Biomarker Testing for Immunotherapy

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ABSTRACT

Immunotherapy is now the fourth pillar of cancer treatment, but the methodology used to determine who will benefit is still a work in progress. PD-L1 is commonly used as a predictive biomarker for immunotherapy, but others—such as immunogenic tumor biomarkers, host environment biomarkers and biomarkers of nonresponse—are being actively investigated. Additionally, research in combining biomarkers is currently being conducted, with new data emerging all the time.

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Immune checkpoint inhibitor (ICI) therapy has rapidly and drastically changed the treatment landscape in oncology. For many malignancies, it has led to unprecedented responses in cancers with historically poor prognoses. However, only a subset of patients respond to treatment with ICIs, underscoring the need for more reliable predictive biomarkers to guide patient selection for this therapy. At the NCCN 2019 Annual Conference, Jarushka Naidoo, MBBCh, Assistant Professor of Oncology, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, discussed the busy and growing area of biomarker testing for immunotherapy.

Biomarkers are defined as "cellular, biochemical or molecular alterations that are measurable in biological media, such as human tissues, cells, or fluids."[1] In oncology, biomarkers are used to identify potential therapeutic targets, provide insight into why a cancer occurred, guide treatment decisions, predict who will respond to treatment, and monitor treatment responses through therapy.[2]

ICIs are still a relatively new treatment option for patients with a variety of solid tumors, and biomarker testing varies depending on cancer type and stage; the NCCN Guidelines provide recommendations for biomarker testing across certain solid tumors. However, in this evolving field, PD-L1 testing via immunohistochemistry (IHC) is generally considered one of the most extensive biomarker tests available, according to Dr. Naidoo.

Current Biomarker Testing for Immunotherapy

For cisplatin-ineligible bladder cancer, patients may receive atezolizumab or pembrolizumab, using 2 different PD-L1 biomarkers. Atezolizumab uses the SP142 antibody with a PD-L1 IHC cutoff of 5% for immune cells only. Pembrolizumab uses a different scoring system, the antibody 22C3, and calculates a PD-L1 combined score of ≥10% (percent of immune cells + percent of tumor cells, divided by percent of tumor cells).

Biomarker testing in lung cancer is simpler. For use of pembrolizumab in the first-line treatment of advanced *EGFR/ALK*-negative/unknown non–small cell lung cancer (NSCLC), the NCCN Guidelines for NSCLC dictate a PD-L1 IHC score >50%, and a score of 1% (tumor cells only for both) in the second line. "But these cutoffs and their relevance are debatable in the new era of chemotherapy plus immunotherapy in lung cancer," she noted.

According to the guidelines, microsatellite instability/mismatch repair deficiency (MSI/MMR) testing should be used in colon and endometrial cancers. And in renal cell carcinoma and metastatic melanoma, all patients should receive immune checkpoint blockade, regardless of a biomarker, Dr. Naidoo said. "But this is a rapidly evolving space, and it's likely to change every couple of months in reality," she added.

Inflamed Tumor Biomarkers

Immunotherapy biomarkers can generally be separated into 3 broad categories: inflamed tumor biomarkers (ie, PD-L1–high), immunogenic tumor biomarkers (related to how likely the tumor is to engender an immune response, such as high tumor mutational burden [TMB]), and factors of the host environment (ie, gut microbial diversity/composition). This last category is new and emerging, according to Dr. Naidoo, and examines characteristics of an individual, separate from the tumor, that can inform whether they are likely to respond to immunotherapy.

PD-L1 was originally identified as a biomarker as a result of a phase I study that revealed a correlation between lack of PD-L1 expression and lack of response to nivolumab in patients with multiple solid tumors,

including lung cancer.[3] This was followed by KEYNOTE-010, a large lung cancer study that validated PD-L1 expression cutoffs of ≥50% and ≥1% in tumor cells only using the antibody of 22C3.[4] Findings from KEYNOTE-010 were confirmed in a subsequent phase III study of first-line pembrolizumab in NSCLC, which showed that patients whose tumor cells expressed PD-L1 at a rate of ≥50%, had an impressive benefit in progression-free survival, as well as an overall survival benefit.[5]

However, there are some key limitations to the use of PD-L1 as a biomarker. First, there may be spatial heterogeneity of PD-L1 assessment even within a lesion that is biopsied. Therefore, the results of PD-L1 testing will depend on the location of the biopsied tumor, the timing of biopsy relative to PD-L1 testing, and the tumor type being tested. Furthermore, depending on measurement of tumor cells, immune cells, or both, cell scoring may also be relevant in an assessment of PD-L1 status. Adding to the lack of standardization, different antibodies have been used with a variety of agents and tumor types, using different platforms and cutoff values (Figure 1). "Hopefully we will see a number of studies to try and harmonize these," she said.

Immunogenic Tumor Biomarkers

"We've all heard the term 'TMB,' but what does it mean and what does it reflect?" Dr. Naidoo asked. TMB is a measurement of the accumulated errors (ie, mutations) in tumor cell DNA. Its rationale as a biomarker for ICI therapy is based on the fact that with every mutational error that occurs in DNA, abnormal proteins or peptides form that can be expressed on the surface of immune cells, called neoantigens. These neoantigens result in tumors that appear more foreign, or less "self," to the immune system and are thus more likely to elicit an immune response.

1. Spatial heterogeneity
- Lesion and specific area to select for biopsy
- Results depend on location and timing

2. Cell Scoring
- Tumor cells, immune cells, or both?
- NSCLC: TC, Urothelial: %TC/IC; IC, RCC/melanoma: N/A

3. Methods of PD-L1 Assessment

IO Agent	Nivolumab	Pembrolizumab	Durvalumab	Atezolizumab	Avelumab
Antibody	28-8	22C3	SP-263	SP142	73-10
Platform	DAKO	DAKO	VENTANA	VENTANA	DAKO
Cells scored	Tumor	Tumor/TC+IC	Tumor	TC+IC, IC only	Tumor
Cutoff value	1%, 5%	1%, 10%, 50%	25%	TC/IC, 5%	None

Figure 1. PD-L1 as a biomarker: key limitations.
Abbreviations: IC, immune cells; IO, immuno-oncology; N/A, not applicable; NSCLC, non–small cell lung cancer; RCC, renal cell carcinoma; TC, tumor cells. Adapted from Drake et al, ASCO 2018.

The relationship between TMB, neoantigen burden, and immunotherapy response has been demonstrated in many studies. A trial of 34 patients with lung cancer treated with single-agent pembrolizumab showed improved progression-free survival among those with a high TMB.[6] When TMB was translated to neoantigen burden, the analysis revealed a similar pattern. Similarly, another study showed that patients with colorectal cancer and a high TMB from MSI also experienced improved responses to checkpoint blockade.[7]

"TMB really is where PD-L1 was several years ago in terms of the need to harmonize the different methods, the different cutoff values, and what they mean," she said. "Some of the factors to standardize in TMB include sequencing depth, the mutations included, and the filtering process."

Another important study from London assessed not only the counts of mutations in tumors and their neoantigen count but also their neoantigen heterogeneity. This study demonstrated that the neoantigens that elicit the strongest immune response originate from highly clonal mutations, or mutations that exist in many different tumor areas.[8] "This is actually being looked at as a biomarker of response in lung cancer in ongoing studies," she said.

Biomarkers of Nonresponse

The third type of biomarkers, those of nonresponse, inform when to avoid giving immunotherapy to select patients. Early studies in lung cancer showed that patients with *EGFR*-mutant NSCLC generally had lower TMB. "[These tumors] are not associated with smoking; it's just a phenotypically different cancer," said Dr. Naidoo. "We observed that these patients can have response rates as low as 0% from immune checkpoint blockade, even if the tumors are high PD-L1 expressors." A meta-analysis of 3 large studies in *EGFR*-mutant NSCLC showed no benefit with checkpoint blockade compared with chemotherapy.[9]

KRAS mutations, which are present in approximately 25% of lung adenocarcinomas and confer a poor prognosis, can co-occur with other mutations, and these genomic subsets have differential responses to immune checkpoint blockade. One study found that patients with a co-mutation between *KRAS* and *STK11* had only a 0% to 7% response rate to checkpoint blockade, whereas those with a co-mutation between *KRAS* and *p53* had a higher response rate of approximately 35%.[10] "The take-home from this study was that co-mutations between *KRAS* and *STK11* are bad actors and these patients shouldn't get checkpoint blockade," she said.

Immunotherapy and the Microbiome

Microbiome is a term for the population of microbes present in a particular biologic niche, such as on the skin or in the gut; this can refer to bacteria, fungi, viruses, and any other organisms not classified as cells.[11] The relationship between the microbiome and immunotherapy is still being explored, but this particular host factor may augment responses and toxicity to ICIs.

"There is 100-fold more gene content in the microbiome than in the human genome, and it has been known to influence both health and disease," said Dr. Naidoo. A seminal study showed that patients with metastatic melanoma who responded to anti–PD-1 therapy had higher gut microbiota diversity than nonresponders.[12] Another study showed that the microbiome is predictive not only of response but also of toxicity: the investigators demonstrated that the presence of a certain species of microbe may be protective against the development of colitis from anti–CTLA-4 therapy in melanoma.[13]

According to Dr. Naidoo, there will certainly be a series of studies examining what biomarkers can be combined and what may be relevant in different tumor types. "I think there's going to be considerable differences between tumor types and patient populations that fall out of these analyses," she said. "This is a very crowded space. But, thankfully, many of us are studying it, and hopefully we will shed some light on these areas."

Disclosures: Dr. Naidoo has disclosed that she has no interests, arrangements, affiliations, or commercial interests with the manufacturers of any products discussed in this article or their competitors.

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References

1. Mayeux R. Biomarkers: potential uses and limitations. NeuroRx 2004;1:182–188.
2. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001;69:89–95.
3. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012;366:2443–2454.
4. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 2016;387:1540–1550.
5. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med 2016;375:1823–1833.
6. Rizvi NA, Hellmann MD, Snyder A, et al. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. Science 2015;348:124–128.
7. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015;372:2509–2520.
8. McGranahan N, Furness AJ, Rosenthal R, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science 2016;351:1463–1469.
9. Lee CK, Man J, Lord S, et al. Checkpoint inhibitors in metastatic EGFR-mutated non–small cell lung cancer—a meta-analysis. J Thorac Oncol 2017;12:403–407.
10. Skoulidis F, Goldberg ME, Greenawalt DM, et al. *STK11/LKB1* mutations and PD-1 inhibitor resistance in *KRAS*-mutant lung adenocarcinoma. Cancer Discov 2018;8:822–835.
11. Bultman SJ. Emerging roles of the microbiome in cancer. Carcinogenesis 2014;35:249–255.
12. Gopalakrishnan V, Spencer CN, Nezi L, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 2018;359:97–103.
13. Dubin K, Callahan MK, Ren B, et al. Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade-induced colitis. Nat Commun 2016;7:10391.