The IO Quiz Show

The Promises and Potential of Predictive Biomarkers for IO

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Thank you. That was a great overview, Sacha, the complexity. I am going to try and bring this back to add a little bit more to just a simple “what’s available now and if you have a patient in front of you, how do you figure out whether you are going to give them anti-PD-1 or anti-PD-1 in combination with something else”, which is really a difficult problem.

Disclosures

These are all my disclosures.

Educational Objective

And the objective, of course, is to try and understand the recent advances in the field of predictive biomarkers, primarily clinically related.

Which of the Following Predictive Biomarkers Were Used as Companion Diagnostics Leading to Approvals for Treatment With Single Agent Anti-PD-1 or Anti-PD-L1?

The first question is: Which of the following predictive biomarkers were used as companion diagnostics leading to approvals for treatment with single agent anti-PD-1 or anti-PD-L1?

Number of T lymphocytes found in the tumor biopsy, PD-1 expression in the tumor, PD-L1 expression on tumor or immune cells or both, tumor mutation burden, interferon-gamma gene signature within the tumor, mismatch repair deficiency, microsatellite high, and then different combinations.

The right answer is three and six, and so we have a very sophisticated audience.

Spectrum of PD-1/PD-L1 Antagonist Activity

Here’s a simple problem. PD-1/PD-L1 antagonists, that’s primarily what we are going to talk about, have now been approved in all the diseases for which I have listed in yellow up there. The problem that we have in the clinic is that first of all, not all patients respond. In fact, for many of these tumors only 20% or so of the patients will develop significant benefit. We don’t know how long to give these drugs for; they are incredibly expensive, so one of the key needs in the clinic is to try and figure out who is going to respond to these and who won’t, and if you are not going to respond, we need to know whether to give PD-1 plus something else or another drug.
Conditions Necessary for Tumor Response to Single-Agent Anti-PD-1 or Anti-PD-L1

In order to develop biomarkers, we want to understand a little bit about the biology which you have already heard from Sacha. But obviously, in order to respond you need to have tumor antigens so you can address that, and so you can think about that as the tumor mutational burden. You have to have a preserved antigen presentation machinery so you have to have Class 1 and beta-2 microglobulin. At some point T-cells have to be primed and activated, so the T-cells have to be there some place hopefully within the tumor microenvironment, so you can look at any of those things as a way to possibly predict for whether PD-1 or PD-L1 will be active in your patient.

Now for the T-cell itself, when a T-cell becomes activated, it up-regulates PD-1. So if it’s an antigen-specific T-cell that expresses PD-1, so you would think, “Well, if PD-1 is there in the tumor microenvironment, that might predict for a response.” When those T-cells start to recognise antigen, they make interferon-gamma so you might say, “Well, if interferon-gamma is there, then maybe PD-1, PD-L1 will work” and then what interferon-gamma does is it actually up-regulates a ligand for PD-L1 and so you might say, “Okay, I'll look for PD-L1 in the tumor microenvironment.” What actually happens, of course, is that PD-L1 then binds to PD-1 and inhibits T-cell function.

Potential Predictors for Clinical Response to Anti-PD-1/PD-L1 Pathway Blockade

You could address any parts of these in order to look for biomarkers of response to single agent anti-PD-1 or anti-PD-L1 and in fact, people have done that. It’s logical you can look for PD-L1 expression either in the tumor or tumor-infiltrating immune cells. You can try and look, as Sacha has shown you, for where the T-cells are, the presence or location of T-cells, and you can look for all sorts of things within that, whether they are activated, they have granzyme-positive, where they are within the tumor.

You can look for a gene signature, you can look for PD-1+ T-cells or PD-1 T-cells that also express CTLA-4; some people have looked at that. You can even look to see whether the T-cells are close to the PD-1+ T-cells or close to where PD-L1 is expressed, which is another way of, maybe, making that assay even more sophisticated.

You can look for the interferon-gamma gene signature that we just talked about before; you can look for T-cell clonality. For example, if the T-cells within the tumor are more clonal that means they’ve seen antigen and they are expanded, and that might predict for a response.

You can look for tumor mutation burden – you are going to hear a lot more about that from Naiyer later – and then you can look for clinical factors. So high LDH may be a poor
predictor; some people have looked at liver metastases, and maybe you might combine all of these in order to get the best predictive score, in some sense, for whether your patient will respond or not.

**PD-L1 Expression Is Heterogenous and Associated With Tils**

This is a much simpler version of what you have already seen, but when you take a piece of tissue and you look at it, the key point here is that it’s very heterogenous. So when you do a biopsy it’s important to remember that depending on where you put that biopsy, you might get a very different result for PD-L1 expression, for example.

When you look within a tumor, T-cells are often heterogenous; they may be surrounding the tumor, and PD-L1 is often expressed close to where the T-cells are, so if you put your needle in the right place you might get the wrong answer as to whether the tumor is PD-L1+ or not. And different diseases have different patterns of expression of PD-L1 and TIL, and Lieping Chen and others have identified really four different patterns. You can have T-cells there and PD-L1 expression, T-cells there without PD-L1 expression or maybe no PD-L1 expression at all. Sometimes there are no T-cells at all also.

In trying to develop anti-PD-1 and anti-PD-L1 assays, each company has developed their own assay for PD-L1, because that’s what we were thinking would be the best predictor for a response to either PD-1 or PD-L1 blockade.

**Comparison of PD-L1 Platforms NSCLC, 13 pathologists, 90 slides**

So a study was done. It was by a number of pathologists led by David Rimm, looking at non-small-cell lung cancer. There were 13 pathologists who looked at these slides and 90 different slides, and what you can see from this picture here is that not all the PD-L1 assays give you the same information. Some of them correlate quite well. For example, the Merck 22C3 assay and the Bristol, the Dako 28-8 assay look very similar in looking at staining for both T-cells, for tumor cells and immune cell PD-L1 expression. But the Genentech assay, the SP142 assay, does not correlate well with the other two. The third one is an investigational assay.

If you do a PD-L1 assay using the Genentech assay, that may not give you the right answer, for example if you are going to use pembrolizumab. So it becomes very, very complicated, and you need to use the assay that’s associated with that drug in that specific setting.

A different patient with a different disease you might need to use a different assay.
Which of the Following Is True Regarding the Use of PD-L1 as a Predictive Biomarker in Metastatic Melanoma and NSCLC?

Which of the following is true regarding the use of PD-L1 as a predictive biomarker in either metastatic melanoma and NSCLC? The higher the expression of PD-L1, the higher the response; patients with less than 5% tumor expression of PD-L1 would not benefit from anti-PD-1 single agent treatment in melanoma. In the randomized study of ipi-nivo versus nivo versus ipilimumab, improved overall survival was seen for the ipi/nivo arm compared to nivo in tumors with high PD-L1 expression, and the last is pembrolizumab as a single agent is approved for first-line treatment of NSCLC in patients with PD-L1 expression greater than 1%.

So, the correct answer is number one – all the other, two, three, and four are actually incorrect, and I will show you some of the data to support that.

Objective Response to Anti-PD-1 by PD-L1 Expression

This is the Merck assay; this is the 22C3 assay, and this is looking at melanoma and this is different stainings with different tissues and positive is really two-plus, so anything that's zero – zero, one is considered negative.

You can see that it can be variable and obviously depending on which tumor you take and what part of the tissue, this assay could be very different.

Objective Response to Pembrolizumab by PD-L1 Expression Level (22C3 Antibody Assay) in Melanoma

However, when you look at this assay and you look at response in melanoma, there’s a clear correlation between higher expression of PD-L1 in response rate, PFS, and overall survival, and you can see here as you go up on the melanoma score all of those three endpoints of activity increase.

Response to Nivolumab Versus Dacarbazine by PD-L1 Status in Melanoma

However, in melanoma, when a randomized study was done comparing anti-PD-1 to dacarbazine, it didn’t matter whether your tumor expressed PD-L1 or not. In both arms, regardless of PD-L1 expression, anti-PD-1 nivolumab was better than dacarbazine. So in melanoma, even though you do better the higher the PD-L1 expression, if by this assay you’re PD-L1 negative, you should still get anti-PD-1. So it’s not a useful assay for selecting patients for a single agent anti-PD-1 in melanoma.
CA209-067: Ipi/Nivo or Nivo Versus Ipi Post-Hoc Analyses of OS by PD-L1 Status

On the other hand, if you look at the randomized study of ipilimumab, nivolumab versus nivolumab versus ipilimumab, in that case PD-L1 expression, perhaps, was important as a predictive biomarker.

In the patients who had high level expression of PD-L1 – this is now a retrospective analysis and a study that really wasn’t even meant to compare the combination to nivo alone – the patients who had high expression of PD-L1 just measured by greater than or equal to 1%. There appeared to be no effect on overall survival by adding ipilimumab to nivolumab, and that’s important because ipilimumab adds a lot of toxicity to single agent anti-PD-1.

On the other hand, the patients who were PD-L1 negative did appear to benefit. This wasn’t statistically significant; it was close, but those were the patients that appeared to get the most benefit from adding ipilimumab. So in melanoma if you are going to give somebody single agent anti-PD-1, you don’t care about PD-L1 expression.

But if you are thinking about giving a combination, you probably ought to be giving the combination to the patients who are PD-L1 negative – that’s one way of looking at this.

PD-L1 Expression in NSCLC and Response to Pembrolizumab

In lung cancer a very different story – a different disease, different assay, slightly different story. You can see here on one half of this graph that the higher the expression of PD-L1, the higher the response rate, and if you look at the curves on the right, it’s only the patients that had PD-L1 expression greater than or equal to 50% that appeared to have the long, durable responses.

Pembrolizumab Versus Chemotherapy in NSCLC, PD-L1 > 50%: PFS and OS in the ITT Population

On that basis, Merck decided to do their randomized study of chemotherapy versus pembrolizumab in patients who had PD-L1 expression greater than 50%, and indeed, this study was positive for both progression-free survival and overall survival. In this case, PD-L1 expression is used to select whether patients get anti-PD-1 or not, and it’s a very useful biomarker.

You ask the question, what happened to the patients who had PD-L1 expression from 1–49%? They weren’t in this trial, and maybe Naiyer can speak more to that a little bit later.
First-Line Nivolumab vs Chemotherapy for Stage IV or Recurrent NSCLC (PD-L1 ≥ 5%) Did Not Improve OS

Now, BMS ran a very similar study but instead they selected their patients on the basis of PD-L1 expression greater than 5% using their assay, and in this case it did not improve overall survival.

How do you explain this? I still don’t have a good explanation for this. This doesn’t mean that anti-PD-1 did not work in this setting. Nivolumab probably is better than no treatment in this setting but it wasn’t better than chemotherapy.

Nivolumab Plus Ipilimumab in First-Line Treatment of NSCLC: Efficacy Across Tumor PD-L1 Expression Levels

And then with the addition of ipilimumab to nivolumab – this is data that I think Mark Socinski and Matt Hellman presented – this is, now, not a randomized trial. This is looking at different phase 2 cohorts who got nivolumab alone or the combination in NSCLC, and the interesting part of this is that unlike melanoma where you see most of the effect of adding ipilimumab to the PD-L1 negative population, in lung cancer it looks like the biggest effect of adding ipilimumab to anti-PD-1 is in the PD-L1+ group.

Again, different disease, a different assay and a different conclusion about which group of patients respond and what this tells you is that when you use PD-L1 as a biomarker for predicting response, you have to know the assay, you have to know the indication, the disease, and the results are very, very different.

PD-L1+ Tumor-Associated Immune Cells Are More Predictive of Nivolumab Outcome in SCCHN Than Tumor PD-L1 Expression

Now one more interesting thing is that both the assays – both the Merck assay and the Bristol assay – are designed to detect PD-L1 expression on tumor cells, but that may not be where the story is.

This is retrospective data from the head and neck randomized trial. I think it was CheckMate 141, presented by Bob Ferris at ASCO, and the point that I want to make here is that at least in the retrospective look here in head and neck cancer where nivolumab was better than chemotherapy, it wasn’t PD-L1 expression; it was expression of PD-L1 on the tumor-associated immune cells that was the strongest predictor for a survival effect from anti-PD-1. So depending on the tumor and what you’re looking at, it could be the assay, the disease context or looking at either tumor cells or immune cells or both in the predictive value of that test for outcome to anti-PD-1 or anti-PD-L1.
We’ll move through the rest of this a little bit more quickly. **Nonsynonymous Mutation Burden Predicts Clinical Activity of Pembrolizumab in NSCLC**

This is work from Dr Rizvi showing that mutation burden can predict for a response in NSCLC; the higher the mutation burden, the more likely you are to benefit, and you can see from the curves here that the patients who had real benefit from anti PD-1s were the ones who had high nonsynonymous tumor mutation burden.

**Impact of TMB on the Efficacy of First-Line Nivolumab in Stage IV or Recurrent NSCLC**

The analysis of that randomized trial I showed you of nivolumab versus chemotherapy – this was a reanalysis of the data looking at tumor mutation burden in PD-L1 – and it turns out that the patients who had high tumor mutation burden and PD-L1+ greater than 50% did the best. That’s the purple curve over there on the left.

If you had high tumor mutation burden and low PD-L1 you still did relatively well, but if you had a low tumor mutation burden you didn’t do very well at all compared to chemotherapy. So tumor mutation burden is an important biomarker in NSCLC, in fact maybe even more important than the PD-L1 assay.

**Pembrolizumab in MMR-Deficient Cancer**

Now does this apply to any other tumors? And the answer is yes. The people at Hopkins decided to look at whether patients with GI malignancies who had high tumor mutation burdens as assessed by microsatellite-high tumors which have DNA mismatch repair deficiency, whether those patients would have a high chance of responding to anti-PD-L1.

This data was published in New England Journal and showed that basically if you had microsatellite instability, you had a high tumor mutation burden and also a high chance of responding to anti-PD-1 as a single agent.

**Patient Survival and Clinical Response to Pembrolizumab Across 12 Different Tumor Types With MMR Deficiency**

This eventually led to essentially a registration for anti-PD-1 in patients who had microsatellite instability or DNA mismatch repair, and you can see the very impressive waterfall plots here, the very impressive progression-free and overall survival in a number of different tumors.

**Interferon-Gamma and Expanded Immune Signatures Correlate With Response to Pembrolizumab in Melanoma**
What about interferon-gamma gene signature? Tony Ribas and his group have shown that if you have a high interferon-gamma gene signature you are likely to have clinical benefit from anti-PD-1. You can see that here.

**PFS and OS in Patients With Melanoma and Interferon-Gamma Signature Score Above and Below the Cutoff**

And then in this next slide you can see that the patients who had higher PFS and higher overall survival were the ones who had a high interferon-gamma gene signature.

I have shown you that PD-L1 can predict for response in the right context, tumor mutation burden can predict for response in the right context, interferon-gamma gene signature can predict for a response in the right context; the question is how do you use these and how do you use them together.

There’s an abstract here being presented that in fact if you have a high tumor mutation burden and high PD-L1, regardless of disease, you have a higher chance for response. If you have one or the other, you have an intermediate chance for a response, but if you have a low tumor mutation burden and very little interferon-gamma, a low interferon-gamma gene signature your chances of responding are very, very low.

**Which of the Following Does NOT Predict for Response or Resistance to Single-Agent Anti-PD-1 or Anti-PD-L1?**

In the last couple of minutes I just want to go over a few other things. Here’s a question to start with. Which of the following does not predict for a response or resistance to single agent anti-PD-1 or anti-PD-L1? A mesenchymal-angiogenesis gene signature, a myeloid cell gene signature, the gut microbiome, patterns of proteins present in peripheral blood, tumor cell signaling pathways or defects in the tumor antigen presentation machinery or the oral microbiome?

A very sophisticated audience – that’s right, it’s the oral microbiome.

The point that I want to make in the next few slides is that there are all these other things that we use that individually can predict for a response to PD-1 or anti-PD-L1 or for lack of response.

**Transcriptome Map of Angiogenesis and Immune-Associated Genes in RCC Tumors**

This is data in a renal cell trial that we participated in, in which an RNA-Seq was done to get a gene signature. You could look for an angiogenesis gene signature, a myeloid gene signature or a signature of T-cell effector function within these tumors and you could
take populations that had, for example, a T-effector high signature and then classify them by
the way they had low myeloid or high myeloid inflammation.

**Addition of Bevacizumab to Atezolizumab in First Line Was Associated with Improved
Benefit in the T-EffectorHigh Myeloid InflammationHigh Subgroup**

What was interesting here, this is now in a randomized trial of atezolizumab,
atezolizumab plus bevacizumab versus sunitinib, that the group that had high T-effector and
low myeloid inflammation did very well with either atezolizumab plus bevacizumab or
atezolizumab alone and they did better than the patients who got sunitinib.

But in the subgroup that had a high T-effector gene signature but high myeloid
inflammation, atezolizumab by itself did not do well. That was a group that actually needed
bevacizumab in order to have a better outcome, so this might be a way of perhaps predicting
what combinations might be best in this setting for these patients.

**Loss of PTEN Promotes Resistance to T-Cell-Mediated Immunotherapy**

Data from MD Anderson has shown that PTEN loss, for example, can be associated
with low T-cell infiltration and lack of response to anti-PD-1. There are other cell signalling
pathways like the beta-catenin pathway that was mentioned that also correlate with lack of
T-cell infiltration and possibly lack of response to single-agent anti-PD-1.

**Association of the Diversity and Composition of the Gut Microbiome Are Associated
With Enhanced Responses and Improved PFS in Metastatic Melanoma Patients on
Anti-PD-1 Therapy**

And this is data from Jen Wargo and there were several papers published on this in
the last month showing that certain bacteria in the gut can be associated with a higher or
lower response rate to anti-PD-1. It can be associated with, for example, lower T-cell
infiltrates within the tumor.

What we have now is a situation where for clinical use PD-L1 assay is out and
available, the use is very context-dependent. You can look at tumor mutation burden and in
fact tumor mutation burden as assessed to some degree by microsatellite instability or DNA
mismatch repair can help to select patients for clinical trials.

But we have all of these other things which correlate partly with those; interferon-
gamma gene signature, the mesenchymal angiogenesis gene signature, the myeloid cell
gene signature, the microbiome, and we have yet to learn how to put all of these biomarkers
together in order to really develop a sophisticated predictive score for patients who receive
anti-PD-1.
The other problem of course is that even if we do this, it still doesn’t tell us for those who don’t respond what that agent, what that next agent should be, what you should add to anti-PD-1 or anti-PD-L1 to improve outcome.

**Predictive Biomarkers Are Critical for Development of Novel Immune Therapies**

In the future my guess is that what we will be using as a patient will come in, will be getting multi-parameter tumor IHC, a piece of tumor as you’ve seen here. We might do DNA sequencing and RNA sequencing for tumor and immune cells; we might measure the microbiome, the bacteria in the microbiome; we might actually look at some protein profile in the peripheral blood, do some sort of integrated bioinformatic analysis and then the company will send you back a note that says: “Your patient has x% chance of responding to PD-1.”

But an even more sophisticated approach might be to do that and then to do a second biopsy while you’re on treatment and do the same analysis and although I don’t have time to show you the slide, there is some data to suggest that what you see after you start treatment or early after treatment, may be even more predictive for a response than what you have at baseline, and so that early treatment biopsy might tell you whether you should continue with anti-PD-1 alone, maybe change to some other treatment or maybe add something to the anti-PD-1. That’s I think where the future is headed.

**Early On-Treatment Changes in Peripheral B Cells May Predict for Severe ICI Toxicity**

I just wanted to show one more slide. This is data from our group, from Madhav and Kavita Dhodapkar, where we looked at peripheral B cell subsets three weeks after starting treatment with ipi/nivo, and it turns out that if you have a decrease in the number of B cells – this is now looking at peripheral blood – and an increase in one specific subset of B-cells, the CD21 low B-cell subset, that that alone is a strong predictor for developing Grade III or IV toxicity from ipi/nivo subsequently.

You might be able to look at the peripheral blood, look at B cell changes and then we are writing a trial now to give rituximab to these patients prophylactically to see if we can prevent toxicity. There may be biomarkers that not only predict for a response but could predict for toxicities of these agents.

**Conclusions**

I’ll stop here. In conclusion, predictive biomarkers are critical to optimize activity and reduce toxicity of these treatments. PD-L1 testing and DNA mismatch repair, MSI-high tumors are used in current practice to select for single-agent anti-PD-1 and anti-PD-L1 treatment.
Several of the PD-L1 assays produce similar results but not with the exception of the Genentech SP142 assay. There are multiple other biomarkers that are present that could further select for a response to single-agent anti-PD-1 or PD-L1.

Combinations of biomarkers may prove superior but how those biomarkers interact with each other still remains undefined, and we still don’t have that critical information that we need. If anti-PD-1 alone is not going to be useful enough, how do those biomarkers guide the selection of one of the thousands of combination agents that are out there and available?

And finally I think pre- and post-treatment biopsies would be the best way to select patients for their best individual treatment in the future. Thank you for your time.