Could Programmed Death-Ligand 1 Copy Number Alterations be a Predictive Biomarker for Immunotherapy Response?

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Immune checkpoint inhibitors that target programmed cell death protein-1 (PD-1) or programmed death-ligand 1 (PD-L1) have durable responses and significantly improve survival in patients with advanced non-small cell lung cancer (NSCLC) that lack driver mutations. However, the benefit of anti–PD-1 or anti–PD-L1 (anti–PD) therapy is limited to a subset of NSCLC tumors. Most patients with NSCLC are nonresponders and current biomarkers to select patients for immunotherapy are based on PD-L1 protein expression on tumors. Given the high rates of nonresponders and potential for inducing immune adverse events, the use of anti–PD therapy as first-line therapy should ideally be limited to patients most likely to benefit and better predictive biomarkers for anti–PD therapy are highly desired.

Tumor PD-L1 protein expression as measured by immunohistochemistry (IHC) is the only approved predictive biomarker to select patients with NSCLC for anti–PD therapy and there is a clear relationship between higher PD-L1 protein expression in tumors and increased overall response rate. However, PD-L1 IHC method is influenced by various factors, including, but not limited to, the location and quality of biopsies, the antibodies used for detection, and the quantification method. In addition, less than half of the patients with metastatic NSCLC with PD-L1 levels greater than or equal to 50% have even a partial response to pembrolizumab, and tumors with high PD-L1 expression may not respond to anti–PD therapy if cytotoxic T lymphocytes are lacking.

As PD-L1 IHC expression has its limitations, much work has been done to identify other clinically useful tools to predict response to anti–PD therapy. Elevated tumor mutation burden (TMB) increases tumor antigenicity, is independent of PD-L1 expression, and has been associated with improved response to anti–PD therapy. TMB was found to be a predictor of response, independent of PD-L1 expression, in patients with NSCLC treated with nivolumab monotherapy and a combination nivolumab and ipilimumab. Biomarkers related to the tumor immune microenvironment (TIME) have also been proposed as important factors to predict the response to anti–PD therapy. These include the biomarkers based on the abundance of intratumor cytotoxic T lymphocytes and expression profiles of T-cell activation. A pancancer, T-cell inflamed gene-expression profile that indicates an active T-cell TIME has been found to correlate with response to pembrolizumab.

Alternatively, PD-L1 copy number alterations could be used to predict responses to immunotherapy; however, there are limited studies in this area. PD-L1 is encoded by the PD-L1 gene (CD274; OMIM 605402) located on chromosome band 9p24.1. Copy number gains at this locus in the genome can lead to amplification of PD-L1 and PD-L2 genes and JAK2 and activation of the JAK-STAT pathway. In Hodgkin’s lymphoma, PD-L1 copy number gains are seen in more advanced-stage disease and are associated with worse outcomes. Patients with Hodgkin’s lymphoma with PD-L1 copy number gains and increased PD-L1 expression have superior progression-free survival when treated with anti–PD therapy. In NSCLC, PD-L1 copy number gains are associated with smoking-related tumors and amplification of PD-L1 has not been observed in tumors with mutant EGFR expression or ALK expression. In

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surgically resected NSCLC, PD-L1 amplification is associated with poor survival.22 Like PD-L1 protein expression, PD-L1 copy number changes are independent of TMB.22 Finally, PD-L1 copy number gains are associated with PD-L1 protein positivity in many solid tumors including NSCLC.22,23

In this issue of the Journal of Thoracic Oncology, Bozinovski et al.24 investigated tumor PD-L1 copy number alterations, as measured by reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR), as a possible biomarker that could be used to supplement PD-L1 IHC staining as a predictor of response to anti-PD therapy. Using surgical samples of tumor and normal adjacent lung parenchyma tissue, investigators measured PD-L1 gene copy number and mRNA expression by RT-qPCR. Expression levels of inflammatory mediators (interferon-γ, interleukin [IL]-6, IL-1, matrix metalloproteinase–9) and immunosuppressive mediators (IL-10, transforming growth factor–β) were used as surrogate markers of an immunologically cold tumor microenvironment.24 They found that PD-L1 copy number loss was associated with reduced expression of these inflammatory mediators, however there is no clinical response or survival outcomes data reported. The data suggests that a low PD-L1 copy number could be further developed to be a biomarker for a less inflammatory TIME and therefore predict a lack of response to anti-PD therapy.24

To date, there has been one published study evaluating the relationship between PD-L1 copy number and response to immunotherapy. Inoue et al.25 evaluated PD-L1 copy number, measured by fluorescence in situ hybridization in 200 patients with advanced NSCLC who were treated with nivolumab. In that study, very similar progression-free survival and overall survival outcomes were observed between patients with PD-L1 disomy and polysomy. PD-L1 amplification was associated with response to nivolumab with an 80% overall response rate, compared with only 18% overall response rate among patients with PD-L1 polysomy or disomy, though treatment numbers in the amplification group were quite small (n = 5).25 This represents a major challenge in using PD-L1 copy number gains as a realistic biomarker for immunotherapy treatment selection; PD-L1 copy number gains are found in relatively few NSCLC tumors. Approximately only 2% to 3% of surgically resected NSCLC tumors have PD-L1 amplification and 13% have PD-L1 polysomy, with loss of PD-L1 copy number being more frequent in NSCLC.22,26 In the Bozinovski study, 17% of NSCLC tumors were found to have a copy number loss whereas 7% had a copy number gain, thus, providing actionable data on only a quarter of potential patients if copy number alterations were to be used as a predictive biomarker.24

The Bozinovski study also evaluates PD-L1 copy number alterations in tissue obtained from bronchoscopic needle biopsies and compares variation in PD-L1 copy number and PD-L1 mRNA expression within multiple samples from the same patient’s primary tumor and metastatic lymph node tissue and between different lymph nodes using RT-qPCR.24 The authors observed a close correlation between PD-L1 tumor proportion score by IHC and PD-L1 mRNA expression. In addition, they found that there was less heterogeneity in PD-L1 copy number across multiple biopsy sites than PD-L1 mRNA expression,24 suggesting that PD-L1 copy number might serve as a more reliable biomarker than PD-L1 staining or PD-L1 mRNA expression. Previous work has reported that PD-L1 mRNA as measured by RT-qPCR correlates with IHC staining in tumor tissues, but that normal tissues can express PD-L1 mRNA without staining positive for the protein by means of IHC.27,28 PD-L1 copy number alterations have been previously found to correlate with tumor PD-L1 expression levels.29 In addition, consistent and reproducible PD-L1 gene copy number alterations between primary tumor biopsies and synchronous regional lymph node metastases have been reported in prior studies.22,29 Furthermore, studies of surgically resected specimens have revealed that there is less intratumor variation in PD-L1 copy number than variation in PD-L1 protein expression.29 Altogether, these data suggest that PD-L1 copy number may be a better, more consistent ancillary biomarker to PD-L1 protein expression than PD-L1 mRNA expression.

PD-L1 copy number variation may have advantages as a more practical independent biomarker for anti-PD therapy response than PD-L1 protein levels. Advanced-stage patients being evaluated for chemotherapy and immunotherapy are the ones for whom mutation testing and PD-L1 assessment are the most critical, and these are the patients from whom tissue is most often obtained by means of needle biopsy. Whereas there have been studies to demonstrate that PD-L1 IHC expression can be obtained from small biopsy samples from fine needle aspirate or cytology specimens,30,31 there are many instances in which there is insufficient tissue to perform these tests.32,33 Genetic tumor profiling using either RT-qPCR or next-generation sequencing (NGS) to evaluate PD-L1 copy number data could be done concurrently with mutation testing on small biopsy specimens.34

PD-L1 amplification above a certain copy number can be detected using NGS.26 Large studies of solid malignancies across many tissue types, sequenced by NGS, reveal that the prevalence of copy number gains varies on the basis of tumor type; however, PD-L1 amplification can only be detected in less than 1% of solid tumors.26 Polysomy can be difficult to detect using NGS and a cutoff of six or more copy number alterations has been
used to define PD-L1 amplifications. Given the cost and challenges of NGS, RT-qPCR testing is a more practical, simple, and cost-effective approach than NGS to obtain PD-L1 copy number information.

At this point in time, detection of altered PD-L1 copy number may represent a potential biomarker to predict response to anti-PD therapy. PD-L1 copy number has the benefit of more consistent intratumor measurement than PD-L1 IHC or mRNA expression and can be accurately measured in small needle biopsy specimens. Additional studies and more clinical data are needed to directly correlate these findings to clinical response outcomes before PD-L1 copy number alterations could be considered as an adjunct to the IHC staining. However, until the TIME is considered and incorporated into biomarkers of patient selection a critical piece of the puzzle is missing. The ideal biomarker to predict response to anti-PD therapy will evaluate both expressed PD-L1 protein on tumor cells and detect the presence and activation of tumor-infiltrating lymphocytes. Until then, tumor PD-L1 protein expression as detected by IHC staining remains our best biomarker to predict anti-PD therapy response.

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References


