Editorial

Harmonization study of antibodies and platforms for programmed death ligand 1 immunostaining in non-small cell lung cancer: does shuffling couples settle the troubles?

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Immune-checkpoint inhibitors (ICIs) resulted in a revolution in the treatment of progressed non-small cell lung cancer (NSCLC). Blockade of programmed death 1 (PD-1) and its programmed death ligand 1 (PD-L1) has demonstrated better survival rates than cytotoxic chemotherapies. PD-L1 expression in tumor cells identified using immunohistochemical (IHC) analysis has been utilized as an inclusion criterion or a predictive factor of clinical outcomes in previous ICI clinical trials involving NSCLC patients. KEYNOTE-024 trial, for example, demonstrated improved efficacy of pembrolizumab compared with chemotherapy in previously untreated patients with NSCLC expressing PD-L1 in 50% or more of tumor cells (1). KEYNOTE-010 showed significant efficacy in second-line setting in patients with NSCLC consisting of 1% or more PD-L1 expressing cancer cells (2).

Based on the clinical trials mentioned above, the United States Food and Drug Administration (US-FDA) approved testing antibodies for IHC analysis of PD-L1 used in all clinical studies of ICIs as companion/complementary diagnostic agents (Nivolumab: 28-8, Dako/Agilent; Pembrolizumab: 22C3, Dako/Agilent; Atezolizumab: SP142, Ventana; and Durvalumab: SP263, Ventana). Patients with PD-L1 expression of tumor cells and/or tumor infiltrating immune cells may benefit from ICIs treatment, and this treatment decision is realistically based solely on PD-L1 IHC framework. Several studies have compared the four, aforementioned, diagnostic anti-PD-L1 antibodies using staining assays (3-6). A collaboration study involving the International Association for the Study of Lung Cancer and the American Association for Cancer Research together with pharmaceutical companies and two diagnostic companies (Dako/Agilent and Ventana/Roche) revealed that PD-L1 positivity detected using SP142 antibody was relatively low compared to those detected with other three clones (28-8, 22C3, and SP263) (Blueprint project) (3). Other studies also showed consistent results (4-6).

Datasheets of the US-FDA-approved diagnostic antibodies mention that each antibody is optimized for its coupled platform and do not guarantee accurate staining results with other developer platforms (7-9) (approved combination of antibodies and platforms are summarized in *Table 1*). Except for high-volume centers, the cost associated with multiple staining platforms would be extremely high for most institutions. This burden can be minimized if all diagnostic antibodies approved by regulatory authorities can be applied to existing staining platforms in each facility. Neuman *et al.* demonstrated PD-L1 IHC analysis on Ventana BenchMark XT using prediluted antibody from Dako 22C3 PharmDX kit harmonized with Dako Autostainer Link 48 (10). However, other combinations of antibodies and staining platforms have not been studied well.

Table 1 PD-L1 assay combination of diagnostic antibodies and IHC platforms

Agent	Diagnostic antibody	IHC platforms	US-FDA diagnostic status
Nivolumab	28-8 (Dako/Agilent)	Autostainer Link 48	Complementary
Pembrolizumab	22C3 (Dako/Agilent)	Autostainer Link 48	Companion
Atezolizumab	SP142 (Ventana)	BenchMark ULTRA	Complementary
Durvalumab	SP263 (Ventana)	BenchMark ULTRA	Unknown

PD-L1, programmed death ligand 1; IHC, immunohistochemistry; US-FDA, the United States Food and Drug Administration.

Adam *et al.* compared the tumor positivity of PD-L1 in 41 cases by staining with diagnostic anti-PD-L1 antibodies (22C3, 28-8, SP142, SP263, and E1L3N, which is used in laboratory studies) (11). They combined these five antibody clones and three automatic staining platforms (Dako Autostainer Link 48, Ventana BenchMark ULTRA, and Leica Bond III) to assess the compatibility of the resultant combinations. All combinations were assessed by a single pathologist to avoid interobserver variability. Most combinations were harmonized with approved methods. SP263 antibody achieved the highest compatibility with all the staining platforms. Thus, their results demonstrate the potential of the non-approved combinations of diagnostic reagents with staining platforms; however, larger studies are warranted to reveal the concordance between procedures and preferable antibody-staining platform combinations.

Adam *et al.* did not conclude that all the combinations of antibodies and staining platforms are appropriate for evaluation of PD-L1 expression in daily clinical practice (11). Concordance ratio between the shuffled-paired methods used for PD-L1 staining was high (around 80–90%) (11); however, if the applicability of antibody/staining platform combinations expands based on similar studies, the discrepancy between the staining results obtained using the initially approved and newly introduced methods might increase with the repetition of these procedures. This increasing discrepancy ultimately will lead to inappropriate therapy decisions based on inaccurate PD-L1 expression status. Prospective trials and/or large retrospective studies including various staining modalities are warranted to test new combinations of diagnostic antibodies and staining platforms to predict clinical outcomes.

Inter- and intra-observer concordance was not examined in the study conducted by Adam *et al.* (11). Scheel *et al.* assessed the difference among the evaluations of PD-L1 expression performed by 13 pathologists (4) and demonstrated existence of interobserver concordance among all trained pathologists. Comprehensive instructions for interpreting PD-L1 staining results for each staining procedure are crucial to maximize concordance between pathologists while using other combinations of diagnostic reagents and staining platforms. Moreover, the availability of authorized staining controls is indispensable for maintaining the quality of laboratory developed tests. Aggregated analysis of PD-L1 expression by high-volume centers or laboratories can be performed to reduce the discordance between observers and between IHC methods; however, regional health insurance and the geographical situation should also be taken into account.

At present, the clinical decision for treatment with ICIs is based on the framework for 22C3 PD-L1 IHC analysis. On the contrary, recent next-generation sequencing (NGS)-based predictive biomarker methods have been developed for immuno-oncology approaches. In the CheckMate227 trial (12), high tumor mutation burden (TMB) measured with FoundationOne™ demonstrated a relatively high precision for predicting the clinical efficacy of nivolumab and ipilimumab. The next step involving issues related to biomarkers for immunotherapy may be the collision between the framework of PD-L1 IHC and that of TMB using the NGS-based multiplex gene assay. The time interval from tissue submission until results and cost-effectiveness are crucial and these factors would determine the feasibility in clinical practice of each region.

Adam *et al.* suggested that PD-L1 staining can be harmonized across five antibodies with three platforms (11). They also pointed out that more specimens should be assessed to reveal the validity of these antibody-staining platform combinations. Interobserver variation should be minimized by a detailed description of the difference between the applied staining methods. The issue regarding consistency of these US-FDA-approved couple of antibodies and platforms can be resolved not only by comparing the staining result of shuffled pairs but also by searching for an alternative, including centralized evaluation of PD-L1 expression and utilization of other biomarkers.

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Footnote

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

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