What the Oncologist Needs From the Pathologist for Immune Therapies

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The advent of active and tolerable immunotherapy drugs has completely transformed the care of patients with advanced cancer. Although significant work remains to be done, many patients in the clinic are benefiting and often achieving long-term survival in diseases where that phrase used to be aspirational only. And yet, many patients do not benefit from these drugs, and the costs of the therapies are great. As indications in common tumor types continue to broaden and novel combinations are developed, immunotherapy use will undoubtedly continue to rise. And given that we lack knowledge about when safely stopping treatment is advisable, we are putting a tremendous financial strain upon the health care system. The search for predictive biomarkers proceeds apace. In this review we will discuss the current state of knowledge that helps clinicians to choose therapy and how pathologists can help provide information that is clinically actionable.

THE COMPLEX CLINICAL ARENA OF IMMUNOTHERAPY

Currently available immunotherapy drugs in the clinic are checkpoint inhibitors: either CTLA-4 blockers (ipilimumab and tremelimumab) or antibodies that block the programmed death receptor-1 (PD-1)–programmed death ligand-1 (PD-L1) axis (nivolumab, pembrolizumab, avelumab, durvalumab, and atezolizumab). To make the scenario even more complex, different drugs are approved for various indications across histologies and lines of therapy; some are approved based on PD-L1 level and some are PD-L1 agnostic.

It is beyond the scope of this article to review the full spectrum of use of checkpoint inhibitors in clinical practice, but hopefully a panoramic view focusing on lung cancer will give a general overview. These drugs are currently not approved for neoadjuvant use, although some studies have been published and others are in progress.[1] These drugs are likewise not approved for adjuvant use in patients at high risk for relapse, although there, too, studies are underway. Durvalumab is currently approved for use after definitive chemoradiation in locally advanced non–small cell lung cancer, where a substantial improvement in progression-free survival (PFS) and overall survival has led to its rapid adoption as the new practice standard.[2] This approval is not based on PD-L1 level, and thus PD-L1 testing in locally advanced non–small cell lung cancer is not required in the algorithm for decision-making.

It is probably not necessary for a medical oncologist to try to explain in a pathology journal that PD-L1 staining has been slightly problematic for pathologists. Different companies developed different antibodies for testing as companion diagnostics during the early-phase studies of their drugs, requiring further projects to try to harmonize results.[3,4] Although clinical cutoffs have been recognized (>50%, 1%–49%, <1%), interpretation of the stains is not perfect and correlation between pathologists is good but not great.[5] As most patients with metastatic lung cancer—a very large cohort of patients—will eventually receive immunotherapy, the question is how beneficial PD-L1 staining really is in the lung space if almost all patients receive checkpoint inhibitors first or second line?

Up-front testing of advanced lung cancer with PD-L1 is necessary, as a very high level of PD-L1, higher than 50%, allows patients to receive single-agent pembrolizumab with a high confidence of response.[6] In the pivotal Keynote 024 trial that led to approval, this patient group had a response rate of 44.8%, compared to 27.8% with chemotherapy alone. Even more encouragingly, the median PFS was 10.3 months compared to 6 months. Although some questions remain about whether chemoimmunotherapy in especially high–PD-L1 patients might give a further benefit over pembrolizumab alone, the reality is that the vast number of patients and their families would easily choose immunotherapy monotherapy and pass on the cytotoxic drugs.

It is important to again note that besides using tissue to make a histologic diagnosis and obtain the PD-L1 level, pathologists will also need to wisely use the biopsy sample to make sure that there is adequate material to perform mutational testing. Some oncogene-addicted tumors have high PD-L1 levels, but the impulse to start these patients on immunotherapy must be cooled until the mutational testing results come back; driver-mutated lung cancer has very poor responses to immunotherapy regardless of PD-L1 expression.[7,8] In addition, there are emerging data indicating that

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exposing driver-mutated lung cancer patients to immunotherapy early in their treatment course and following that with a tyrosine kinase inhibitor exposes them to a very high risk of pneumonitis.[9] Thus, it is more imperative than ever to get both the PD-L1 expression levels and the mutational profile before launching into therapy.

If there is scant tissue, what should be prioritized? Currently, PD-L1 needs to be performed on tissue. Increasingly, cell-free DNA testing seems to be accurate in identifying actionable mutations in many patients with advanced lung cancer. Recently reported were the results of the Nile study comparing the use of a cell-free DNA platform in newly diagnosed non–small cell lung carcinoma (NSCLC) patients who had standard-of-care mutation testing on tissue.[10] The use of circulating tumor DNA (ctDNA) increased the discovery of actionable mutations as listed in the National Comprehensive Cancer Network guidelines from 60 to 89 of 282 patients. The positive predictive value of ctDNA versus tissue was 100% and the turnaround time was better, 9 versus 15 days. Although some of the tissue testing that was performed did not test for all actionable mutations listed in the National Comprehensive Cancer Network guidelines, tissue exhaustion remains a significant issue in hospitals where sequential stand-alone gene testing is performed; thus, this study reflects what often is seen in the real world. As small biopsies continue to be an issue in the lung cancer population, having the ability to send plasma testing in the event that tissue is scant should allow for better identification of patients with targetable mutations and allow treatment with either US Food and Drug Administration (FDA)–approved agents or enrollment in clinical trials. (And to be redundant: avoid early exposure to immune checkpoint agents when started with ignorance of the patient's true mutational profile.)

TUMOR MUTATIONAL BURDEN

Because of the imperfect nature of PD-L1 staining as a biomarker in the clinic, much attention has been spent on finding other markers that could hopefully prognosticate as well as generate hypotheses on how to get around primary immunotherapy resistance. Tumor mutational burden (TMB) has emerged as a potential candidate to fill that space. In some early studies in melanoma and lung cancer patients who were treated with checkpoint inhibitors, there was a correlation between elevated TMB and clinical response.[11,12] The proposed explanation was that increased mutations led to increased neoantigen formation and an increase in the number of potential targets to be recognized by the patient's T cells.

So, how useful is TMB as a biomarker in the clinic? Hellmann et al[13] reported on lung cancer patients with high TMB treated on CheckMate 227, where patients were randomized to nivolumab versus nivolumab/ipilimumab versus chemotherapy (if the patient's PD-L1 was 0, chemotherapy was added to the nivolumab arm). The FoundationOne CDx assay was used to determine TMB, defined here as the number of somatic, coding base substitutions and short insertions and deletions (indels) per megabase of genome examined. It is important to note that although 94.8% of patients had biopsy material available, only 57.7% had valid TMB data, underscoring the fact that if this marker moves into the clinic, patients might need a number of repeat biopsies to get adequate tissue for analysis. Patients with high TMB (defined as >10 mutations per megabase in this study) treated with the combination of ipilimumab and nivolumab had a significantly better PFS at 1 year than patients treated with chemotherapy (43% versus 13%). Benefit of IO with high TMB was seen whether the patient had high or low PD-L1 levels. And as is often seen in immunotherapy trials, responding patients often remain on study and have ongoing benefit. However, currently TMB is not used to make decisions in the clinic and nivolumab and ipilimumab are not US Food and Drug Administration–approved for high TMB patients, as it remains unclear that the benefit in PFS will translate to overall survival improvement. For hospitals where genomic testing is sent out to reference labs, TMB will be reported, but for those who do in-house next-generation sequencing panel testing, it remains experimental to include TMB (and the definitions of TMB as well as standardization remain works in progress). Oncologists do not yet need that information to guide initial therapy.

If small biopsy samples continue to pose issues for more extended next-generation sequencing testing, can plasma testing help? Gandara et al[14] reported the results of ctDNA analysis in NSCLC patients randomized between chemotherapy with docetaxel and an immune checkpoint drug, atezolizumab, during early clinical trials. The investigators were able to compare blood TMB with tissue TMB and found a positive correlation (the Spearman rank correlation was 0.64). They were also able to confirm that with increasing levels of blood TMB above 16, there was a correlation with benefit from therapy with PD-1 blockade and that it seemed to be a continuous variable; the higher the blood TMB, the better the results. In addition, blood TMB was not associated with high tissue PD-L1 expression and was independently predictive of PFS benefit. As in CheckMate 227, however, results were not always available; results depended on the degree of the patient's tumor burden.

One additional area where ctDNA might be useful is in monitoring response to therapy. Goldberg and Patel[15] reported their results in a small study looking at the longitudinal results of the allelic fraction of cancer-associated somatic mutations in the blood of NSCLC patients treated with immunotherapy. The ctDNA responses correlated strongly with radiographic responses, and, importantly, the median time to initial response was faster for the ctDNA than the radiographic responses, 24.5 days compared to 72 days. Although these results need to be validated in a large prospective cohort, as well as in patients with other tumor types and treated with chemoimmunotherapy combinations, this might conceivably allow clinicians to recognize lack of efficacy in patients early and avoid continued administration of an exorbitantly expensive medication.

Mismatch repair deficiency has also emerged as a rare but very important actionable target in selecting patients for immunotherapy. Like TMB, mismatch repair deficiency leads to the development of a high level of mutations and neoantigens.[16] Studies in patients with mismatch repair–deficient colon cancers revealed considerable activity of IO drugs: response rates of 40% as compared to 0% in mismatch-proficient patients and immune-related PFS rates of 78% versus 11%.[17] For that reason, clinicians will need microsatellite instability status on not just advanced colon cancer patients but other sites as well (pancreas, stomach,

prostate, etc). This information is not just speculative at this time: it leads to insurance approval of drugs and often major responses in patients with advanced disease who very often do not have other significant therapeutic options available. For that reason, the US Food and Drug Administration approved immunotherapy in an organ-agnostic approach for patients with microsatellite instability–high tumors.

FUTURE DIRECTIONS

Investigation into genomic signatures that predict for responsiveness or resistance to immunotherapy drugs remains robust. It includes looking for mutations that correlate with resistance to checkpoint blockade, such as mutations of STK11—when present with KRAS, it strongly predicts for lack of benefit from checkpoint inhibitors.[18] It includes looking at gene expression signatures to differentiate between immunologically "hot" and "cold" tumors. Studies looking at predicting neoantigen load, genes expressed in the IFN-γ pathway, alterations in the JAK-STAT pathway, and stemness all show some promise, as reviewed by Cristescu et al.[19] In fact, negative associations between high stemness tumors and immune recognition of tumor occurred even in the presence of high tumor burden and expression of cancer-testis-antigen.[20] Trials are underway to see if exploiting this new molecular knowledge can increase response rates.

The development of precision immuno-oncology will depend on a continued expansion of our knowledge of the dynamic interplay among tumor genomics, the tumor microenvironment, and very likely the microbiome.[21] A single binary marker will not be found, but rather an algorithm of PD-L1 expression, TMB, and perhaps analysis of inhibitory or stimulatory gene signatures will need to be developed in order to best identify patients who will be best served by the ongoing immunotherapy revolution. Oncologists and pathologists will need to continue to work together to foster communication, stewardship of valuable and small biopsy specimens, and cost-effective use of technology in the service of expanding patient opportunities.

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