Editorial Commentary

Measuring tumor mutation burden in cell-free DNA: advantages and limits

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The treatment for advanced non-small cell lung cancer (NSCLC) has changed rapidly over the last several years. There are now several commercially available checkpoint inhibitors (CPI) targeting the programmed death ligand 1 (PD-L1)-programmed death 1 (PD-1) pathway. These CPIs have been shown to confer a significant overall survival (OS) benefit in first- and second-line therapy for unresectable/metastatic NSCLC (1-5). While the use of CPI in the clinic is prevalent, the determinants of those most likely to benefit remains imprecise. The only predictive biomarker for OS benefit that is currently used for patient selection is PD-L1 expression, though it is an imperfect biomarker with several limitations (6-8). Thus, the exploration of other potential biomarkers for patient selection to match with CPI therapy continues to be a major focus of ongoing research efforts.

However, while PD-L1 expression remains an important actionable biomarker tested, it is primarily utilized in the first-line setting. A resultant issue is the presence of adequate tumor tissue for additional standard biomarker tests that may direct systemic therapy selection, including *EGFR*, *ROS1*, *ALK*, and *BRAF* alterations. Couple this with the fact that upwards of 30% of patients with NSCLC have insufficient tumor tissue remaining for biomarker testing after the completion of diagnostic testing (9). Additionally, exploratory biomarkers such as tumor mutation burden (TMB), that provide other possible means that can identify patients who are more likely to benefit from CPI monotherapy in NSCLC would be welcome. Several studies have reported that TMB can serve as a surrogate for overall neoantigen load (10). TMB, derived by algorithms applied to whole-exome sequencing (WES), has been reported to associated with clinical benefit. In this case, clinical benefit was defined as improvement of objective response and progression-free survival (PFS) ≥6 months, for several CPIs. An exploratory analysis from the CheckMate-026 trial reported that high tumor tissue-based TMB (tTMB) count was associated with longer PFS and higher overall response rate (ORR) with nivolumab (a CPI) in NSCLC in the first-line setting. Several research papers report that when compared with WES, targeted next-generation sequencing (NGS) might measure tTMB. Furthermore, these studies report that tTMB may be predictive for patients that are likely to derive clinical benefit from CPI therapies in metastatic melanoma, metastatic urothelial carcinoma, and advanced NSCLC.

A reliable predictive biomarker should necessarily correlate with OS and not just with ORR or PFS. tTMB has yet to prove predictive or prognostic value for OS. Some of the most compelling data for tTMB comes from *ad hoc* subgroup analyses of single-arm trials. No standard criteria for mutation discovery and calling has emerged. Discordances between assays, and cut-offs based on tumor type or immunotherapy plague the biomarker field. Moreover, the algorithms used might influence tTMB estimation as they differ widely across gene panels' platforms. In addition, there can be variation between assays in the mutation types considered for tTMB assessment. These may include or exclude synonymous and nonsynonymous base substitutions/single nucleotide variants, short insertions, and/or deletions. In many retrospective analyses that utilized WES, tTMB counted only missense mutations, leaving out other types of mutations. Notably, tumor samples that are formalin-fixed paraffin-embedded (FFPE) can induce deamination artefacts. These artifacts can dramatically impact tTMB results (11).

To overcome some of these problems, some studies have explored the role of TMB in the blood (bTMB) as either complementary to or as a displacement of tTMB. The feasibility and ability to use a simple blood collection to analyse tumor genomes can be advantageous when compared to tumor tissue biopsy collection. Blood offers a readily available source of material for examination. This source could be less susceptible to sampling and tumor heterogeneity biases that are often associated with single-site tumor tissue biopsies. For these reasons, there has been an increasing interest to explore blood to reliably detect mutations in cell-free DNA (cfDNA), including digital droplet PCR, allele-specific PCR, and panel-based NGS. In fact, recently, the first blood-based assay for the detection of mutations in the *EGFR* gene (cobas EGFR Mutation Test v2, Genentech) received Food and Drug Administration (FDA) approval (12). Most blood-based assays used for predictive biomarker detection have relied on PCR-based approaches; however, there are now several recent publications have shown that NGS may obviate those methods.

In a retrospective analysis of two randomized trials that had plasma samples available from more than 1,000 patients given atezolizumab (a CPI) as second-line or beyond therapy for NSCLC, researchers (13) assessed a novel bTMB assay. They reported that in patients with advanced NSCLC, bTMB can predict clinical benefit from treatment with atezolizumab. Further, bTMB was noted to be positively correlated with tTMB (Spearman's rank correlation 0.64; 95% CI: 0.56–0.71). While this study applied a defined cutoff to an independent cohort, its statistical plan was lacking and did not account for testing multiplicity. Therefore, the true significance of the reported PFS and OS association is unclear. The BFAST phase III trial of 580 participants (NCT03178552), may be the first to address bTMB and its association of OS benefit when the results are reported.

Further steps are needed to validate a biomarker: there is a pre-analytical and analytical validation part followed by the clinical validation, which is the crucial point. A reliable predictive biomarker should correlate in a prospective study with OS benefit and not only with ORR or PFS (14). Notably there is no prospective study thus far for tTMB or bTMB confirming a predictive benefit for OS in NSCLC. There remain several aspects that are essential before bTMB could establish itself as a reliable biomarker: harmonising the different techniques and prospectively establishing a cut-off that could predict an OS improvement.

Because of the high cost of sequencing, many blood-based panels have relatively limited genomic content and have not been clinically or analytically validated. Potential advantages for bTMB is lower sample acquisition costs (minimal invasive procedure *vs.* an invasive procedure +/– anesthetics +/– analgesics +/– facility fees for imaging and procedure room utilization) to get "current" status mutational burden and expected faster turnaround time from sample collection to a reported result. With expected lower overall costs, sequential sampling to assess the change of bTMB may provide a more relevant interpretation of how tumor burden is responding to systemic treatment (15-17).

In conclusion bTMB remains a fascinating biomarker with many potential advantages compared to the tTMB. Further studies are warranted to further explore the applicability of this biomarker.

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Footnote

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References

1. Rittmeyer A, Barlesi F, Waterkamp D, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet 2017;389:255-65.
2. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell

Lung Cancer. N Engl J Med 2015;373:1627-39.
3. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med 2015;373:123-35.
4. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med 2016;375:1823-33.
5. Fehrenbacher L, Spira A, Ballinger M, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet 2016;387:1837-46.
6. Rimm DL, Han G, Taube JM, et al. A Prospective, Multi-institutional, Pathologist-Based Assessment of 4 Immunohistochemistry Assays for PD-L1 Expression in Non-Small Cell Lung Cancer. JAMA Oncol 2017;3:1051-8.
7. McLaughlin J, Han G, Schalper KA, et al. Quantitative Assessment of the Heterogeneity of PD-L1 Expression in Non-Small-Cell Lung Cancer. JAMA Oncol 2016;2:46-54. Erratum in: Error in Figure Presentation. [JAMA Oncol 2016].
8. Grigg C, Rizvi NA. PD-L1 biomarker testing for non-small cell lung cancer: truth or fiction? J Immunother Cancer 2016;4:48.
9. Ellis PM, Blais N, Soulieres D, et al. A systematic review and Canadian consensus recommendations on the use of biomarkers in the treatment of non-small cell lung cancer. J Thorac Oncol 2011;6:1379-91.
10. Spigel DR, Schrock AB, Fabrizio D, et al. Total mutation burden (TMB) in lung cancer (LC) and relationship with response to PD-1/PD-L1 targeted therapies. J Clin Oncol 2016;34:9017.
11. Quach N, Goodman MF, Shibata D. In vitro mutation artifacts after formalin fixation and error prone translesion synthesis during PCR. BMC Clin Pathol 2004;4:1.
12. PMA P150047: FDA Summary of Safety and Effectiveness Data. Date of FDA Notice of Approval: June 1, 2016.
13. Gandara DR, Paul SM, Kowanetz M, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. Nat Med 2018;24:1441-8.
14. Gyawali B, Hey SP, Kesselheim AS. A Comparison of Response Patterns for Progression-Free Survival and Overall Survival Following Treatment for Cancer With PD-1 Inhibitors: A Meta-analysis of Correlation and Differences in Effect Sizes. JAMA Netw Open 2018;1:e180416.
15. Weiss GJ, Beck J, Braun DP, et al. Tumor Cell-Free DNA Copy Number Instability Predicts Therapeutic Response to Immunotherapy. Clin Cancer Res 2017;23:5074-81.
16. Cabel L, Riva F, Servois V, et al. Circulating tumor DNA changes for early monitoring of anti-PD1 immunotherapy: a proof-of-concept study. Ann Oncol 2017;28:1996-2001.
17. Raja R, Kuziora M, Brohawn PZ, et al. Early Reduction in ctDNA Predicts Survival in Patients with Lung and Bladder Cancer Treated with Durvalumab. Clin Cancer Res 2018;24:6212-22.

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