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. David Rimm, professor in the Departments of Pathology and Medicine at Yale University School of Medicine, 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. Rimm and I will be discussing the potential for tumor mutation burden as a predictive biomarker for immunotherapy. Over the past few years, we've seen multiple clinical trials looking at clinical benefit in patients who have lung cancer, melanoma, and various cancers.

Dr. Velcheti: Over the past few years we've evaluated tumor mutation burden as a predictive biomarker and there's a lot of promising, exciting data emerging from clinical trials, especially CheckMate 026 in lung cancer with nivolumab and most recently CheckMate 227 with ipilimumab and nivolumab in metastatic non–small cell lung cancer.

Dr. Velcheti: Dr. Rimm, recently, there has been so much excitement around the use of tumor mutation burden to select patients for immunotherapy. There's exciting clinical data and retrospective data from early clinical trials. Can you explain to us, from a pathophysiological standpoint, why tumors with high mutation burden respond to immunotherapy?

Dr. Rimm: The best data is related to the patients with really high mutation burden. That is, microsatellite-unstable patients or patients with mutations in DNA repair genes, where they don't have high mutational burden, they have very high mutational burden. That very high mutational burden generates mutations that generate neoantigens that actually get transcribed and then produced on the surface of the cell and recognized by T cells as foreign.

Dr. Rimm: When they're recognized by T cells as foreign, in a process too long to go into at this point, the immune system then attacks the tumor, and that's how many tumors actually never become clinically relevant because the immune system realizes that they're tumors and kills them before they are even clinically presented.

Dr. Rimm: However, in this case, the tumors are clearly presented. However, the tumor has evaded the immune system, and these particular tumors with very high mutational burden most likely present an antigen, that is a neoantigen created by a mutation that ultimately is the reason that when you turn the immune system back on as you do with the checkpoint inhibitor, you actually have the checkpoint inhibitor reactivate the immune system and kill the tumor.

Dr. Rimm: Now, that's certainly true and the studies have shown patients with very high mutational burden, like those with microsatellite unstable colon cancer or microsatellite unstable uterine cancer, do in fact have a great response rate as high as 70% in this relatively small subset of tumors.

Dr. Rimm: In fact, it's exciting to see that for the first time ever, the FDA approved a pan-histology indication for pembrolizumab for any patient that has one of these highly, very high tumor mutation burden or microsatellite unstable tumors. However, those are not to be confused with the TMB, which refers to tumor mutation burden, which includes both high, very high, and somewhat high and maybe not so high all in the same category.

Dr. Rimm: In fact, there is no definition for what high TMB is. Different sites use different cut points for high TMB, as low as eight mutations per megabase to as high as 16 or 20 mutations per megabase as the low end threshold. Whereas once you get up to 100 or 200 mutations per megabase as you see in the microsatellite unstable tumors, or in the mutations of DNA repair genes, some of those may even be in the 600-800 range. Those clearly everybody agrees on. Those are clearly tumors that have very high tumor mutation burden.

Dr. Velcheti: Do you think, just like MSI, we might see more clinical trials using high TMB and in a tissue agnostic way, do you think we will have a tissue- or site-agnostic drug approval with high TMB?

Dr. Rimm: Well, I'm not so optimistic about that. I think that it might be tried, but my guess is it won't succeed. High TMB means different things to different people, and so if you thought we had a problem with four PD-L1 tests, you've just seen the tip of the iceberg as there's probably somewhere between 50 and 100 different tests for TMB, as we sit here today and maybe more than that. If you include LDTs, everyone can kind of make up their own cut point for what represents high TMB.

Dr. Rimm: Not only, even if they agree on the cut point, they might come to the conclusion of what's a mutation in a different way. In fact, a classic example of that is one of the key promoters of TMB, Foundation Medicine, uses synonymous mutations whereas others use only nonsynonymous mutations, that is mutations that, should you count a mutation that changes a base pair, a nonsynonymous mutation, versus – or changes the codon and gives you a different peptide, versus a synonymous mutation, which changes the base pair but doesn't change the amino acid. You can see that as we sit here today, there's not even agreement on what should be counted as a mutation.

Dr. Rimm: The challenge of clinical trials, new clinical trials using TMB as a criteria for entry raises a lot of questions of what TMB test do you use? Perhaps that's what we'll see is that rather than just using the term TMB, they'll say something like, the Foundation Medicine or the Guardant Health or the NeoGenomics or the Memorial Sloan Kettering, or fill-in-the-blank tumor mutational burden test.

Dr. Velcheti: Right, and also, a high tumor mutation burden in itself, biologically, it seems like a really heterogeneous entity. For example, the KRAS could be one mutation, lung cancer patients don't respond to immunotherapy despite of having high mutation burden. Most recently, we've seen data from renal cell carcinoma where TMB doesn't appear to be predictive for a response in RCC. There's certainly a lot to be learned and I think it's still really early.

Dr. Velcheti: I know you mentioned and talked about this a little bit, but there seems to be a lot of different assays which are currently commercially already available for comprehensive genomic profiling. There are a lot of different platforms by which patients are getting the sequencing information. Clinicians are faced with this information about TMB. Can you comment on the analytical validation of these assays, reproducibility, and what should treating oncologists do with that information?

Dr. Rimm: Sure. TMB, unlike immunohistochemistry, TMB has been promoted in a very borderline ethical manner. That is, I've even heard key opinion leaders say that a certain TMB test is FDA approved, and that's actually false. The test, that is the FM1 test, is in fact FDA approved, but it's only FDA approved for targeted therapy, so for BRAF, for EGFR, for various specific mutations, but it is not approved for tumor mutation burden.

Dr. Rimm: I think while saying the test is FDA approved, that's accurate, but it's not actually completely accurate because it's not true for TMB. There is no FDA approved indication for TMB and there's no drug that requires TMB be tested in order to prescribe the drug, so TMB is not a companion diagnostic test. Even using just the Foundation Medicine test, it is not 100% standardized.

Dr. Rimm: Now, Foundation Medicine has done a great job of standardizing their test, and they have shown data of reproducibility among samples and between samples and every kind of way, so it is analytically validated, but that's only one way to get TMB. As a result of that, the Friends of Cancer Research have organized a harmonization project. Unlike the harmonization project where there were four vendors involved for the blueprint for PD-L1, there are at least almost 20 involved parties including nine diagnostic companies, at least four academic sites, and a number of pharmaceutical industry members as well.

Dr. Rimm: This began in May of 2018 and as of now, it's a three-step process with an in silico analysis, then an empirical analysis, and finally a clinical analysis. I doubt if we'll see it end for at least another year or so before we finally see the reporting. But I think this kind of analytic validation and clinical validation ultimately with implementation of some sort of unifying standards are required before TMB gets to the point where we'd even consider it for use as an indication.

Dr. Rimm: The problem with this is that if you order a targeted therapy test from a number of these companies, they give you the TMB data for free. I think its value is what you pay for it. That is, I don't think it has any value at this point. It's only been

shown in clinical trials and as Dr. Velcheti pointed out, in some clinical trials, it's been shown not to be a predictor, where in other clinical trials, it is.

Dr. Rimm: In those clinical trial settings, the TMB is only applicable to the test that was used in that clinical trial, because as stated before, for example, the Memorial Sloan Kettering test is very different from the Guardant test is very different from the Foundation Medicine test. Each of those has been used in different clinical trial settings or reported in different clinical trial settings.

Dr. Rimm: As a practicing oncologist, you have to be careful to weigh carefully the value of that information, since it's never been shown to be a companion diagnosis test. There have only been associations with outcome and in fact, it's never even been shown to be associated with overall survival. In all of those tests to date, they all show association with progression-free survival, but usually the overall survival test fails, which suggests that the TMB itself may not be a great test.

Dr. Rimm: A recent paper in *Science* addresses this issue and compares TMB to genomic expression profiling and finds the area under the curve of TMB in many studies is close to 0.6. That's the area under the receiver operator characteristic curve, that is used to compare tests. 0.6 is not very good. 0.7 is a little bit better, which is what some of the gene expression profile studies showed. A great test we want to have somewhere in the range of 0.8 or 0.9.

Dr. Rimm: These tests are sort of of borderline value even in the best case, and in the worst case are of no value at all because of their lack of standardization.

Dr. Velcheti: That's a great point, Dr. Rimm. I really find that very interesting that in multiple studies now, there's been progression-free survival benefit, but there's no overall survival benefit as of yet. Do you think that there's a biological explanation for that? Is that a feature, a biological feature of these tumors that have high TMB? Are they more prone to immune escape under pressure?

Dr. Rimm: Exactly. I think you really hit on something really interesting there. It could be if you think about how tumors evolve, the more mutations they have, the more quickly they evolve because the more pathways are interrupted. Patients with high tumor mutational burden are likely to evolve more quickly, which also means evolving resistance to all sorts of drugs including immune therapy.

Dr. Rimm: It may be that the reason we don't see the benefit for overall survival but often see it for progression-free survival is that in fact, the immune therapy drug works for a little while, but because it's a high tumor mutation burden tumor, it evolves quickly and therefore evolves a mechanism for evasion of the immune therapy. That may be why we see the benefit only in the progression-free survival as opposed to overall survival.

Dr. Velcheti: Also what I found interesting was in the CheckMate 227, in the chemotherapy and the control arm, the chemotherapy arm actually did very poorly compared

to historical control. High TMB patients who were receiving chemotherapy did very poorly, so it kind of just may potentially be a poor prognostic marker, but I don't think we know enough yet. Is that right, Dr. Rimm? Any thoughts on that?

Dr. Rimm: Yeah, so related to that, I would say that we have to be very careful when we talk about predictive versus prognostic value and remember that prognostic value is associated with the outcome independent of therapy, and the predictive value is what we'd hope any biomarker would be or what the companion diagnostic test all have to have predictive value. That means there's a statistical test that has to be performed that has to show significant interaction.

Dr. Rimm: Although not published yet, I do believe that they showed interaction on CheckMate 227, but most studies, in fact I know of no other independent study including 568 and a number of other studies that have looked at TMB where the interaction test actually showed an interaction between TMB and response to therapy, which is sort of the statistical requirement for evidence of a companion diagnostic test showing predictive, not prognostic value.

Dr. Velcheti: Dr. Rimm, can you comment on any novel immunotherapy biomarkers that you're excited about, any potential biomarkers that could have clinical impact in the near future?

Dr. Rimm: Well, I think that one that comes ... You know, this is where TMB might have its greatest strength, even though it still needs to be proven, validated, and shown to be predictive. But if all those things were true, if you could do that then the TMB is something you could potentially do from the peripheral blood. In fact, we see two trials that are moving in that direction and the results of those trials, I think Dr. Velcheti is leading one of them and can comment when I'm done here, but those trials are potentially very interesting.

Dr. Rimm: There's a few other blood-based markers that have shown some promise and in fact, specifically some targeted therapies. STK11, if you have a mutation in STK11, that might be detected in the peripheral blood and then you're probably not a candidate for immune therapy because patients with that mutation essentially always do not benefit from the immune therapy. Those are sort of a few things that are going on. Clearly, a blood-based assay would be great, or a radiologic assay would be great, if we didn't have to get a biopsy of the tissue.

Dr. Rimm: However, we're still in very early stages on that. Other novel approaches include looking at the PD-L1 expression on tumor cells, however that hasn't really borne out at this point, although some companies are working on it.

Dr. Rimm: There are people that are looking at exosomes in circulating blood to see if there's information in the exosomes that might provide information, predictive, ultimately predictive information, but unfortunately, we're still really early on blood-based markers. I don't think, certainly with targeted markers, there's a lot

of progress with finding circulating-free DNA and looking for known mutations, but immunotherapy has been a little trickier with biomarkers in the circulating blood.

Dr. Velcheti: Dr. Rimm, thank you very much for your valuable insights into the field of oncology. Thank you very much for joining us on this podcast. Be sure to check out the other podcast episodes from the series of Biomarkers for Immunotherapy. For more information, please visit educate.ascopost.com. Thank you very much.