Immune checkpoint blockade has emerged as a promising and distinct treatment strategy and has caused a paradigm shift in oncology. Significant response and survival benefit have been observed in a fraction of patients who were treated with immune checkpoint inhibitors (ICIs) in several malignancies including melanoma (1-3), non-small-cell lung cancer (NSCLC) (4-7), gastric cancer (8), urothelial carcinoma (9), Hodgkin's lymphoma (10), and head and neck squamous cell carcinoma (11). Additionally, because clinical response to ICI treatment has been seen to vary from patient to patient, a predictive marker that provides insight on patient response is urgently needed. Currently established companion diagnostics include the HercepTest™ immunohistochemistry (IHC) staining for breast and gastric cancers, fluorescence in situ hybridization (FISH) assays to disclose anaplastic lymphoma kinase (ALK) translocations for NSCLC, and mutation analyses for the epidermal growth factor receptor (EGFR) gene in NSCLC and BRAF for melanoma. Individual differences in treatment efficacy of ICIs may be due to the complex interaction of the tumor microenvironment; where tumor, immune, and stromal cells closely interact. Thus, it has been difficult to establish simple determinants that would predict the efficacy of ICIs like those currently used in targeted therapy.

Immune checkpoints are regulated by many signaling processes that are in part controlled by key players such as programmed death 1 (PD-1), programmed death ligand-1 (PD-L1/CD274), programmed death ligand-2 (PD-L2/CD273), cytotoxic T lymphocyte antigen-4 (CTLA-4), lymphocyte activation gene 3 (LAG-3), and T cell immunoglobulin and mucin domain 3 (TIM-3). Among these interactions, blockade of the PD-1 or its corresponding ligand, PD-L1, has been thoroughly investigated.

The mechanism by which T cells survey and assess cellular antigens depends, in part, on the interaction of inhibitory and activating domains that maintain the immunological synapse. Tumor-specific neopeptides that are created by some of tumor nonsynonymous mutations are recognizable by T cells (12). Thus, ongoing investigations aim to utilize these neopeptides and allow for their recognition and downstream T cell activation using anti-PD-1/PD-L1 immunotherapy. In the case of the PD-1 pathway, activated T cells express PD-1 on their surface and may result in exhausted or inactivated phenotype when PD-1 engages either PD-L1 or PD-L2 (13). PD-L1 is expressed by a variety of cells including those in the tumor microenvironment such as antigen-presenting cells, endothelial cells, and tumor cells. Two anti-PD-1 antibodies, nivolumab and pembrolizumab, and a PD-L1 inhibitor, atezolizumab, were recently approved by the US Food and Drug Administration (FDA), and have recently been applied to patients with a variety of advanced cancers.

Several predictive biomarkers for PD-1/PD-L1 axis blockade have been suggested. The overexpression of PD-L1 protein on tumor cell surface and/or tumor-infiltrating immune cells (TIICs) was associated with better response to the therapy (2,4,7,9). A PD-L1 IHC test using the clone 22C3 antibody was approved by the FDA as a companion diagnostic for selecting patients with NSCLC for pembrolizumab while another PD-L1 IHC test using the clone 28-8 antibody was approved as a complementary assay for nivolumab in NSCLC. Clone SP142 has also been approved for the detection of PD-L1 on TIICs for locally
advanced or metastatic urothelial carcinoma. However, other studies revealed that the predictive value of PD-L1 expression for the use of PD-1 inhibitors were low (3,5), implying that a proportion of PD-L1-negative patients could have responded and benefited from PD-1/PD-L1-directed immunotherapy. Furthermore, evaluating PD-L1 expression levels using IHC analysis poses several issues that may affect treatment planning. First, PD-L1 protein expression is heterogeneous both spatially (14) and temporally (15). Furthermore, tumor cells, endothelial cells, and TIIICs may stain for PD-L1 within the tumor microenvironment. Thus, these context-dependent results of PD-L1 expression, especially when examined among small biopsy specimens, may be skewed and not represent true PD-L1 expression status. Second, standardization of PD-L1 staining and tumor tissue preparation has not been established. Finally, which antibody clone and cutoff point for IHC evaluation should be used has yet to be determined. Although performing and assessing PD-L1 IHC is relatively simple, the aforementioned problems in heterogeneity, reproducibility, and standardization has made it more difficult to compare data and should thus be addressed for the future.

Another candidate predictive biomarker for the blockade of the PD-1 axis is mutation burden of tumor cells. Although it has been well established that the likely cause of melanoma and NSCLC are mutations that arise from ultraviolet radiation and tobacco smoking, respectively, its relevance to anti-PD-1/PD-L1 immunotherapy response was unknown. Recently, higher somatic nonsynonymous mutation burden was reported to be associated with greater efficacy and clinical benefit of pembrolizumab monotherapy (16). Furthermore, mismatch-repair deficiency determined by the microsatellite instability PCR analysis has been shown to predict the treatment benefit of pembrolizumab in a cohort that mainly consisted of patients with colorectal cancer (17).

An additional probabilistic predictor of response to PD-1/ PD-L1 inhibitors is the presence of tumor-infiltrating antigen-specific CD8-positive T cells. Recently, cancers have been proposed to be stratified into four different tumor microenvironments based on the presence of TIIICs and tumor PD-L1 expression status (18). PD-L1-positive tumors that contain TIIICs are classified as a type I tumor microenvironment. This environment is characterized by tumor immunogenicity and is most likely to respond to checkpoint blockade. In melanoma patients with pre-existing tumor-associated CD8-positive T cells, pembrolizumab therapy was shown to inhibit PD-1/PD-L1 mediated adaptive immune resistance and conferred tumor regression (19). It should be noted that oncogene-driven PD-L1 expression, which is diffuse and constitutive, is distinct from adaptive inflammation-driven PD-L1 expression. Although EGFR activating mutations (20) and ALK translocations (21) in NSCLC were shown to increase PD-L1 expression, both of these gene alterations have been reported to be associated with low response to PD-1/PD-L1 inhibitors, with objective response rates of 3.6% in EGFR-mutant or ALK-positive patients versus 23.3% in EGFR wild-type and ALK-negative/unknown patients (15). The decreased presence of the type I tumor microenvironment was suggested to be responsible for the low treatment benefit seen among tumors harboring these gene alterations. Because EGFR-mutant or ALK-rearranged NSCLCs are more common among never- or light-smokers and have less nonsynonymous mutations than smoking-related tumors (22), the immunogenicity of the tumors harboring the EGFR or ALK gene alterations are relatively low, resulting in less tumor recognition by immune cells.

In a recent issue of Genes Chromosomes & Cancer, Budczies and colleagues (23) reported the landscape of PD-L1 copy number alterations (CNAs) in 22 major cancer types using The Cancer Genome Atlas (TCGA) RNAseq and CNA datasets. They found a strong correlation between PD-L1 CNAs and mRNA expression levels for most cancers. Notably, they observed that the mutation load was significantly higher in tumors with PD-L1 copy number gains than in tumors with normal PD-L1 copy number among eight individual cancer cohorts including the lung adenocarcinoma and lung squamous cell carcinoma cohorts. Interestingly, higher mutation load was also found among PD-L1 deleted tumors when compared to PD-L1 normal tumors in seven out of the 22 cancer types. Whether immunotherapy using checkpoint inhibitors is effective in PD-L1 deleted tumors with a high mutation burden seems to be a topic that requires further investigation.

As written in the article by Budczies and colleagues (23), CNAs of the PD-L1 gene have received surprisingly little attention until now. We previously reported the prevalence, clinicopathological characteristics, and prognostic implications of PD-L1 copy number gains in NSCLC using FISH (24). PD-L1 amplification and polysomy were observed in 3.1% and 13.2% of patients, respectively, and were independently associated with PD-L1 protein overexpression. PD-L1 copy number gains were more commonly observed among smoking-related tumors, and strikingly, PD-L1 gene amplification was found to be exclusive to EGFR
mutations and ALK expression, both of which were reported to be negatively associated with response to PD-1/PD-L1 inhibitors (15). Furthermore, our data indicated that tumor PD-L1 copy number status was more consistent and reproducible than tumor PD-L1 protein expression detected by IHC when primary tumors and synchronous regional lymph node metastases were comparatively analyzed.

Similar to Budczies and colleagues (23), Ock and colleagues (25) carried out comprehensive analyses of immunogenomic properties in TCGA datasets and evaluated the RNA expression levels of PD-L1 and CD8A. They observed that the type I tumor microenvironment defined by high PD-L1 and CD8A expression were especially common among lung adenocarcinomas (67.1%) and lung squamous cell carcinomas (63.5%) as well as cancers derived from lymphoproliferative tissues and kidney clear cell carcinoma. Importantly, a high mutation burden and PD-L1 amplification were independently associated with the type I tumor microenvironment in a multivariate analysis. Taken together, PD-L1 copy number gains, in particular PD-L1 amplification, appear to represent the genomic instability of tumor cells in several cancer types. Although PD-L1 copy number gains, which lead to diffuse and constitutive PD-L1 expression, are one of many mechanisms of innate immune resistance, they also reflect the type I tumor microenvironment with high mutation load in tumor cells.

The search for a biomarker that could accurately predict ICI response has been widely and enthusiastically performed so that rational and full use of the promising and costly therapy can be achieved. Given the complexity of the dynamic interaction of the immune system and tumors, predicting the response of ICI treatment using a single biomarker might not be possible. PD-L1 protein expression has relatively limited power to predict response to PD-1/PD-L1 inhibitors. At present, the wide use of next-generation sequencing data in the clinical setting for evaluating the mutation burden of each patient is not possible. Additionally, it is difficult to precisely predict true neo-epitopes which can be recognized as non-self by T cells from total nonsynonymous mutations tailored to each patient's tumor. As for evaluation of TIICs, inaccurate results might be reported because testing is carried out on a small tissue specimen in a proportion of patients with advanced cancers such as NSCLC. However, evaluating PD-L1 copy number gains can be relatively simple using FISH even on small biopsy specimens. Moreover, PD-L1 copy number screening has been suggested to be helpful in assessing accurate PD-L1 protein expression, mutation burden, and specific tumor microenvironments. Thus, conclusively, we believe that the predictive significance of therapy response should be prospectively assessed in clinical trials. We hope to further evaluate the predictive value of PD-L1 copy number gains in our upcoming clinical trial of patients with advanced NSCLC using PD-1/PD-L1 ICIs.

**Acknowledgements**

This work was supported by MEXT, SRF, and AMED in part.

**Footnote**

*Provenance:* This is a Guest Editorial commissioned by the Section Editor Xia Fang (Department of hematology, Shanghai Tongji Hospital, Tongji University School of Medicine, Shanghai, China).

*Conflicts of Interest:* The authors have no conflicts of interest to declare.


**References**


