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. Borghaei and I will be discussing the role of MSI and deficient DNA mismatch we are testing in selecting patients for immunotherapy. The approval for pembrolizumab recently was based on a clinical trial which had close to 150 patients who tested positive for deficiency in the mismatched repair proteins and MSI unstable biomarkers. The study had 90 patients with colorectal cancer and 59 patients had various tumors, one out of 14 other tumor types.

Dr. Velcheti: The response rates from that trial were roughly in the range of 30-40% and for durable as expected with immunotherapy. Similar results were seen with nivolumab leading to recent approval for nivolumab and ipilimumab in [inaudible] tumors. This is a very interesting time in terms of drug approval, and traditionally we have had drug approval based on tumor type or line of therapy or based on biomarkers and particular tumor types like EGFR and ALK in non–small lung cancer. The diagnostic approval for MSI is a landmark in my opinion in drug development. And this tissue-agnostic approval makes sense. Dr. Borghaei, what are your thoughts on using biomarkers for selecting for a drug across chemotypes? Can you comment on such an approach?

Dr. Borghaei: Sure. Again, thank you for the discussion and invitation. I think this actually is very exciting. I agree with you. This is a landmark approval. Sort of a tissue-agnostic or site-agnostic approval as long as the biomarker is there, the drug should work. And to me, that's the sign of a real biomarker. I would call this a more of tumor intrinsic type of a phenomena. The tumor is telling you something because of this mismatch repair mechanism. And I think it's very interesting that the story with the mismatch repair and responsiveness immunotherapy seems to go along with what people have been hypothesizing about the use of these agents and namely tumors that are associated with having more neoantigens or no more mutations, seem to be a little more responsive to immunotherapy.

Dr. Borghaei: I think the MSI story tells us that and I think the regulatory approval of using checkpoint inhibitors that we have now in anybody that has a microsatellite instability is a good indicator of one such tumor intrinsic factor that makes it responsive to the immunotherapy drugs that we're using at this point. I think this was a great finding. I think it's great for the patients and I think a testament to that is that almost immediately, once the studies came out, a lot of the next-gen sequencing platforms that we would commonly use started reporting microsatellite instability as a part of the standard panel because everybody

recognized that it makes sense that those tumors would be responsive to the PD-1 or the PD-L1 inhibitor.

Dr. Velcheti: In the past, especially for BRAF, for example, we think different biological functions and clinical relevance of BRAF inhibitors in colon, let's say, is pretty disappointing. But in lung cancer and melanoma, BRAF inhibitors works pretty well in patients with BRAF mutation. Tissue-agnostic development for biomarkers have we not necessarily the great strategy in every situation, but perhaps something like MSI and perhaps even TMB might be a noble approach for patients that have rare tumor types that might be a great opportunity to get a drug for them. Dr. Borghaei, could you explain to us from a pathophysiological standpoint, what is microsatellite instability and mismatch deficiency, and where did these patients respond to immunotherapy?

Dr. Borghaei: A mismatch repair genes are basically responsible for correcting small errors that happen during DNA replication, and all the cells that undergo an active division. So the theory is that as a result of some enhanced errors in terms of base pairing that happened during cell cycle, we have over the evolutionary process, acquired these system, basically, mismatch repair, homologous DNA repair mechanism where we have checks and balances in place. And these particular proteins and genes go back and identify a particular error that has happened during the replication and repair those. There are several of these genes such as MSH2, MLH1, a number of others, and we have known for many years now, a couple of decades that in certain diseases like Lynch syndrome and diseases like colon cancer, deficiencies of these DNA repair mechanisms are associated with colon cancer.

Dr. Borghaei: The reason why we think some of these tumors actually respond to immunotherapy is thought to be because once you have these repaired deficiencies, more mutations can accumulate within the tumor and borrowing from the term mutational burden story higher levels of mutations within a tumor seems to introduce the immune system with the potential of encountering more neoantigens that at least some of those could be immunogenic and, therefore, start the process of antigen presentation. And then you come in with a PD-1 or a PD-L1 and you activate the T cells around and you activate the immune system that's already somewhat activated because of the neoantigen load that it's facing, and that can be associated with a bit of responsiveness to the PD-1, PD-L1 inhibitors that we have. In a sort of high-level view, that's the way I look at the field and that's how I understand the mechanisms involved.

Dr. Velcheti: How do you test for mismatched repair deficiency in MSI?

Dr. Borghaei: I think traditionally, they relied on using immunohistochemistry and again, that goes back to the discovery of the Lynch syndrome and all of that. But more recently, as I said, a lot of the next-gen sequencing platforms that we have been using commonly to detect the typical EGFR or KRAS, BRAF mutations are reporting the microsatellite instability genes as part of their overall panel. And I

think that the detection sort of relies on an actual PCR method, which some people actually feel it's a lot more sensitive and specific than standard IHC although I've seen studies that suggest IHC can be just as good as the DNA-based assays but clearly, the PCR assays have not become commonplace in detecting MSI instability. And I see that sort of routinely reported as part of the next-gen sequencing platforms that we use for our patients here.

Dr. Velcheti: So Dr. Borghaei, in the trials most of the studies used IHC or PCR, what are your thoughts on NGS-based assays to test for MSI? Is it possible that you would be picking up some clinically insignificant microsatellite loci using a more comprehensive NGS-based platform? You kind of begin to wonder if they have any functional relevance.

Dr. Borghaei: I think the possibility of detecting small variance of unclear significance is there. But I think the risk of that is probably low. To me, I don't want to miss is a patient that truly does have it and not getting the appropriate drug. So I think from my perspective, although it is very important to have the patient who will respond to it based on the MSI story, I think we also have to be careful to select test or testing methods—again can be plural here—that would capture as many patients as we possibly can just because of the significant clinical efficacy data that we have seen coming out of the field. So yeah, there could be an error in terms of picking up variance of undetermined significance. But, how to reconcile that, I think there are some centers that sort of put policies in place where patients have to have a couple of different levels of testing done to qualify for something. But again, every test that we introduced that is associated with the cost is associated with delay and reporting. And therefore delay in starting treatment. So I think we have to draw the line at some place and say we rely on this test, this is validated, we're going to go ahead and use it to the advantage of our patients.

Dr. Velcheti: I totally agree with you. So the big question right now is who do we test for MSI? Of course, the common tumors like colorectal and uterine, but is it current clinical practice or recommendation to test patients of all tumor types for MSI?

Dr. Borghaei: So fortunately, I do not sit on any committee that decides that for the country.

Dr. Velcheti: [laughter]

Dr. Borghaei: So again, the data suggests that in the lung cancer that something close to 1 maybe 2% of the patients will have the MSI high. I sort of feel that we do capture quite a number of those patients in lung cancer because of other testing or because of the way the clinical practice is moving. And by that I mean the combinations with chemotherapy in the front-line setting. So I think in lung cancer we are getting a lot of coverage with our patients with the oncology drugs. I think certainly in cases that you mentioned, the colorectal patients, uterine cancer, gastric, maybe even pancreatic cancer, testing would make a lot of sense. Particularly diseases where we have not been able to have new drugs introduced. Or diseases where standard immuno-oncology protocols, and by

standard I mean using PD-L1 for instance, has not shown to be effective. I think it makes sense to do the MSI testing again to capture as many of those patients as we can. Out of pancreatic cancer patients, I'm really running out of options I think I would like to have that MSI data available to me because, as you mentioned, that gives you 20-30% response rate. So, why not?

Dr. Velcheti: Right. So are there DNA repair markers that could potentially also predict increased mutations and perhaps increase the response to immunotherapy?

Dr. Borghaei: Sure. So the one that we are a little bit biased towards at Fox Chase just because we have investigators who have a lot of interest in it is the POLE mutation. This is a mutation in the epsilon chain of the DNA polymerase gene that has been shown to be associated with a hypermutated state. The other DNA repair mechanisms that is very well known to us is of course the BRCA mutations that we know a lot from the breast cancer world. And there is a lot of good data with regard to BRCA mutations and responsiveness to some even standard hypoxic chemotherapy.

Dr. Borghaei: So there are other markers that are under investigation at this point. I think the issue is that the incidence of a POLE mutation in general oncology practice seems to be limited. On the other hand, if they can develop a data set that for instance, tell us in gyn malignancies where I see most of the data with POLE, that that particular mutation is a truly predictive of immunotherapy response, then I think we need to do the same thing that you and I were talking about just a few minutes ago, and that's designing the prospectively randomized patients based on the biomarker who truly establish the utility of the potential biomarker for one disease site. Even if it is one disease site at a time, I think that by itself represents an advancement and an improvement above and beyond what we're doing right now.

Dr. Velcheti: Thank you very much Dr. Borghaei for your valuable insights into the field of Biomarkers for Immunotherapy. Thank you very much for joining us on this podcast. Be sure to check out other podcast episodes to familiar yourself with immunotherapy biomarkers. For more information, please visit educate.ascopost.com Thank you very much.