Can tumor mutational burden determine the most effective treatment for lung cancer patients?

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“These studies have led to the first tissue-agnostic approval for anti-PD-1 therapy across unresectable or metastatic solid tumors with microsatellite instability or mismatch repair deficiency”

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Immunotherapy has revolutionized the treatment of cancer, and this success is represented by immune checkpoint inhibitors (ICI) (including anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-CTLA-4 antibodies and their combinations), which show higher efficacy than standard therapies in several cancers, including lung cancer. However, the majority of patients do not respond to immunotherapy, and biomarkers for predicting immunotherapy clinical response are urgently needed. Most tumor types show response rates below 40% to PD-1 inhibition and the objective response rates of each tumor type are reported to be highly correlated with the tumor mutational burden (TMB) of each tumor type [1]. In addition to TMB, multiple other factors are reported to affect ICI effectiveness,

including *PD-L1* expression, the degree of cytotoxic T-cell infiltration, mutational signature, antigen presentation defects, interferon signaling, tumor aneuploidy, T-cell gene expression signatures and microbiota [2].

The initial evidence supporting the correlation between high nonsynonymous mutational burden and improved clinical benefit obtained with immunotherapy was demonstrated by using whole-exome sequencing in advanced non-small-cell lung cancer treated with anti-PD-1 antibody and their matched normal DNA [3]. Later clinical trials have confirmed high TMB correlates with enhanced immunotherapy responses in other cancer types, such as head and neck cancer and urothelial carcinoma [4]. Tumors with DNA mismatch repair deficiency can display high microsatellite instability and accumulate substantial numbers of somatic mutations. A high objective response rate has been demonstrated across mismatch repair-deficient tumors to anti-PD-1 therapy [5,6]. These studies have led to the first tissue-agnostic approval for anti-PD-1 therapy across unresectable or metastatic solid tumors with microsatellite instability or mismatch repair deficiency.

Originally, TMB was detected with whole-exome sequencing, while recently, targeted gene panel sequencing has been widely used in clinics for TMB assessment. There is no consensus definition of TMB, different clinical practices use different definitions with different detecting methods. Foundation Medicine (MA, USA), defined TMB as the number of base substitutions (including synonymous mutations) in the coding region of targeted genes. Germline DNA was not sequenced but filtering for both oncogenic driver alterations and germline variants was carried out using public databases. The Memorial Sloan Kettering Cancer Center (NY, USA) approach quantified nonsynonymous mutations using sequencing data from both tumor and germline DNA. Several other different target gene panels have also been reported and used in clinics [7].

Targeted gene panel sequencing with formalin-fixed and paraffin-embedded samples appears to be a more feasible and straight-forward approach for TMB assessment in clinics. However, formalin fixative is known to induce various crosslinks, which are the main source of sequencing artifacts, notably through DNA fragmentation, denaturation and cytosine deamination. Blood TMB (bTMB) was assessed using cell-free DNA from blood, bTMB has recently emerged as an effective predictive biomarker for ICI response prediction [8]. For accurate bTMB quantification, mutations derived from clonal hematopoiesis of white blood cells should be well controlled [9]. The TMB cut-off values associated with improved survival varied markedly between cancer types, and there may not be one universal definition of high TMB [10].

Previously, it has been reported that TMB shows imperfect correlation with ICI response in that mutation load distributions overlap considerably between responders and nonresponders [11]. In addition, TMB does not correlate with the immunotherapy clinical response in some tumor types, including Hodgkin's lymphoma and renal cell carcinoma [4]. Furthermore, no clinical study has confirmed an overall survival advantage in high-TMB patients compared with low-TMB patients after immunotherapy. As reviewed recently, TMB also has some inherent technical issues that could dampen its clinical utility [12,13]. Here, we summarize that the following factors should be carefully considered for further improvement of TMB-based immunotherapy biomarkers.

Mutation type

Mutations are not the same, and some types of DNA mutations can be more efficient in ICI response prediction compared with others. For example, APOBEC mutation signature has been reported to predict immunotherapy response more effectively than total TMB [14]. Different types of mutations could have different effects on the coding peptide, which could lead to differences in peptide hydrophobicity and/or immunogenicity. Neoantigen quality has been proposed to assess the immunogenicity of neoantigens, and the overall quality of neoantigens should replace simple TMB in future immunotherapy response prediction. In addition, some specific mutation itself can affect immunotherapy response, for example, *JAK1*, *JAK2*, *b2M*, *SKT11*, *SERPINB3* and *SERPINB4* mutations [15,16]. The rational inclusion of these mutations in TMB quantification needs to be carefully designed.

Other tumor antigenicity

Cancer germline (also known as cancer testis) antigens are normally expressed in germ cells and trophoblast tissues and are aberrantly expressed in a variety of human malignancies. Cancer germline antigens are important sources for tumor antigenicity, and this antigenicity should also be considered for future improvement of TMB. Specific alternative splicing in cancer cells can encode for a *de novo* protein, which is not expressed in normal tissues, and this novel protein can evoke an immune response as a tumor antigen. DNA structural alterations and so called 'noncoding' regions could also encode for a novel tumor specific peptide/protein, and these types of tumor antigenicity should also be considered in future biomarker design.

Mutation sampling

Clonal and subclonal TMB can have different effects on immunotherapy response prediction [17]. Due to intra-tumor heterogeneity, different regions of the tumor could have different mutational burdens. This regional effect should be carefully considered, especially for primarily localized cancer. Low tumor purity can also influence mutation calling, and consequently influence TMB assessment. For clinical practice, especially tissue TMB detection, minimum tumor purity level is required.

Sex differences

Recently, Wang *et al.* reported that the predictive power of TMB in lung cancer immunotherapy response is influenced by patient's sex, and for male lung cancer patients, TMB is a relatively poor biomarker in immunotherapy response prediction [18]. This sex difference in TMB's predictive performance is probably caused by the enhanced immune response in females compared with males [19]. Future development of immunotherapy biomarkers should consider sex differences and special efforts should be paid to improve the performance of immunotherapy predictive biomarkers for male lung cancer patients.

Antigen presentation status

TMB reflects tumor antigenicity. To evoke an immune response, this tumor antigenicity should be presented to the immune cells for reorganization and specific killing. Recently, antigen presentation gene expression signature has been combined with TMB to generate a novel biomarker, tumor immunogenicity score. In both correlation with pan-cancer ICI objective response rates and ICI clinical response prediction for individual patients, tumor immunogenicity score consistently showed improved performance compared with TMB and other known prediction biomarkers for ICI response [20].

In conclusion, immunotherapy revolutionized the treatment of lung cancer, and has demonstrated success in some originally incurable late-state lung cancer. TMB is a widely used clinical biomarker for ICI response prediction; however, TMB itself is not sufficient for the selection of lung cancer patients for immunotherapy. Several factors need to be considered for the future improvement of TMB-based biomarker, including: mutation type, mutation sampling, other tumor antigenicity, sex differences and antigen presentation status. With all the above factors considered, we may generate a robust predictive biomarker for ICI in lung cancer.

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