

Mechanisms and Clinical Applications of Tumor Mutational Burden in Non-Small Cell Lung Cancer

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Abstract:

Over the past few years, ICIs have rapidly emerged as primary standard treatments for advanced NSCLC, offering new therapeutic directions for the disease. However, some remain unresponsive to ICIs or develop resistance. Therefore, the development of predictive biomarkers is critically important for accurately identifying patients most likely to respond to immunotherapy. TMB serves as a promising biomarker in cancer immunotherapy, effectively assessing the tumor's neoantigen load. Tumor cells with high TMB can generate neoantigens, activating T cells to attack tumor cells and produce an immunotherapy response. Therefore, the presence of a high TMB in patients with NSCLC treated with ICIs correlates with sustained clinical responses and improved long-term outcomes. This paper elucidates the mechanism of action of TMB as a biomarker in immunotherapy for NSCLC, evaluates its clinical utility (demonstrating significantly improved ORR, PFS, and OS in patients with high TMB when combined with monoclonal antibodies) and identifies its limitations (including insufficient testing standardization and inability to directly reflect mutation burden). These findings aim to provide additional theoretical reference for clinical practice.

Keywords: NSCLC; TMB; Immune Checkpoint Inhibitors; Neoantigens.

1. Introduction

Among the histological categories of lung carcinoma, NSCLC is the most prevalent, constituting the vast majority (80–85%) of all cases. It is characterized by high malignancy, high mortality, and poor prognosis. Currently, there are multiple approaches

for the therapeutic strategy of advanced NSCLC. As the standard initial therapy for advanced NSCLC, Immune checkpoint inhibitors (ICIs) significantly improve patient prognosis and reduce mortality rates. PD-1 and CTLA-4 function as immune checkpoint molecules located on T cells, serving to suppress and downregulate T cell-driven immunological activity.

Immune checkpoint inhibitors, including antibodies targeting CTLA-4, PD-1, and PD-L1, function by blocking these co-inhibitory signals, thereby restoring anti-tumor immune reactivity [1]. Therefore, ICIs block immune checkpoint signaling, thereby releasing the suppression of immune cells by tumor cells. This process augments the capacity of the immune system to identify and target malignant cells, thereby suppressing further tumor progression.

However, these drugs are not effective for all patients. Only 20-40% of patients reap the benefits of this therapy, and some are likely to experience toxicity. It is evident that predicting effective biomarkers is crucial [1,2]. TMB has garnered significant attention from researchers as a key indicator reflecting the mutation burden of tumor cells within the tumor genome.

This study, through retrieval of high-quality literature from databases such as PubMed, aims to elucidate the mechanisms by which TMB induces neoantigen production and predicts the efficacy of ICIs in NSCLC, evaluate its clinical application value, and identify its limitations.

2. Definition and Mechanism of TMB

TMB is the total number of single nucleotide variants, insertions and deletions, frameshift mutations, or copy number alterations detected in the whole exome or targeted gene panel coverage regions of a tumor genome, measured in mutations per megabase (mut/Mb). It reflects the genetic instability and mutational burden of tumor cells, serving as one of the key biomarkers for evaluating the efficacy of immunotherapy.

Mutational accumulation is a prominent feature of tumors, with major mutations primarily reflected in defects in DNA damage repair and abnormalities in DNA replication. High TMB may be caused by mismatch repair (MMR) defects associated with microsatellite instability (MSI) [3]. In DNA damage repair, existing literature indicates that when genes responsible for repairing mismatched base pairs generated during DNA replication undergo inactivating mutations, errors arising during DNA replication cannot be repaired. This leads to the accumulation of mutations in tumor cells, manifesting as high TMB [4]. In DNA replication, the MMR pathway plays a role in correcting replication errors, which commonly found in microsatellites (a DNA sequence, often composed of short, repetitive nucleotide units, typically located in the non-coding regions of the genome) [5]. DNA polymerases are key enzymes that recognize and eliminate errors during DNA replication, with POLD1 and POLE involved in correcting errors in the lagging strand and leading strand replication processes, respectively. Mutations in the

POLE exonuclease domain can lead to hypermutation of TMB >10 mut/Mb or a hypermutation phenotype >100 mut/Mb [6]. It is evident that abnormalities in DNA replication or damage repair can lead to increased mutations, thereby significantly elevating the TMB.

Additionally, prolonged exposure to certain chemicals, contact with radioactive substances, excessive ultraviolet radiation, or smoking may also increase TMB in NSCLC. These factors, either individually or in combination, can influence tumor immunogenicity and response to immunotherapy. Age is also strongly linked to TMB, a connection largely attributable to the accumulation of somatic mutations over time, which drives a progressive rise in TMB with increasing age [7].

Tumors with high nonsynonymous TMB (mutations that cause changes in amino acid sequences) manifest a massive number of abnormal proteins. These aberrant polypeptides are perceived as foreign by the body's defense mechanisms through engagement with T cell receptors (TCRs), thus marking them as neoantigens [8]. As novel tumor-specific protein epitopes, the MHC-mediated surface expression of neoantigens on malignant cells serves as a critical signal for T cell activation and the elicitation of an immune response. A specific group of neoantigens is identified by the immune system as foreign, which subsequently elicits T cell-driven effector functions and initiates an antitumor immune response. Therefore, high TMB is often associated with DNA replication abnormalities or malfunctions in DNA damage repair, which lead to increased mutation accumulation in tumor cells. These mutations give rise to a greater number of neoantigens that, upon recognition by T cells, can stimulate an antitumor response.

3. TMB Detection Methods

Early studies on TMB were predominantly based on whole exome sequencing (WES), where TMB typically refers to the number of mutations per genomic exonic coding region (exome), expressed as mut/exome. However, while WES serves as the gold standard for detecting TMB, it is time-consuming, costly, demanding in terms of sample requirements, and procedurally complex. Furthermore, the interoperability of WES data is compromised by tumor heterogeneity, artifacts associated with tissue preparation of formalin-fixed, paraffin-embedded (FFPE) samples, and variations among tests offered by commercial providers [5]. Therefore, current clinical practice increasingly favors the use of targeted next-generation sequencing panels (NGS panels) for detecting TMB. Compared to WES, targeted gene panels enable deeper sequencing and enhance mutation detection sensitivity while

using smaller amounts of DNA [3]. However, targeted sequencing covers fewer genetic testing sites than exome sequencing. Furthermore, different platforms employ varying detection methods and sequence coverage lengths for exonic regions. Therefore, TMB constitutes a key genomic metric, representing the cumulative load of somatic variants per megabase (Mb) of interrogated tumor DNA. This quantitative measure is standardly denoted using the unit mut/Mb. NGS panel TMB detection should exhibit high consistency with WES TMB detection. It is currently generally accepted that an NGS panel ≥ 0.8 Mb can effectively assess the TMB level in tumor tissue [5].

4. Clinical Application

4.1 bTMB

Clinically, detecting tTMB often presents challenges such as difficulty in obtaining tumor tissue or insufficient tumor cell content in paraffin-embedded samples. In these situations, ctDNA in peripheral blood can be used to measure blood-based TMB (bTMB). Compared to tTMB, using ctDNA to detect bTMB avoids the trauma and risks associated with tissue biopsy. Simultaneously, multiple samples can be taken to monitor treatment efficacy and tumor changes in real time.

In 2022 B-FIRST trial, Foundation Medicine was used to detect bTMB by analyzing single nucleotide mutations across 1.1 Mb of genomic sequence. A bTMB ≥ 16 (equivalent to approximately 14.5 mut/Mb) was defined as high bTMB [9]. It demonstrates that patients with high bTMB exhibit a higher objective response rate (ORR) (27.1% vs 10.5%) and longer overall survival (OS) (29.1 months vs 13.4 months) when treated with atezolizumab, the findings imply that bTMB is associated with improved outcomes following immunotherapy.

4.2 Combine TMB Prediction with ICIs

Immune checkpoint molecules negatively regulate the tumor-killing activity of cytotoxic T cells while suppressing neoantigen-mediated antitumor responses, thereby aiding tumor cells in evading immune attacks. In clinical practice, by using ICIs, they can specifically bind to immune checkpoint molecules, thereby reinstating the tumor-killing capacity of cytotoxic T lymphocytes and reinvigorating the host's antitumor immunity. However, the clinical application of current ICIs is facing a bottleneck - their overall response rate (ORR) is only about 20%. Tumors with high TMB usually carry a richer variety of neoantigens, which can more effectively activate T cells, enhance tumor immunogenicity, and ultimately provide the possi-

bility of improving the sensitivity to ICIs treatment.

Rizvi et al. were the first to demonstrate that NSCLC patients who have high TMB achieved longer progression-free survival (PFS) following immunotherapy [10]. The study divided patients into high-TMB (≥ 178 mut/Mb) and low-TMB (< 178 mut/Mb) cohorts. Results showed that patients who have high TMB demonstrated superior outcomes compared to those who have low TMB, including overall response rate (ORR: 45% vs. 15%), progression-free survival (PFS: 9.7 vs. 3.8 months), and overall survival (OS: 24.6 vs. 12.7 months). This study establishes the foundation for TMB as an immunotherapy biomarker, while also demonstrating that the combination of TMB and ICIs often yields superior clinical efficacy. Results from the Hellmann-led CheckMate 227 open-label Phase 3 trial demonstrated that nivolumab together with ipilimumab markedly prolonged PFS, improved ORR, and extended OS among patients having advanced NSCLC and high TMB (≥ 10 mut/Mb) [11]. However, the combination regimen showed no clear benefit among patients having low TMB (< 10 mut/Mb). Further analysis indicates that monoclonal antibody therapy demonstrates greater efficacy advantages in individuals with elevated TMB.

4.3 Combine TMB Prediction with Other Biomarkers (PD-L1 Expression)

A phase 3 trial (CheckMate 026) categorized patients into three groups based on TMB levels: low burden (0 to 100 mut), intermediate burden (100 to 242 mut) and high burden (243 or more mut), then conducted exploratory biomarker analysis of TMB [12]. The findings revealed that in patients who have high TMB, the response rate to nivolumab treatment reaches 47%. In contrast, the response rate in the chemotherapy group is only 28%. And patients with high TMB also have longer PFS (9.7 months). But patients with low TMB only have 5.8 months. Research also indicates no significant correlation between TMB and PD-L1 expression levels (Pearson correlation coefficient = 0.059). In addition, in the nivolumab group, the response rate (75%) of patients who met both high TMB and PD-L1 expression level $\geq 50\%$ was higher than that of patients who only have one of those factors. Specifically, the response rate was 32% for patients with only high TMB, $> 50\%$ for those with only PD-L1 expression level $\geq 50\%$, and only 16% for patients with neither of these two factors.

4.4 Combine TMB Prediction with Chemotherapy Drugs

A key objective of the KEYNOTE-189 trial was to de-

termine whether tTMB levels correlate with enhanced clinical benefits from combination therapy involving pembrolizumab, pemetrexed, and platinum agents in previously untreated NSCLC. Research indicates that for patients with tTMB ≥ 175 mutations per exon group, combination therapy with pembrolizumab improved outcomes in this subgroup. Patients with tTMB < 175 demonstrated less pronounced efficacy when receiving combination therapy compared to those with high TMB [13]. Chemotherapy drugs (such as platinum-based agents) can increase TMB by inducing DNA damage. This DNA damage leads to more mutations, which in turn boosts the production of neoantigens, thereby enhancing the efficacy of immunotherapy.

Overall, TMB represents a standalone predictive factor for estimating the efficacy of ICI therapy in individuals with NSCLC, aiding in the identification of individuals more likely to benefit from immunotherapy.

5. Limitation

Different studies include diverse categories of mutations in TMB calculations. For instance, WES quantifies the aggregate count of somatic non-synonymous mutations in tumor tissue specimen; The NGS panel quantifies the aggregate count of base substitution mutations and insertion/deletion mutations within somatic coding regions. Therefore, more valuable clinical trials are needed to determine its threshold. Different testing methods such as WES, WGS, and NGS panels vary in coverage and accuracy, which can also influence TMB measurement results. Currently, three types of tumor categorization have emerged from various studies: tumors with significantly high TMB (> 10 mut/Mb), tumors with low TMB (< 5 mut/Mb), and tumors that do not fall into either of these two categories. TMB analysis lacks standardization across different assays, with positive thresholds ranging from ≥ 7.4 to ≥ 20 mut/Mb when measured via NGS [8].

Furthermore, TMB analysis is not useful for all invalids, and the percentage of patients who signed on to the clinical trials based on TMB is lower than that based on PD-L1 expression. For instance, in the Checkmate 568 trial, 88% of patients were assessable for PD-L1 expression, however, merely 34% had a detectable TMB [8].

TMB only reflects the quantity of mutations and cannot accurately predict dynamic immune responses. It also does not directly indicate whether these mutations will generate new antigens recognized by the immune system. Current research also explores neoantigen load (NAL) as a biomarker. Compared to TMB, NAL directly reflects the quantity of tumor-derived mutated antigens recognized by the immune system to be foreign, exhibiting a more

straightforward correlation with immune therapy response [14]. Neoantigen generation is not associated with germline and non-synonymous mutations, and thus these mutations are excluded from TMB calculations. However, it is often difficult to avoid these data biases in clinical practice, so false positives frequently occur in clinical data. Clinically, these false positive data should be avoided as much as possible, but the process is extremely time-consuming and labor-intensive.

6. Discussion

Given that neoantigens stem from mutations, a higher mutation count (equivalent to elevated TMB) correlates with an increased likelihood of tumor cells displaying neoantigens via their surface HLA molecules. Consequently, the probability of MHC-presented neoantigens being immunogenic also rises, which in turn boosts their recognition by T cells and interaction with cancer cells. However, theoretically, neoantigens recognized by T cells may originate from low-mutation environments (though this is less likely); similarly, despite an elevated TMB, there is no direct correlation with the automatic generation of neoantigens that are capable of eliciting an immune response [15]. Therefore, a high TMB does not necessarily guarantee that immunotherapy will be effective; A low TMB does not universally preclude a positive response to immunotherapy, as some individuals still derive significant benefit. These also explains why the response rate among patients with TMB-high (≥ 20 mut/Mb) tumors remains approximately 45% [16].

Numerous factors influence the capacity of T cells to identify and destroy tumor cells, and the generation of immunogenic neoantigens is only one of these factors. For instance, deactivating mutations occurring in antigen presentation pathways may also disrupt the process by which cells present peptides to the immune system, thereby affecting recognition.

7. Conclusion

TMB is a key biomarker for immunotherapy in NSCLC. TMB plays a crucial role in NSCLC immunotherapy by accumulating mutations, generating new antigens, and enhancing immunogenicity. Patients with high TMB demonstrate greater benefit from monoclonal antibody therapies such as Nivolumab and Ipilimumab, exhibiting significantly improved ORR, OS, and PFS. Concurrently, combining TMB with PD-L1 expression provides superior predictive value for the efficacy of monoclonal antibody treatments. However, TMB as a single biomarker has certain limitations. For instance, TMB is a dynamically

changing indicator that can be influenced at any time by multiple factors such as the tumor microenvironment and treatment processes. This means that results from a single-time-point assessment may not fully reflect a patient's response to immunotherapy. Second, although high TMB is typically associated with better responses to immunotherapy, many patients who have high TMB remain unresponsive, and those who have low TMB may also benefit from immunotherapy. Therefore, this has also prompted researchers to explore the combined use of TMB with other biomarkers—such as tumor-infiltrating immune cells and immune microenvironment markers—to enhance the predictive accuracy of immunotherapy efficacy. Future research directions should also include further standardization of TMB detection technologies, enabling TMB to become a biomarker that makes NSCLC treatment more precise, personalized, and effective.

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