Editorial

Improving our knowledge in PD-L1 testing in lung cancer: the archival sample is 'promoted'!

The therapeutic approach for the second-line treatment of patients with advanced non-small-cell lung cancer (NSCLC) without actionable mutations has been recently revolutionized by the approval of immune checkpoint inhibitors. Nivolumab, pembrolizumab and atezolizumab improved overall survival (OS) in patients with advanced pretreated NSCLC with both squamous and nonsquamous histology compared with single-agent docetaxel (Table 1) [1–5]. In the studies with nivolumab and atezolizumab, patients were not selected on the basis of programmed death ligand-1 (PD-L1) expression, on the contrary in the study with pembrolizumab (KEYNOTE-010), patients were included only if they were positive for PD-L1 expression [tumor proportion score (TPS) on at least 1% of tumor cells (TCs)] on the basis of a companion diagnostic test [PD-L1 immunohistochemistry (IHC) 22C3 pharmDx assay].

In this update of KEYNOTE-010 study, Herbst et al. [6] compared treatment response with pembrolizumab by PD-L1 expression in archival and newly collected tumor samples. The median time between sample collection and PD-L1 assessment was 250 days for archival samples and 11 days for new samples. The first finding of this analysis is that with a longer median follow-up (31 months), pembrolizumab continued to improve OS over docetaxel [hazard ratio (HR) 0.66; 95% confidence interval (CI) 0.57–0.77] in the intention-to-treat population. The second and most important finding of the study is that pembrolizumab led to an improvement in OS compared with docetaxel irrespective of tumor sample type (archival versus new collected), in both the TPS $\geq$ 50% and TPS $\geq$ 1% populations. In particular, for patients with TPS $\geq$50%, the OS HR for pembrolizumab versus docetaxel comparison was 0.64 (95% CI 0.45–0.91) and 0.40 (95% CI 0.28–0.56) for patients enrolled based on PD-L1 expression in archival samples and on newly collected tumor samples, respectively. For patients with TPS $\geq$1%, the OS HR was 0.74 (95% CI 0.59–0.93) and 0.59 (95% CI 0.48–0.73) for archival and newly collected tumor samples, respectively.

The KEYNOTE-001 study has first demonstrated a clear association between PD-L1 tumor expression and antitumor efficacy of pembrolizumab in patients with metastatic NSCLC [7]. However, the comparability of the response to pembrolizumab in patients with archival and newly collected tumor samples by PD-L1 expression was not evaluated. Indeed, this is an important question, because previously collected archival tissue is often the most convenient and easily accessible tissue source for biomarker testing in the second-line setting. The results of this updated analysis of KEYNOTE-010 confirm that the PD-L1 test from the archival sample of the tumor can be used for evaluating the eligibility of patients to pembrolizumab treatment. These results are in agreement with those of the ATLANTIC study, a phase II study of durvalumab in patients with advanced heavily pretreated NSCLC [8]. In this study, both archival and recently acquired samples were available for 112 patients: concordance with recent samples was highest with archival samples less than 3 years old (76.2%).

Of course, there are a number of key questions remaining to be addressed: which is the optimal IHC assay to assess PD-L1 expression? Are cytological materials adequate for PD-L1

Table 1. Randomized clinical studies with immune checkpoint inhibitors in pretreated patients with advanced non-small-cell lung cancer

Study	Author	Phase	Treatment	Pts	OR (%)	PFS (months)	OS (months)
CheckMate 017	Brahmer, 2015	III	Nivolumab versus docetaxel, (squamous)	272	20 versus 9, $P = 0.008$	3.5 versus 2.8, $P < 0.001$	9.2 versus 6.0, HR: 0.59, $P < 0.001$
CheckMate 057	Borghaei, 2015	III	Nivolumab versus docetaxel, (non-squamous)	582	19 versus 12, $P = 0.02$	2.3 versus 4.2, $P = 0.39$	12.2 versus 9.4, HR: 0.73, $P = 0.0015$
POPLAR	Fehrenbacher, 2016	II	Atezolizumab versus docetaxel	287	15 versus 15	2.7 versus 3.0	12.6 versus 9.7, HR: 0.73, $P = 0.04$
OAK	Rittmeyer, 2017	III	Atezolizumab versus docetaxel	850	14 versus 13	2.8 versus 4.0, HR: 0.95	13.8 versus 9.6, HR: 0.73, $P = 0.0003$
KEYNOTE 010	Herbst, 2016	III	Pembrolizumab 2 versus pembrolizumab 10 versus docetaxel	1034	18% (.0005) versus 18% (.00002) versus 9%	3.9 (HR 0.88) versus 4.0 (HR 0.79) versus 4.0	10.4 (HR 0.71) versus 12.7 (HR 0.61) versus 8.5

OR, objective response; PFS, progression-free survival; OS, overall survival; HR, hazard ratio; pts, patients.

assessment? What will be the role of pembrolizumab in the second-/third-line setting? What will be the role of PD-L1 in the next future?

Different PD-L1 IHC assays have been developed for each PD-1/PD-L1 inhibitor, including 28-8 assay (Dako, Carpinteria, CA, USA) for nivolumab and SP142 assay (Ventana Medical Systems, Tucson, AZ, USA) for atezolizumab, approved both as complementary diagnostics tests and not required for the treatment, and 22C3 (Dako, Carpinteria, CA, USA) for pembrolizumab, approved as companion diagnostics test and required for the eligibility of patients [9–11]. Moreover, SP263 assay (Ventana Medical Systems, Tucson, AZ, USA) has been developed for durvalumab, recently approved for the treatment of patients with locally advanced NSCLC after completing chemo-radiotherapy and 73-10 assay (Dako, Carpinteria, CA, USA) was developed for avelumab [12–14]. The Blueprint project, a pivotal academic/professional society and industrial collaboration that assessed the feasibility of harmonizing the clinical use of these commercial PD-L1 IHC assays by using real life clinical lung cancer samples, showed highly comparable staining by the 22C3, 28-8 and SP263 assays to detect PD-L1 expression on TCs, less sensitivity with the SP142 assay and higher sensitivity with the 73-10 assay [15]. Therefore, results from the Blueprint confirm the interchangeability among three different assays (22C3, 28-8 and SP263) for use in scoring expression of PD-L1 on TCs (on the basis of TPS).

For the second question, a major issue for the clinical practice is that all PD-L1 tests have been developed and approved only for histologic samples, while 30%–40% of patients with metastatic lung cancer are still currently diagnosed only by cytological materials, through less invasive procedures. A high degree of agreement (85%–95%) on PD-L1 expression levels was recently observed between histologic and cytologic specimens in 86 paired Formalin-Fixed Paraffin-Embedded samples of cytologic cell block and histologic material from lung malignancies, using 28-8 and 22C3, suggesting that PD-L1 assessment on cytologic material is feasible and could be an alternative when histologic samples are not available [16]. However, the technique should be standardized before recommended for clinical practice.

The role of pembrolizumab in the second- or third-line therapy of advanced NSCLC patients will be likely decreasing in the next future, after the results of clinical trials demonstrating the efficacy of pembrolizumab in the first-line setting [17–19]. In particular, the KEYNOTE-024 study showed that pembrolizumab significantly prolonged progression-free survival and OS compared with platinum-based chemotherapy as the first-line therapy of patients with advanced NSCLC and PD-L1 expression on at least 50% of TCs, reinforcing the importance of PD-L1 testing at diagnosis in all patients [17]. Following these results, we witnessed a major revolution in the diagnostic and therapeutic algorithm that now requires PD-L1 testing at diagnosis for all patients with any histology and the use of pembrolizumab in the first-line setting as single agent for patients with PD-L1 $>$ 50% or in combination with chemotherapy for all the other patients eligible to the combination of immunotherapy and chemotherapy.

Finally, PD-L1 is to date the only molecular factor able to guide the choice of an immunotherapy for patients with advanced NSCLC, but a number of PD-L1 testing limitations can confound its use as a predictive biomarker, including the heterogeneity and dynamics of PD-L1 expression, the absence of consensus regarding the relevance of geographic patterns of expression of PD-L1 or its expression on tumor or inflammatory cells within the tumor microenvironment. Therefore, a number of additional factors are under investigation, including the tumor mutation burden, tumor-infiltrating lymphocytes, and immune gene signatures that may identify tumors with preexisting immune activity and correlate with response to anti-PD-L1/PD-1 [20].

In conclusion, the results of this updated analysis of KEYNOTE-010 confirm that PD-L1 expression is preserved following months of storage and suggest that re-biopsy in patients who have received prior anticancer treatment may not be required for clinical assessment of PD-L1 expression. Therefore, the evaluation of PD-L1 expression at baseline in addition, in the future, to genomic and immune profiles will help to define the best therapeutic strategy for each patient with advanced NSCLC.

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