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 role of PD-L1 testing to select patients for anti–PD-1/PD-L1–based treatments. In the early days of PD-1 drug development, there were clear suggestions that PD-L1 protein expression was a predictor for reference to anti–PD-1 drugs. However, during the course of the drug development there were significant gaps in biomarker assay development and incorporation into these clinical trials. There were multiple PD-1 and PD-L1 agents being developed parallelly, and there were multiple PD-L1 diagnostic assays being developed by each of the former partners, and it created a lot of confusion in the field regarding the utility of PD-L1. Dr. Rimm, any thoughts on what we could learn from all these early trials with PD-1 and PD-L1, and should there be a better way to harmonize diagnostic assays across drugs that are mechanistically similar, and how do we incorporate this into early-phase drug development, and how can we improve clinical trial design for drug development?

Dr. Rimm: First, thanks for including me in this. I think that this is really a classic case of important selective factor for predicting responders to therapy combined with a lack of agreement on how that should be processed or how that should be rolled out to be useful for patients. And what happened was we ended up with each company in a race to get their drug to patients, but realizing that this is a kind of drug or class of drug that really doesn't benefit all patients but a relatively small subset, and so different companies took different approaches to find that small subset, although they all sort of centered around the approach of immunohistochemistry, mostly because that's an approach that can be used by the most labs around the country.

Dr. Rimm: If you look at the availability of different kinds of testing, one of the most universal types of testing is immunohistochemistry, and so it's perhaps not a surprise that all four or five of these drug companies, as they were rolling out their drugs, companioned an immunohistochemistry test with the drug rollout. Now, what we saw was that some of them, the test actually was required and some drug trials were designed such that the test would be required and ultimately approved by the FDA as a required test; whereas others, they gave all patients the drug and they tested them all but the test was kind of secondary. In those cases, the test was then designated by the FDA as a complimentary diagnostic test. This was the introduction of a new term and important for oncologists and pathologists to realize the difference between a companion diagnostic test, which means required for prescription of the drug, and a complimentary diagnostic test, which is interesting and perhaps nice to have for

patient management, but not required and often not reimbursed, and that's much less common.

Dr. Velcheti: An important aspect the PD-L1 story pertains to 2 guidance documents from the FDA regarding development of companion biomarker tests during drug development. The first guidance was issued in 2014, and then updated in 2016 to urge co-development even earlier in the drug development process. The guidance documents are available on the FDA's website. In summary, these guidances make clear that the FDA expects that an in vitro diagnostic, or IVD, companion diagnostic test that is intended for use with a specific therapeutic product would be approved through an appropriate submission. If the corresponding therapeutic product is already FDA approved, its labeling would be revised to stipulate use of the specific companion IVD test once that is approved. Further, FDA's guidance document states that "FDA may decide to approve a therapeutic product even if an IVD companion diagnostic device is not yet approved or cleared when the therapeutic product is intended to treat a serious or life-threatening condition for which no satisfactory alternative treatment exists and the benefits from the use of the therapeutic product are so pronounced as to outweigh the risks from the lack of an approved or cleared IVD companion diagnostic device."

Dr. Velcheti: I know there have been a lot of efforts from various academic groups and industry collaborations and you led some of these efforts to harmonize all of these assays and optimize these assays. Can you please comment on some of this work that was done to harmonize and optimize these PD-L1 assays?

Dr. Rimm: The first study was the Blueprint Study. Quick on its heels was a study from the NCCN where the NCCN, with the help from BMS, sponsored an academic study where eight academicians, actually a total ultimately of 13 academicians, read the slides, all practicing, board-certified pathologists, and they tested only three of the four FDA-approved tests, and then they also included an LDT. In that study, the so-called NCCN study, was statistically powered and showed that the assays were essentially all equivalent. The LDT, the 22C3, and the 28-8 assays were all essentially equivalent. The SP142 assay was shown to be less sensitive than the other three assays.

Dr. Rimm: This was concordant with what was shown in the Blueprint 1, even though it was underpowered, but that same organization that did Blueprint 1 then organized phase two of the Blueprint, and then in a beautiful study of 25 pathologists from maybe 12 or 13 countries across the world, got together all of those pathologists and trained them on how to read the slides. Then they did a statistically powered number of slides that were read both on glass and digitally. It included both normal biopsies and cytology specimens. Basically what they found was very similar to what had been found in the previous tests. They actually included the Pfizer-indicated test, that is the 73-10 assay. They found that that assay was actually a little more sensitive than the SP263, 28-8, and 22C3, but that again, SP142 was less sensitive.

Dr. Rimm: They also found, as was found in the NCCN study, that the pathologists were very good at reading the expression of PD-L1 in tumors for the so-called tumor proportion score, whereas the pathologists were not very good at all, even after a day and a half of training in the case of the Blueprint 2, at reading the immune cell, or IC, score. The immune cell proportions are generally lower and, depending on how they're divided, generally harder for the pathologists to read. In both the NCCN study and the Blueprint 2 study, suggested that that was not reproducibly performed by pathologists. This is a bit of an issue since, as we've moved beyond lung, we've seen that the immune cell score, or newly defined combined proportion score, CPS, has become more important than even a companion diagnostic test.

Dr. Velcheti: That's an issue because a lot have become mostly available PD-L1 assays to both tumor and stromal PD-L1 expression, and it is a challenge in the clinic, to interpret those results for clinical optimization of patient care. Dr. Rimm, I know you've done a lot of work on objective quantitative evaluation of various biomarkers. Currently, PD-L1 is measured and reported as a proportion of tumor cells staining parts for PD-L1. It seems very subjective. Do you think there's any potential for automation here or objective and quantitative evaluation of PD-L1?

Dr. Rimm: The short answer is yes. Just to sort of give the context, some of the things can be done quite effectively by pathologists just estimating because that's what we do. We don't actually count every cell. We estimate the percentage of cells that are positive for a given marker. In the tumor proportion score, we estimated quite well. It was the immune cell scores that we had trouble estimating, and that's where perhaps a quantitative tool would be helpful or, in fact, maybe required to ultimately accurately and reproducibly find that subpopulation of patients.

Dr. Rimm: As we sit here today, there are a number of investigators that are trying to use that same substrate, the same slide that the pathologists looked at, and apply quantitative image analysis tools to that slide to maybe make a better percentage. There have been a few exploratory or pilot scale papers coming out on that, but I don't know of anything that's about to be introduced to the clinic. The other sort of quantitative or objective method would be to use fluorescent multiplex testing, which allows not only assessment of the tumor cell and immune cell PD-L1, but could also assess the immune cell environment. It seems like the tumor microenvironment, that is the presence of CD8 cells or the presence of macrophages or the presence of other T cells and the expression of various molecules on those T cells ultimately may be important for subclassifying patients. Again, those are still in the early stages of development, but it's something to look out for. and in fact, I wouldn't be surprised if in a year or two from now we see FDA submissions from companies that are using a more objective method, whether it be chromogenic or fluorescence to try to take this assay from an only variably reproducible assay to one that is much more objective and hence much more reproducible.

Dr. Velcheti: That is fascinating. Dr. Rimm, thank you so much for your valuable insight into the field of biomarkers and immunotherapy and discussing the role of PD-L1 as a biomarker in clinical practice.

Dr. Velcheti: Thank you very much for joining us on this podcast. Be sure to check out the other podcast episodes in the series Biomarkers for Immunotherapy. For more information, please visit educate.ascopost.com. Thank you very much.