Editorial

Characterizing advanced or metastatic adenocarcinoma of lung—one step closer?

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Comment on: Rangachari D, VanderLaan PA, Shea M, *et al.* Correlation between Classic Driver Oncogene Mutations in EGFR, ALK, or ROS1 and 22C3-PD-L1 ≥50% Expression in Lung Adenocarcinoma. J Thorac Oncol 2017;12:878-83.

Submitted Mar 17, 2017. Accepted for publication Mar 31, 2017.
doi: 10.21037/tcr.2017.04.11
View this article at: http://dx.doi.org/10.21037/tcr.2017.04.11

First-line treatment of metastatic non-small-cell lung cancer (NSCLC) has been evolving. Standard treatment of platinum-based combination chemotherapy has yielded an overall survival (OS) of 10 months (1). With the advent of tyrosine kinase inhibitor (TKI) targeting mutant epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK), OS of over 16 months is possible (2,3). However, only less than a half of patients are eligible for targeted therapy. The prevalence of *EGFR* mutations in lung adenocarcinoma ranges from 12% in non-Asian cohorts to 44.1% in Southeast-Asian cohorts, and *ALK* mutations at around 5% regardless of ethnicity (4-7). Among them, 6.6% are known to carry other concurrent gene mutations that may hamper efficacy of targeted therapy (8). There is a significant unmet need in identification of additional targetable mechanisms that will improve outcomes in patients with NSCLC.

While driver mutations are important in lung cancer oncogenesis, immune evasion also plays a significant role. Through expression of programmed death-ligands 1/2 (PD-L1 or PD-L2) on tumor cells, and their subsequent binding to programmed cell death 1 (PD-1) receptor expressed on activated T-cells, T-cell mediated anti-tumor immune response is much dampened. The interruption of PD-1/PD-L1/PD-L2 pathway provides a window for therapeutic intervention by restoring immune responses to tumor cells.

The clinical efficacy of anti-PD-1 antibody pembrolizumab in the setting of first-line treatment of advanced or metastatic NSCLC was demonstrated in the KEYNOTE-024 trial (9). Three hundred and five patients with no *EGFR* and *ALK* mutations, and a high PD-L1 expression in at least 50% tumor cells were recruited. Pembrolizumab was superior in prolonging both progression-free survival and OS, when compared to investigators' choice of platinum-based combination chemotherapy with a hazard ratio (HR) of 0.5 [95% confidence interval (CI): 0.37–0.68, P<0.001] and 0.6 (95% CI: 0.41–0.89, P=0.005) respectively. The response rate was significantly higher than chemotherapy (44.8% with pembrolizumab *vs*. 27.8% with chemotherapy). In those with high PD-L1 expression on trial entry screening, only 6% of patients had concomitant *EGFR* or *ALK* mutations, suggesting the possibility of tumors with high PD-L1 expression belonging to a distinct entity.

While assays for *EGFR* and *ALK* mutations could be performed on a variety of specimens including cytology and even plasma samples (10), assessment of PD-L1 expression on these samples is still under development. Although standardized immunohistochemistry (IHC) assays are available, they are drug-specific and are only validated in tissue biopsies and rarely cytology samples (11).

Rangachari *et al*. (12) tried to provide an answer on

the feasibility of assaying PD-L1 expression in cytology specimens and the possibility of characterization of NSCLC patients into distinct entities. They examined the occurrence of *EGFR*, *ALK* and *ROS1* mutations and PD-L1 expression level in a sample of 71 biopsy-patient pairs of lung adenocarcinoma with a variety of clinically available specimens, which included surgical specimens (29.6%), small biopsies (35.2%), fine-needle aspiration (FNA) cell blocks (22.5%) and cytology cell blocks (12.7%). Clone 22C3 pharmDx kit was used in PD-L1 IHC and tumor proportion score (TPS) calculation (over 50% of complete or partial membrane staining in at least 100 viable tumor cells taken as positive). They observed a PD-L1 positivity rate of 29.6%, unaffected by biopsy type (P=0.7768). In addition, high PD-L1 expression rarely co-occurred with other common oncogenic mutations (P=0.0073). Of the 19 patients with *EGFR*, *ALK* or *ROS1* mutations, only 1 patient (5.3%) with *ALK* mutation was found to have high PD-L1 expression.

The above study suggested that it may be technically feasible for small biopsy and cytology cell block specimens to be used in 22C3 PD-L1 IHC assay. Yet, some important limitations of using cytology cell block in diagnosis, mutation detection and IHC staining were pointed out by Miyoshi *et al*. (13). In their study, 35 paired samples of pleural biopsy and pleural fluid with malignant diagnosis were used in comparison of diagnostic sensitivity. They reported a significantly lower diagnostic rate from pleural fluid cell blocks when compared with pleural biopsy specimens (71.4% *vs*. 94.2%, P=0.008), likely due to heavily blood-stained specimen and hypocellularity. These two factors occur commonly in daily clinical practice (11) and contribute to the inherent weakness of cytology and small biopsy specimens.

The rate of PD-L1 prevalence and co-occurrence with *EGFR* or *ALK* mutations observed by Rangachari *et al*. is close to that observed in KEYNOTE-024, which reported prevalence of high PD-L1 level at 30.2% and co-occurrence rate of 6%. The only caveat would be the small number of patient sample, where there were only 19 cases of driver mutations and one case of co-occurrence, which implies a risk of such results observed due to chance. Given the fact that the use of PD-L1 22C3 PharmDx staining kit is currently not yet validated on specimens other than formalin-fixed paraffin-embedded sections, a larger-scale paired cytology-tissue validation study, involving multiple centers and ethnicities, is required to formally establish the role of cytology cell block specimens in PD-L1 predictive marker assay.

To combat the shortcomings of PD-L1 IHC including subjectivity, the semi-quantitative nature and the unproven role on cytology specimens, Chargin *et al*. have tested a novel approach of PD-L1 expression detection by flow-cytometry on tissue and cytology samples of 12 NSCLC cases. They demonstrated highly correlated results between flow-cytometry and traditional tissue IHC (14). With the maturation of PD-L1 assays, millions of cancer patients will be spared from enduring the pain of core biopsy.

While PD-L1 expression level is predictive of the response and outcome of anti-PD1 immunotherapy, it is an imperfect biomarker to dichotomize patients for treatment selection. In KEYNOTE-001 trial, patients with PD-L1 IHC TPS <1% still gained considerable benefit with an objective response rate (ORR) of 8% and durable response in around 10% (15), showing potential interplay of other predictive factors. To assess the predictive role of PD-L1 expression and other novel markers, Rizvi *et al*. performed a retrospective whole-exome sequencing on 34 lung cancers treated with pembrolizumab, with or without prior treatment (16). They identified that the load of non-synonymous mutations was highly predictive of durable clinical benefit (DCB), with a high sensitivity of 86–100% and specificity of 67–75% using a cutoff level of ≥178 non-synonymous mutations. When comparing the receiver operating characteristic curve of non-synonymous mutation ≥178 against PD-L1 expression ≥50%, the former appears to be more sensitive and specific. To propose a solution to the limited predictive value of PD-L1 expression level in treatment outcome, they also tried to combine the use of both non-synonymous mutation load and PD-L1 expression level. Patients with simultaneously high non-synonymous mutation load and PD-L1 expression ≥1% had a high DCB rate of 91%, compared to 10% for those with low mutational burden. Even in patients with low PD-L1 expression of 1–49% who are now clinically considered to be ineligible for pembrolizumab, DCB was still observed in 75% of patients with high non-synonymous mutation burden. Non-synonymous mutation load can emerge as a more powerful predictor and the combined use with PD-L1 expression can best predict disease control with treatment of pembrolizumab. Larger studies are warranted, especially on their predictive role in untreated patients and the relationship between non-synonymous mutation load with other driver mutations.

Apart from PD-1/PD-L1 signaling pathway, other immune checkpoints are being harnessed as therapeutic

targets. Combined cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and PD-1 pathway inhibition was shown to yield promising results in CheckMate 012, a phase I study recruiting 77 chemotherapy-naïve stage IIIB or IV NSCLC patients. Impressive response rate of 92% was observed for those with high PD-L1 expression, and hence a confirmatory phase III trial CheckMate 227 is currently on the way (17). Tim-3, another immune checkpoint receptor responsible for induction of immune tolerance in T-cells, was also shown to be upregulated in PD-1 treatment-resistant tumors in mouse models, and would worth further research (18).

While it's tempting to stratify the large cohort of untreated advanced or metastatic lung cancer patients into distinct treatment groups, namely the group with driver oncogenes (those harbouring *EGFR/ALK/ROS1* mutations, to be treated with respective TKI), the immunotherapy group (those harbouring PD-L1 TPS ≥50%, to be treated with pembrolizumab) and biomarker negative group (to be treated with conventional platinum-based combination chemotherapy), the search for better biomarker for immunotherapy is still on the way. With current classification according to PD-L1 expression levels, overlap of the immunotherapy group with the driver oncogene group is present (6% of patients with high PD-L1 expression harbours *EGFR* or *ALK* mutation simultaneously) (9) and sheds a grey area of contention on which first-line treatment to be used in these patients. As first-line EGFR and ALK targeted therapy give ORR of around 70% (2,3), and the fact that PD-L1 expression may even increase with EGFR targeted therapies (19), treating the minority of patients carrying both actionable mutations and high PD-L1 expression with targeted therapy first, followed by immunotherapy in the second-line setting will be a reasonable approach. Lastly, it may even be possible to sensitize patients to immunotherapy. Preclinical studies showed promise in inducing PD-L1 expression by fractionated radiotherapy, thus creating a window for inhibition of PD-1/PD-L1 interaction in otherwise ineligible cases (20). Future directions should focus on harmonization of various PD-L1 IHC assays, identification of better PD-L1 assay techniques, discovering better predictive biomarkers for immunotherapy and exploring the synergy between radiotherapy and immunotherapy in human subjects.

Where are we now? We are now, at least, half step closer to characterizing lung cancer into clinically applicable treatment groups. Exciting development on better diagnostics and treatments is ahead of us.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Wei Xu (Division of Respiratory Disease, Department of Geriatrics, the First Affiliated Hospital of Nanjing Medical University, Nanjing, China).

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2017.04.11). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Cheung KM, Chow JC, Cho WC. Characterizing advanced or metastatic adenocarcinoma of lung—one step closer? Transl Cancer Res 2017;6(Suppl 3):S498-S501. doi: 10.21037/tcr.2017.04.11

