C1 ⊣ FOS_JUN → CANCER PROGRESSION NOTCH1 → HES1 ⊣ NR3C1 NOTCH1 → HES1 ⊣ NR3C1 → NFKBIA ⊣ NFKB1 → HIF1A → FOXM1 → CANCER PROGRESSION

You can see that most of these things are resistant. There are a couple of sensitives in here. It's interesting because we always see in these complex cancers combinations of things that are both sensitive and resistant.

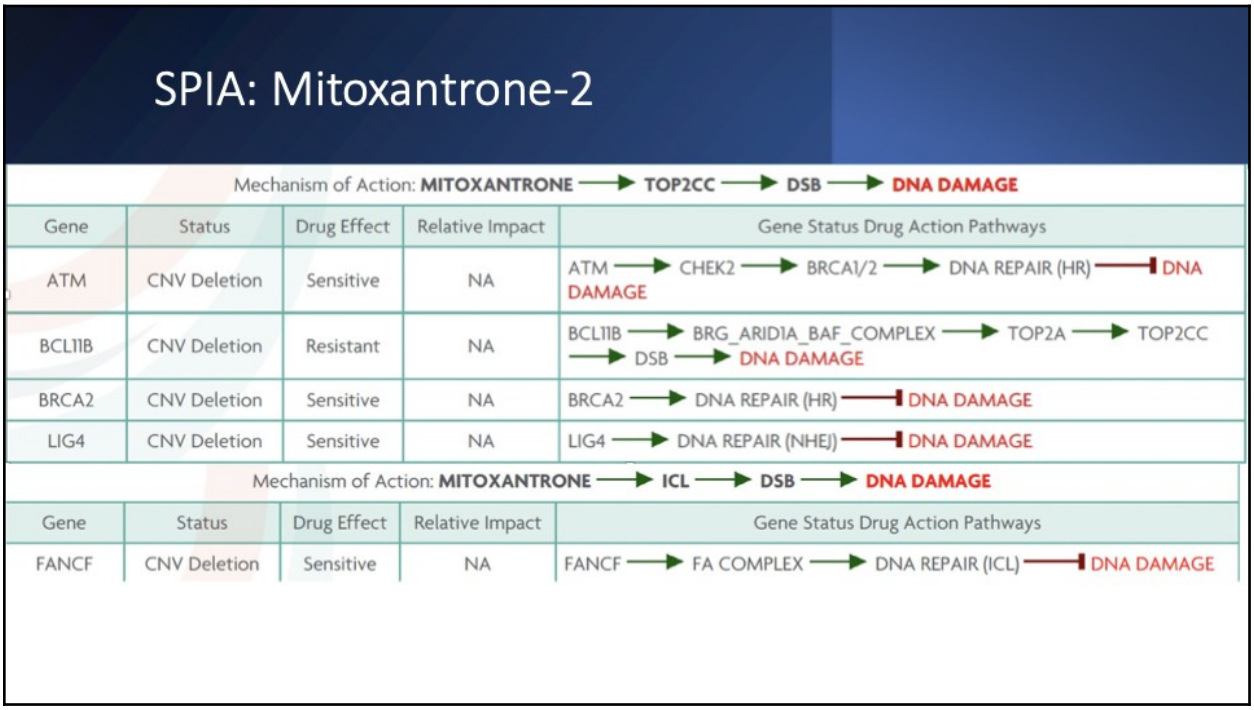
Signaling Pathway Impact Analysis: Mitoxantrone-1

Mechanism of Action: **MITOXANTRONE → DAMAGED_DNA**

Gene	Status	Drug Effect	Relative Impact	Gene Status Drug Action Pathways
CDK9	CNV Amplification	Sensitive	100	CDK9 → SMAD2_SMAD4_SMAD3 ⊣ BRCA1 → CHEK1 → RAD51 → DNA REPAIR(HRRP) ⊣ DAMAGED_DNA → PARP1 ⊣ PKM2 ⊣ AKT1S1 ⊣ MTOR_RPTOR_MLST8_RHEB → CANCER PROGRESSION*
CDKN1B	CNV Deletion	Sensitive	78.76	CDKN1B → CDK5R1_CDK5 → ATM → DNA REPAIR(MMRP) ⊣ DAMAGED_DNA → PARP1 ⊣ PKM2 ⊣ AKT1S1 ⊣ MTOR_RPTOR_MLST8_RHEB → CANCER PROGRESSION*
HSP90AA1	CNV Deletion	Sensitive	3.08	HSP90AA1 → PIM3 → CDKN1B → CDK5R1_CDK5 → ATM → DNA REPAIR(MMRP) ⊣ DAMAGED_DNA → PARP1 ⊣ PKM2 ⊣ AKT1S1 ⊣ MTOR_RPTOR_MLST8_RHEB → CANCER PROGRESSION*
TP53	CNV Deletion	Sensitive	2.5	TP53 ⊣ MNAT1_CDK7_CCNH → CCNB1_CDK1 ⊣ DNA REPAIR(HRRP) ⊣ DAMAGED_DNA → PARP1 ⊣ PKM2 ⊣ AKT1S1 ⊣ MTOR_RPTOR_MLST8_RHEB → CANCER PROGRESSION*
CSNK1E	CNV Amplification	Sensitive	1.67	CSNK1E → PER1 → CHEK2 → MDC1 → NBN_RAD50_MRE11A → DNA REPAIR(TLP) → DAMAGED_DNA → PARP1 ⊣ PKM2 ⊣ AKT1S1 ⊣ MTOR_RPTOR_MLST8_RHEB → CANCER PROGRESSION*
ICMT	CNV Amplification	Sensitive	1.31	ICMT → HRAS → PIK3CG → SET ⊣ PRKDC_XRCC6_XRCC5 → DCLRE1C → DNA REPAIR(NHEJP) ⊣ DAMAGED_DNA → PARP1 ⊣ PKM2 ⊣ AKT1S1 ⊣ MTOR_RPTOR_MLST8_RHEB → CANCER PROGRESSION*

Michael Castro 13:05

Then we get to Mitoxantrone. You start to notice that all the things that were resistant are now sensitive. In fact, a lot of the things that were causing resistance to docetaxel are causing sensitivity to Mitoxantrone.



There's a BRCA2 deletion, which is not germline. It's a somatic change. There is also an ATM deletion, so DNA checkpoints are around. We have the Fanconi pathway down at the bottom. It's also known to impact the response to DNA damaging agents.

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

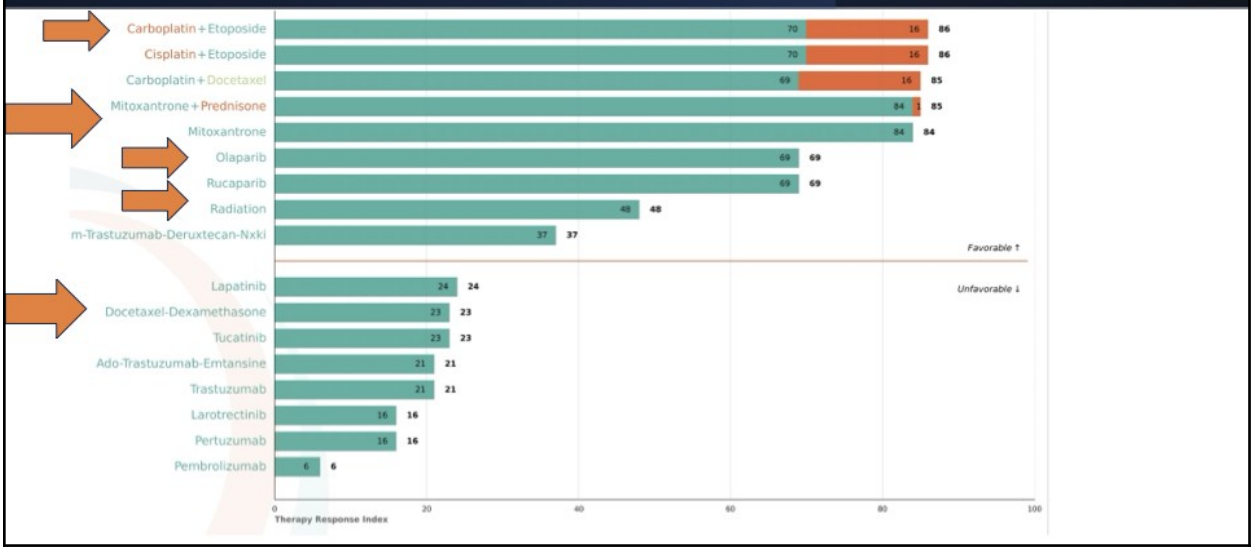
SPIA: Mitoxantrone-3

Mechanism of Action: **MITOXANTRONE** → **TOP2CC** → **DSB** → **DNA DAMAGE**

Gene	Status	Drug Effect	Relative Impact	Gene Status Drug Action Pathways
NBN	CNV Deletion	Sensitive	NA	NBN → RAD50-MRE11A-NBN → DNA REPAIR (HR) ⊣ DNA DAMAGE
PRKDC	CNV Deletion	Sensitive	NA	PRKDC → DNA REPAIR (NHEJ) ⊣ DNA DAMAGE
RAD52	CNV Deletion	Sensitive	NA	RAD52 → DNA REPAIR (HR) ⊣ DNA DAMAGE
RB1	CNV Deletion	Sensitive	NA	RB1 → PRKDC_XRCC5_XRCC6 → DNA REPAIR (NHEJ) ⊣ DNA DAMAGE
TET2	CNV Deletion	Sensitive	NA	TET2 ⊣ CPGMET ⊣ BRCA1/2 → DNA REPAIR (HR) ⊣ DNA DAMAGE
TOP2A	CNV Amplification	Sensitive	NA	TOP2A → TOP2CC
WT1	CNV Deletion	Resistant	NA	WT1 → DNMT3A → CPGMET ⊣ XRCC5 → DNA REPAIR (NHEJ) ⊣ DNA DAMAGE
XRCC3	CNV Deletion	Sensitive	NA	XRCC3 → RAD51 → DNA REPAIR (HR) ⊣ DNA DAMAGE
XRCC4	CNV Deletion	Sensitive	NA	XRCC4 → DNA REPAIR (NHEJ) ⊣ DNA DAMAGE

Michael Castro 13:47
There's TOP2A amplification. Everybody knows this is Mitoxantrone and also happens to be an etoposide- and anthracycline-sensitizing pathway.

Therapy prediction index...



Michael Castro 14:00

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

Here is the therapy prediction index that is the main result of Cellworks. It shows about a fourfold difference between Mitoxantrone and prednisone, and docetaxel.

If you hadn't done this biosimulation, you would be giving your patient docetaxel, and when that didn't work, you would give them cabazitaxel, which is also, by the way, not going to work in this patient for similar reasons. They're both tubulin-targeting agents. The drugs that really do work, which are the ones on top here, are not going to be assessed.

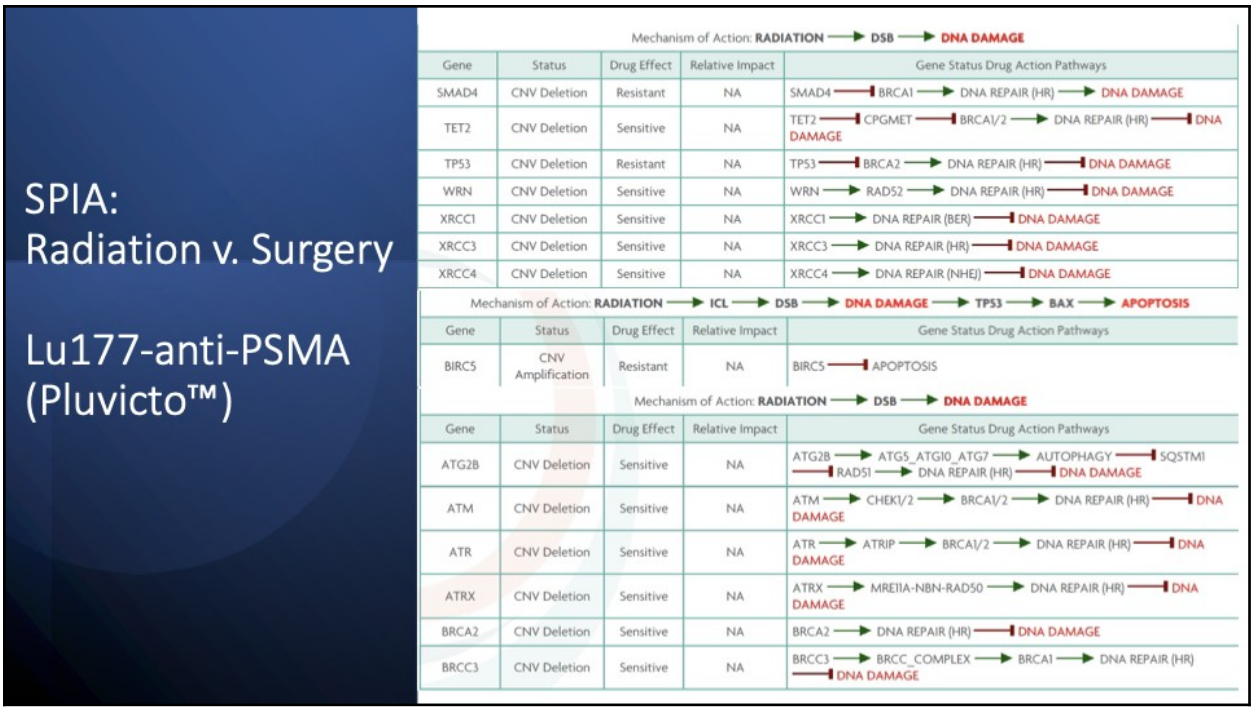
By the way, we find out that even though this patient doesn't have germline BRCA1 or 2, they have this really nice responsiveness to PARP inhibitors. Got a great response here to platinum drugs and BP16 also. Hits topoisomerase 2a, and so you can get an additive effect. Radiation is sort of middling.

Mechanism of Action: OLAPARIB ⊣ PARP1/2 → DNA REPAIR (HR) ⊣ DNA DAMAGE

Gene	Status	Drug Effect	Relative Impact	Gene Status Drug Action Pathways
ATM	CNV Deletion	Sensitive	NA	ATM → CHEK1/2 → BRCA1/2 → DNA REPAIR (HR) ⊣ DNA DAMAGE
ATR	CNV Deletion	Sensitive	NA	ATR → CHEK1/2 → BRCA1/2 → DNA REPAIR (HR) ⊣ DNA DAMAGE
BRCA2	CNV Deletion	Sensitive	NA	BRCA2 → DNA REPAIR (HR) ⊣ DNA DAMAGE
CDKN1B	CNV Deletion	Resistant	NA	CDKN1B ⊣ CDK4_CCND1 → BRCA1 → DNA REPAIR (HR) ⊣ DNA DAMAGE
CHD1	CNV Deletion	Sensitive	NA	CHD1 → DSB → DNA REPAIR (HR) ⊣ DNA DAMAGE
FANCF	CNV Deletion	Sensitive	NA	FANCF → FA COMPLEX → DNA REPAIR (HR) ⊣ DNA DAMAGE
FANCI	CNV Deletion	Sensitive	NA	FANCI → FANCD2 → FA COMPLEX → DNA REPAIR (HR) ⊣ DNA DAMAGE
FANCM	CNV Deletion	Sensitive	NA	FANCM → FA COMPLEX → DNA REPAIR (HR) ⊣ DNA DAMAGE
NBN	CNV Deletion	Sensitive	NA	NBN → MRE11A-NBN-RAD50 → DNA REPAIR (HR) ⊣ DNA DAMAGE
RAD51B	CNV Deletion	Sensitive	NA	RAD51B → RAD51 → DNA REPAIR (HR) ⊣ DNA DAMAGE
RAD52	CNV Deletion	Sensitive	NA	RAD52 → RAD51 → DNA REPAIR (HR) ⊣ DNA DAMAGE
RAD54B	CNV Deletion	Sensitive	NA	RAD54B → RAD51 → DNA REPAIR (HR) ⊣ DNA DAMAGE
RB1	CNV Deletion	Sensitive	NA	RB1 → BRCA1 → DNA REPAIR (HR) ⊣ DNA DAMAGE
REV3L	CNV Deletion	Sensitive	NA	REV3L → DNA REPAIR (TLS) ⊣ DNA DAMAGE
SMAD4	CNV Deletion	Sensitive	NA	SMAD4 → BRCA1 → DNA REPAIR (HR) ⊣ DNA DAMAGE
TET2	CNV Deletion	Sensitive	NA	TET2 ⊣ CPGMET ⊣ BRCA1/2 → DNA REPAIR (HR) ⊣ DNA DAMAGE
TP53	CNV Deletion	Resistant	NA	TP53 ⊣ BRCA2 → DNA REPAIR (HR) ⊣ DNA DAMAGE
WRN	CNV Deletion	Sensitive	NA	WRN → RAD52 → DNA REPAIR (HR) ⊣ DNA DAMAGE

Michael Castro 15:14
Just to show you the signaling impact of various pathways, on olaparib, you can see that those are mostly all sensitive.

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]



Mechanism of Action: RADIATION → DSB → DNA DAMAGE

Gene	Status	Drug Effect	Relative Impact	Gene Status Drug Action Pathways
SMAD4	CNV Deletion	Resistant	NA	SMAD4 ⊣ BRCA1 → DNA REPAIR (HR) → DNA DAMAGE
TET2	CNV Deletion	Sensitive	NA	TET2 ⊣ CPGMET ⊣ BRCA1/2 → DNA REPAIR (HR) ⊣ DNA DAMAGE
TP53	CNV Deletion	Resistant	NA	TP53 ⊣ BRCA2 → DNA REPAIR (HR) ⊣ DNA DAMAGE
WRN	CNV Deletion	Sensitive	NA	WRN → RAD52 → DNA REPAIR (HR) ⊣ DNA DAMAGE
XRCC1	CNV Deletion	Sensitive	NA	XRCC1 → DNA REPAIR (BER) ⊣ DNA DAMAGE
XRCC3	CNV Deletion	Sensitive	NA	XRCC3 → DNA REPAIR (HR) ⊣ DNA DAMAGE
XRCC4	CNV Deletion	Sensitive	NA	XRCC4 → DNA REPAIR (NHEJ) ⊣ DNA DAMAGE

Mechanism of Action: RADIATION → ICL → DSB → DNA DAMAGE → TP53 → BAX → APOPTOSIS

Gene	Status	Drug Effect	Relative Impact	Gene Status Drug Action Pathways
BIRC5	CNV Amplification	Resistant	NA	BIRC5 ⊣ APOPTOSIS

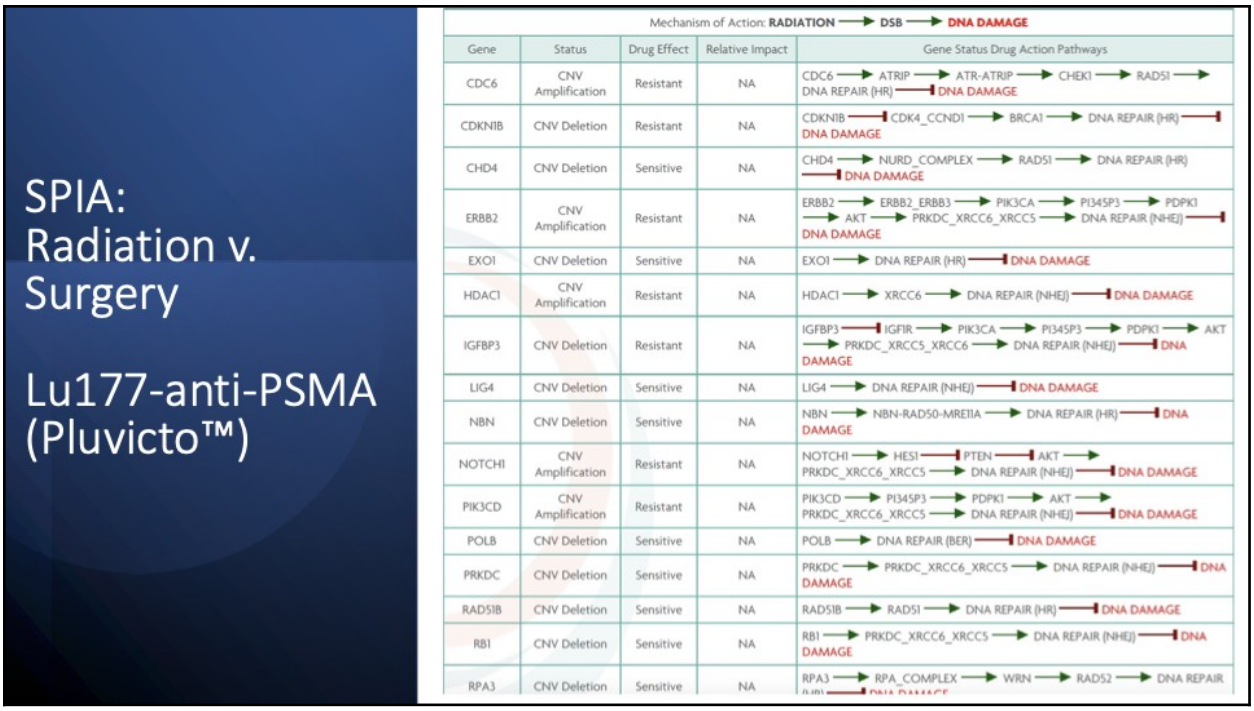
Mechanism of Action: RADIATION → DSB → DNA DAMAGE

Gene	Status	Drug Effect	Relative Impact	Gene Status Drug Action Pathways
ATG2B	CNV Deletion	Sensitive	NA	ATG2B → ATG5_ATG10_ATG7 → AUTOPHAGY ⊣ SQSTM1 ⊣ RAD51 → DNA REPAIR (HR) ⊣ DNA DAMAGE
ATM	CNV Deletion	Sensitive	NA	ATM → CHEK1/2 → BRCA1/2 → DNA REPAIR (HR) ⊣ DNA DAMAGE
ATR	CNV Deletion	Sensitive	NA	ATR → ATRIP → BRCA1/2 → DNA REPAIR (HR) ⊣ DNA DAMAGE
ATRX	CNV Deletion	Sensitive	NA	ATRX → MRE11A-NBN-RAD50 → DNA REPAIR (HR) ⊣ DNA DAMAGE
BRCA2	CNV Deletion	Sensitive	NA	BRCA2 → DNA REPAIR (HR) ⊣ DNA DAMAGE
BRCC3	CNV Deletion	Sensitive	NA	BRCC3 → BRCC_COMPLEX → BRCA1 → DNA REPAIR (HR) ⊣ DNA DAMAGE

As far as radiation goes, I put this in here because people who are facing prostate cancer, as an initial diagnosis, are essentially confronting the surgery versus radiation issue.

At ESMO (the European Society for Medical Oncology), there was a very interesting concept of using this new radio-antibody Pluvicto in the upfront setting. Should we do that or not? This analysis can help us understand the relative utility of these various agents for individual patients. Here, the same thing that creates sensitivity to the DNA-damaging agents also creates sensitivity to radiation. On the other hand, the efficacy of radiation requires the intactness of the non-homologous end joining pathway, which has some deficits.

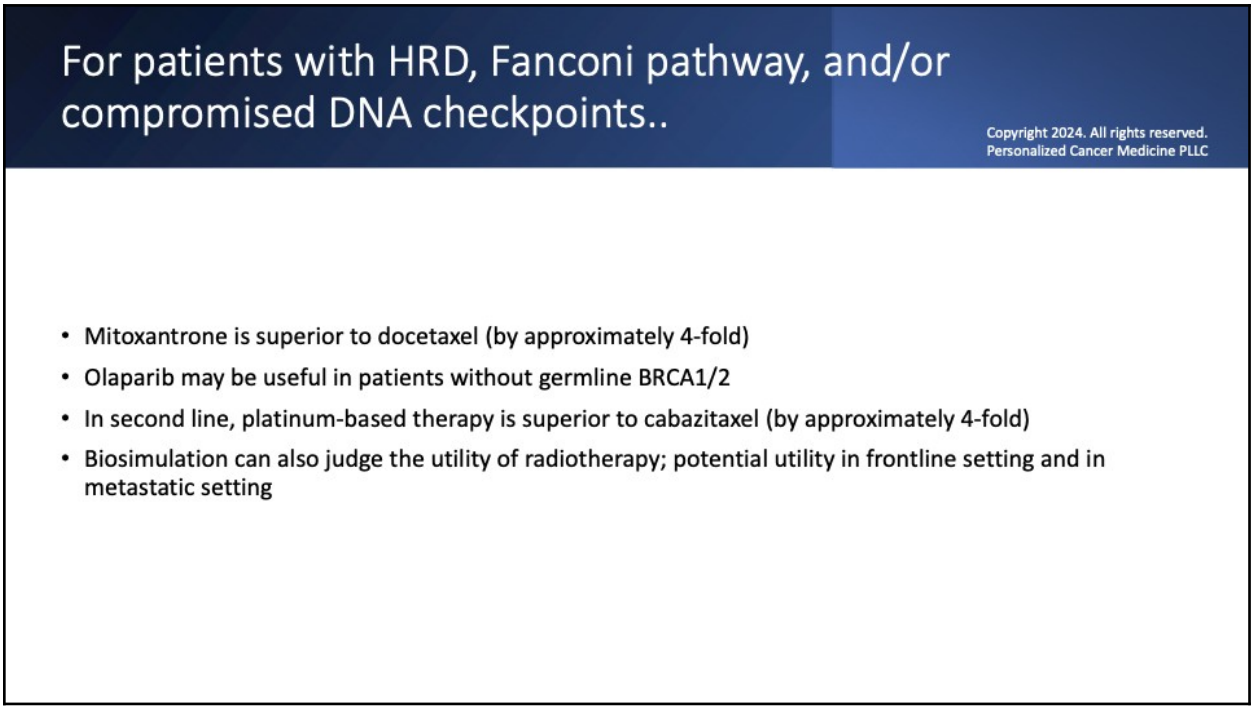
“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]



Mechanism of Action: **RADIATION** → **DSB** → **DNA DAMAGE**

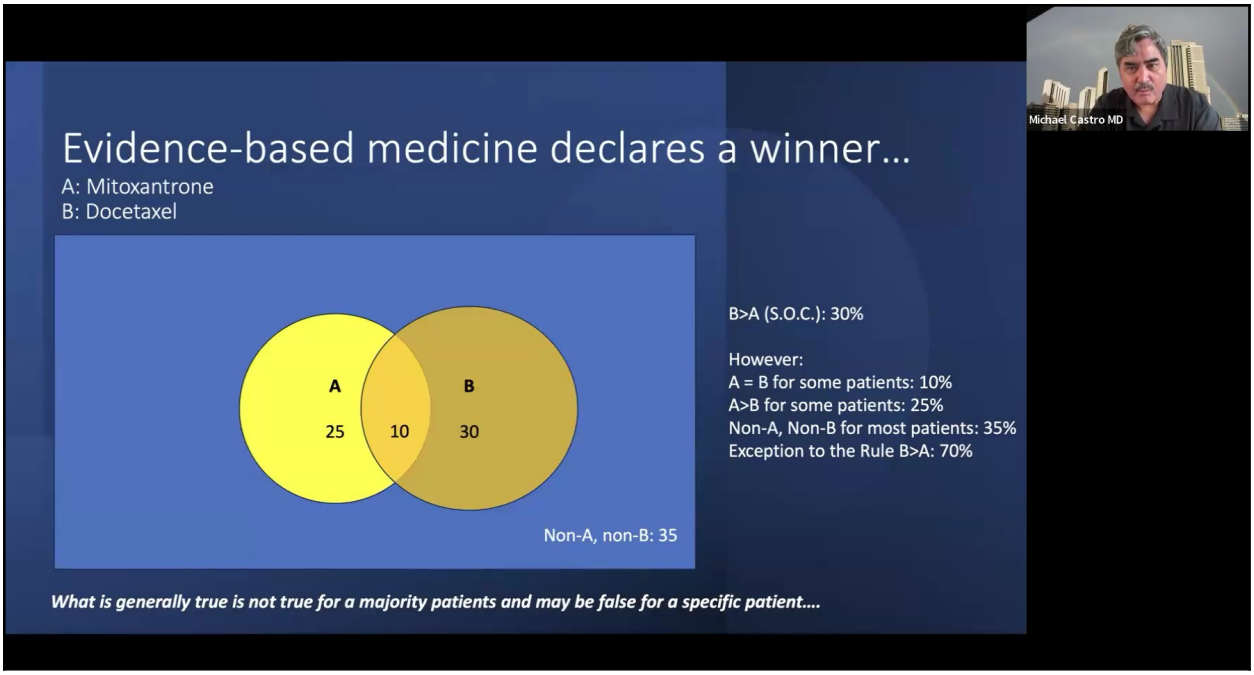
Gene	Status	Drug Effect	Relative Impact	Gene Status Drug Action Pathways
CDC6	CNV Amplification	Resistant	NA	CDC6 → ATRIP → ATR-ATRIP → CHEK1 → RAD51 → DNA REPAIR (HR) ⊣ DNA DAMAGE
CDKN1B	CNV Deletion	Resistant	NA	CDKN1B ⊣ CDK4_CCND1 → BRCA1 → DNA REPAIR (HR) ⊣ DNA DAMAGE
CHD4	CNV Deletion	Sensitive	NA	CHD4 → NURD_COMPLEX → RAD51 → DNA REPAIR (HR) ⊣ DNA DAMAGE
ERBB2	CNV Amplification	Resistant	NA	ERBB2 → ERBB2_ERBB3 → PIK3CA → PI345P3 → PDPK1 → AKT → PRKDC_XRCC6_XRCC5 → DNA REPAIR (NHEJ) ⊣ DNA DAMAGE
EXO1	CNV Deletion	Sensitive	NA	EXO1 → DNA REPAIR (HR) ⊣ DNA DAMAGE
HDAC1	CNV Amplification	Resistant	NA	HDAC1 → XRCC6 → DNA REPAIR (NHEJ) ⊣ DNA DAMAGE
IGFBP3	CNV Deletion	Resistant	NA	IGFBP3 ⊣ IGF1R → PIK3CA → PI345P3 → PDPK1 → AKT → PRKDC_XRCC5_XRCC6 → DNA REPAIR (NHEJ) ⊣ DNA DAMAGE
LIG4	CNV Deletion	Sensitive	NA	LIG4 → DNA REPAIR (NHEJ) ⊣ DNA DAMAGE
NBN	CNV Deletion	Sensitive	NA	NBN → NBN-RAD50-MRE11A → DNA REPAIR (HR) ⊣ DNA DAMAGE
NOTCH1	CNV Amplification	Resistant	NA	NOTCH1 → HES1 ⊣ PTEN ⊣ AKT → PRKDC_XRCC6_XRCC5 → DNA REPAIR (NHEJ) ⊣ DNA DAMAGE
PIK3CD	CNV Amplification	Resistant	NA	PIK3CD → PI345P3 → PDPK1 → AKT → PRKDC_XRCC6_XRCC5 → DNA REPAIR (NHEJ) ⊣ DNA DAMAGE
POLB	CNV Deletion	Sensitive	NA	POLB → DNA REPAIR (BER) ⊣ DNA DAMAGE
PRKDC	CNV Deletion	Sensitive	NA	PRKDC → PRKDC_XRCC6_XRCC5 → DNA REPAIR (NHEJ) ⊣ DNA DAMAGE
RAD51B	CNV Deletion	Sensitive	NA	RAD51B → RAD51 → DNA REPAIR (HR) ⊣ DNA DAMAGE
RB1	CNV Deletion	Sensitive	NA	RB1 → PRKDC_XRCC6_XRCC5 → DNA REPAIR (NHEJ) ⊣ DNA DAMAGE
RPA3	CNV Deletion	Sensitive	NA	RPA3 → RPA_COMPLEX → WRN → RAD52 → DNA REPAIR

That's why radiation came out as somewhat inferior, let's say not as active, as the DNA-damaging agents.



Michael Castro 16:54
Basically, Mitoxantrone looks like it's about fourfold superior to docetaxel. I've seen at Cellworks, by the way, that this happens to be true in patients with homologous recombination

deficiency in general. We are giving the wrong treatment to these patients. This patient did not have germline therapy, and this would have been missed. That's what makes it such a great example. Some HRD, some Fanconi pathway, some compromised DNA checkpoints, and together all of those things, makes the DNA-damaging agents and the PARP inhibition viable therapies, whereas the standard of care, docetaxel or Cabazitaxel, really don't have much.

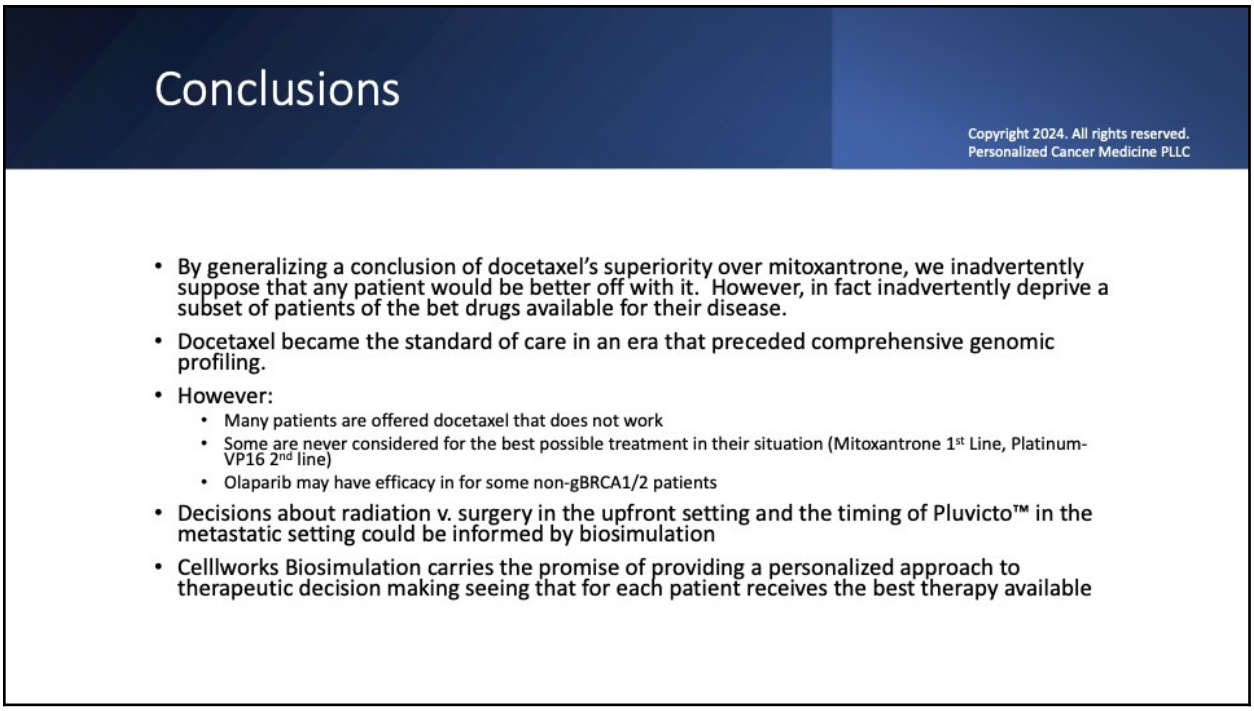


Michael Castro 17:45
In evidence-based medicine we do these huge randomized trials. They have to be huge in order to find tiny differences. During the conduct of the trial, the investigators cannot tell who's winning. That's the problem of marginal therapy. You need a statistician and 1000 patients to tell you that something's better.

But here's the problem: even though it's true that, as I've illustrated here, B is better than A because it's got a bigger circle, it's only true for 30% of patients. A is equal to B, the other drug could have been effective 10% of the time. A was in fact better than B the opposite 25% of the time, and neither therapy was effective for 35% of the patients. When you add up all of those numbers, what's generally true is that docetaxel being better than Mitoxantrone happens to not be true for 70% of the patients.

This is an interesting thing about the way we work in cancer, where many of our therapies are marginal. The fallacy of marginal benefit is that we over-celebrate these trials.

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]



Michael Castro 19:22
In the era of biosimulation, we can do a lot better than that by individualizing the treatment for specific patients. Companies like BostonGene that are doing whole exome, and who have been extremely gracious in terms of sharing the raw data, the copy number. We're in this era of transitioning to transcriptome. They're going to be leading the way here, and I at least am optimistic that I can do a better job for my personal patients doing this.

I felt some obligation to get this news out to this group. Because I think you guys can appreciate this and are in a position to raise consciousness about it.

We're going to need to do a prospective randomized trial with Cellworks biosimulation in order to change medicine. I don't know where the money for that is coming from.

I see myself as being involved with Cellworks to try to raise consciousness about the fact that we're mistreating patients, where we are coming up with the exact wrong therapy. Since I'm a member of this profession, I have to admit I’m “guilty as charged”, because we've been stuck in the evidence-based medicine fallacy of marginal therapies.

Robert Gurmankin 21:44
This is very interesting, especially since there are people on this call who I've been reaching out to on these issues.

When you have heavily pretreated patients, you have even more heterogeneity of the cancer. When you're taking biopsy samples, and running your biosimulations, you may get two different samples and get two totally different simulations. So how do you work with that?

Michael Castro 22:26
It's one of the great challenges. Tumor heterogeneity increases over time because of genomic instability. There's a relationship between the degree of genomic instability and our ability to run after this disease. From a practical point of view, allele frequency is something I use to try to sort that out.

My latest patient is a fellow with liver cancer. He has in his circulating tumor DNA, two genes with mutation rates in the 30% range. Everything else is below 1%. Four mutations below 1%. They're all really bad oncogenic drivers. It's got these other two in the 30% range. I can neglect those four for the time being. As I re-measure, as I follow this fellow, and going forward, we may be selecting out those resistant clones.

You treat the thing that has the most life threatening impact in terms of disease burden, knowing that there are other clones you may not be treating, and you're going to have to come and deal with them. It’s like a chess game. You're not going to get this in one shot. You have to be ready with your mid-game strategy and your end-game strategy, and adopt an attitude where you look at it in this dynamic way, as opposed to having some sort of algorithm fixed by the NCCN.

Robert Gurmankin 24:36
How can I put that into practice? I'm scheduled for a bone biopsy next week. Michael Hensley at BostonGene has been very kind, and they are willing to do sequencing.

I’ve also been trying to reach out to Dr. Apfel (at Sage Medic) who got the message from his office about calling about functional testing, but the more data the better.

Michael Castro 25:04
I'm working with Chris Apfel myself. He's a good guy, and he's also got a great team.

It depends on knowing the individual patient situation: How much bone metastases? How many visceral metastases? What is the burden of disease in each site? What does the liquid biopsy show us in terms of heterogeneity? The other thing about liquid biopsy is it gives you the sense of diversity of metastatic behavior in the body. You might not get a mutation that's in the lung, for example, if you biopsy in the BOM (bone marrow). But you could see that with a liquid biopsy, so I'd like to do them both because they're complementary.

Robert Gurmankin 26:07
To possibly have you work on mine, I would do that through BostonGene?

Michael Castro 26:17
BostonGene is doing a great job, and I'm really happy to work with them.

Allen Morris 26:25

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

I heard you say the phrase “synthetic lethal nodes”. I've never heard that before. Did your group invent that concept? Or is that well known in molecular biology?

Michael Castro 26:49
I invented that concept.

Synthetic lethality [as opposed to “synthetic lethality node”] is very well defined. It's been in the literature for probably at least a dozen years.

When I get the results of the genomic analysis, I'm looking for how many things I can treat, and then I try to target as many as possible.

I've had some remarkable successes, including a case that was published in the Frontiers of Oncology last fall (“Network-targeting combination therapy of leptomeningeal glioblastoma using multiple synthetic lethal strategies: a case report”), about a person with relapsed glioblastoma. leptomeningeal spread with a terrible prognosis, who not only got a complete remission, but is alive 27 months later, with no evidence of disease.

> *Summary Excerpt: Network targeting of disease-specific nodes represents a useful principle for designing combination cancer therapy. In this case of a patient with relapsed leptomeningeal glioblastoma, comprehensive molecular diagnosis led to the identification of a disease network characterized by multiple disease-specific synthetic lethal vulnerabilities involving DNA repair, REDOX homeostasis, and impaired autophagy which suggested a novel network-targeting combination therapy (NTCT). A treatment regimen consisting of lomustine, olaparib, digoxin, metformin, and high dose intravenous ascorbate was employed using the principle of intra-patient dose escalation to deliver the treatment with adequate safety measures to achieve a definitive clinical result.*

Why is she doing so well? Her situation was unique in that she had about a dozen synthetic lethal nodes that were all successfully targeted. This idea of network targeting and combination therapy came to mind.

I thought I invented this [in addition to synthetic lethal nodes]. It turns out that [network targeting] was invented by an Australian mathematician, Robin Williams. It's the idea that the network is like the Paris Metro or the London Underground, if you've been to those places. They have such amazing redundant systems. If one station goes down, you take a different line and can go around.

Normally what we do in oncology when we have a network like that, we drop a bomb in the middle and hope that that's going to do it. That's not really the way it works because people will just say, “You have to take a different train and go around.” That's what the cancer is doing. On the other hand, if you create a small explosion in multiple stations, key stations along the way,

whether those are **synthetic lethal nodes, oncogenic nodes, or master regulator nodes,** the network collapses. If you can hit enough of those nodes in the network, it doesn't recover.

If you hit Achilles in the heel, he can still sit up and fire his arrow at you. But if you hit him in both heels and both elbows, he's not going to get up. That's the idea.

The synthetic lethal node is an important node. It is the key for how we're going to go forward in combination therapy for cancer. Because of all of these toxic treatments that we have, you get to the problem of polypharmacy (using five or more medications). Eventually the human body can only take so much. But if you're doing synthetic lethality and normal tissue can handle things, then you can stack therapies, one on top of each other, without really ever getting anywhere near the MTD (maximum tolerated dose).

That patient I just mentioned, who had the great remission, had no toxicity, which is even more remarkable.

I've written a couple of publications about this in the last year. If you're interested, here they are:

Publications

E. C. Smyth", D. Watson2, M. P. Castro, et al. **Integration of genomic aberrations to predict clinical outcomes for patients with gastroesophageal adenocarcinoma receiving neoadjuvant. chemotherapy.** ESMO Gastrointestinal Oncology January 22, 2024, DOI:https://doi.org/10.1016/j.esmogo.2023.08.009

Michael P Castro, Kristin Dittmar, **Network targeting combination therapy (NTCT) of synthetic lethal (SL) vulnerabilities in *9p21* deficient glioblastoma (GBM): a case report,** *Neuro-Oncology Advances*, 2023;, vdad162, https://doi.org/10.1093/noajnl/vdad162

Castro MP, Sipos B, Biskup S and Kahn N. **Network-targeting combination therapy of leptomeningeal glioblastoma using multiple synthetic lethal strategies: a case report.** (2023) Front. Oncol. 13:1210224. doi: 10.3389/fonc.2023.1210224 https://www.frontiersin.org/journals/oncology/articles/10.3389/fonc.2023.1210224/full

Engineering Level Molecular Diagnosis: A Necessity for CancerTreatment (and for Moonshot Success.) Michael P. Castro, *Cancer Letter* 2023, 49(29), https://cancerletter.com/trials-and-tribulations/20230721_7/

Use of proton pump inhibitors (PPI) in glioblastoma (GBM) is detrimental to overall survival in a national real-world evidence (RWE) database. Michael P. Castro, Jameson Quinn, Asher Wasserman, Mark Shapiro, Timothy J. Stuhlmiller, Santosh Kesari. DOI: 10.1200/JCO.2023.41.16_suppl.e14027 *Journal of Clinical Oncology* 41, no. 16_suppl (June 01, 2023) e14027-e14027.

Use of Computational Biosimulation to Identify NSCLC Patients Receiving Immunotherapy Who Will Benefit from the Addition of Chemotherapy. JA Wingrove, MA. Klein, D Watson,… MP Castro. ASCO Annual Meeting, 2023: Abstract 406716

The impact of mismatch repair deficiency (MMRD) on survival of temozolomide (TMZ)-treated patients with MGMT methylated (m-MGMT) glioblastoma (GBM): a Cellworks computational biosimulation pilot study. Michael Castro et al. *Neuro-Oncology*, Volume 24, Issue Supplement_7, November 2022, Pages vii116–*vii117,* *https://doi.org/10.1093/neuonc/noac209.441*

Targeting chromosome 12q amplification in relapsed glioblastoma: the use of computational biological modeling to identify effective therapy—a case report. Castro MP, Khanlou N, Fallah A, Pampana A, Alam A, Lala DA, Roy KGG, Amara ARR, Prakash A, Singh D, Behura L, Kumar A, Kapoor S. Ann Transl Med 2022 | https://dx.doi.org/10.21037/atm-2022-62 https://atm.amegroups.com/article/view/104196/pdf

Targeting the Complex Protein Network of MYCN-amplified Anaplastic Ependymoma: A Case Report. Castro MP. J Cell Signal. 2022;3(4):179-192. https://www.scientificarchives.com/article/targeting-the-complex-protein-network-of-mycn-amplified-anaplastic-ependymoma-a-case-report

Michael P Castro, Unintended effects of proton pump inhibitors (PPIs) in patients with glioblastoma (GBM): A double-edged sword, *Neuro-Oncology Practice*, Volume 9, Issue 4, August 2022, Pages 344–345, https://doi.org/10.1093/nop/npac035

Castro, M., Pampana, A., Alam, A. *et al.* **Combination chemotherapy versus temozolomide for patients with methylated MGMT (m-MGMT) glioblastoma: results of computational biological modeling to predict the magnitude of treatment benefit.** *J Neurooncol* (2021). https://doi.org/10.1007/s11060-021-03780-0; https://rdcu.be/cmb23; https://link.springer.com/content/pdf/10.1007/s11060-021-03780-0.pdf.https://rdcu.be/cmb23

Michael P. Castro, Mohammad Afshar, Coralie Williams, et al. **Utility of Serial Transcriptomic Analyses to Characterize the Resistome and to Refine Treatment Selection for Metastatic Colon Cancer: Case Report. Clinical Colorectal Cancer 2021, 20(1): 96-99.**DOI : https://doi.org0.1016/j.clcc.2020.10.003 https://www.clinical-colorectal-cancer.com/action/showPdf?pii=S1533-0028%2820%2930141-9

The impact of mismatch repair deficiency (MMRD) on survival of temozolomide (TMZ)-treated patients with MGMT methylated (m-MGMT) glioblastoma. Michael Castro, Fabio Iwamoto, Manmeet Ahluwalia, et al., *Neuro-Oncology*, Volume 24, Issue Supplement_7, November 2022, Pages vii116–vii117, https://doi.org/10.1093/neuonc/noac209.441

Use of proton pump inhibitors (PPI) in glioblastoma (GBM) is detrimental to overall survival in a national real-world evidence (RWE) database. Michael P. Castro, Jameson Quinn, Asher Wasserman, Mark Shapiro, Timothy J. Stuhlmiller, Santosh Kesari. ASCO Annual Meeting 2023, Abstract e14027.

Allen Morris 31:06
I'm guessing that your synthetic lethal nodes are largely the homologous DNA repair pathways, such as BRCA and ATM, but are there other ones? Like, [P53] that are your “synthetic lethality nodes” that you hit simultaneously for your combinatory synthetic kill?

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

Michael Castro 31:40
You're absolutely right. We already know about the HR pathway (homologous repair, used by cells to repair DNA breaks that occur on both strands of DNA). The HR pathway includes Fanconi repair (a complex DNA repair mechanism involving homologous recombination, nucleotide excision repair, and mutagenic translesion synthesis) and the DNA damage checkpoints. Nucleotide excision repair (the main pathway to remove bulky DNA lesions from DNA) is also extremely important for certain types of drugs, for example, the nitrosoureas in neuro-oncology. (Nitrosoureas are often used in chemotherapy for brain cancers such as glioblastoma because they can cross the blood–brain barrier.)

Normally a p53 mutation, for example, is a bad thing because we rely on p53 to trigger apoptosis (cell death) under DNA damage. (The p53 gene is a tumor suppressor gene, i.e., its activity stops the formation of tumors.) If your p53 is knocked out, or functionally disabled, then you're not going to trigger apoptosis. But, on the other hand, you need p53 to express XPC (a gene that provides instructions for making a protein that is involved in repairing damaged DNA) for nucleotide excision repair. If you don't have that, it turns out certain drugs, including platinum agents, are much more effective.

It becomes another one of those things where the mutation for one drug could be bad, but for another drug could be good. I try to be extra aware of those.

There are other things too, besides the DNA damage repair, and one of them has to do with energy. For example, if you have STK11 (serine/threonine kinase 11, a biomarker and potential target of interest, especially for patients with lung cancer), you're going to have difficulty with triggering autophagy (the degradation of the cell that removes unnecessary or dysfunctional components) for energy production.

One of the things I did in the GBM (glioblastoma) case was to target ATP synthesis (part of the cellular respiration process for energy production) at the level of both the ATP pump on the cell membrane using digitalis (a heart medicine) and metformin (which controls the amount of glucose in your blood), targeting of complex I in the mitochondria (the first enzyme of the respiratory chain).

By the way, this is the ultimate in translational medicine because all of my colleagues say, “This is speculative. There's no evidence for this.” They're absolutely right. No one has done clinical trials of this stuff. But if you read enough molecular biology, these are the things which PhDs in the labs have been spending their whole careers on. It's taken as fact. It's not speculative in the laboratory. It's speculative in the clinic. It may be speculative, but it illuminates an otherwise dark room.

Another area that is interesting for synthetic lethality is in the area of oxidative stress, because oxidative stress has to be extremely tightly managed, otherwise it kills the cell. It seems that some oxidative stress is oncogenic (drives cancer) because it activates oncogenes and can

disable tumor suppressor genes. But if it gets out of hand, then it's going to lead to cell death. So the cell's ability to defend against oxidative stress is usually upregulated, but it can be targeted. That's another area that I am always paying attention to these days.

Frank Nothaft 35:35
I'm a molecular biologist or bioinformatician, depending on how people look at me.

I definitely relate to your point here about a lot of this being known in the molecular biology literature, but not in the clinical space.

Have you tried to do any synthetic trial replication studies, to see if you can explain some of the trials where you see tremendously variable strong response/non-response?

For instance, one that comes to mind is pembrolizumab (Keytruda, an immune checkpoint inhibitor), especially as pembrolizumab has moved into TMBH (tumor mutational burden high, tumors that have a high number of mutations appear to be more likely to respond to certain types of immunotherapy) and MSIH (microsatellite instability high, a high number of genetic mutations indicating possible response to immunotherapy), where you see some patients who have tremendous response and other patients who don't.

It feels like the model that you're looking at could be very insightful in trying to explain what the drivers of response/non-response are in a case like that through a synthetic validation. Obviously, it wouldn't replace full clinical validation, but it'd be an interesting study in the middle.

Michael Castro 36:54
You're going to see some publications in the near future in non-small cell lung cancer on prospective retrospective studies where the outcomes are known. We get the genomic data in a blinded fashion, we put it through the model, and then we predict the outcome. We're working with the statistician Drew Watson. Drew is the person who invented Oncotype DX for breast cancer. He's been a delight to work with. He can take a given parameter and come up with a way of weighting that with exponents, according to its relationship with survival, and then in the end, create an equation which will tell you whether or not immunotherapy is going to work, and what the patient's survival will be, given a certain set of inputs.

We are addressing the clinical conundrum in lung cancer about whether or not you should give chemotherapy simultaneously. It seems that if Cellworks gives you a high score, then you don't need chemo. And if you have a low score, then you should get chemo, and that's independent of TMB (tumor mutational burden). This statistical analysis has a huge chi square (a high probability that there really is a significant difference) compared to TMB and PD-L1 (the target of pembrolizumab/Keytruda).

When I was glancing through the list of things I showed you in that last case, the patient had PTPRD deletion (protein tyrosine phosphatase delta, which plays a critical role in regulating cellular function and is a central feature in signaling cascades involved in driving cancer, which

is frequently inactivated in glioblastoma). PTPRD is a phosphatase (an enzyme that removes a phosphate group from a protein) that reverses step three, phosphorylation (the addition of a phosphate group to a molecule, vital for the cellular storage and transfer of free energy). Step three poisons the tumor microenvironment and reprograms macrophages (a type of white blood cell that surrounds and kills microorganisms, removes dead cells, and stimulates the action of other immune system cells), to cause M2 polarization (macrophage polarization, the process by which macrophages react to specific microenvironmental stimuli and signals), which washes the immune response. You've got to deal with step three in that patient if you're going to consider giving pembrolizumab.

Not everything is actionable. For example, beta-catenin (the central component of the signaling pathway which plays a key role in the regulation of cell proliferation, differentiation, and cell death) is a problem in that it's changing around the cytokine milieu of the tumor microenvironment. (Some cytokines stimulate the immune system and others slow it down.) How are we going to overcome that? We don't have drugs for everything.

One of the things that I've started doing, almost out of a sense of therapeutic necessity, or what I call “the therapeutic imperative”, is finding ways to manage the immune response. For example, one of the things that gets upregulated is the AHR, Aryl hydrocarbon receptor (a transcription factor that regulates gene expression), and that activates TDO2 (Tryptophan 2,3-dioxygenase 2, which plays a key role in regulating oxidation) and IDO1 (Indoleamine 2,3-dioxygenase-1, which plays an important role in immune tolerance), which are the things that make for quashing the immune response. It turns out that you can block this with a substance used in traditional Chinese medicine called Tanshinone. That's pretty wild. I've become knowledgeable about how to do that. I'm giving tanshinone to my patients who have that biology because otherwise I have no hope that pembrolizumab is going to do anything. It usually doesn't for most patients, unless they have very high mutagenic cancers, or something else.

The MSI high you mentioned is a little bit of an easier question because in that situation, the MHC1 apparatus gets knocked out, or the choreography that gets inserted into the membrane is knocked out. (The major histocompatibility class I antigen presentation pathway plays an important role in alerting the immune system to virally infected cells.) When we look at the fancy copy number analysis from our NGS (next generation sequencing) associates, we find out that HLA genes (human leukocyte antigen system, an important part of the immune system) are being deleted, and so those are not too useful for antigen presentation (recognition by the immune system).

There's lots of different things to learn in each patient's case. We try to make the best of it once we get that knowledge.

Ian Lewington 42:04
Good morning from New Zealand, your international audience.

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

When I was diagnosed with de novo stage 4 (metastatic) advanced prostate cancer, I ended up with docetaxel (chemotherapy) and ADT (androgen deprivation therapy) and had a favorable response. Obviously it didn't eliminate it. I have a relatively low tumor burden at the moment. It is looking like I'm starting to get resistance sort of two-and-a-half, three years later. I've had a liquid biopsy, which shows very low mutation levels now, even though I had TP53, P10, and tested that olaparib wouldn't work.

Is the testing you do useful for someone like myself, where you've been fairly heavily pretreated?

When you're looking at the next lines of treatment, what next sort of ADT you should use, or a different chemo combination, at what point should I be letting the tumor burden get higher before I am tested? When would you test?

Michael Castro 43:28
It's a practical issue. It's going to depend on reviewing the radiology and seeing if there is anything we can see to put a needle into and get a biopsy from. The Cellworks model is not informative for what ADT you should be on. I would simply explore those options in the way we usually do.

We have a bunch of very effective drugs that seem to have slight differences in their mechanisms of action, and can be useful if used in sequence. I'm not sure that any one sequence is better than another.

As far as chemotherapy, I would definitely be interested in that. For what Cellworks is doing, we really need a high quality NGS (next generation sequencing). To get a high quality NGS you need enough DNA in order to get a copy number. I would do a liquid biopsy now. And I would definitely be interested in scanning your body to see if there is anything that's useful to biopsy. Of course, prostate cancer is unusually challenging because it has its predilection for bone, and it’s much less certain what the outcome will be when we put a needle into bone, compared to putting it into a liver metastasis or lung metastasis.

Some of it depends on the skill of the person who's doing the biopsy.

My radiologist spent two hours with a patient the other day, because she was sure she didn’t get it, even though she could see radiographically that the needle was in the right place. She knew that there wasn't enough tumor in that specimen. So she kept at it for two hours. That's a great doctor. Not everybody has that bandwidth to be able to do that thing for a patient.

In the end, I would talk with your doctors about this, and ask if you could see the scans yourself.

Ian Lewington 46:08
I've seen the scans. I have only bone mets, no organ involvement. The largest met has only got SUVs (sugar uptake values) of about 2 at the moment. So it would all have to be off the bone.

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

Michael Castro 46:25
Can you see them with an MRI (magnetic resonance imaging) scan?

Ian Lewington 46:28
I'm not sure. I've only had PSMA (prostate-specific membrane antigen) scans.

Michael Castro 46:34
I would do an MRI next and see if any of them are targetable with a needle. Especially sometimes they can be sticking out of the bone a little bit or more towards the surface. Those are the ones that are more interesting to go after.

Vanessa Hugo 46:55
I am a GBM caregiver.

Your approach is pretty novel. Where do you see it fitting within the continuum of care for GBM patients, where we are getting TMZ (Temozolomide) to MGMT unmethylated patients (O6-methylguanine DNA methyltransferase, a biomarker for TMZ response) in the newly diagnosed stage, and then we have no standard of care at the recurrent stage?

Michael Castro 47:31
GBM is a special subject. I became a neuro-oncologist around age 60 or so, because of certain very special patients that I was involved with who changed the trajectory of my career by getting me involved in what I'm doing now. I'm especially interested in trying to do something with this group of patients.

The more you know, the sooner you know it, the better. I would encourage everybody to get the deepest possible molecular diagnosis as soon as possible with GBM, and then to use whatever tools you have. Cellworks is a pretty extraordinary tool compared to other tools, because if you're going to have hundreds of chromosomal abnormalities, and you give this to a practicing oncologist, they will just throw their hands up or shrug their shoulders and say, “What do you expect me to do with that?” They don't know the biology. Even if they know a pathway, or maybe a few pathways. For example, DNA repair has about 497 genes in it. That's an awful lot of genes. There are more than 2500 transcription factors that work in groups. They compete against each other to upregulate and downregulate gene expression. Wow, it's an overwhelming complexity in our cells.

To take the next step in human history, as far as understanding these complex diseases go, we're going to need an AI level, oracular (predictive) tool that can show us the complex proteogenomic networks that we're dealing with.

Then the question becomes: “How are you going to take out that network?”

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

Another analogy that I have is thinking about the way the military is conducting things. They're going to hit the communications, they target the radar, they get the fuel depot, then they hit the airstrip. Then they attack from five directions at once. It's overwhelming. They're attacking all of the network of the enemy. If you do that, effectively, they're not going to recover. There's nothing to recover from. That's the concept in cancer. Then it becomes an issue of: how can you do that without killing the patient?

That's why the concept of synthetic lethal targeting, where you can stack multiple therapies simultaneously, without impacting normal tissues much, is the key forward.

You mentioned glioblastoma. I have several case reports.

Vanessa Hugo 51:00
I've read the one you mentioned.

Michael Castro 51:10
There's another one in the relapsed setting. I have some other ideas, by the way, on how to keep going with this disease.

The standard of care is a very weighty thing. If you don't do it, people think you're a bad doctor.

There are 10% or 13% of patients with MGMT unmethylated disease who do seem to have a benefit from that treatment. If you can understand who those people are, and not deny them a drug that works, that's a virtue. Cellworks can do that. One of my projects for this year is to try to get that data out.

On the other hand, we have to develop a will, as a profession, to stop doing things that don't work. We're wasting time. We're causing toxicity. We're harming patients. We have to take a strong look in the mirror and develop the will to stop giving treatments that don't work. I established this rule in my practice a number of years ago. I can tell you, it makes me a radical. The simplest thing is not doing things that don't work.

> [AM editorial: “Do no harm”]

But I also believe in a lot of the papers I read. If you don't read those papers, then you will never think about these things. Most of my colleagues don't read. [AM: Hear, hear].

Vanessa Hugo 52:40
I really look forward to seeing that research on which unmethylated patients responded to TMZ.

Brian McCloskey 52:49
My question is around multi-omics. Right now you have a model that's incorporating genomics and transcriptomics. It has a certain amount of hopeful predictability in terms of predicting drug sensitivity. But that's only part of the equation. As we've talked about, prostate cancer is a cold

cancer, and the immune system changes over time when advanced prostate cancer patients are exposed to multiple different treatments. I've worked very closely with BostonGene to look at how my tumor has evolved over time. I don't know if you're familiar with their tumor evolution report, but it's pretty awesome. Part of that is looking at the immune profile. I've gone from an immune desert, when I had my first surgery, a prostatectomy in 2016, to a fibrotic immune profile.

How do you factor these additional datasets into your existing model in terms of understanding the likelihood that a patient is going to benefit from any one of these combinations of drugs?

Where does proteomics fit into that?

We can start with immune profiling and then maybe get into proteomics or other analyses that can add a higher degree of predictability to your model.

Michael Castro 54:28
These are really terrific questions.

The evolution of the tumor microenvironment is a really fascinating thing because it's reflecting the evolution of the cancer under some therapeutic stress.

Fibrosis, normally, to a large degree, is mediated by TGF-beta (transforming growth factor-beta, a secreted cytokine, which controls many physiological and pathological processes during cancer evolution).
[AM editorial: TGF-beta mediates EMT (Epithelial Mesenchymal Transition), the latter the molecular underpinning of the Cancer hallmark for invasive capability/phenotype. Invasion is the Hallmark that makes a cancer diagnosable by a pathologist under the microscope.]

There may be other things, like CXCR4 (Chemokine Receptor type 4, plays a critical role in therapeutic resistance by directly promoting cancer cell survival, invasion, and initiation), that are potentially targetable. TGF-beta can be targeted with Losartan, which is an ordinary antihypertensive that's been studied in pancreatic cancer. In the lowest dose, 25 milligrams, it doesn't cause hypotension. In stage three pancreatic cancer, for patients who were unresectable, they had a higher downstaging, and higher R0 resection (resection for cure or complete remission) rate by adding Losartan to FOLFIRINOX. Unfortunately, in stage four, whatever is going on in the pancreas is already beyond just giving Losartan, but it has a role for blocking TGF-beta. TGF-beta is practically ubiquitous in human cancer. It's almost always involved, and it is a real challenge. I use Losartan very liberally, and without too many problems.

[AM, pathologist’s editorial]: Pancreatic Cancer, specifically PDAC, notoriously has a fibrotic Tumor Microenvironment (TME). So for all comers in Stage 3 PDAC, Losartan showed benefit, no biomarker needed to select patients in order to show benefit. Whereas, I predict for prostate cancer patients without bioselection, Losartan would not show benefit. But since Brian’s Boston Gene report suggests he is in a fibrotic immune profile, he could be one of a small subset of

Prostate Cancer patient’s who might benefit from Losartan, with the promise of slowing down or stopping his fibrotic progression. But Dr. Castro, correct me if I am wrong.]

As far as this transition to proteomics, everything we're doing with genomics is really trying to figure out what's going on with the proteins. The idea of DNA goes to RNA goes to protein – it’s a lot more complicated than that. A lot of things that have no problems in the world of DNA, like the MYC oncogene (a transcription factor with a wide array of functions affecting cellular activities such as cell cycle, cell death, DNA damage response, and blood cell production), for example, is a dominant player, because its ubiquitin (targets proteins for degradation) turnover (the processes of protein synthesis and degradation) is downregulated. It gets sustained in various ways. If it's not mutated, if it's not amplified, it still takes over the cell.

The more we know from the world of proteomics, the more humble we get about genomics only. The profiles from one company I use are really useful in breast cancer. 10% of those patients who are HER2 negative are HER2 activated. We have false negative tests. Because when you treat those patients with anti-HER2 agents, even though they don't have HER2 amplification, they respond.

That's a major discovery. There will be more and more. The transcriptome (RNA) is much closer. I noticed from looking at the transcriptome that there are a lot of things that you see in the genome that you don't have to pay attention to.

I had a GBM case recently, and there were something like 15 tumor suppressor genes that were missing. They had mono-deletions. How am I going to deal with that? It's too many things to deal with. In the transcriptome, 14 out of 15 had normal expression levels, so the normal allele (one of two or more versions of a genetic sequence at a particular region on a chromosome) was compensating. So that's an extremely powerful tool for narrowing the mission or focusing in the right place. We'll have more abilities.

Brian McCloskey 58:02
Proteomics would trump anything that you're doing with DNA and RNA. Or do you need the full model? Because I am one of those patients who actually has the whole central dogma: DNA, RNA, and proteomics.

The problem is, I'm trying to make a decision: where do I place my weight? Do I look at all the data in its entirety to piece together something that is more than just proteomics? Proteomics are the gold standard.

Michael Castro 1:00:07
Proteomics is super important when we're thinking about developing CAR-T cells or CAR-NK cells, because you want to be sure that the antigen is sitting on the surface. Similarly, for the antibody drug conjugates, which are using the antibody as a smart bomb, we're delivering the chemotherapy to the right place. It's nice to know that that antigen is really sitting there on the surface.

But I'm not aware that there are any proteomics that are in the 5000 protein range. I understand that there are about 5000 proteins that run a cell. But we can get the entire transcriptome. I hope we get the microRNA as well. We can get the entire genome. I don't think the proteome is there yet to replace those other things, and we don’t have enough experience with the transcriptome yet as inputs, but that's the project that we have this year at Cellworks. I'm sure other people are working on that too.

Brian McCloskey 1:01:20
I'll just add a point of validation that nowhere in my DNA or RNA did HER2 show up as a gene that was a cancer driver. But in my proteomics, I had a moderate expression of HER2. So there doesn't necessarily seem to be a direct correlation based upon the data that we have with DNA and RNA to proteomics.

Michael Castro 1:02:03
If you do something that affects the turnover of a protein, you can prolong its half life and stability in the cell, and it can take over in an oncogenic manner. The turnover of HER2 from the surface of the cell, where it goes into the lysosome (where digestive enzymes break down excess or worn-out cell parts) for destruction is a heavily choreographed process. If you either accelerate that or block it, then HER2 accumulates on the surface of the cell.

By the way, immunohistochemistry (staining of tissue samples, selectively identifying antigens in cells and tissue by exploiting the principle of antibodies binding to the stain) is proteomics. It's 1930s proteomics, but it's still good. That's why if pathologists can see it, that's a target.
[AM Editorial: Hear, Hear. That is what I have been trying to tell you all, in PCL, Cancer Patient Lab].

Brad Power 1:03:40
For a layperson who doesn't understand molecular biology, please helicopter up to the big picture level. At various times you've called this complex and speculative.

Where does this sit in its maturity? Where is this in the hype cycle?

You mentioned AI. Immediately there's a question about the hype cycle. We've had people on this forum before who've said that the modeling of biology is incredibly complex, and quite difficult with a fraction of the numbers of variables which you've cited.

Who is this for? What patients should be availing themselves of your services, given the early stage research lab nature of what you're doing?

Michael Castro 1:04:27
It comes down to a matter of philosophy. What I do is not very comfortable for many people because it is outside the box. It is vanguard medicine. It is taking off the training wheels and trying to figure out a solution to the problem.

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

Since I'm in the twilight of my career, I've decided I don't want to just continue doing things that don't work because I made this pact with myself to stop doing that and to find solutions. The more I do that, the more I can seem to be able to be effective. But it does mean that you make yourself a guinea pig, and you can either walk the plank as a patient – forgive the crudity of this analogy – but I do see it that way. Getting metastatic cancer is your ticket to the next world.

You can either go with a standard of care and the NCCN and consensus medicine, or you can ask, “Is there anything else that's been learned about cancer that could help me? Is there anything that anyone could learn about my tumor that I can leverage as a therapy?” The answer to those two questions is a resounding, “Yes.”

The forefront of cancer research is decades ahead of the clinic. Unfortunately, most of what is being learned by the great public investment in cancer will never be translated into therapy, because that translation is driven by one thing, and that's profit. We live in a society where the first words are, “Show me the money.” If you can’t, then it’s not investable.

If it's investable, you need intellectual property, you need a development plan, you need the right team, you need clear steps to getting something that can generate a charge of $20,000 to $30,000 a month per patient. Then you're going to develop that therapy in isolation because that's how things get on the market.

But that is not the way to treat cancer. Because once you get the view that I've gotten from working with Cellworks about the complexity of the disease, you can see how meaningless most of what we do is. Current drug development efforts are so naive as to be laughable.

There's an old saying that “people who live in glass houses shouldn't throw stones”. So I have to be careful how I put these ideas out in the world, because it's going to offend a lot of people. But the truth is: if you're the patient, you don't have time for all of these shenanigans.

The person who should work with me is somebody who number one believes in science. Number two is willing to go outside the box, and even be in an uncomfortable relationship with other doctors who say, “There's no evidence for what Michael Castro is doing. Therefore, I wouldn't do it.” But one thing that's happened to me as I've gotten older is, I’ve been close to a number of people who have passed away from this disease, including friends. It cuts pretty close to home, even though I haven't yet gotten a diagnosis. I realize it could happen. It's a 50% chance, in fact, that the average male is going to get a malignant disease. So I always put myself in the patient's shoes and say, “Knowing what I know, what would I do?”

I've been extremely fortunate to be practicing oncology at a time in history when I've seen people who are nine-tenths into the grave have complete remissions and go back to meaningful lives. I've seen it multiple times in my career with the Cellworks biosimulation tool.
[AM editorial: Dr. Castro needs to enter his (GBM) complete remissions into Zak Kohane’s “miraculous cure” database].

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

There's an old saying, “All models are wrong, but some of them are useful.” That is true of Cellworks as well. This is an incomplete model, but it's a lot more than anybody else is working with. It's so much further ahead than any clinician can do on their own that looking for its utility is interesting.

What got me interested in this whole thing to begin with was when I would get a report from Cellworks I would see things that I simply didn't know before. It was like, “That's interesting. What do I do with that one?” It comes down to your philosophy about how you want to be treated, and where your comfort level is.

Remember, in the movie, “The Matrix”, that scene where he says, “You can swallow the red pill, or the blue pill. If you swallow the blue pill, you wind up in your bed, knowing you're receiving the very best care possible, from the very best doctors. And if you swallow the red pill, we're going to show you the way it really works.”

If you're at Stanford, UCSF, or City of Hope, all these great institutions, you're getting the blue pill treatment. You're getting consensus medicine NCCN guidelines, or somebody's idea of a clinical trial. The clinical trial may or may not be useful for you. I'm not against clinical trials at all, but not all of them are good for all patients. One wants to be shrewd about it.

Allen Morris 1:11:58
How many clinicians are also molecular biologists? How many people actually wear those two hats (one in the clinical medicine world and one in the basic science world) in a real real way? I suspect that you are one of the few. A unicorn.

Michael Castro 1:12:38
Pathology now has molecular pathology.

I've been arguing to people at ASCO, “You've got to create molecular oncology as a certification.”

Allen Morris 1:13:00
Molecular pathology is a board certified subspecialty of pathology.
I estimate there are less than 300 board-certified molecular pathologists in this country.

[Background: There are 14 subspecialty board certifications in pathology, but in practice I estimate there are about 120 subspecialties in pathology].

This speaks to one of my bugbears: that there has always been a chasm between

a. Molecular pathology: the closest field to science that medicine has, and
b. Molecular biology: the closest thing to clinical medicine that science has.

This Pacific Ocean chasm, I think, is best captured by the respective fields disparate credos:

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1. Molecular biology: All the molecular mechanisms which apply to the most rudimentary pseudo-life forms, an obligate intracellular parasite, the viruses apply identically to us Humans.

2. Clinical Medicine: What applies to the closest models of human disease,
whether cancer cell line models, such as LNCaP(**L**ymph **N**ode **Ca**rcinoma of **P**rostate) for the specific patient cohort mHSPC (metastatic hormone sensitive PCa); or animal models, such as Macaque Monkey gold standard model for Covid, does not necessarily, and usually does not apply to humans.

I believe the above credo divide is profound.

I applaud you for being the inventor of the [phrase] “synthetic lethal node.”

[Dr. Castro, I want to present to you a phrase I think I invented. Admittedly, I have not done a provenance search.]

The phrase is “primordial molecular node”. And coincidentally, it concerns

1. NF-kB: the very first molecular node you listed in your learning session:
2. Prostate cancer: not only PC, but the 1st forgotten historic treatment of Prostate Cancer,
3. Prednisone

[Background: There is a primordial molecular node, NF-kB (discovered in the context of the immune system).

NF-kB was discovered in David Baltimore’s lab back in 1977. Believe it or not, I was there. It was discovered in the context of immunology, specifically B lymphocyte cell function. This discovery led to a Nobel Prize.

Back then, how did a molecular biologist (not microbiologist) figure out what a gene does? They did knockout mice experiments. A knockout experiment silences a specific gene. So, when they did knockout mice experiments, and they knocked out this gene, NF-kb, unbelievably, the mice did not develop lymph nodes, period. How profound. So this one gene knocked out/silenced and one doesn't have lymph nodes.
Background: Lymph nodes are the major organ of our adaptive immune system. And moreover, that is not all that is silenced, knocked out. The mice also had no osteoclasts. Osteoclasts are a

cell, one of two that forms our skeletal system: the yang of the yin and yang, molecules involved in bone formation and remodeling, respectively.
This molecule, NF-kB, I believe, is at the crossroads of the evolution of the adaptive immune system in jawed chordates and why I chose to deify it with the term “primordial osteoimmunologic node”.]

Michael Castro 1:15:12
NF-kB is a big issue. You need NF-kB in your functioning immune system in order to mount an immune response. But on the other hand, NF-kB is a major driver of cancer. Particularly the lymphomas, myelomas, but also solid tumors, because it happens to be downstream of Akt. (The Akt signaling pathway, or PI3K-Akt signaling pathway, is a signal transduction pathway that promotes survival and growth in response to extracellular signals. Key proteins involved are PI3K – phosphatidylinositol 3-kinase – and Akt – protein kinase B.)The PI3K-Akt pathway is dysregulated in at least 90% of every disease I treat, maybe 100%.

Allen Morris 1:16:28
Because of this nexus, there's a term called “osteoimmunology”. It is in the dustbin of history. You are this rare bird that is bridging molecular biology and molecular pathology.
So, I will ask you a question, I have asked other experts, with no answer to date.

Do you know if osteoimmunology is taking off or not?

Furthermore, there is another Prostate cancer curiosity: Prostate cancer of all the cancers is very bone-centric; moreover in a uniquely osteoblastic (as opposed to osteolytic) fashion. Why?

Michael Castro 1:17:02
There must be something in there.

[AM editorial: If Dr. Castro does not know, barring the likes of Dr. Ida Deichaite, nobody knows. And thus I believe, it must be a “curiosity”.]

Allen Morris 1:17:23
I pose these curiosities to expert prostate cancer experts, and they don't even attempt to answer them, because “the eye sees what the mind knows”. Their mind doesn't have the foundational knowledge for their eyes to see, because the foundational knowledge does not exist.
[I admit this an oblique allusion to one of my curiosities, which I believe could be solved By a Tower of Babel Molecular Hackathon aimed at NF-kB and Osteonecrosis of the Jaw (OJN).]

On to another question.
There's a patient here, Robert Gurmankin, who asked you about bone biopsies and whether or not you can extract your magic out of it. He is a fascinating case as he's one of the rare

advanced prostate cancer patients who is mismatch repair deficient. He tried Keytruda, which I thought would most likely work. However, he and his doctors believe Keytruda failed, and not just failed, but actually showed “hyperprogression”.

Please comment on differentiating pseudoprogression versus hyperprogression vis-a-vis radiology RECIST criteria (Response Evaluation Criteria in Solid Tumors, a set of published rules used to assess tumor burden in order to provide an objective assessment of response to therapy), concerning Keytruda (an Immune Checkpoint Inhibitory) in a biomarker selected patient, specifically the uncommon Mismatch Repair deficient patient.

Michael Castro 1:19:20
Pseudoprogression is very common, and it's a conundrum in radiology. It can't be solved in radiology except by doing serial scans over many months. We need the circulating tumor DNA assay, either an MRD (minimum residual disease) test, like Signatera (from Natera), or the BilliontoOne test, called Northstar Response, which is a fantastic technology. They can tell you a 0.1% difference, getting worse or better, with their technology. If you aren't familiar with BilliontoOne, I would turn you on to them.

[AM translation: Pseudoprogression is not only very common, it is what is expected in an immune responsive patient. Whereas, hyperprogression is ?; in any case it is not even solvable by radiology in a short period, a few months. But Dr. Castro, please correct me if I am wrong].

Frank Nothaft 1:20:00
As somebody with a network biology background, especially working on transcription factors, one of the methodological challenges that we face with these very large, many nodal models is overfitting because fundamentally, if you look at the whole genome plus the whole transcriptome, you're looking at 20,000 genes times two.

How are you all approaching that problem?

Because that's always been a confounding issue with these whole exome/whole transcriptome cancer predictive models of progression.

Michael Castro 1:20:59
The ability to think about cancer from a hallmark behavior perspective: things that are causing proliferation, things that are upregulating DNA repair, things that are changing the apoptosis (cell death) pathways. The histogram index that I showed you before – what does that really represent? That represents the net cell number, that cell number is the difference between things that are growing and still alive vs. after cell death. You can have various ways of getting there. We're hoping at Cellworks ultimately to validate that.

But the model itself, I'm not sure we can do it without somebody like Drew Watson, who's going to come in and then give us weightings for specific individual phenotypes. When I'm talking

about phenotypes, I'm talking about 130 different pathways. And those pathways are themselves complex.

What you're saying is absolutely true. It is a very high level of complexity.

The company has been doing it for 15 years. It's been in incubator mode in Bangalore, India, where the cost of hiring a PhD is about 25% of what it is in Silicon Valley. It probably wouldn't be possible to do this development in the U.S. because of that.

Ultimately, we'll need to have some validation.

But if you can understand one pathway, and you can understand another pathway, and you start putting it all together, it's the integration and predictive value that becomes tricky in the end.

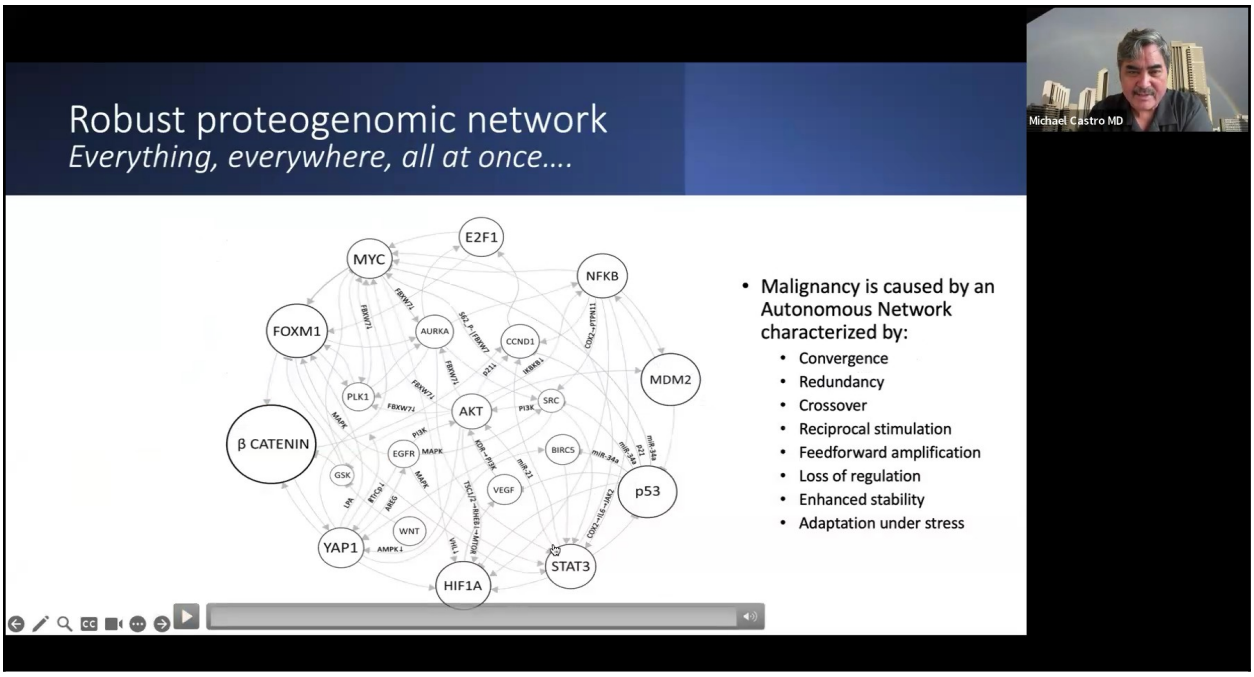
Frank Nothaft 1:23:28
If you look at transcription factor biology, you're looking at binding complexes of multiple transcription factors.

Michael Castro 1:23:45
Plus promoters that affect individual transcription factors are a whole other level of complexity that we haven't even sorted through yet. Transcription factors are at the end of signaling pathways that are also complexly dysregulated in the tumor. It's mind boggling.

That's why, finally, to have the network view of cancer is a completely revolutionary and modern view of what the disease is. I can say cancer is a complex proteomic network, and people will say, “Yeah, yeah, yeah. Yeah. Tell us something new.” It's really complicated. We need to embrace this idea of this individual thing that's feeding back on itself.

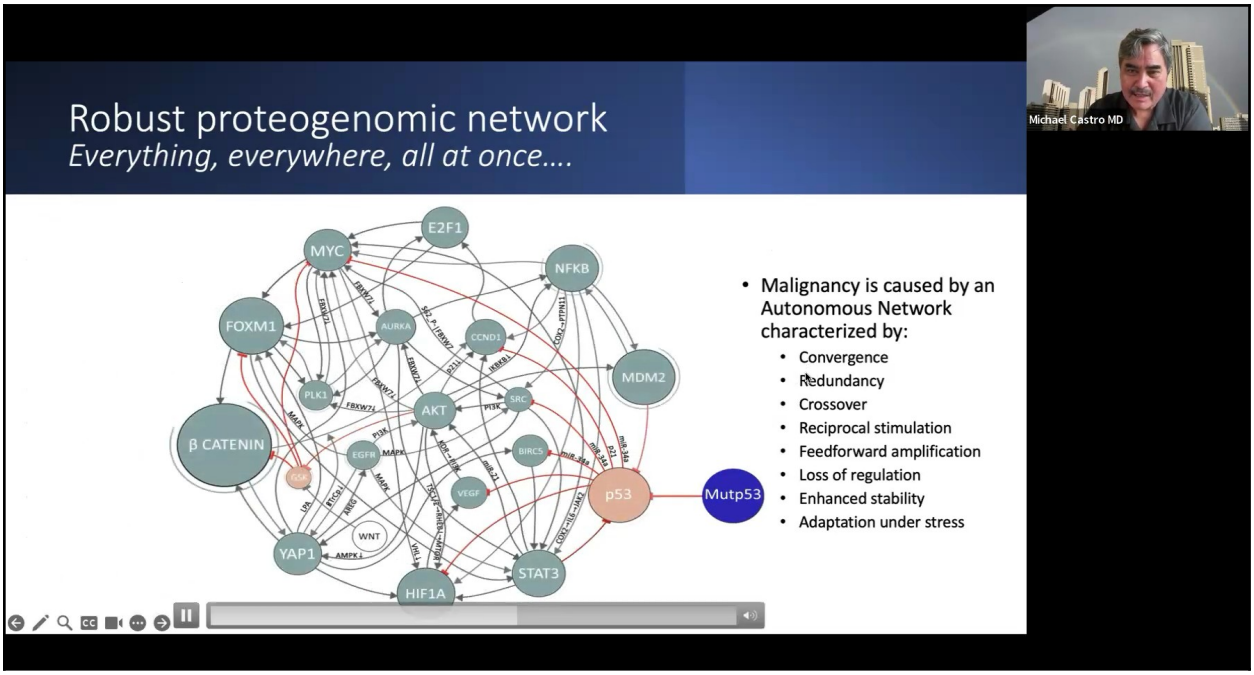
I would love to show you an animation that I had created to explain this concept of what cancer is.

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]



What I've done here is to make a little diagram, putting the transcription factors on the periphery and the signaling pathways on the inside. Here's NF-kB, by the way.
[AM editorial: Did you say NF-kB? The osteo-immunologic primordial node; the epicenter of the adaptive immune system.]

This is what I began to understand from working at Cellworks.



What you're seeing here is: I've listed on the right side, something that has redundancy, convergence, crossover, reciprocal stimulation, feedforward activation.

This makes the tumor network completely autonomous. Once it gets going, there's nothing that's going to stop it unless you have some particular therapy that can take down the key nodes in this network.

From the point of view of thinking about how we should be treating cancer, we have to start with an understanding that looks more like this, and not simply take one single oncogene and say, “Okay, how are we going to block that?” And “What disease should we develop this drug in?” The way we're doing it now is so silly and naive. We have to get smarter than that.

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

Post Meeting Email Discussion on Validity and Viability of the Biosimulation Approach

On Tue, Mar 5, 2024 at 3:36 PM Frank Nothaft <fnothaft@alumni.stanford.edu> wrote:

I thought it was an interesting talk. The method has a lot of biological intuition, but I worry that it will have statistical challenges: it’s very easy to overfit genome-wide models to noise. I’ll be very interested to see their validation results when they are available.

I did find the allusion that pharma won’t be interested in this work to be a bit odd. It seems like a tool like what he’s developed would be of very high interest to a variety of pharma teams that plan/design trials. Especially if he’s identifying hypotheses for medications that work synergistically in combination; that’s a very big area for pharma companies these days and was a very common ask of us at Tempus.

While medical research has made huge strides in adopting an “open access” approach the last few years—I think the pandemic really helped push the adoption of open access along—it still definitely lags behind computing and bioinformatics. It’ll be interesting to see the effect of the generative AI craze on open access. On one hand, there’s never been more of a demand for openness! But in practice, I’ve noticed that a subset of people are definitely feeling more protective of IP / content given the risk of the various generative AI companies scraping data which winds up incorporated in their models. My bet is on the open side, but time will tell!

Best,

Frank

—

On Tuesday, March 5, 2024 at 05:51:01 PM PST, Brad Power <bradpower@cancerpatientlab.org> wrote:

The interesting question to me is whether Dr. Castro has laid out a path to model disease.

When we had a session with Marc Birtwistle of Clemson, he said that the combinations and permutations of multiple variables makes modeling disease nearly impossible.
I have accepted that it won't be done for decades in the future.
But Dr. Castro asserts he's using AI to break through this barrier.
He could be over-representing their capabilities today, but if he's directionally correct, we have learned that AI systems get better and better with time.

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

Given your bioinformatics expertise, do you think he's got a line of sight through an AI-enabled model that could eventually work to personalize treatment recommendations with non-toxic targeted combinations?

Thanks,
Brad

—

On Mar 5, 2024, at 6:40 PM, allen morris <allmorris@yahoo.com> wrote:

Hi Brad and Frank,

Though, I am beyond interested in Frank's take on the promise or lack thereof for targeting "synthetic lethality nodes"
from a nuts and bolts, practicing, computational biologists view;

Allow me to do an end around, on you guys.

I speak to the holy grail of the N of 1 experiment: The miraculous cure for what an expert would agree is an incurable cancer.
Btw: Not Ewing's Sarcoma or NED for many chronic diseases such as myeloma, CLL, CML, etc.
Sidenote: The only registry of miraculous cures, I just discovered courtesy of Brad and Glenn Sabin, is the Zak Kohane's registry of est. 100 patients. This registry should include Marty Tenenbaum and see below: Dr. Gary Onik and Dr. Castro’s GBM patients.

Dr. Gary Onik had cryosurgery done to a bone metastasis with GM-CSF and Immune Checkpoint Inhibitor injection into the resultant autovaccination lysate for incurable stage 4 metastatic Prostate carcinoma and cured himself. No evidence of disease x 4+ years and not on ADT or any other treatment. Unheard of.
That is ultimate: N of 1. The proof is in the pudding. Sign me up, barring the sticker shock of what he charges.

Dr. Michael Castro, despite not having run any evidence-based translational research, testified that he got NED on a GBM patient with no additional therapy x 40+ months of followup. But he sounded like an understating physician-scientist to me. That is Proof of Principle.

GBM presumably IDH wild type and pancreatic, presumably PDAC with a median survival measured in months, <12 months, unfortunately for the

patients, is the ideal patient to run the N of 1 experiment looking for the miraculous cure or at least the long term, for real, outlier survivor;
not a CLL, Myeloma, CML, usual ER+ breast cancer, the vast majority of prostate cancer, or papillary thyroid cancer patients.
For the latter, N of 1 experimentation without at least a "contemporary" control, barring some futuristic AI stuff, I believe, is folly, unless you guys can educate me otherwise.

Allen

—

On Mar 6, 2024, at 11:21 AM, Mark Taylor <mark@patientledoncology.com> wrote:

I think it's a valid approach. You can now test to see what pathways need hitting. Metavectum test is purely based on this approach and allows you to target genes that are upregulated on circulating tumour cell tests so it leaves some guessing out. Datar, Rgcc onconomics plus and Nextgen expression profiler allows you to do the same and most even suggest what drugs to use . Gbm cusp9 study gives an indication that if you hit seven targets with chemo it can bring promise with some very good long term responders. But without a cytotoxic drug alongside I’m still looking for good evidence.

in practice, with non mutated wildcard genes, what I’ve observed is a wack o mole approach, where you hit a few upregulated pathways and others pop up as you do it. So it’s not clear how effective it can be in all cases. With some intelligence maybe we could predict the genes that upregulate as you down regulate the ones regulated. Kind of like wacko a mole with a prediction engine :)

I’m keen to hear more as this is an area I’m researching. But I’m yet to find convincing evidence it works without a cytotoxic drug in combination. Keen to be convinced otherwise.

—

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

On Wed, Mar 6, 2024 at 12:14 AM Frank Nothaft <fnothaft@alumni.stanford.edu> wrote:

I’ll try to reply to both of you ;-) These topics are well in theme with my reading for tonight (I’m rereading Nassim Taleb’s Incerto, specifically “Fooled by randomness").

From a computational perspective, modeling genome-wide networks has been a tractable computational problem for a number of years now. There were a number of problems that were challenging to tackle about a decade ago, but I’d say they’ve been largely solved through the combination of cloud computing and more efficient numerical algorithms. You can always think up a more elaborate model, but as long as you’ve got the $$$, then you can find a computer to build/run it. ;-)

IMO, the big challenges these days are:

• Having the right data for the question you’re trying to answer
• Systemic validation of predictive models

It’s a pretty good business these days to build a “genome-wide network” model and then use it for drug discovery. That’s *loosely* how I’d describe Recursion and Insitro’s business model’s (use a combo of large public data + robotized labs to build these models) or the Regeneron Genetics Center’s thesis (learn how genes work from enormous public genomic/clinical datasets, and then validate our learnings in mice), and that’s what the OpenTargets project has been doing in open source/open data for years now (on a much smaller scale, mostly driven by mining the literature).

It gets harder once we move into clinical response. If we want to build a model that predicts clinical response of combo therapies using genomic signatures, the good news is that we have (arguably) lots of cancer genomics data, but the bad news is that a limited amount of that data has comprehensive clinical outcome data, and that data is presumably biased to represent the standard of care. As such, we’re probably able to build a decent model to predict whether a patient will respond better to medicine A vs. medicine B (let’s say, they’re eligible for two different PARP inhibitors).

But! It’d be surprising from both an epistemic and a statistical perspective if our model was able to predict whether a patient will respond well to a novel combination therapy: we didn’t use data about responses to combo therapies in our model (epistemic limitation) and training on high dimensional data (all the genes in the genome) with few samples (small number of patients) leads to overfitting (makes our model worse at generalizing to new data/questions).

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

We could think of ways to make our model handle the combo therapy prediction task, e.g., we supplement it with functional screening data, or we reframe the combo therapy prediction task as “predict all therapies that this patient is likely to respond to, and then filter out any combinations of therapies that are likely to have mutual toxicity”. Or, we could do something even more unconventional like pair a highly robotized lab (CRISPR + RNA-seq at scale) with propensity matching techniques that allow us to extrapolate lab results to real-world patient trajectories (that’s actually a pretty cool idea, I should look into that).

If at the end, we do finally build a model that we feel pretty confident in, it’s just statistically hard to run an experiment that’s well powered to show results (unless we restrict ourselves to testing a limited number of combinations, in which case we’re just running a standard combination therapy trial, or if we’re confident that we can show outstanding results on otherwise incurable disease, to Al’s point).

None of these problems are impossible, they’re just very hard to string together and do well!

Where I’ve seen people be most successful is using large datasets to build *small artisan models* ;-) that are well conditioned for rapid validation. E.g., I see that patients with a severe mutation that knocks out gene XYZ survive much longer than the average patient, so I build a computational model that explains this (by correlating the loss of gene XYZ with the expression of other genes), and then I quickly validate that by CRISPRing that gene out of several mice and seeing if my model replicates.

I’ll have to take a look at Marc’s talk! I think this all is conceptually doable, I think it just takes *quite a bit* of statistical care (and well defined data, don’t get me started on “clinical phenotyping”). For now, I’ll just wait to see validation data on the method; as they say “In God we trust, all others must bring data” ;-) (my apologies, this got a little long winded).

Best,

Frank

—

On Wed, Mar 6, 2024 at 12:21 PM allen morris <allmorris@yahoo.com> wrote:

Brad et. al.,

I see the French Connection has blown up. Fantastic. Thank you for including me in the "real" connected brain.

I see you have included Marc Birtwistle,

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

Can you ask him a semantic question for me/us.

I know Dr. Castro coined the phrase "synthetic lethality node". He told us.
This is his breakthrough: uncloaking a combination of synthetic lethal nodes, then targeting them with known, off the shelf treatments.

Can you ask Dr. Birtwistle, not whether he has heard of "synthetic lethality",
a catchy phrase that is now in the lexicon x more than a decade, invented to explain how one of the current breakthroughs in treatment works, the PARP inhibitors vis a vis blocking double stranded break DNA (homologous) repair.

but if there are any similar notions, such as "vulnerability cascades", "achille's heel pathways, super vulnerable onco-drivers or tumor suppressors, etc.
bandied about, and thus there is a supersaturated solution in the research cosmos and something, for example, that many AI computational groups are aiming to uncloak. Or is the Gene Hack Man approach, a truly novel approach.

Interestingly, Dr. Castro said that his "synthetic lethality nodes" are not just homologous repair foci, so, I submit, though the phrase is catchy, it is semantically incorrect.

P.S. Frank. I have to admit your biocomputational lingo is largely French to me.
You will have to recommend a primer for me to get up to speed. Something on the 1st year undergraduate level.

Allen

—

Razelle Kurzrock, MD

Mar 6, 2024, 5:58 PM

to
Mark,
Brad,
Brian,
Frank,
Marc,
Michael

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

, allen,
gabriel.
gavazzi

Read

Sicklick… kurzrock, … IPREDICT… Nature Medicine, 2019

And

Sicklick… Kurzrock. IPREDICT. Genome Medicine 2021

We show safety and efficacy in the clinic

Best,

Ray

Sent from my iPhone

Please excuse iPhone typos

Razelle Kurzrock, MD

Professor of Medicine

Associate Director, Clinical Research

Linda T. And John A. Mellowes

Chair of Precision Oncology,

MCW Cancer Center and

Linda T. and John A. Mellowes

Center for Genomic Sciences and

Precision Medicine

Founding Director, Michels Rare

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

Cancers Research Laboratories

Froedtert and Medical College of Wisconsin

Chief Medical Officer,

Executive Committee,

Equal Opportunity and Diversity Officer,

Worldwide Innovative Network (WIN) for Personalized Cancer Therapy (non-profit)

Adjunct Professor,

University of Nebraska

—

> On Thu, Mar 7, 2024 at 4:04 PM Marc Russel Birtwistle <mbirtwi@clemson.edu> wrote:
>
> Hi Brad and others,
>
> Good to hear from and meet some of you. My apologies on the delayed response; it's been quite hectic of late for me.
>
> There's a ton of interesting conversations and ideas going back and forth that I'm not sure I completely was able to follow yet, but I do see a direct question below that I'd be happy to speak to.
>
> Synthetic lethality---yes it is quite a common phrase that in my understanding describes a genetic interaction where the deletion or disruption of two genes causes death (or inability to grow), but disruption of either individually does little to nothing. That is, the gene pair is essential. Usually it is mechanistically related to parallel pathways or a compensatory mechanism. And yes, a classic case in cancer is PARP inhibitors in BRCA mutants, although I believe the phrase is somewhat loosely applied when drugs instead of genes are involved. If I remember correctly, this happens because without BRCA the only pathways left for DNA double strand break

repair depends on PARP. The female organ cancer specificity of all that is completely baffling to me though.

Synthetic lethals are kind of a holy grail for cancer because if you can find a drug that is super effective only when a certain mutation is present, the selectivity for cancer vs. normal could be huge. I'm not aware of too many that have been found and validated outside of the above example though, despite extensive searching.

Also, Gene Hack Man---hahah nice name for a technique

Best,

Marc

Marc Birtwistle, PhD
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Web: www.birtwistlelab.com

—

From: Brad Power <bradpower@cancerpatientlab.org>
Sent: Thursday, March 7, 2024 5:53 PM
To: Marc Russel Birtwistle <mbirtwi@clemson.edu>
Cc: Brian McCloskey <brian.j.mccloskey@gmail.com>; allen morris <allmorris@yahoo.com>; Frank Nothaft <fnothaft@alumni.stanford.edu>; Michael Castro <michael.castro@personalizedcancermedicine.us>
Subject: Re: Question: "Synthetic Lethality Node"?

Thanks for weighing in on this thread.

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

> One of the big take-aways I got from your discussion with our community was that disease is so difficult to model, that it will be a long time before a comprehensive model will be diagnostic and predictive.
> If you have time, please see Dr. Castro's thesis (slides attached), and share your thoughts on the feasibility of this approach of identifying multiple nodes that are on that pathway and hitting them with drug combinations.
>
> Best,
> Brad

—

On Tuesday, March 12th, 2024 at 1:28 PM, Marc Russel Birtwistle <mbirtwi@clemson.edu> wrote:

> Hi Brad, thanks for sharing, and thanks Michael for your slides. Without seeing the presentation itself it is somewhat hard to judge, but it looks like a large amount of prior knowledge is integrated within a computational omics biology model which is then combined with a set of patient data to predict drug responsiveness. Are there any papers on the work? I wonder how the models are built and what math is used. I also wonder how much validation of the predictions coming out of the model on the Therapy prediction index is there? It's a really challenging problem for sure and it will require testing and evaluation of many types of approaches by many people I think.

—

From: **Michael Castro, MD - PCM** <michael.castro@personalizedcancermedicine.us>
Date: Tue, Mar 12, 2024 at 5:35 PM
Subject: Re: Question: "Synthetic Lethality Node"?
To: Marc Russel Birtwistle <mbirtwi@clemson.edu>
CC: Brad Power <bradpower@cancerpatientlab.org>, Brian McCloskey <brian.j.mccloskey@gmail.com>, allen morris <allmorris@yahoo.com>, Frank Nothaft <fnothaft@alumni.stanford.edu>

Dear Marc,

The math is based on mass action and Michaelis Menten differential equations. Please have a look at the website where there are a large number of references for your interest: cellworks.life.

Regards,
M~

“Personalizing Treatments with Biosimulation" (Michael Castro, MD) [#88]

Michael P. Castro, MD | Board-certified in Neuro-oncology and Medical Oncology

Personalized Cancer Medicine, PLLC
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