Editorial Commentary

Blood-based tumor mutation burden: continued progress toward personalizing immunotherapy in non-small cell lung cancer

Vincent K. Lam, Jianjun Zhang

Department of Thoracic/Head and Neck Medical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

Correspondence to: Jianjun Zhang, MD, PhD; Vincent K. Lam, MD. 1400 Holcombe Blvd, Unit 432, Houston, TX 77459, USA. Email: JZhang20@mdanderson.org; VKLam@mdanderson.org.

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Tumor mutation burden (TMB) measures the number of somatic coding alterations in a tumor. Increased presence of these alterations contributes to immunogenicity through the generation of neoantigens targeted by T cell responses. Accordingly, a higher TMB is associated with favorable response rate and survival across multiple cancer types (1). TMB is independent of PD-L1 and is emerging as a promising immunotherapy biomarker. In non-small cell lung cancer (NSCLC), multiple studies have affirmed the utility of TMB as a marker for response to immune checkpoint inhibitors (ICI) (2-5). For example, advanced NSCLC patients with high TMB (≥10 mutations per Mb) treated with front-line combination ICI therapy had improved progression-free survival (PFS) compared to standard chemotherapy (HR 0.58, $P<0.001$) while patients with lower TMB did not (3).

Whole exome sequencing (WES) is the gold standard for calculating TMB. However, routine WES is currently clinically impractical and thus various cancer gene panels (CGP) have been investigated as surrogates for determining TMB. Accurate estimation of TMB is dependent on many factors such as the size of the sequenced CGP, type of genomic alterations captured, sequencing depth, and tumor purity and ploidy (6). Perhaps reflective of this heterogeneity, the TMB cut point associated with improved clinical benefit from ICI in NSCLC varies between studies (2,3). Additionally, clinical utilization of tissue-based TMB testing is hampered by the requirement for an invasive biopsy, occurrences of specimen insufficiency for next-generation sequencing, and long turn-around time. In fact, up to 30% of NSCLC patients do not even have adequate tissue available for standard molecular testing (7). Our group and others have also demonstrated that single biopsies insufficiently represent intra-tumor heterogeneity (ITH) (8,9). Therefore, more recent efforts are underway to evaluate the feasibility of blood-based TMB (bTMB) as a predictive biomarker (7,10,11). bTMB measured from circulating tumor DNA (ctDNA) sequencing enables non-invasive, rapid testing that may also more accurately depict the tumor genomic landscape since it is less impacted by ITH (12,13).

Wang and colleagues substantially contribute to these efforts with their recent publication in *JAMA Oncology* (14). The authors computationally determined an optimal gene panel size for TMB estimation by comparing TMB calculated from randomly-generated CGPs *in-silico* with those from WES data of 9,205 samples across multiple tumor types in The Cancer Genome Atlas (TCGA). Based on those results, a novel gene panel (NCC-GP150) was designed that covered the whole exon regions of 150 cancer-related genes. TMB calculated with this gene panel correlated strongly with WES-based TMB; similar to that of well-established clinical CGPs. The predictive utility of NCC-GP150 was then evaluated *in-silico* with the seminal Rizvi *et al.* cohort of advanced NSCLC patients treated with anti-PD-1 therapy (5). Technical and clinical

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validation of blood-based NCC-GP150 was accomplished by correlating bTMB with (1) matched tissue WES-based TMB in a small NSCLC clinical cohort and (2) clinical outcome in a separate cohort of advanced NSCLC patients treated with ICI.

The findings are an encouraging advance for the potential use of bTMB as a predictive immunotherapy biomarker. The authors demonstrated that TMB calculated from the NCC-GP150 panel strongly correlated with WES data (r^2=0.96) and was indeed able to distinguish patients with improved PFS when applied to data from the Rizvi *et al.* clinical cohort (HR 0.36, P=0.03 for TMB > median). With patient samples, NCC-GP150-based bTMB correlated well with WES-based TMB (Spearman r=0.62). A bTMB cut point of 6 mutations per Mb was determined to have optimal sensitivity and specificity and successfully identified those with better objective response rates (39.3% versus 9.1%; P=0.02) and PFS (HR 0.39; P=0.01) in the separate cohort of 50 advanced NSCLC patients. Consistent with prior studies, bTMB was independent of PD-L1 expression (3,4,7).

The authors should be commended for their systematic approach to developing and validating a novel bTMB assay. This study provides rationale for the development of a smaller, more cost-effective CGP for estimating TMB. There are several important technical aspects worth discussing. First, we have to keep in mind that the number of genes in a CGP is not the main determinant of adequate coverage for TMB calculation. Computational modeling by Chalmers *et al.* estimated that a CGP with a coding region footprint of less than 0.5 Mb resulted in unacceptable concordance with the WES reference (15). The size of the coding region covered by NCC-GP150 was not clearly described. Without this key parameter, it is difficult to fully place this assay in context among the current major investigational bTMB assays that comprise 300–500 genes and cover ~1.0 Mb (7,10). Second, it is unclear whether the TMB calculations were normalized for tumor purity and ploidy, which may have profound impact on TMB estimation. Third, the NCC-GP150 ctDNA assay did not use matched DNA from white blood cells as a germ line control. Therefore, there could exist potential 'false positive' somatic mutations arising from clonal hematopoiesis, in which mutations present in aging hematopoietic stem cells can be misinterpreted as tumor-related (16). Particularly, commonly mutated cancer genes in NSCLC such as *TP53*, *KRAS* and *JAK2* harbor mutations associated with clonal hematopoiesis with a non-negligible prevalence of 3–10% in solid tumors (17,18).

Additionally, the retrospective nature of the clinical validation in this study may inherently introduce selection bias and other uncontrolled variables that could impact the assessment of bTMB and associated clinical outcomes. For example, the clinical validation cohort may be unbalanced for important factors that influence ctDNA shedding such as tumor burden, visceral metastasis, and *EGFR* or *TP53* mutations or amplifications (19,20). While it is known that low allelic frequency (<1%) in non-shedding tumors is associated with a higher rate of ctDNA technical discordance and a lower positive predictive value (21), there is no consensus on whether ctDNA variant allele frequencies <1% should be universally excluded in determining bTMB. Moreover, known genomic determinants of immunotherapy response in NSCLC such as targetable driver mutations (e.g., *EGFR*, *ALK*) and *STK11* ideally should be stratified in any comparison of clinical outcomes (22,23). Finally, the observed threshold for high TMB in NCC-GP150 (6 mutations per Mb) stands in contrast to the optimal cut points of 16 and 20 mutations per Mb previously described in other major bTMB assays. The reasons for this discrepancy were not discussed by the authors, but it is likely due in part to the potential technical and clinical considerations we have summarized above.

These issues exemplify the pressing need for standardization of key parameters for calculating TMB. Requirements need to be defined to guide technical development of TMB assays (both tissue and blood-based) and inform the design of prospective studies necessary for clinical validation. Indeed, a TMB Harmonization Working Group comprised of the key stakeholders has been assembled to define a standard methodology for assessing and reporting TMB (24). In addition to tissue-based TMB, this standardization effort and others will undoubtedly need to also address parameters that are important and unique to bTMB such as minimum variant allele frequency thresholds and clinical stratification of pathologic and genomic determinants of ctDNA shedding. If successful, we will be able to leverage the current rapid pace of adoption of ctDNA for molecular profiling and disease monitoring in NSCLC to accelerate clinical development of bTMB and introduce a valuable immunotherapy biomarker to our armamentarium.

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Footnote

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