“How MSI and Other Tests Can Guide Immunotherapies for Cancer Treatment” (Heather Tomlinson) [#43]

February 8, 2023
Brian McCloskey and Brad Power

“Microsatellite instability (MSI) … means that you have areas within the DNA of the tumor that are prone to errors during DNA replication.” Heather Tomlinson

“When you have MSI (microsatellite instability) high, or in some cases, TMB (tumor mutational burden) high, you have a larger number of mutations not only in your tumor, but everywhere in your body. You have all of these mutations that exist, which means you have a broader immune response as well.” Heather Tomlinson

Meeting Summary

Advanced cancer patients are searching for treatment options, and one of the most promising treatment areas is in immunotherapy -- awaking the patient's immune system to do its job: recognizing cancer cells as bad guys and eliminating them. Immunotherapy has the distinct advantage of being a system (the immune system) fighting a system (the cancer system), which is more powerful and likely to create a durable response than the typical approach of using one drug to attack one biomarker.

But before patients take a drug, they'd like to know if it’s likely to work. A "companion diagnostic" can help determine that before a patient goes through the treatment.

Heather Tomlinson is well qualified to guide advanced cancer patients in decisions about tests for immunotherapies. She has focused her career on companion diagnostic development and commercialization. She has a PhD in pharmacology and recently led the team at a diagnostic company (Promega) that got FDA clearance for a diagnostic test (OncoMate® MSI) that identifies “microsatellite instability” – when a short, repeated sequence of DNA is different from what it was when it was inherited. Dr. Tomlinson recently co-founded a community (MSI Insiders) to bring valuable information to patients to help them gain access to precision medicine sooner in their treatment journey.

Are there tests which can help patients and their physicians decide if an immunotherapy is likely to be effective?

Tests for immunotherapy response include PD-1/PD-L1, microsatellite instability and mismatch repair deficiency, and tumor mutational burden.

- **PD-1/PD-L1**: PD-1/PD-L1 (Programmed Death-Ligand 1) was the first biomarker for immunotherapy. PD-1 is a receptor protein found on certain immune cells which when activated, “tunes them down,” acting as a kind of “brake” to keep the body's immune responses under control. It can be turned on by binding to PD-L1, its corresponding ligand which is upregulated by many cancer cells. These cells use PD-L1 to activate PD-1 and thereby shut down parts of the immune system (a ligand is a molecule that typically binds to another molecule to activate or deactivate it). Certain immune therapies work by shutting down PD-1, or by masking PD-1 from its PD-L1 ligand. In cancers

which use this mechanism to slow down the immune system, inactivating this braking mechanism allows the immune system to operate more effectively.

- **Microsatellite Instability (MSI) and Mismatch Repair Deficiency**: Microsatellites, which are short, repeated DNA sequences, occur throughout the genome. They are prone to replication errors, which are often fixed by the DNA mismatch repair system. But when the DNA mismatch repair system is not working well (for reasons which include being inactivated by certain genetic traits such as Lynch syndrome, or by some cancers), errors can accumulate and leave telltale signatures in the genome, tracings called “microsatellite instability”. High MSI is a useful predictor for how well a cancer will respond to certain therapies, including immunotherapies, and it was the first tissue diagnostic biomarker approved by the FDA. In prostate cancer, 3-5% of cancers are “MSI high”.
- **Tumor Mutational Burden (TMB)**: MSI is a form of mutation (that is, change in DNA), detectable in a particular manner as described above. But there are many forms a mutation can take, and TMB is a more generalized statistic of how many changes are in the DNA of cancer cells. As before, tumors with a high number of mutations appear to be more likely to respond to certain types of immunotherapy. In 2020, tumor mutational burden was approved as a tissue-agnostic biomarker (in contrast to tissue-specific, which might only have utility for a particular type of cancer such as, say, lung cancer).

How effective are these tests as predictors of immunotherapy response in advanced prostate cancer?

When a patient has mutations in the genes of certain cells, that can be a signal to the immune system that those cells are “different” from normal cells in that person’s body and that can sometimes activate the immune system to attack those cells. When there are a lot of mutations in the cells of a tumor, those tumors are called “hot tumors,” and they are more likely to actively engage the immune system. Prostate cancer tumors are often “cold tumors,” with fewer mutations, and that is one reason that immunotherapies can be less effective in prostate cancer tumors. There are also fewer tests to indicate whether – and which – immunotherapies will be effective.

Tumor testing for MSI or DNA mismatch repair deficiency is recommended for patients with metastatic prostate cancer. Prostate cancer patients who test “MSI high,” and who have already undergone docetaxel and one novel hormone therapy, will often be recommended to get the immunotherapy Keytruda/pembrolizumab as a subsequent systemic treatment. Other immunotherapies include Jemperli, Tecentriq (atezolizumab), Imfinzi, Bavencio, and Opdivo. A cancer patient is more likely to respond to an immunotherapy if the tumor is “hot”. A hot tumor is a tumor that shows evidence of T-cells within the tumor microenvironment. This evidence usually comes from a stain (IHC) on a slide of the tumor tissue, or RNA analysis.

What other immunotherapy tests and treatments are in development?

Most clinicians are hesitant to use immunotherapies in prostate cancer because they want evidence from a Phase III trial. But work is ongoing to improve immunotherapies in prostate

tumors and scientists continue to look for better ways to measure, dose, and vary treatment frequency, and also to mix existing immunotherapies with other drugs to make them more effective. (Pharma companies continue to look at drugs which take advantage of the weaknesses in cells with mismatch repair deficiencies.)

Vaccines, which train the immune system to attack certain types of infectious malefactors, are perhaps the oldest immune therapy in the history of medicine. But more recently, they have become more highly specific, “personalized”, if you will. A personalized vaccine does not train everyone’s immune system to protect everyone against the same disease (as a polio vaccine trains everyone against polio). Instead, it is made for a particular person, to train their immune system against a particular concern (often cancer). This is a complicated process, because the vaccine maker will need to find bright recognizable flags on the cancer which it can use to train the immune system, even though many cancers do not present such ornamentation in any grand way. A further complication arises because of the interactions between the cancer and the patient’s own, very specific, immune system, characterized by the markers called “HLA type” (for human leukocyte antigen). In practice what that means is that even though two people may have cancers with the same metaphorical bright flags, in one patient that flag may be bright red, and in the other, nearly invisible. Real advances in this area have only come after years – decades – centuries – of work understanding the immune system and developing tools (including computers and software) to harness it. But in the future, many expect there will be tools to rapidly develop effective vaccines for many patients, with many cancers, regardless of HLA type. Vaccines typically work by training multiple parts of the immune system simultaneously. And while personalized vaccines are pretty specific, other approaches can be even more bespoke. These include taking one component of the patient’s immune apparatus – typically some T-cells – and rebuilding them to attack something particular on the patient’s cancer.

Perhaps in the not-too-distant future these approaches will be complemented by very early blood tests, which measure such things as circulating tumor DNA (ctDNA) which can tell presymptomatic patients, perhaps years before a cancer may develop, that something is wrong and perhaps should be dealt with. Then, when there is no time urgency at all, vaccines, T-cells or other immune therapies can be deployed to remove the cancer in its very earliest stages, before it is a danger, indeed, before it is even clear whether it would ever become a threat.

Spatial analysis of a tumor’s interaction with the immune system is also being used, and will likely be on the list of important future tests to alert clinicians to how effectively the tumor is sequestering itself from the immune system, and which immune cells are most effective at infiltrating the battlezone.

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Meeting Notes

SUMMARY KEYWORDS

msi, biomarker, hla, tumor, ricardo, mutations, immunotherapy, test, prostate cancer, peptides, patients, tmb, trial, companion diagnostic, approved, vaccine, type, drug, diagnostic, question.

SPEAKERS

Eric Hall, Ricardo Salgado, Heather Tomlinson, Saed Sayad, Brad Power, Richard Anders, Rick Stanton

Brad Power

I'd like to introduce Heather Tomlinson. Heather is a friend who's been doing work with MSI Insiders. I'm sure she'll explain about that. I was intrigued because in general, prostate cancer patients are on a treadmill of treatment options. But the one treatment option that seems to offer a durable response is immunotherapy. Nigel Brockton of AICR said, "You're fighting a system with a system." It's not one drug targeting one biomarker, it's a system, an immune system, fighting this cancer, which is a system, so you have a much better chance at getting a durable response. If prostate cancer patients are looking for immunotherapy as a treatment option, how would they know that they're likely to respond? What are the diagnostics that would tell them? One of those is MSI, where Heather has been working, and she can tell us about some of the other options that would help tell someone if they're likely to respond to an immunotherapy before they take that treatment option. That's the setup, at least from my point of view.

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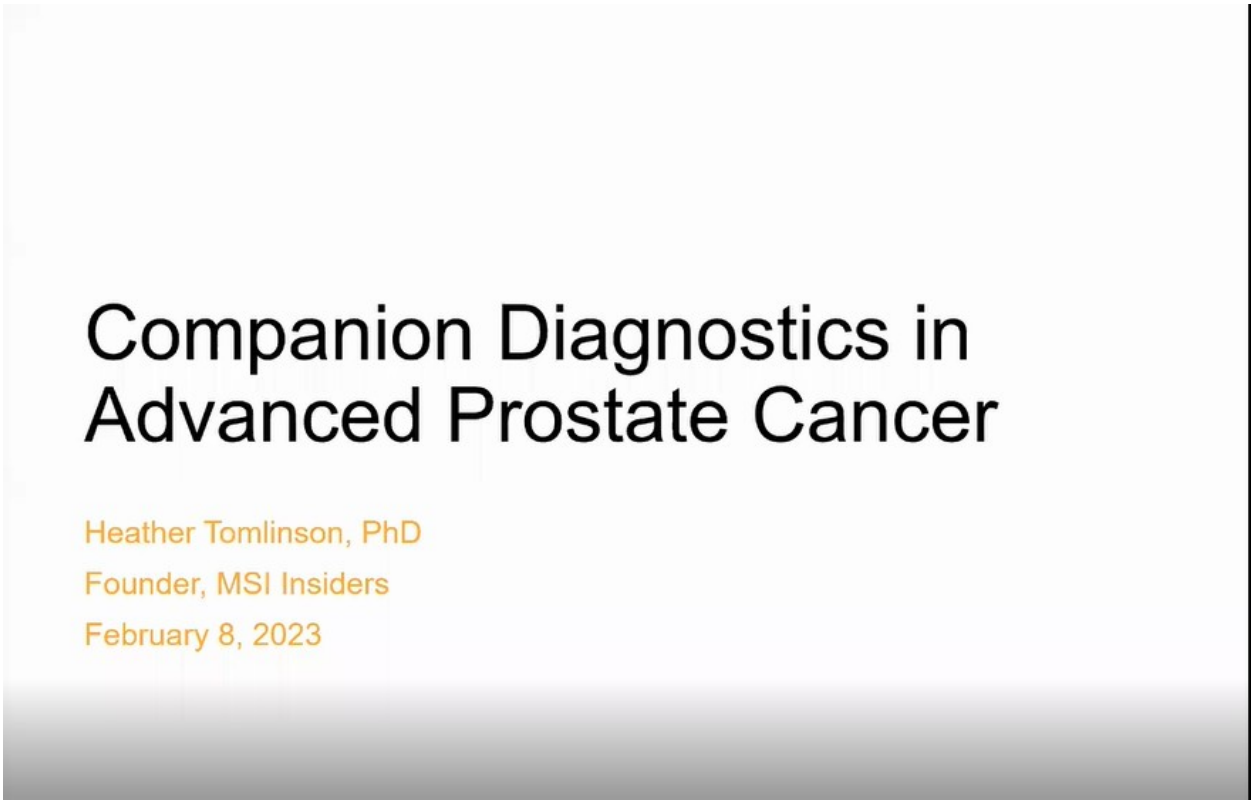

Heather Tomlinson 02:14

I've recently started a patient advocacy and patient education group called MSI Insiders. My background is in clinical diagnostic testing. I worked for companies that make diagnostic tests for the majority of my career. Most recently, I was leading the clinical diagnostic team at Promega Corporation. Promega is a biotech that's located in Madison, Wisconsin. At Promega, they didn't previously have FDA- or CLIA-approved diagnostic tests, but because of my work with a huge team, we helped to make it happen.

One of the things that I did while I was there was to close a companion diagnostic agreement with Merck to develop our test as a companion diagnostic for Keytruda. Through that, I learned quite a bit about some of these biomarkers we're going to talk about today and some of the exciting shifts that we're starting to see in cancer treatment, looking where historically testing and treatment were based on where the cancer was in the body. We're moving more towards the future where it doesn't matter where the cancer originated in the body. What matters is the biomarkers and the genomics that make the tumor that enable us to strategically treat it.

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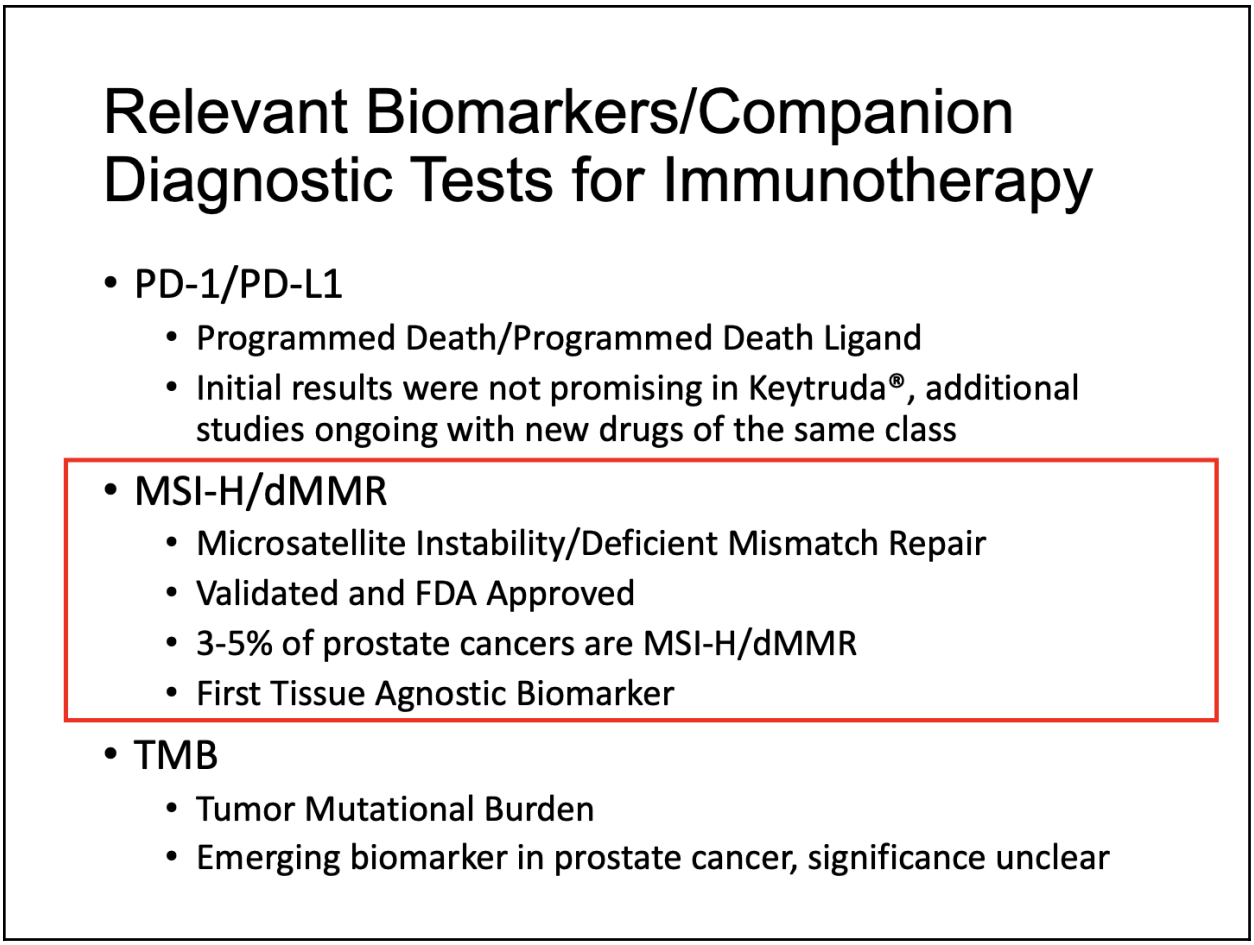
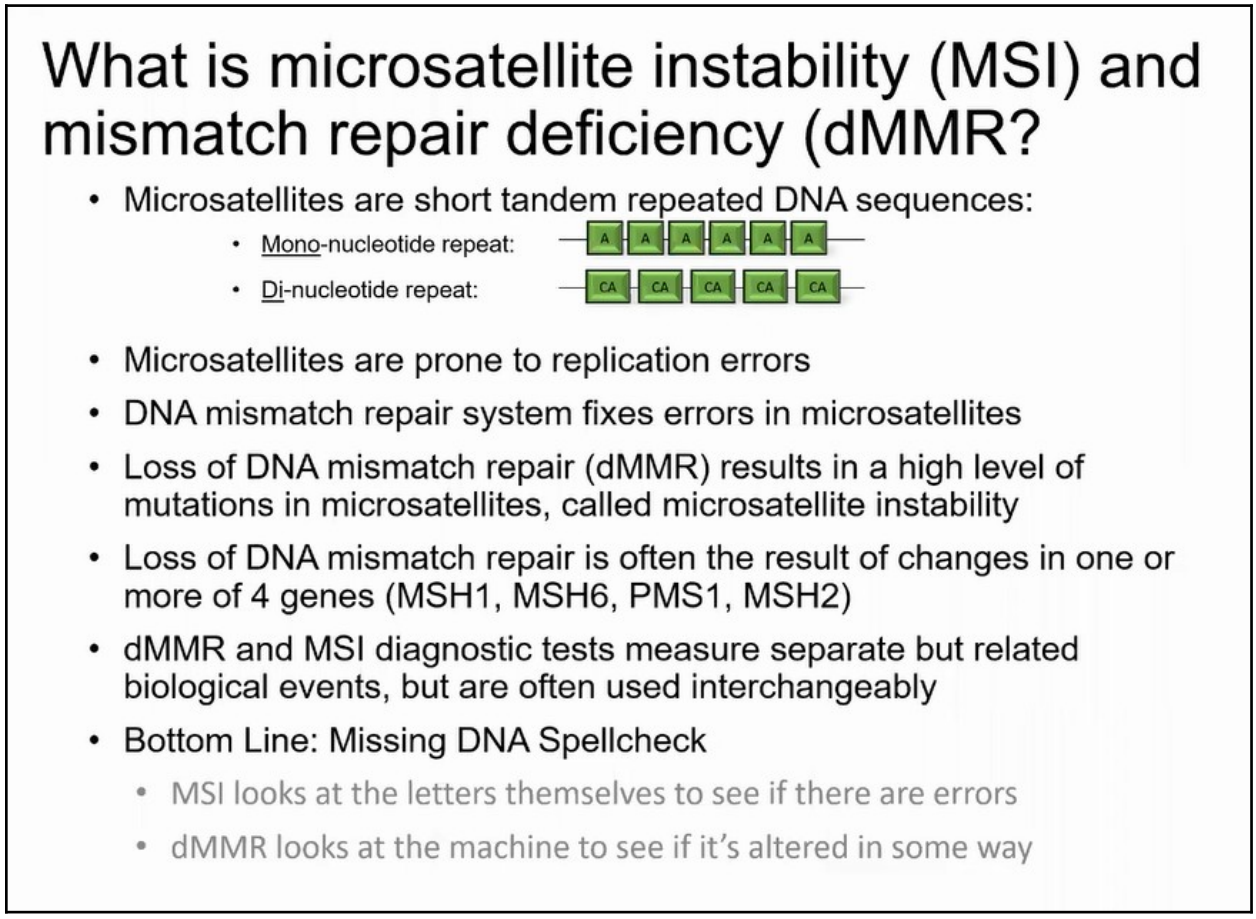


There are currently three relevant biomarkers for immunotherapies. Immunotherapy is essentially removing the brakes of the immune system. I'm sure you've all heard about it. You remove the brakes of the immune system so that the immune system can target and attack the cancer in ways that are unprecedented. That's where the analogy of you having a system fighting a system comes from. When you remove the brakes off the immune system, then that system can fight the tumor in a much more effective way. The first biomarker for immunotherapy was PD-1, PD-L1. PD-L1 is a ligand that's upregulated in cancer cells. When you combine a tumor that has a large number of PD-L1 molecules on a surface with the PD-1 antibody, then that results in a very effective removal of the brakes. This is considered potential for prostate cancer, but PD-L1 is a common biomarker in prostate cancer markers. It's unclear at this point in time how effective understanding the PD-L1 status by doing a diagnostic test to quantify PD-L1 is with prostate cancer patients. Keytruda, which was the 1st PD-L1 immunotherapy on the market, didn't have great results in prostate cancer, but there are other drugs in that class and there are ongoing studies to understand how they might be more effective.

The second immunotherapy approach considers the MSI high mismatch repair deficiency. This is my area of expertise. When you look at this, and when you look at this entire slide, it just kind of looks like alphabet soup. My objective is to kind of explain what these words mean and hopefully bring it down to a level where you're competent having conversations about it with your treatment team. This is a validated and approved biomarker for prostate cancer, and 3% to 5% of prostate cancers are MSI high, so they fall in this category. This was the first tissue agnostic biomarker approved by the US FDA. This was the first time in 2017 when the FDA

said, "Let's rethink this paradigm. Is it important to assess cancers based on where they initiate in the body? Or should we really be just looking at what they're made up of?" That led to the first tissue diagnostic approval for Keytruda in MSI high cancers. In 2020, tumor mutational burden (TMB) also was approved as a tissue agnostic biomarker. This is considered more emerging in prostate cancer. You could get your doctor to prescribe based upon TMB and be within the FDA realm, but in general, prostate cancers don't necessarily have a lot of mutations. The significance is unclear in prostate cancer, and I'll talk about why that is.



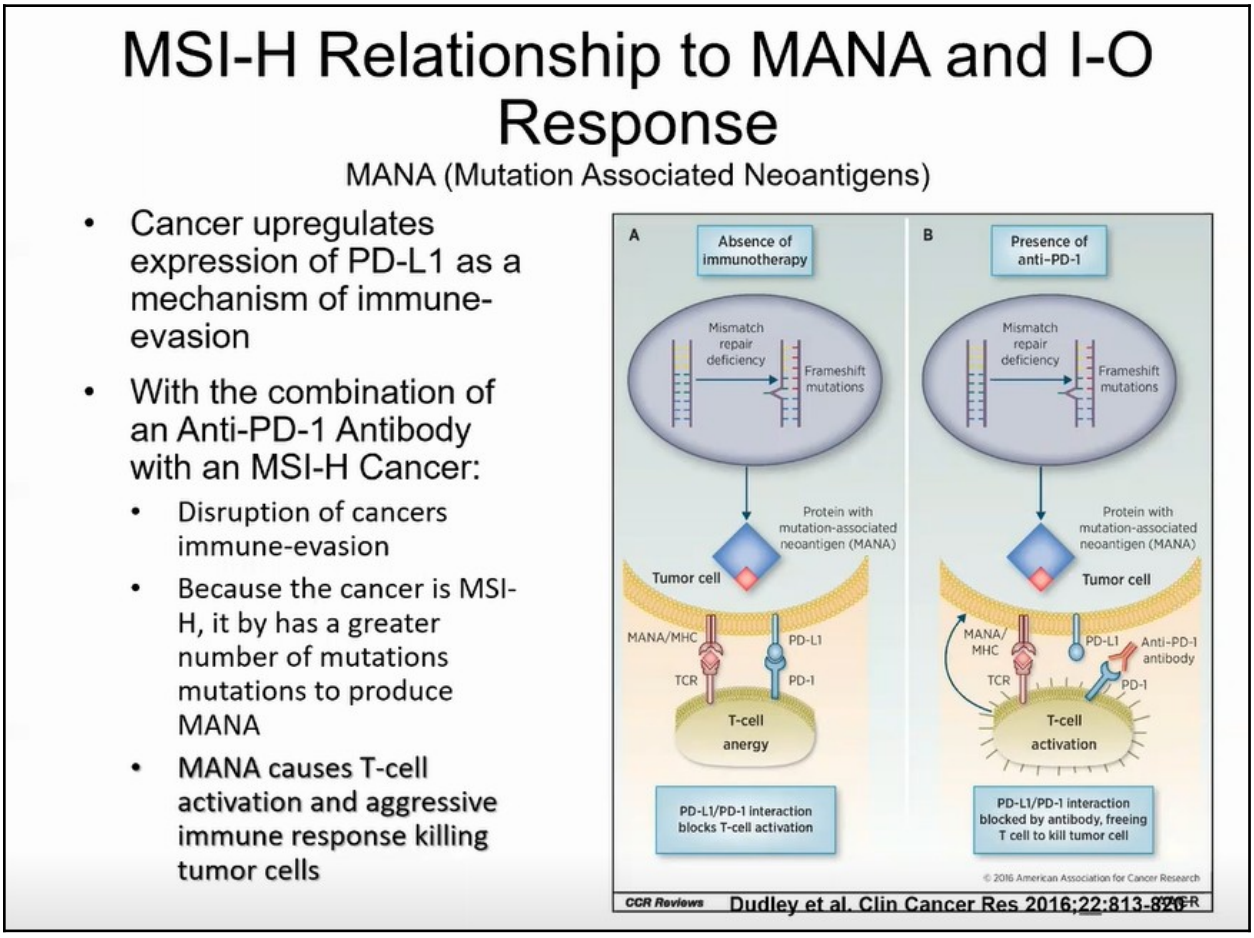
Just to get a little bit into what all these letters mean. Microsatellite instability just essentially means that you have areas within the DNA of the tumor that are prone to replication errors during DNA replication. Whenever our DNA is replicating there are always mistakes made.

Heather Tomlinson 12:29

A good analogy is a typewriter. What normally happens when you're typing with a typewriter is that you have a spell checker. Spell Check will go back and make those corrections for you or alert when there's an error. With MSI high, you have errors that occur, and they're not being corrected. Errors occurring during DNA replication are completely normal. What's not normal is that you don't have this repair mechanism. The repair mechanism is called mismatch repair. That's essentially DNA mismatch repair, or dMMR. When you're looking at MSI and dMMR, they're measuring separate events, but they're used interchangeably. MSI is essentially looking at the paper to see if there are mistakes. It's a functional assay that’s showing you there's a problem with how this typing is going because I can't understand anything. dMMR is looking at

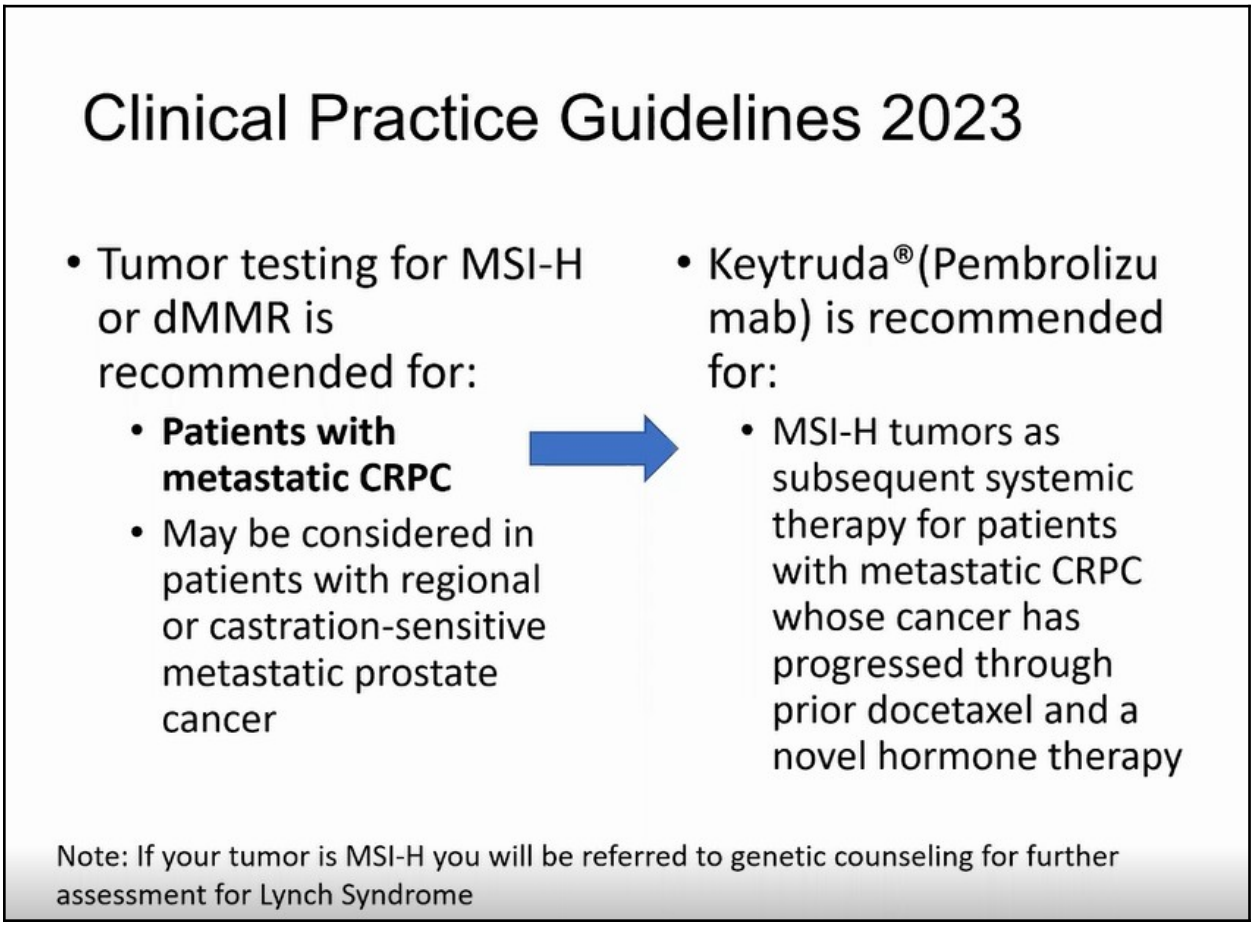
the mechanism within the typewriter that's causing it not to make the corrections. The outcome is essentially the same. You have errors that are occurring during DNA replication. To take that one step further with tumor mutational burden, when you have MSI high, within this analogy, you are looking at the sentences as a whole, and the letters that are within words. When a single letter is missing, everything will shift left or right and cause the sentence not to make any sense at all.

Tumor mutation burden is looking more at point mutations. It takes into consideration all kinds of mutations that are happening during DNA replication. But it's easier to see a single point mutation for example, when you're typing and you just change one letter, than if you leave out an entire part of a sentence or part of a word. That's the difference between TMB and MSI.



Let's bring in the third immunotherapy biomarker, or PD-L1, which is probably one that you've heard a lot about. When you have cancer, it upregulates expression of PD-L1 as a mechanism of immune evasion. The cancer wants more PD-L1 there to essentially block the immune system from functioning as it normally would. When you have a PD-1 antibody like Keytruda, Jemperli, or Opdivo, essentially you can put the brakes on. When you have MSI high, or in some cases, TMB high, you have a larger number of mutations not only in your tumor, but everywhere in your body. You have all of these mutations that exist, which means you have a broader immune response as well, in other words, broader antibodies that are working to fight

the tumor. This combination of putting the brakes on the PD-1 mechanism of immune evasion and having MSI high means you get a one-two punch that can really help fight the cancer. TMB takes into consideration MSI and single point mutations. But again, prostate cancers aren't known to be typically very mutation heavy. From the TMB perspective, it's not totally well understood with prostate cancer specifically.



Looking at the clinical practice guidelines, tumor testing for MSI or mismatch repair deficiency, used essentially interchangeably, is recommended for patients with metastatic prostate cancer and may be considered with regional or castration sensitive metastatic prostate cancer. Once you determine that your prostate cancer is MSI high, then Keytruda is FDA approved and recommended as a subsequent systemic treatment for patients who've already gone through docetaxel and a novel hormone therapy. One other thing I just wanted to note here is that MSI is associated with a form of hereditary cancer, although not all MSI high tumors are hereditary. But if you do have an MSI high diagnosis, you would be referred to genetic counseling for further assessment.

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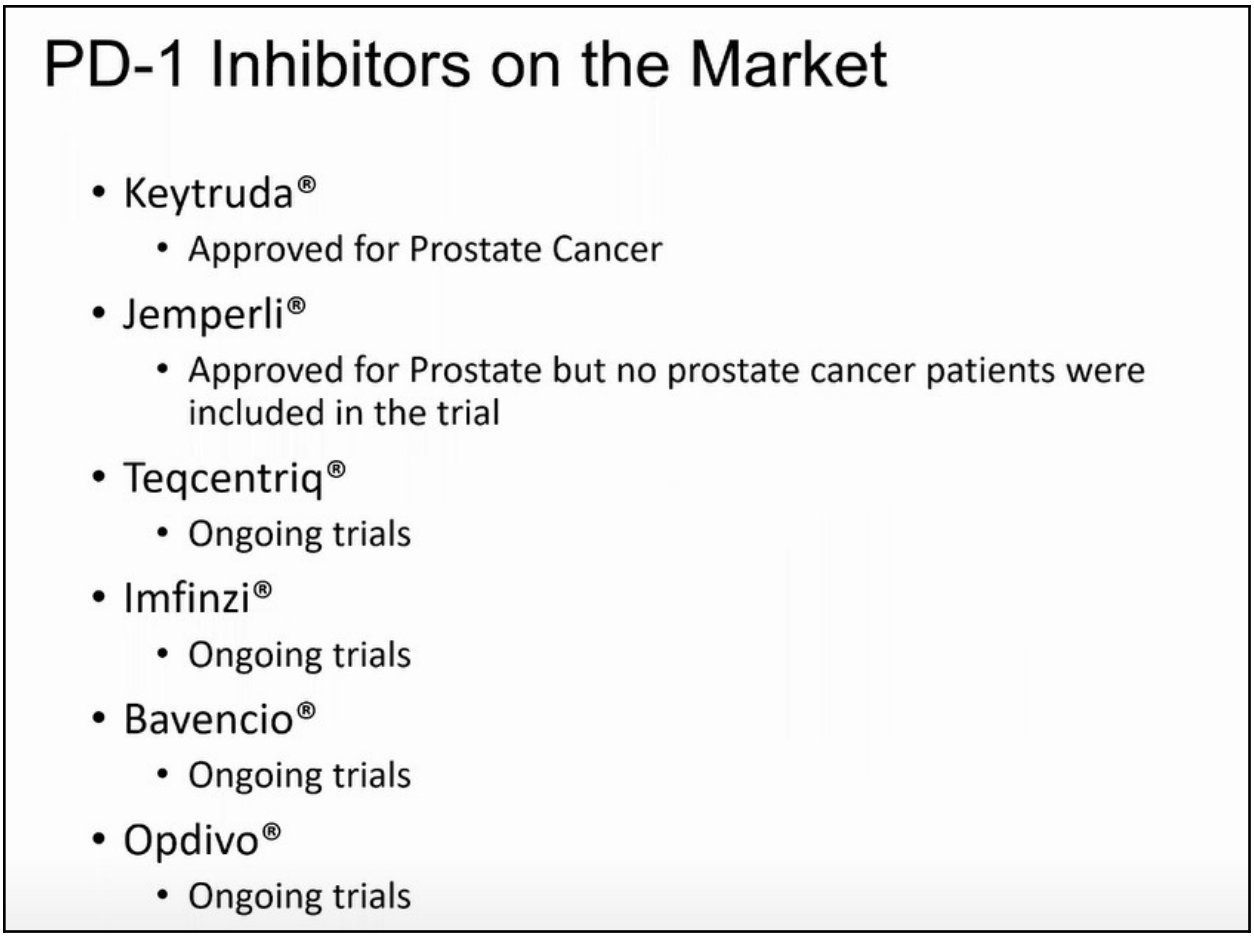

Looking at the PD-L1 inhibitors on the market, Keytruda is approved for prostate cancer for MSI high patients and for TMB high patients. In the Keytruda trial for MSI high, they only included 2 prostate cancer patients. They did not include any prostate cancer patients. Jemperli, which is a drug by Glaxo, is approved for prostate cancer, but no prostate cancer patients were included in the trial. Then there are a number of other PD-1 inhibitors on the market that are approved for other indications where we have ongoing trials to look at their efficacy in prostate cancer. I listed those here.

Brad Power 18:30

Prostate cancer is typically a cold tumor and has a low mutational burden.

Ricardo Salgado 18:56

Hope resides in immuno-oncology. Unfortunately, just like prostate cancer, oncogene-driven lung cancer like ALK, which is what I got diagnosed with, are usually characterized with low PD-L1. MSI, for me, is actually stable, which is defined as an MSI sensor of zero, and TMB of 0.8 mutations per megabase, instead of the average non-small cell lung cancer at 5.3. What I've seen is that there are preliminary tests and trials, and they immediately don't work. However, I've continued to hear more, not necessarily from clinicians, but the scientists that are doing all the work, that maybe now we have a more precise way to measure, and we should not discard these options because maybe in combination and creatively we can actually be more precise.

These alternatives are considering dosage and frequency versus a one size fits all type of model. That's why I'm still very curious about this path, even though most clinicians are hesitant because they want a phase three and want everything to be perfect. That's not the reality right now.

Heather Tomlinson 20:17

I think you're very smart to push there because when you look at what's coming down the pike in terms of clinical trials, and where this field is going, understanding how the drugs work and what tumors they work most effectively against is important. But as you mentioned, the combination of Keytruda plus Lenvatinib, which is a tyrosine kinase inhibitor, is one trial that's just opening up now that has promise, and they're hoping to be able to treat everyone regardless of whether you have a biomarker or not. Opdivo, which typically they've focused only on colorectal cancer for a number of reasons, isn't typically used outside of colorectal and for prostate cancer is looking more promising than Keytruda did. Understanding why is difficult because these PD-1 antibodies are very similar to one another. As they've been available longer, and the trials have gone on, they're starting to see differences in how people respond to them. Understanding why is kind of the next objective.

Saed Sayad 21:48

Are there any statistics about using these drugs?

Heather Tomlinson 21:59

The trials have been very small. It's surprising that they've gone through with such approvals on so many tumor types without a lot of information by tumor type. The objective response rate is 40% in patients up to 36 weeks that have MSI high and 0% at the same time point with those that are not MSI high. Fewer than half were responding.

Saed Sayad 22:50

How many cases?

Heather Tomlinson 22:51

This was in approximately 250 cases, only two of which were prostate cancer. That's one of the positives of this new model. We're looking at biomarkers instead of disease type, right? Where the tumor is in the body is that you can do the trials more quickly and get them available and on the market for a larger number of people. But there's not that much data by tumor type.

Saed Sayad 23:28

My second quick question is that I've heard the way they designed the monoclonal antibody for PD-L1. They try to create some sort of randomness and the design of the ligand. Do you have any information about this? When they want to design instead of the fixed sequence, they create some randomness and it gives that molecule the chance.

Heather Tomlinson 24:07

There are certain parts that have to be conserved for it to act the way that we want it to act. But the randomness that you're talking about could be some of the reasons why we're seeing Opdivo or Bavencio interacting differently and more positively for prostate cancer than Keytruda did. I think their objective would be to try to make the parts that they can as broad as possible, but still have it be effective for the mechanism they're hoping to drive towards.

Saed Sayad 24:52

Do we know exactly if you're using this drug that is targeted vs broad application?

Heather Tomlinson 25:06

Within the pharma companies, they might know more, but nothing has been published and nothing is available specifically about that right now, in terms of, “This is a sequence within this antibody that could mean X, Y, or Z.” There's nothing about that. There's a lot of intellectual property around those types of things as well.

Rick Stanton 25:36

You're associated with a company?

Heather Tomlinson 25:39

No, I am not. I worked for Promega that made an MSI diagnostic test. That was my primary function when I was there. But I left six months ago and started the MSI Insiders advocacy group.

Rick Stanton 25:57

Let's say a patient like me comes to you. Is there anything that you would do for us? I have MSI low like Ricardo. My TMB is low, as most of us are. I've analyzed the RNA seq for Brian and I, and our T-cell infiltration in the tumor microenvironment from deconvolution of RNA seq shows we have very little. I'll ask two questions. Is there anything that you could do for us with that? That's the first question which would pertain to all of us. The second question is, have there been thoughts of prerequisites to change that cold tumor into a hot tumor? Any comments on especially the first one? And then the second question?

Heather Tomlinson 27:22

Brad did say you're a very educated group of patients. I would wholeheartedly agree with that. Most patients aren't even getting this type of testing. The fact that you know your MSI score or your TMB score is huge. Looking at the trials that are available and some of the trials that are available for all comers, not having the biomarkers at this point means you're not going to get these immunotherapies as a standard drug. But as you say, now that we've understood that immunotherapy is such a powerful tool, and there are now trials for vaccines that can look to push your tumor more into that hyperactive state, that mutated state, getting the vaccine that would create a kind of MSI high environment and then be able to treat with immunotherapy is certainly on the horizon. You would definitely need a very cutting-edge doctor involved in the trial because it's quite experimental. But you’re spot on about how we then take advantage of this. If we're not the candidates now, can we become the candidates for the future?

Rick Stanton 28:55

A few years ago, there was hope of turning a cold tumor hot by modulating the innate immune system TLR-4 or TLR-9 agonist. By kicking the innate immune system, it would tend to activate the adaptive immune system. But ultimately, we need an HLA molecule that will be able to present our mutation to our immune system to get recognized. I would think that a personalized vaccine that understands whole exome sequencing, the HLA sequence, the predicted binding from your mutations, would go after that.

Heather Tomlinson 30:08

Are you aware of anyone that's doing specifically that? Is that something that you're interested in pursuing personally?

Rick Stanton 30:21

All of us should be.

Heather Tomlinson 30:25

Are you able to access that kind of technology currently?

Rick Stanton 30:28

I haven't yet. I probably need to ask.

Ricardo Selgado 30:33

Specifically, are you talking about a self-pay vaccine? That's widely available. I think finding the vaccine partner is super easy. The challenging part is if you combine it with IO because that IO carries some toxicity. These kinds of biomarkers are telling you this is cold. Where the field is going, and we're trying to intercept, is what are the tests that you can proactively take to give you a sense of what is the right IO component to complement a neoantigen vaccine? More importantly, what is the right dosage and what is the right sequence? You shouldn't be just the one fit for all types of things. I think that's probably what Rick and I are super interested in learning.

Rick Stanton 31:21

Are you doing anything along those lines, Ricardo?

Ricardo Selgado 31:25

We proactively have done a whole exome and RNA seq, and we did a little bit of whole genome seq to test it. We do have predicted peptides, and we just have them on reserve and ready to go. The other stuff that we have done with this is to very proactively look at TCR gene therapy that's also very promising. It's a little more toxic than a vaccine. But I would pursue a tumor from CtDNA that told me before anything shows up on a scan, and if the tumor from CtDNA would tell me something's going on, I would pursue a TCR strategy. It's just that finding the right TCR from your TCR repertoire that matches your HLA is very difficult. But if you're able to find that, then you can use that, and there's plenty of trials around that. What we've done is look for that and

start identifying that TCR. In summary, all that work has been done. And it's just ongoing in the TCR is much more behind the neoantigen. There's plenty of vaccine companies out there that you can partner up with to do something like that.

Richard Anders 32:37

Ricardo, you said that there are vaccine companies that will make bespoke vaccines for patients?

Ricardo Salgado 32:45

Yes, there are.

Richard Anders 32:48

What do they cost?

Ricardo Selgado 32:49

They range anywhere from $40,000 to $100,000 in the US and abroad they cost $40,000.

Richard Anders 32:55

These are approved in the US? They can do that outside the aegis of a trial?

Ricardo Selgado 33:00

There are some that will. Some within a trial, some without. They'll do it with SPI and other ones. But it's rare.

Richard Anders 33:13

There is something in cancer vaccines that I’ve been following, based on the concept of the regitope (T-regitope).For each HLA, there is a whole class of antigens which are believed to activate regulatory T cells and which downregulate the killer T-cell mechanism. Killer T-cells will be a major part of the killing mechanism in a cancer vaccine, so you want them to be effective. A conventional cancer vaccine, so a theory goes, might be tweaking not just epitopes to promote an active killer T-cell immune response, but also, via the regitope, epitopes that downregulate the immune response. If that’s true, a typical custom cancer vaccine might be working at cross-purposes to itself. There are definitely companies which work with drug companies to help map the regitope in particular situations.. I don't know if that's clinically available but if people are really interested in it, I could make inquires to see..

Brad Power 34:30

You're having a graduate level of conversation, and we might have entry level students in the conversation. There are at least three terms that you used, and for those who didn't hear the Willy Hoos presentation on personalized vaccines, he defined a number of these terms, but I would ask some of our experts here to define "peptide", "TCR" and "HLA." There might have been some other words that were thrown around that are well known to you, but might not be understood by a lay audience. I don't know who wants to do that. Richard, you said “peptide”. Why don't you define that one? Rick, you said "HLA", so why don't you define that one?

Ricardo, you said, "TCR" so why don't you define that one? Then we'll at least have a common foundation, and then we can go back into the details.

Richard Anders 35:34

I said all of them, but I don't think I said any of them first. I think Rick might have said "peptide," but I'm happy to define that one. Proteins are made out of strings of amino acids, and short strings of amino acids, little pieces of amino acids are called "peptides." It's just a way of saying a short protein or protein fragment. The reason peptides are important is because in certain parts of the immune response, what the immune system pays attention to is peptides interacting with "HLA" and "TCRs."

THIS SECTION CAN BE OMITTED OR MOVED TO RICK’S PART, PUT AT THE TOP, DELETED OR LEFT. I’ll give a quick summary of the other terms and others can go into more detail if they wish. HLA stands for Human Leukocyte Antigens (also referred to as the human version of the – more jargon – MHC or major histocompatibility complex), which are specific markers we each have in certain patterns on different cells in our body. In part they serve a powerful and surprising function – to enable our immune system to access the inner workings of a cell to ensure things are OK. For a loose analogy I’ll use in a bit, think of an HLA molecule as a particular decorative pike, that pointy thing on the top of a metal fencepost. But instead of a metal fencepost, it’s on top of a thin hollow metal straw which is stuck into a cell, with one end inside and the pike end sticking out.

The common perception of the immune system is that it surveys the goings on of things that are roaming around in the blood or on the surface of cells and whatnot, and when it finds something, antibodies and other floating immune cells race over and neutralize them. But a lot of the action happens when the invader manages to burrow inside a cell and then, from its cellular foxhole, hijack the cell, grow in number, and gets ready to break out and cause more damage. One part of the HLA system protects against this. Remember those piked straws from above? In effect, the straw makes a connection from the inside of the cell to the outside, drawing out (and this a very loose metaphor, the process is quite different) core samples of proteins from the cell innards. When the protein reaches the top of the straw it may be impaled (or not) on top of the spike (like heads displayed on pikes in the gruesome old days). As mentioned above, these spikes come in different patterns, and while each person only has a few different patterns in their body (and that is determined by their genetics), and only one spike pattern is on each straw, when you look across the entire human race there are many thousands of different patterns and it would be extremely rare for two people picked at random (siblings aside) to have identical combinations. The repertoire of straw/spike pairings in a particular person determine that person’s HLA type.

Continuing this metaphor of pikes and heads to HLA types and cell protein debris, different “pikes” can display some types of heads - er, proteins, but not others, and so a protein that one person’s pike might show proudly could not be displayed at all by another person with a different pike pattern. But once the protein “head” is stuck on the pike, passing T-cell soldiers, each trained to look for very specific patterns (via, in fact, their T-cell receptors, or TCRs), will inspect the macabre pike/head combination and see if it matches the pattern it has been trained to find. That’s loosely speaking, the general idea.

Brad Power 36:25

Rick: HLA?

“How MSI and Other Tests Can Guide Immunotherapies for Cancer Treatment” (Heather Tomlinson) [#43]

Rick Stanton 36:30

The following is a more thoughtful and in-depth discussion added after the meeting.

Here are some links on HLA:

https://www.youtube.com/watch?v=t81EjpkCiss

https://www.nature.com/articles/4402235

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7185345/

Let’s start with some definitions as the immune system is quite complex!
Antigen: A toxin or foreign substance which can be recognized as “non-self”, and induce an immune response in the body.

TAA (Tumor-Associated Antigen): These are typically peptides (short amino acids, or short fragments of a protein, typically about 13-30 amino acids in length). These TAAs are of great interest to cancer patients (and their immune systems!) as these TAAs can result from tumor genetic mutations which can be recognized as “non-self” (foreign) by our immune systems and used to kill our tumors.

MHC (Major Histocompatibility Complex): MHC molecules in humans are termed HLA (Human Leukocyte Antigen) and are encoded on chromosome 6 of the human genome. For human discussions, consider MHC and HLA one in the same. HLA comes in types, HLA-I, HLA-II and HLA-III. For this discussion, I’ll focus on I and II.
MHC class I (or HLA class I): Classical MHC class I molecules are ubiquitously expressed on all mammalian cells (and cancer cells!). The function of these molecules is to sample and present peptides on the cell surface, and upon antigen recognition - bind to T-cells and natural killer cells (NK). Classical HLA I genes are: HLA-A, HLA-B, and HLA-C.
MHC class II (or HLA class II): Classical MHC class II molecules are expressed on Antigen Presenting Cells (APCs) including dendritic, macrophage, and B cells. Classical HLA II genes are: HLA-DR, HLA-DP, and HLA-DQ.
Human diversity: There are roughly 59+ different HLA-A proteins found in humans. 118+ different HLA-B. 124+ HLA-DR. Each of these HLA genes (class I and II) and the different subtypes can present different sets of peptides (but not all peptides!) For example one person might have an HLA-A1 (but not HLA-A2:A59), and another person might have an HLA-A2 (but not HLA-A1, HLA-A3:A59). This matters as not all peptides can be presented for T-cell recognition. A peptide can be thought of as a short strand of amino acids similar to a hot dog, and not all HLA types (the hot dog bun) can bind to a particular hot dog! They have to match. This is a similar analogy to Richard’s pike analogy above.

Why is the above important?

> When you get a whole exome sequence you should be able to get a good definition of your HLA type (your set of a few hot dog buns). Bioinformaticians can query what mutations you have. You may have three mutations that are fairly well known to be driving your cancer, but you have probably another 30 mutations that are called variants of unknown significance. They are mutations, but we don't typically associate them as driving the cancer (we just don’t know their function - hence: unknown significance). One of those mutations, unlike perhaps a well known driver mutation, may fit in that hotdog bun really well, and so you must run a little analysis to determine what mutations you have (not just driver mutations assessed from a selected gene panel), and what is their binding affinity to your HLA type (the hot dog bun types you have). This analysis will estimate which mutations can actually activate your adaptive immune system - and this knowledge will drive the definition of a personalized vaccine for a particular patient, as well as provide understanding of prediction of response to a variety of immunotherapy approaches.

Heather Tomlinson 43:35

Going back to what Ricardo was saying, you need to understand specifically which immunotherapy you can treat if you have a vaccine that would modify how your immune system functions. Finding that match is the difficult part. I don't know anyone who's done it. Ricardo, I think you're much more experienced than I am at this level of complex cases and complex therapies. I applaud you for what you've done and sharing it here with the group.

Brad Power 44:15

If I'm thinking of Eric Hall, or Chad or Mike on this call, or Kevin, will they have gotten these three tests? You said the PD-L1, the MSI, and tumor mutational burden, is that just sort of a standard part of any kind of gene sequencing test?

Heather Tomlinson 44:39

MSI would be part of the standard test. TMB may or may not, and PD-L1 typically is an immunohistochemistry test. It's a protein-based test. Those of you who really understand your diagnostic profile, did you have to ask for PD-1? Did you get her to ask for it? I would say, typically, you would want to make sure you're getting all those results.

Ricardo Salgado 45:09

My reports on this came from MSK which is part of MSK Impact and their next generation sequencing capacity. They told me that my MSI status is stable, which they defined as an MSI score of zero. It's low, which is zero to 100, as well. Number two, it does tell you the TMB right there, it was 0.8 mutations per megabase. Instead of the normal non-small cell lung cancer that's non mutated, which was 5.3. They had another report in parallel, which said that I was PD-L1 negative. I think those are the three markers that you're looking for. And I didn't ask for them, they came.

Heather Tomlinson 45:51

But that's MSK, probably the very best place in the United States to be treated. Not everyone gets that automatically.

Brad Power 46:05

One advice for patients would be to check the testing that's been done on you and see if you haven't got that trio, maybe get the one like the PD-1 and PD-L1 when you have a chance. That's done from immunohistochemistry? Is that if they have FFPE tissue, then they can just go in, and stain it and look at it?

Heather Tomlinson 46:27

For PD-1 and mismatch repair deficiency, those can be done via staining, by IHC. MSI and TMB are done on nucleic acid, which you can get from FFPE, just by scraping a part of the tumor.

Brad Power 46:53

Before we move from diagnostics, is there anything on the horizon that would be a next generation test for companion diagnostics for immunotherapy? Is there anything you've seen, that's the next best thing that's in the lab that might be coming out soon?

Heather Tomlinson 47:04

From that perspective, you'd go to the meetings where the pharma companies are looking to identify biomarkers and next gen tests that would help them get their drugs approved, and it's really centering around this area of DNA repair and errors within DNA. The next one that's getting the most attention is homologous recombination repair deficiency. Looking at different ways that the DNA is being repaired, and how we can disrupt that. Also there's kind of push towards a more of a hot state of a tumor which would involve HRD, or homologous repair deficiency.

Brad Power 47:42

If a patient said, “I want to then get that test,” is that straightforward? It's from the tissue that I have, and it's FFPE? Is it just yet one more test to request?

Rick Stanton 47:59

It's not straightforward. You can request from a pathologist an IHC assessment and he/she will give you a vague four-word response, "between zero and 5% TILs observed." You have no idea of the spatial compartments. Zero to 5% is kind of a big difference.

Brad Power 48:36

Spatial would be on that list of in-the-wings tests that might be useful because you were talking about neighborhoods and whether there's immune infiltration. That would be a test of the future that will eventually be available to patients.

Eric Hall 49:20

I had a question about the trio of biomarkers to get. I had whole exome sequencing from Caris. I had the MSI, TMB, and PD-L1 tests done, not that I'm positive for them. I don't have the PD-1. I think that was the other one you had in your presentation. Do I need to get that? Is that different from PD-L1?

Heather Tomlinson 49:49

No. So PD-1 is the diagnostic test and then if you have PD-L1 positive you have the opportunity to be treated with a PD-1 inhibitor. That's the drug. That's a good piece of information that you can use for discussing with your physician. PD-1 antibodies like Keytruda and Jemperli are not approved for PD-L1 positive prostate cancer, but they might be willing to have some conversations with you about what opportunities would exist.

Ricardo Salgado 50:22

They're approved for PD-L1 negative?

Eric Hall 50:29

I had the test, but it was a negative result.

Heather Tomlinson 50:33

Negative. Okay, then that wouldn't be an option for you, but you got the right test, so that's good.

Eric Hall 50:41

That's ultimately my question. Did I get the test?

Heather Tomlinson 50:45

Yes.

Brad Power 50:48

We love drug combinations. I wonder if anybody's looking at a cocktail of immunotherapies? In the same way that someone said something about a variety of ways to hit the tumor. Could you have Opdivo, Keytruda, and one of the others as a cocktail, and have a better immunotherapy response?

Heather Tomlinson 51:22

I'm not aware of anyone doing that. It's more looking at hormone therapy plus immunotherapy or immunotherapy and other agents. I'm not aware of any trial that looks at multiple immunotherapies in a single patient. One of the challenges there would be the dosing and understanding how to make them all effective, but without overpowering the patient's immune system. It's certainly an interesting idea to explore.

Ricardo Salgado 51:55

I haven't seen two PD-L1 inhibitors. What we have seen is combining a PD-L1 inhibitor with a CTLA-4. You'll have nivolumab (Opdivo) with ipilimumab, for example. I just attended the 2023 Cancer Immunotherapy Winter School, and there was this doctor, Sumit Subhudi from MD Anderson, who was there. He has interesting statistics around combining the things. He contends that if you're able to combine them, the key there is quoting a trial. He said that if you

just do nivolumab (Opdivo) in melanoma, you're going to have an overall response of 55%. But if you combine nivolumab plus ipilimumab, we're seeing a 71% response is the same thing with non-small cell lung cancer. If you just apply nivolumab, you'll get a 44% response in a clinical trial. If you combine nivolumab plus IPI, you’ll get 66%. It was not insignificant on those combinations that he was presenting. But you have the toxicity. He's an expert at dealing with all the toxicity. It's more toxic. You want to get him close to death, and get that durable response, which is a little bit intimidating. What I'm hearing thematically is that there are tests that you could actually see. If you can be more precise on yourself, you can pursue precision medicine more effectively. You can take some calculated risks. But this is FDA approved and ready to go. This would be in a clinical trial type of format, but that's what I learned.

Richard Anders 53:41

You gave a list of a bunch of diagnostics that you said were approved. Were they actually approved, or were they cleared? I ask because – I know you know this – "cleared" is a different, usually simpler, regulatory burden than "approved." I'm just wondering what the level of regulatory oversight is on these.

Heather Tomlinson 54:18

Anything that has a companion diagnostic on a label has been "approved." It's a more rigorous process that those must go through. One of the challenges with companion diagnostic testing is that the FDA doesn't enforce that the lab uses an FDA-approved diagnostic test for that indication. You can go through all the trouble to get a companion diagnostic status, but then the clinical lab can just go ahead and do something off-label that does the same thing but hasn't gone through the same level of regulatory rigor. Right now, Foundation Medicine is mainly the approved companion diagnostic tests for TMB MSI high. Roche antibodies are approved for dMMR.

Richard Anders 55:08

What about the MSI tests?

Heather Tomlinson 55:13

The MSI test that's approved right now is through Foundation Medicine and that measures MSI in a different way. The Promega assay that I was working on is going through the approval process right now. It's cleared as a diagnostic test, but not approved as a companion diagnostic.

Richard Anders 55:32

One other question, which I think you mentioned. Is anybody checking the mutation profile of PD-1 or PD-L1 to see if some of some of the drugs are better at hitting those receptors? The reason I'm asking that is, Heather, you mentioned earlier the different drugs that hit PD-L1 might not hit a mutated version of PD-L1, and yet that mutated version may be the one on your cell. If you're taking immunotherapy for a mutated version of PD-L1, it might not be the right drug, and you might be able to find another one, but I don't know if anyone's doing that.

Heather Tomlinson 56:17

“How MSI and Other Tests Can Guide Immunotherapies for Cancer Treatment” (Heather Tomlinson) [#43]

Rick (Stanton) is here making comments that no mutations are really noted in patients for PD-L1. That's the drug mechanism versus the patient mechanism. That's probably part of the reason there, but anything I'm aware of in that area is just purely on the research and academic level right now.

Richard Anders 56:41

Rick, when you say none are noted, does that mean the tests don't look at them, or they have tested it, and there are no mutations?

Rick Stanton 56:50

The latter.