Moderator: Welcome to Predictive and Prognostic Biomarkers in Immunotherapy: A four-part podcast series presented by the publishers of *The ASCO Post* and Harborside Medical Education. Our moderator, Dr. Vamsidhar Velcheti, Director of Thoracic Medical Oncology at NYU Langone, and his guest, Dr. Hossein Borghaei, Chief of Thoracic Medical Oncology at Fox Chase Cancer Center, will discuss current recommendations, emerging data, clinical application, and expert guidance on using biomarker testing to choose appropriate immunotherapies for individual patients.

Dr. Velcheti: In this podcast, Dr. Hoss Borghaei and I will be discussing the evolving role of biomarkers in immuno-oncology treatments. Immunotherapy, especially the PD-1/PD-L1 inhibitors have revolutionized the treatment of several cancers, especially melanoma, lung cancer, kidney and bladder cancer, and others.

Dr. Velcheti: What is exciting about these drugs is that they have a potential to induce long-term responses and potentially cures in a subset of patients. These drugs, however, don't work in all patients. Now we have clinical trials exploring the benefit of combination-based immune therapy approaches to various cancers. There has been an incredibly exciting time in the field of drug development; however, the field is moving at such a fast pace, and often biomarker development is playing catch up and it's becoming increasingly hard to validate diagnostic assays to identify patients who will benefit most from these approaches. Dr. Borghaei, it would be great to hear your thoughts on this and about the major challenges in the field right now in terms of biomarker development and incorporating biomarkers to select patients for immunotherapy.

Dr. Borghaei: Sure, thank you for the invitation and the discussion. I think we are facing quite a challenge in the world of oncology in general, and the field that I deal with, mostly non–small cell lung cancer, because we do have really effective drugs. We have examples where we do have potential biomarkers that are highly effective in predicting who is going to respond and who's not going to respond to these targeted therapies, as we call them. On the other hand, for the majority of our patients who do not have a molecularly driven tumor, we have been hampered by lack of a really good biomarker that can be predictive. Now, that prediction can be for responsiveness to specific drugs, lack of responsiveness to specific drugs, or even at times predicting who's going to have too much toxicity from specific drugs. I think having access to this kind of biomarker, used as a general term, would be highly desirable in a field like oncology where, unfortunately, a lot of our drugs have serious side effects.

Dr. Borghaei: You can imagine if we can limit the number of patients who are not expected to respond to immunotherapy, but could still have side effects, if we can figure out exactly who the patients are who would have the advantage. Research in this area has traditionally been a little bit difficult because you need large cohorts of patients. You need adequate tissue samples, or in some cases blood samples. It requires expenditure of a lot of bioinformatics and other techniques and to really teasing out potential lead that is approaching a gene or otherwise to

establish it as a biomarker for one of the several elements that I mentioned earlier.

Dr. Borghaei: And we haven't paid a lot of attention to that, even though a lot of us have complained about it, and I think in my personal view some of that had to do with the fact that at least in the world of non–small cell lung cancer in the metastatic setting, we really didn't have good drugs for a long time. We didn't have adequate treatment for our patients, so if a patient in the general patient population showed any efficacy, we were willing to use the drug to get better outcomes, but I think now with the emergence of checkpoint inhibitors and all the targeted therapies, I think the story is a little bit different. I think the research to identify the biomarkers has lagged behind the clinical development of many of these agents, and I think we need to go back to the basics a little bit and try to do the really hard work that's necessary to identify factors that could be predicative of either response, or lack of responsiveness or increased toxicity.

Dr. Velcheti: If you look at the early days of PD-1 drug development, there was a clear signal that patients who had high levels of PD-L1 were the ones who benefited most from these drugs. But there was such a significant gap in biomarker development in the initial trials, and also multiple drugs targeting PD-1/PD-L1 agents were being developed and the biomarker assay development was unparalleled, but the multiple independent PD-1/PD-L1 assays created a lot of confusion in the field and at some point questioned the utility of PD-L1. Dr. Borghaei, any thoughts on what we could learn from the PD-1 development story and how we could actually improve clinical trial design to incorporate biomarkers into the early drug development process? Clearly, it looks like we need some innovation here.

Dr. Borghaei: I agree, I think we definitely need some innovation, and I think we are beginning to see some of that for some of the factors that hopefully we'll talk about, like tumor mutational burden and other factors or other markers, I should say. I agree with you, I think the PD-L1 story is very interesting to me, because, look, we've heard at many different meetings and we've seen in many different publications that PD-L1 is not a perfect biomarker.

Dr. Borghaei: Now what do we mean by that? We mean there are patients with high PD-L1 who don't respond to checkpoint inhibitors. We have patients with low PD-L1 expression that do respond, and we have a whole mix between. We've seen data that suggests PD-L1 is a heterogeneous factor, meaning that some parts of a tumor can be staining positively for PD-L1 expression when you test the specific antibody and some parts could not possibly test positive, and within the same tumor sample, so there have been issues with that sort of question of the validity of PD-L1 as a biomarker.

Dr. Borghaei: On the other hand, if I'm looking at the data sort of without a lot of bias, the overwhelming amount of clinical efficacy data that we have supports the fact that PD-L1 can be a predictor of response to the checkpoint inhibitors, but all

the deficiencies and issues that come with an IHC-based assay and the heterogeneity of expression. So I think looking at the clinical data that's been published—either with IO alone or chemo plus IO—I think it's fairly clear that a high level of PD-L1 expression is associated with better clinical efficacy in some studies. That includes overall survival. I think there are companies that sort of follow that protocol in terms of testing the biomarker and sticking with what the testing or it was showing them. And I think the clinical development of those compounds have been in a completely different track than others that did not embrace the biomarker development program.

Dr. Borghaei: And you're right that very early on, there was a hint that PD-L1 can be predictive. But for all the reasons that you mentioned; you know, multiple platforms, they find antibodies, I think the field got a little bit confusing and difficult to compare studies with different drugs and different PD-L1 testing. Up until recently, we didn't really have a unifying study, but the Blueprint Project sort of put that to rest. So I am a strong proponent of using a biomarker and looking for biomarkers for these drugs, especially the immunotherapy drugs. I think at this point, I will use PD-L1 as a potential biomarker for selection of patients who might or might not have clinical efficacy.

Dr. Velcheti: For our listeners, The Blueprint project is an ongoing effort of members of the International Association for the Study of Lung Cancer, also known as IASLC, to study the performance of various PD-L1 immunohistochemistry assays. The study was encouraged by the FDA and several professional organizations, including ASCO and AACR. Phase I of the Blueprint Project was published in the Journal of Thoracic Oncology. Investigators reported that three of four assays studied yielded similar PD-L1 expression on tumor cells, but showed variation among reporting PD-L1 expression on immune cells. Phase II of the Blueprint Project was presented at the 2017 World Conference on Lung Cancer and verified results from the phase I study. In particular, the phase II investigators reported that three assays, including Dako 28-8, Dako 22C3, and Ventana SP263 showed similar levels of PD-L1 expression on tumor cells, but variability in levels of PD-L1 expression on immune cells. A fourth assay, Ventana SP142, stained fewer tumor cells. Investigators acknowledged the interchangeability of three assays is possible, though added that clinical cutoffs for positive status or high PD-L1 expression levels or negative status or low PD-L1 expression levels could be more important than choice of assay. The Blueprint Project is ongoing.

Dr. Velcheti: Dr. Borghaei, in your view, what are the most promising immunotherapy biomarkers at this time? What should oncologists do in routine clinical practice?

Dr.Borghaei: ~~You're asking a couple different questions there.~~ In the clinical practice, I think that the test that's been available and has a lot of regulatory approval and other issues associated with it is PD-L1 testing.

Dr. Velcheti: Current clinical guidelines recommend PD-L1 testing as a key step in planning therapy for a variety of advanced solid tumors, including refractory melanomas, kidney cancers, and lung cancers to name a few. So right now, best clinical practice is to use the recommended companion tests as indicated in the prescribing information for the immune checkpoint inhibitor that you are considering as treatment.

Dr. Borghaei: I think the emerging biomarkers for me includes tumor mutational burden. Tumor mutational burden is a potential biomarker that has a significant amount of data associated with it in terms of retrospective analysis. So far, we don't really have a prospectively designed study that's been reported out, although, at least one or two studies are pending that would elect patients strictly based on TMB. At this point, TMB suffers from a couple of deficiencies. One is that, as I emphasized in the beginning, we don't really have survival data associated with high versus low TMB. Number two is that that distinction, high versus low, is not uniformly defined.

Dr. Velcheti: Let me call out specifically some of those studies in advanced lung cancer showing a relationship between tumor mutational burden and response to therapy with a PD-L1 inhibitor. CheckMate 012, presented at the 2018 ASCO Annual Meeting, and Checkmate 568 and 227, presented during the 2018 AACR Annual Meeting are among such studies. An updated analysis of the CheckMate 568 trial presented at AACR showed that tumor mutational burden greater than or equal to 10 mutations per megabase of DNA distinguished patients who responded to nivolumab plus ipilimumab from patients who did not respond. Further, the study showed that high tumor mutational burden was associated with response, regardless of PD-L1 level. Tumor mutational burden is an emerging biomarker independent of PD-L1 levels although it warrants further study and standardization of testing.

Dr. Borghaei: It sort of resembles where we were in the beginning of PD-L1 testing with different platforms and different antibodies. Everyone has a different cutpoint, the testing is a little bit different, the platforms are a little bit different, so some unification of the TMB analysis is needed and that work is already in progress with all the involved parties. Then once we have a defined cutpoint, I think applying a defined cutpoint in a uniformly measured way on a standard platform, and prospectively randomizing patients that would be treated with IO or non-IO or different variations of a design like that would be needed to establish TMB as a valid and reasonable biomarker for a selection of patients who are on immunotherapy.

Dr. Borghaei: I think the point that I see is that more likely in the next few years we'll be looking at a composite of a couple of different markers to truly figure out who should or should not be treated with immunotherapy based on what we get out of tumor- or perhaps blood-based assay. I know you have been involved yourself in projects involving using blood-based assays to detect tumor mutational burden or perhaps randomize patients based on that, which I think would be a huge step forward, given the difficulties that we have sometimes in

acquiring adequate tissue, especially in the field of lung cancer, where tumors are sometimes not accessible.

Dr. Velcheti: Also it appears, like TMB and PD-L1, they could be identifying different biologies in the cancer and could be complementary. What do you say about that?

Dr. Borghaei: The complementary aspect I think I am beginning to see in some of these retrospective studies, but from what I have seen so far, it does look like we are identifying different patient populations. They are coming from different pools so not everybody with high TMB has high PD-L1 and vice versa. Although, again there is some retrospective analysis from a randomized phase 3 study that to me suggests that patients who truly benefit from single-agent immunotherapy are those that have high levels of PD-L1 expression and high TMB.

Dr. Borghaei: I think that would be a particularly interesting subgroup of patients for further study. The other point that I have to emphasize on, I don't know how you feel about it, is that the majority of data that we're seeing with TMB is done in the era of single-agent IO and in some of the diseases that we're dealing with, the era of using single-agent IO is coming to a close, because the majority of patients seem to be getting some sort of an IO combination, either with chemotherapy, there might even be the potential of an IO-IO combination. I think again, keeping all of that in mind and making sure the biomarker is applicable to a changing treatment landscape in oncology and in lung cancer specifically, I think would be quite useful.

Dr. Velcheti: I think it's a major challenge in the field trying to incorporate biomarkers in routine day-to-day clinical practice. When you talk about normal biomarker approaches, do you see anything exciting in the horizon?

Dr. Borghaei: I am very excited about the blood-based TMB analysis, which again I know you have been involved with. I think there are number of circulating biomarkers that could potentially become valid. There are a number of different publications recently looking at a general immune profiling, trying to identify a T-cell intricate gene expression profile of a tumor. There's a recent publication in science I believe, where a pan-tumor biomarker was looked at that incorporated a number of different factors, including TMB and sort of a T-cell gene expression profile. Unfortunately, because of the limited time, it's a little bit difficult to go into details of that. I think those are the kind of promising potential biomarkers that, as you suggested, need to be incorporated in clinical trials for us to have the kind of data that we need to feel comfortable assigning treatment based on a specific signature.

Dr. Velcheti: Now with all the combination immunotherapy, clinical trials, and combination with radioimmunotherapy agents, there is increased risk for autoimmune toxicity, and I think it's probably time for us to not just explore predictive biomarkers for response or efficacy, but perhaps also to identify those patients who would have really bad outcomes in terms of toxicity with these drugs. Are you aware of any approaches looking at biomarkers to predict toxicity?

Dr. Borghaei: I have not come across anything that I would consider to be ready for prime time in terms of identifying specific biomarkers for toxicity. Again, I am aware of efforts that are underway, but anything that I can tell you I am using in my everyday clinical practice, I really haven't seen any.

Dr. Velcheti: Dr. Borghaei, thank you very much for your valuable insights into the field of biomarkers for immunotherapy. There's clearly a very exciting time in oncology and we will certainly see more novel approaches for selecting patients for immunotherapy in the near future. Thank you very much for joining us on this podcast. Be sure to check out other podcast episodes from the series of Biomarkers in Immunotherapy. For information, please visit educate.ascopost.com. Thank you.