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CONSENSUS

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Expert consensus on the detection and clinical application of tumor mutational burden

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ABSTRACT As an emerging biomarker, tumor mutational burden (TMB) has attracted increasing attention from clinicians in predicting the efficacy of tumor immunotherapy. Currently, TMB is detected primarily by whole-exome sequencing or targeted panel sequencing on high-throughput sequencing platforms. However, the lack of uniformity in detection methods, threshold settings, and reporting formats, as well as the significant differences in TMB values among different cancer types, have hindered the standardized application of this biomarker in clinical practice. This consensus focuses on the definition, standardization of detection, clinical significance, and limitations of TMB, and provides consensus recommendations for the clinical application of TMB in real-world practice in China. This consensus is aimed at helping clinicians and laboratory personnel understand the clinical significance and testing standards of TMB, promoting more accurate interpretation of test results, and improving patient care.

KEYWORDS Biomarkers; tumor mutational burden; tumor immunotherapy; targeted panel sequencing; whole-exome sequencing

Introduction

Immunotherapy based on immune checkpoint inhibitors (ICIs) has significantly improved objective response rates (ORRs) and overall survival (OS) in patients with advanced malignancies. However, the lack of effective population-based biomarkers has resulted in an overall response rate below 20% for ICI monotherapy, which is often accompanied by immune-related adverse events. Therefore, an urgent need exists to identify accurate and reliable biomarkers for screening patients who might potentially benefit from immunotherapy. Tumor mutational burden (TMB), defined by the number of tumor genetic variants, correlates with ICI efficacy. The concept of TMB originated from a study published in *Nature* in 2013 examining more than 7,000 specimens from 30 cancer types[1]. That study analyzed mutation profiles through whole genome sequencing (WGS) and whole-exome sequencing (WES) technologies and described the number of mutations per megabase (Mb) in various cancer samples. In 2015, the first study exploring the correlation between TMB and immunotherapy efficacy in non-small cell lung cancer (NSCLC) was published in *Science*[2]. In that study, patients with NSCLC with above-median TMB showed prolonged progression-free survival (PFS). Since then, multiple large studies have confirmed the predictive role of TMB in ICI treatment. In 2017, a study in *Genome Medicine* involving 100,000 patients with solid tumors explored the correlation between targeted capture sequencing panel and WES results for TMB detection, and confirmed the feasibility and reliability of panel detection of TMB[3]. The 2019 Chinese Society of Clinical Oncology guidelines and the National Comprehensive Cancer Network guidelines for molecular pathological testing in advanced lung cancer both included TMB. In 2020, the U.S. Food and Drug Administration (FDA) approved pembrolizumab as a monotherapy for patients with inoperable or metastatic tumor mutational burden-high (TMB-H) solid tumors that progressed on prior therapy. Pembrolizumab became the first anti-tumor drug using TMB as a biomarker to be approved worldwide. However, the lack of unified standards for TMB detection and evaluation in clinical practice markedly limits

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its clinical application. To promote a unified understanding and standardization of TMB testing and its clinical application, the East China Lung Cancer Group, Youth Committee (ECLUNG YOUNG, also known as the Yangtze River Delta Lung Cancer Collaboration Group) has developed this expert consensus on the clinical use of TMB, based on the characteristics and practices in the field of thoracic oncology in China, and jointly formulated an expert consensus on the clinical application of TMB. To enable the highest possible accuracy and reliability of ICI treatment prediction, this consensus provides definitions for TMB, sample type, detection method, gene panel inclusion criteria, bioinformatics analysis methods, threshold setting, and the clinical significance of TMB testing. Additionally, the expert group provides opinions regarding the selection of various efficacy prediction biomarkers including TMB in tumor immunotherapy. The recommendation grades were used in the principles and methods for developing the ECLUNG consensuses/guidelines[4].

TMB definition

Consensus 1: TMB is defined as the average number of somatic nonsynonymous mutations contained in a specific genomic region per megabase. TMB reflects not only tumor genomic mutations but also neoantigen production ability, which is associated with immunotherapy efficacy. However, TMB evaluation is affected by a variety of factors, and clarifying the scope of its use for precise application is important (strongly recommended).

TMB, the number of somatic nonsynonymous mutations contained in a given genomic region per Mb[3,5,6], indirectly reflects a tumor's ability to produce neoantigens[3,7,8]. When tumor cells undergo somatic nonsynonymous mutations, new antigens may be produced and recognized by the body's immune system, thus triggering an immune response. TMB-H typically indicates that tumor cells carry large numbers of somatic nonsynonymous mutations and are therefore likely to produce cancer-specific neoantigens[3,8]. These neoantigens are processed and loaded onto major histocompatibility complexes, where they are recognized by the immune system and trigger a T cell-mediated antitumor immune response[8]. Therefore, for some tumor types, the neoantigen burden or TMB is expected to be an important clinical biomarker for guiding ICI therapy decisions[9-12]. A growing body of clinical data suggest that patients with TMB-H cancers are relatively likely to benefit from ICI therapy. However, many challenges exist in the TMB evaluation process. A variety of factors, such as sample type, sample quality and quantity, genome coverage, sequencing platform, bioinformatics analysis procedures, and threshold setting, can significantly affect the evaluation process and experimental results[13]. Different types of samples, such as tumor tissue samples and blood samples, may have different TMB test results. Poor sample quality, such as low proportions of tumor cells or nucleic acid degradation, interferes with test accuracy. The number of detected mutations varies according to genome coverage. Differences in sequencing platforms and bioinformatics analysis procedures may lead to bias in the identification and calculation of mutations. The change in the threshold setting directly affects the determination of TMB-High or TMB-low (TMB-L). In addition, tumors with different organ and tissue origins significantly differ in the magnitude (i.e., mutations per megabase) and nature of TMB[14]. Therefore, clarifying TMB application scenarios and scope is important to ensure accurate clinical application of this biomarker for predicting the efficacy of tumor immunotherapy.

Standardization of TMB assays

Consensus 2: We recommend using recently paraffin-embedded tumor tissue samples for TMB detection. The tissue to be tested should first undergo pathological quality control to ensure that it contains sufficient malignant tumor cells (strongly recommended).

Consensus 3: We recommend prioritizing use of nucleic acid extraction kits approved by the National Medical Products Administration (NMPA) or qualified by laboratory performance verification for genomic DNA extraction. Additionally, TMB testing laboratories should establish appropriate quality control standards and operating procedures for DNA samples according to actual needs, and should strictly control the purity, concentration, and degree of fragmentation of DNA samples to be tested (recommended).

Consensus 4: When targeted sequencing panels are used for TMB evaluation, we recommend evaluating their consistency with WES results. In principle, the coverage of the targeted sequencing panel should not be below 1.0 Mb, and the minimum effective sequencing depth is 200×. We recommend that targeted sequencing panels for TMB testing maximally cover the patient's other molecular genetic information, including driver mutations that could guide targeted therapy, positive predictors of immunotherapy associated with the development of gene variants, and possibly negative predictors of immunotherapy (recommended).

Consensus 5: For TMB detection based on targeted sequencing panels, WES should be used as the gold standard. Additionally, somatic mutations that affect protein coding should be included, and somatic mutations with a detection mutation frequency >5% should be ensured to achieve accurate, stable TMB detection values. The ICI efficacy follow-up database should be used to standardize genome alignment and mutation detection algorithms. We recommend using control samples or Chinese population databases to rule out germline variants (strongly recommended).

Consensus 6: Significant differences exist in TMB values among cancer types, and the threshold should be determined according to the clinical efficacy of ICIs, to maximize the identification of patients who might potentially benefit from ICI treatment. Importantly, TMB thresholds vary among targeted sequencing panels and detection systems (recommended).

Consensus 7: In addition to focusing on TMB calculation principles and values, the TMB report should interpret the significance of immunotherapy for specific cancer types. Systematic evaluation of mutations in various driver genes detected by the panel is also necessary to comprehensively interpret patients' tumor biological characteristics. Application of the Molecular Tumor Board (MTB) for clinical decision-making is strongly recommended (strongly recommended).

Principles of sample collection and processing

In clinical practice, tissue TMB (tTMB) testing is widely used, whereas the accuracy of blood TMB (bTMB) testing requires further verification[15]. Therefore, this consensus primarily establishes principles for TMB sample collection and processing according to the published literature and consensus.

Specimen types and recommended requirements for TMB testing

(a) Formalin-fixed, paraffin-embedded (FFPE) tumor tissue: FFPE tissue is a commonly used specimen type in TMB testing. We recommend using 10% neutral formalin fixative solution for FFPE tissue. The volume of the fixation fluid should be more than 10 times that of the sample tissue. The optimal fixation time is approximately 24 h for surgical specimens and 12 h for needle biopsies. For TMB evaluation, recent tissue blocks within 3 years, preferably within 1 year, or paraffin sections prepared within 6–8 weeks, with a section thickness of 4–5 μm, are recommended[5,10]. At least 5 sections are necessary for surgical tissue specimens. A minimum of 10 sections is necessary for biopsies or small specimens. The tissue specimen should contain at least 200 viable tumor cells, or 10% of the tumor, to ensure sufficient material for testing and quality control. Insufficient numbers of tumor cells can affect the accuracy of the test results. If necessary, techniques such as microdissection can be used to enrich the tumor cell population or remove necrotic tissue, thereby enhancing the sensitivity of TMB detection, depending on patient consent.

(b) Fresh tumor tissue: Fresh tissue, although usable, is not routinely recommended, because of difficulties in assessing the tumor cell proportion[13]. Frozen sections can be used for initial quality control. For surgical specimens, a minimum of 10 mg (approximately the size of a grain of rice) is required. For biopsies or small specimens, at least one piece of tumor tissue that is visible to the naked eye is needed. Importantly, the accuracy of TMB detection and calculation is affected by the proportion of tumor cells, and FDA-approved TMB assays require a tumor cell proportion of at least 20%[16-19].

(c) Normal control: Most TMB assays require a normal control to provide and filter out germline variation information[20]. Normal tissue or peripheral blood can be used as a control. If a peripheral blood sample is used as a control, 2–5 mL EDTA anticoagulated peripheral blood should be collected. After collection, the sample should be gently inverted and mixed 8–10 times to prevent blood clotting. We recommend that specimens be transported to the testing laboratory at a low temperature of 4°C and processed within 2 h to avoid hemolysis. If processing is not possible within 2 h, or long-distance transportation is required, we recommend storing or transporting samples at room temperature in a dedicated cell-free DNA collection tube.

(d) Lesion selection: Both the primary tumor lesion and distant metastatic lesion tissue can be used for TMB evaluation[21]. Given tumor heterogeneity, several studies have explored the detection of TMB in paired primary lesions, distant metastases, and lymph node metastases of lung adenocarcinoma[22]. TMB is significantly lower in lymph node metastases than in primary lesions, whereas the difference between distant metastases and primary lesions is relatively small. In addition, significant intratumoral gene mutation heterogeneity may be present among regions of the same tumor, thus potentially leading to large variations in TMB values[23,24]. Currently, no studies have specifically explored heterogeneity in TMB samples and correlations with clinical outcomes. Therefore, we recommend preferential use of primary tumor lesion tissue and distant metastatic lesion tissue for TMB detection.

Nucleic acid extraction and quality control

In TMB detection, nucleic acid extraction is a key step. We recommend using nucleic acid extraction kits that have been approved by the NMPA or whose performance has been validated by laboratories. Nucleic acid extraction should be performed in laboratories meeting the stringent requirements for high-throughput sequencing[5,10], to ensure the quality and stability of nucleic acid extraction, and to prevent problematic kits or laboratory conditions from affecting subsequent test results[25]. DNA purity, concentration, and fragmentation should be rigorously evaluated before TMB detection. The quality of the tumor tissue DNA library is critical. Insufficient

concentrations, particularly those below 5.0 nmol/L, can lead to inflated or TMB-L and consequently result in false-positive or false-negative results. Clinical genetic testing laboratories should select an appropriate platform for nucleic acid quality control. We recommend using micronucleic acid fluorescence quantification to determine nucleic acid concentrations, and evaluating nucleic acid fragmentation with agarose gel electrophoresis or a nucleic acid fragment bioanalyzer. The amount of nucleic acid required for TMB detection with large panel genetic testing is 20–200 ng, depending on the sequencing method. For WES-based TMB assays, we recommend a nucleic acid amount of at least 250 ng. Clarifying the nucleic acid requirements would help ensure test accuracy and reliability.

Panel design and platform selection for TMB assays

Method selection for TMB detection

TMB assays are important for evaluating the efficacy of tumor immunotherapy. Currently, 2 main methods are used, WES and targeted panel sequencing, each with its own advantages and limitations[5,10,26]. WES directly sequences the entire exome region of the tumor genome and therefore accurately reflects TMB and is considered the gold standard. However, complexity and cost limit widespread use of WES in routine clinical practice[27-29]. Targeted panel sequencing focuses on a comprehensive set of tumor-associated genes, combined with bioinformatics algorithms, to quickly and accurately identify alterations in the tumor genome[16,24]. Several studies have confirmed that targeted panel sequencing results show high consistency with WES results; this method therefore provides a viable alternative for TMB detection[30,31].

Panel size in TMB detection

The size of the targeted sequencing panel is an important factor influencing several key metrics in TMB assessment, including confidence intervals, thresholds, assay sensitivity, specificity, and positive and negative predictive values. Different panels cover different genomic regions, thus resulting in variations in TMB values[32]. The Dana-Farber Cancer Institute has constructed large (300-gene), moderately sized (48-gene), and small (15-gene) panels, and has found that small panels are poor predictors of overall mutational load, whereas large panels successfully recapitulate the WES mutational load[33]. Some research institutions have used random mutation models to mimic non-random mutations in real-world cancer genomes and intratumoral heterogeneity in public databases, and have found that the coefficient of variation (CV) of TMB assessed with targeted sequencing panels is inversely proportional to the square root of the panel size and the square root of the TMB. When the tumor TMB threshold was set to 10 mutations per megabase (mut/Mb), CV increased with decreasing panel coverage (from 4 Mb to 2 Mb, 1 Mb, 0.5 Mb, and 0.25 Mb, with CVs of 22%, 26%, 32%, 45%, and 63%, respectively). When the panel size was less than 0.5 Mb, the CV sharply rose. At less than 1.0 MB, TMB assessment accuracy significantly decreased, thus hindering effective identification of patients who might benefit from immunotherapy[34-36]. To study the effects of selected genes on the CV of TMB, researchers in Germany, the United Kingdom, and Switzerland have simulated 3 panels: panel A, containing oncogenes and tumor suppressor genes; panel B consisting of randomly selected genes; and panel C excluding oncogenes and tumor suppressor genes[32]. Analysis of The Cancer Genome Atlas (TCGA) data indicated a 6% greater TMB variance for panels B and C, and a 15% greater TMB variance for panel A, than observed with a random mutation model. Therefore, the panel design should cover a wide range of genetic elements and should not be limited to oncogenes and tumor suppressor genes. The Genomics Evidence Neoplasia Information Exchange, initiated by the American Association for Cancer Research, has analyzed real-world TMB data from approximately 19,000 patients with cancer. When the TMB was 0–5 mutations per Mb, approximately two-thirds of the samples could not be accurately assessed with small panels. Panel sizes greater than 1.0 Mb are therefore necessary to accurately assess TMB[37]. Researchers in the Cheng laboratory[31] at Jilin Cancer Hospital have evaluated panels covering more than 1.0 Mb and verified 98.7% concordance in TMB calculations by using their OncoTOP algorithm and WES across 2,864 samples of 18 cancer types. In a study by Zhang's team[38] at Sun Yat-sen University, large-panel-based TMB assessment findings were highly concordant with WES TMB detection results in lung cancer populations (Spearman R = 0.81). In the first one-third of the TMB-H population, both panel and WES TMB were predictive of the response to ICI therapies (PFS HR: 0.45/0.43). In an analysis of 3 FDA-approved targeted sequencing panels, the panel with ≥1.0 Mb coverage met the clinical requirements for TMB evaluation. Therefore, we recommend an ideal coverage of targeted sequencing panels for the evaluation of TMB between 1.0 and 3.0 Mb[39]. In addition, targeted sequencing panels can introduce bias arising from changes in the covered genomic regions. The TMB panel should contain comprehensive molecular genetic information from the patient, such as driver gene mutations (e.g., *EGFR*, *ALK*, *ROS1*, *RET*, *NTRK*, *BRAF*, *ERBB2*, *KRAS*, and *MET*), to guide targeted therapy. Additionally, both positive predictors of immunotherapy response [e.g., deficient mismatch repair (dMMR)/microsatellite instability (MSI), *POLE*, *POLD1*, and *BRCA1/2*] and potential negative predictors [e.g., programmed death receptor-1 ligand 1 (PD-L1), *PIK3CA*, *JAK1/2*, *PTEN*, and *STK11*] should be considered, because they are associated with mutation-driven mechanisms[40]. Rebiopsy and genetic testing should be minimized to avoid delays in treatment[41-46].

Sequencing coverage depth

Sequencing depth is critical for both panel-based and WES-based TMB assays. The standard sequencing depth for WES is typically 100×, at which allele mutations with frequencies greater than 15% can be reliably detected, thus ensuring TMB assessment accuracy. In contrast, panels can achieve higher sequencing depths, thus resulting in detection of more low-frequency variants[42-44,47]. To effectively increase the ability to detect low-frequency variants at specific loci, we recommend a sequencing depth of at least 200× for targeted sequencing combinations[4]. The average depth of coverage for the FDA-approved MSK-IMPACT sequencing panel is 200×. In 2019, the European Society for Medical Oncology (ESMO) guidelines clearly stated that the depth of coverage required for accurate assessment of TMB should be greater than 200×[13]. A Chinese research team has shown that panel assays show higher consistency with WES at 500× sequencing depths and significantly correlate with patient immunotherapy efficacy[45-47,48].

Cost-effectiveness analyses

Targeted panels outperform WES in cost-effectiveness, with lower costs, higher sequencing depth, faster turnaround, and better coverage of disease-relevant genes, thus enhancing variant detection, and conferring advantages in routine clinical use and large-scale screening. WES offers broader coverage for novel variants in complex cases but has higher costs, more generated data (increasing analysis expenses), and potentially lower gene-specific coverage. This method might save costs by replacing multiple sequential tests or as prices decline to near-panel levels.

In advanced NSCLC, WGS-TMB (with cost and scope similar to those of WES) is less cost-effective than PD-L1 testing for immunotherapy selection. For WGS-TMB to achieve comparable cost-effectiveness to PD-L1 testing, it would require either a ≥24% reduction in sequencing costs or an improvement in TMB's predictive value for immunotherapy response. Combined TMB-PD-L1 testing would require a 40%–50% reduction in sequencing and drug costs to achieve cost-effectiveness comparable to that of single PD-L1 testing[49]. Decision-tree models favor PD-L1 over TMB/no testing; tissue-based TMB is the most economical TMB method but remains more expensive than no testing, and its economic value depends on its costs and utility[50].

Standardization requirements for TMB algorithms

The core aim of the TMB algorithm is to accurately align and calculate somatic mutations that affect protein-coding sequences. To ensure high accuracy and reliability of TMB calculation results, several potential problems in sequencing data processing and mutation analysis at the algorithm level remain to be solved.

Quality control of sequencing data

From the generation of raw data by the sequencing platform to the completion of somatic mutation detection, data typically undergo 4 critical stages: quality control, data filtering, genome alignment, and mutation detection. Because raw sequencing data often include problems such as low-quality readouts, linker contamination, and insertion and deletion errors, data quality must be evaluated through bioinformatics analysis. Moreover, strict quality control standards must be established for parameters such as data efficiency, error rate, Q30, GC content, mapping rate, average sequencing depth and uniformity of the target region, to ensure that these problems do not interfere with TMB detection. Specifically, these criteria include data availability of >99%, error rate <0.1%, Q20 score >90%, Q30 score >85%, GC content of 42–55%, and mapping rate >95%[4].

Upstream data preprocessing workflows

Standardization of upstream data preprocessing is crucial for ensuring the accuracy, reliability, and reproducibility of subsequent variant calling. Eliminating technical biases (e.g., sequencing errors, adapter contamination, and PCR duplicates) and correcting data quality through standardized steps are essential to generate high-quality BAM files suitable for variant calling. Relevant workflows adhere to internationally recognized protocols, such as GATK Best Practices and ENCODE standards[51], while adapting to different sequencing types (e.g., WGS, WES, or targeted region sequencing). Key steps in sequence include quality control of raw sequencing data (FASTQ) by using FastQC to evaluate metrics such as the Phred quality score distribution, GC content, and adapter contamination rate, combined with MultiQC for aggregating multi-sample quality reports and defining thresholds (e.g., average Phred score ≥20)[52]; sequence trimming and filtering *via* Trimmomatic or Cutadapt to remove adapter sequences, trim low-quality ends (e.g., LEADING:3 or TRAILING:3), and filter short reads (e.g., length <36 bp) to decrease alignment noise[53]; alignment to the reference genome (e.g., hg38 or GRCh38) with tools such as BWA-MEM (for WES) with specified parameters (e.g., seed length and mismatch penalties) to generate SAM files; conversion of SAM to BAM format with SAMtools (to reduce storage), and sorting by chromosomal position to facilitate subsequent steps[54]; marking PCR duplicates *via* Picard's Mark Duplicates to avoid bias in variant calling from duplicate counts; base quality score recalibration (BQSR) with GATK's BaseRecalibrator with known variant databases (e.g., dbSNP or 1000 Genomes) to correct for the inherent base quality biases of sequencers, thus yielding recalibrated BAM files; and post-preprocessing quality validation with Qualimap or GATK's Collect HsMetrics (for WES) to assess key indicators (e.g., alignment rate ≥95%, duplicate rate <10%, and average coverage depth meeting experimental design) to ensure suitability for variant calling. Additionally,

we discuss standardization principles for reproducibility, including selecting tool versions (e.g., GATK 4.2.6 or BWA 0.7.17) to avoid version-related discrepancies, transparently reporting parameters with clear justifications for adjustments (e.g., for tumor *vs.* normal tissues), recommending workflow management tools (e.g., Snakemake or Nextflow) for automation, and providing reusable scripts (e.g., *via* GitHub repositories) to facilitate replication[54,55].

TMB algorithm and mutation analysis

After the initial filtering of raw sequencing data, a variety of bioinformatics analysis tools play integral roles in genome sequence alignment and mutation detection. Commonly used software for next-generation sequencing (NGS) alignment includes BWA[55], Bowtie[56], SOAP[57], BFAST[58], ELAND[59], MAQ[60], and SHRiMP[61], among which the BWA software with the SMEM algorithm for alignment with the human reference genome is the most widely used. For mutation detection, commonly used algorithms include VarScan2[62], which relies on statistical methods, and Strelka[63] and Mutect2[64], which are based on Bayesian models. In addition, the acceleration algorithm Genomic Variant Caller (GVC)[65], developed with artificial intelligence (AI) technology, can adapt to data from various platforms through the AI model, thereby ensuring efficient and accurate detection results. Moreover, the detection speed is 4–8 times faster than that of traditional software, thus providing strong support for accelerating the application of NGS in clinical practice (**Supplementary material**).

Evaluation of TMB algorithm accuracy

Panel-based TMB detection is performed by calculating the number of somatic mutations in the exons covered by the panel divided by the exon coverage area of the panel. However, a discrepancy exists between calculated TMB values and TMB values obtained through WES detection[66-69]. Tumor purity is a non-negligible factor in the calculation of TMB values. Anagnostou et al.[70] have found that with decreasing tumor purity, the frequency of somatic mutation sites also decreases. Correction of TMB values by tumor purity correction parameters enables better differentiation of efficacy in patients treated with ICIs. In general, the purity of tumor cells should not be below 20%. When the purity of tumor cells is low, a larger tumor purity correction parameter is required.

Filtering out germline variants is essential for accurately determining TMB. Ideally, all germline variants, including single nucleotide polymorphisms (SNPs; population allele frequency ≥1%) and mutations (population allele frequency <1%), should be excluded from the TMB calculation, to avoid overestimating the TMB values. Consequently, a normal sample to serve as a control must often be obtained from the patient, most commonly by collecting paracancerous tissue or peripheral blood[71]. For example, among the 4 FDA-approved methods for tumor TMB detection, MSK-Impact, Omics Core, and PGDx elio tissue complete all filter the germline mutations detected in tumor samples by detecting the patient's leukocyte control, to ensure that the mutations used to calculate the TMB are all somatic mutations. However, this approach not only increases the cost of testing but also poses ethical issues associated with germline genetic information. Some laboratories that have developed TMB panels capable of screening germline variants by using only tumor tissue samples, often filtered with the help of population-based databases, such as the Genome Aggregation Database (gnomAD), TCGA, Exome Aggregation Consortium (ExAC), 1000 Genomes Project, and Single Nucleotide Polymorphism Database (dbSNP). However, most of these public population databases are based on European and American population data, which are not fully applicable to the Chinese population and therefore might lead to inaccuracies in TMB estimation for Asian and African population. The OncoTOP algorithm, published by Cheng's team[31] , solves the technical bottleneck of traditional TMB detection based on paired samples (**Supplementary material**). By integrating multi-dimensional information such as CNV, MSI, and SNP and mutation frequency, the algorithm achieves ultra-high-accuracy germline variant identification and TMB calculation without control samples. Moreover, the algorithm was developed completely on the basis of a Chinese population database and therefore has wide application prospects[31]. Therefore, we recommend using control samples (peripheral blood or normal tissue) to remove germline variants when determining somatic mutations in TMB or to optimize germline variant filtering by laboratory-constructed Chinese population databases or algorithms[71,72].

Exploration and clinical application of TMB thresholds

Distribution of TMB across cancer types

In a WES analysis of 27 tumor types based on TCGA database, the median nonsynonymous mutation frequency among tumor types significantly differed, by more than 1,000-fold. The TMB in pediatric tumors was low, at approximately 0.1 mut/Mb, whereas some patients with malignant melanoma and NSCLC had more than 100 mut/Mb[1]. In addition, high heterogeneity in TMB values was observed among patients with the same cancer type. In malignant melanoma and lung cancer, the TMB values ranged from 0.1 to 100 mut/Mb across patients, and a DNA-targeted sequencing study based on a pan-cancer cohort of 10,000 patients has yielded similar results[73]. A Chinese team has found similar results in a sample of 2,864 cases from 18 cancer types, with differences in the distribution of TMB among different cancer types[45].

TMB threshold

The median TMB and its range markedly differ across cancer types[11]. A fixed TMB threshold may capture a higher proportion of TMB-H patients with cancer types with inherently higher TMB levels but a lower proportion of TMB-H patients with cancer types with lower TMB levels. Therefore, a unified screening strategy should be applied for all cancer types. Specifically, cases ranking in the top 20% are defined as the TMB-H group. However, the TMB-H thresholds identified across different cancer types substantially vary, from 4.4 to 52.2 mut/Mb[11]. Consequently, establishing cancer type-specific thresholds for defining TMB-H will be essential.

The classification of TMB-H thresholds should not be based solely on population distribution, and whether this classification can correctly indicate immunotherapy efficacy is the key criterion[34]. Budczies et al.[34] have used the WES TMB results from TCGA database as a reference standard and set the cut-off value of TMB at 199 mut/Mb. Correspondingly, panel-based TMB thresholds were defined for 10%–12% of the population with lung adenocarcinoma and 17%–19% of the population with lung squamous cell carcinoma (LUSC). The retrospective FIR, BIRCH, and POPLAR studies initially used the Foundation One LDT method based on genomic DNA targeted sequencing, with thresholds of either 9.9 mut/Mb or the top 75% of the population. These thresholds partially distinguished populations benefiting from ICI treatment[45]. However, 2 prospective clinical trials, CheckMate 568 and CheckMate 227, using the parallel-validated FoundationOne CDx assay, identified 10 mut/Mb as the optimal TMB threshold for predicting immunotherapy efficacy in NSCLC[74]. In addition, a Chinese team led by Liang[45] has found that patients with lung cancer with TMB > 10 mut/Mb had a higher benefit rate from ICI treatment compared to those with TMB ≤ 10 mut/Mb. Zou's team[46] has indicated that patients with melanoma whose TMB exceeds 8 mut/Mb have the highest benefit rate of ICI treatment, and Lin' s team found that patients with gastric cancer with TMB exceeding 8 mut/Mb have significantly higher ORR in ICI treatment than those with TMB ≤ 8 mut/Mb[75]. Therefore, the clinical efficacy of ICIs is the best criterion for determining the TMB threshold.

Methods frequently used for TMB detection

Since TMB was first identified as a potential predictive biomarker for immunotherapy efficacy, multiple testing laboratories and companies have developed TMB detection solutions. Internationally recognized commercial panels include TEMPUS (648 genes, 2.4 Mb genome coverage), Guardant OMNI (550 genes, 2.2 Mb genome coverage), Caris SureSelect XT (592 genes, 1.6 Mb genome coverage), and F1CDx (324 genes, 1.1 Mbgenome coverage), as well as institutionally validated platforms such as MSK-IMPACT (468 genes, 1.2 Mb genome coverage) from the Memorial Sloan Kettering Cancer Center and OncoPanel (447 genes, 1.3 Mb genome coverage) from the Dana-Farber Cancer Institute. In China, several providers offer similar commercial tests, including GenePlus (1,021 genes, 1.6 Mb genome coverage), Burning Rock (520 genes, 1.26 Mb genome coverage), and Geneseeq (425 genes, 1.26 Mb genome coverage)[76]. Notably, Geneseeq's large gene panel has been approved by China's NMPA for TMB testing in formalin-fixed tissue samples from patients with EGFR/ALK-negative non-squamous NSCLC. However, significant discrepancies exist in TMB definitions across experimental and bioinformatics methods (**Table 1**). For instance, F1CDx incorporates synonymous mutations and short intronic indels into TMB calculations, whereas these features are typically excluded by other algorithms. Emerging evidence suggests that indels might generate novel open reading frames capable of producing immunogenic neoantigens; therefore, intronic region analysis and indel inclusion might potentially enhance TMB estimation accuracy. Nevertheless, F1CDx's lack of paired germline sequencing risks overestimating TMB because of incomplete germline variant filtering. In contrast, the FDA-approved MSK-IMPACT platform uses matched blood sequencing to eliminate germline variants and focuses solely on somatic exonic mutations across approximately 500 cancer-related genes. These methodological heterogeneities highlight the challenges in achieving reproducible TMB measurements across testing platforms.

Recommendations for TMB report templates

Information to include in TMB reports

(a) Basic information: Information should include the examinee, including name, sex, age, clinical and pathological diagnosis, genetic testing history, family medical history, and clinical treatment history; sample information, including sample type, date of collection, collection institution, and date of receipt by the testing laboratory; and descriptions of the project, including the assay method, platform used, assay range (e.g., number of genes and panel size), library construction method, and reference genome used.

(b) Test results: Information should include the total number of somatic coding mutations, TMB value, tumor type, TCGA database ranking, and summary of the interpretation of results.

(c) Interpretation of results: Information should include TMB evaluation, such as mutation type, minimum detection frequency, and consistency with WES, as well as interpretation of the results, including whether a clear threshold statement is present. For the various immunotherapies, relevant thresholds and clinical trial evidence should be clearly stated.

(d) Signature: Testing technicians and auditors are required to sign. The final report must be reviewed by a qualified physician or authorized signatory who holds

a senior title or MD degree, has a background in pathology, has at least a master's degree, and has received relevant training.

(e) Limitation description: The limitations of TMB in clinical applications should be explained, with full consideration of factors such as tumor type, sample type, the testing panel, and relevant clinical research results.

In summary, we recommend that TMB reports document the above information in detail for clinical use. Information on the recommended report template is presented below (**Table 2**).

Recommendations for clinical interpretation of TMB reports

We recommend interpreting TMB results according to the specific indications and therapeutic agents. Samstein et al.[11] have demonstrated that the TMB thresholds for benefit from immunotherapy vary across cancer types. For example, in IMvigor210, a clinical trial of atezolizumab for bladder cancer, the TMB threshold detected by the FoundationOne product was ≥16 mut/Mb[17]. In the clinical trial BIRCH/FIR for NSCLC treatment, the TMB threshold detected by the Foundation One product was ≥13.5 mut/Mb in first-line patients and ≥17.1 mut/Mb in second-line patients. The TMB threshold in the POPLAR clinical trial was ≥15.8 mut/Mb[77]. In the CheckMate 012 study of nivolumab combined with ipilimumab for patients with NSCLC, the CheckMate 032 study for small cell lung cancer (SCLC), and the CheckMate 038 study for malignant melanoma, the TMB thresholds detected by WES were ≥307 mut/Mb, ≥248 mut/Mb, and ≥100 mut/Mb[78], respectively. Therefore, as a biomarker for predicting ICI efficacy, the TMB threshold for companion diagnostic products of different drugs has relatively limited reference value. A unified TMB threshold should not be used to assess the therapeutic effects of different ICIs on patients.

Clinical significance of TMB

Consensus 8: TMB, an emerging independent predictor of ICI therapeutic efficacy, is associated with the efficacy of monotherapy or a combination of 2 ICIs in multiple tumor types, and has been demonstrated to be a predictive marker for pan-cancer immunotherapy efficacy. TMB testing is

Table 1 Commonly used TMB assessment products in China and other countries

TMB type	Biospecimen	Laboratory/ institution	Panel name	Number of genes covered	TMB region covered (Mb)	Total region covered (Mb)	Types of exonic mutations included in TMB estimation
Tumor-based TMB	Paired tumor and blood samples	Memorial Sloan Kettering Cancer Center	MSK-IMPACT	468	1.14	1.53	Nonsynonymous
Tumor-based TMB	Paired tumor and blood samples	TEMPUS	TEMPUS Xt	648	2.40	2.40	Nonsynonymous
Tumor sample only	Tumor sample only	Caris Life Sciences	SureSelect XT	592	1.40	1.60	Nonsynonymous, synonymous
			FoundationOne CDx	324	0.80	2.20	
Tumor sample only	Tumor sample only	Dana-Farber Cancer Institute	OncoPanel	447	1.30	1.94	Nonsynonymous
Blood-based TMB	Blood sample only	Guardant Health	GuardantOMNI	500	1.00	2.15	Nonsynonymous, synonymous
Tumor-based TMB	Paired tumor and blood samples	GeneseeQ Co	GENESEEQ PRIME	425	1.00	1.26	Nonsynonymous
Tumor-based TMB	Paired tumor and blood samples	Geneplus Co	OncoD	1,021	1.00	1.60	Nonsynonymous
Tumor-based TMB	Paired tumor and blood samples	Burning Rock Biotechnology	OncoScreen Plus 520		1.00	1.26	Nonsynonymous

TMB, tumor mutational burden.

recommended for patients with solid tumors that have progressed on prior standard therapy for whom no better alternative therapy is available, particularly patients with pre-relapse TMB-H, to help expand the population benefiting from immunotherapy. In cases in which tumor tissue is difficult to obtain or the amount of tumor tissue in paraffin-embedded samples is insufficient, blood may be considered for bTMB evaluation based on circulating tumor DNA. However, the value of bTMB must be clarified by more prospective studies (recommended).

Consensus 9: TMB is a validated predictive biomarker for the efficacy of pan-cancer immunotherapy. However, the level of evidence currently varies across cancer types. We recommend using TMB in cancers that have been widely studied and validated (recommended).

tTMB

TMB has been well established in several studies as a predictive biomarker of ICI efficacy in the treatment of solid tumors[10,16-18]. In 2014, Snyder et al.[7] first reported TMB as a potential biomarker for ICIs in melanoma. Subsequently, the predictive value of TMB in predicting the response to pembrolizumab (an anti-PD-1 antibody) was validated in patients with advanced NSCLC[2].

In the CheckMate 568 study, which evaluated the efficacy of nivolumab in combination with ipilimumab in metastatic NSCLC, higher tumor tTMB (≥10 mut/Mb) was associated with higher ORR (44% *vs.* 12.0%) and longer PFS (7.1 *vs.* 2.6 months)[9]. In the prospective CheckMate 227 trial, PFS was significantly longer in patients with NSCLC with TMB-H (≥10 mut/Mb) than in patients with TMB-L (7.2 *vs.* 5.5 months; OR 0.58; 95% CI 0.41 to 0.81; P <0.001), independently of PD-L1 expression levels[13]. However, the efficacy of TMB-guided monoclonal antibodies is limited, because nivolumab monotherapy showed a trend toward improved outcomes in patients with TMB-H, whereas no significant benefit was observed in patients with TMB-L. In LUSC, an important NSCLC subtype whose genomic characteristics are distinct from those of lung adenocarcinoma, TMB also serves as a predictive marker of immunotherapy efficacy[13]. In a CheckMate 227 subgroup analysis, nivolumab plus ipilimumab, compared with chemotherapy, significantly prolonged PFS in patients with TMB-H (≥10 mut/mb), including those with LUSC[13]. The CheckMate 9LA study confirmed that nivolumab plus ipilimumab and short-course chemotherapy resulted in significantly longer PFS and OS in patients with TMB-H LUSC than in patients with TMB-L[79]. Beyond NSCLC, the efficacy of TMB and immunotherapy has been demonstrated in other cancer types. TMB-H has been associated with immunotherapy efficacy in SCLC, head and neck, breast, and colorectal cancers[23,24,80]. In the CheckMate 275 study, nivolumab-treated patients with urothelial carcinoma with TMB-H (≥13 mut/Mb) had higher ORR and longer PFS/OS than patients with TMB-L[81]. In the KEYNOTE-158 study, which included 10 tumors (e.g., SCLC and endometrial cancer), pembrolizumab-treated patients with tTMB-H (≥10 mut/Mb) had a higher ORR than patients with TMB-L (29% *vs.* 6%)[82]. On the basis of those results, the FDA approved pembrolizumab as a monotherapy for patients with advanced TMB-H (≥10 mut/mb) unresectable and/or metastatic solid tumors that progress after standard therapy. For patients with cancer of unknown primary (CUP), TMB testing can help guide treatment decisions. Historical data have indicated that approximately 10%–20% of patients with CUP have TMB-H[83]. In a study by Maria et al.,[26] in 31 patients with CUP treated with nivolumab plus ipilimumab, the TMB-H (≥12 mut/Mb) patients had a significantly higher ORR than the TMB-L patients (60% *vs.* 7.7%), thus suggesting that patients with CUP with TMB-H are more likely to have a

Table 2 Consensus template for clinical TMB reporting components

Section	Content to include
Basic information	Patient information, including name, sex, age, clinical and pathological diagnosis, genetic testing history, family medical history, and clinical treatment history
Sample information	Sample type, adequacy, and quality metrics
Assay details	Platform, panel/exome, coverage (Mb), and approval status
Variant types	Types included (e.g., nonsynonymous SNVs or indels)
Filtering criteria	Allele frequency, read depth, and germline/artifact filtering
Control/reference	Reference genome and matched normal/in silico controls
TMB value	Mutations per megabase (mut/Mb) and panel size
Confidence intervals	95% CI or prediction limits
TMB thresholds	High/low cut-off and clinical interpretation
Quality assurance	QC metrics and validation status
Additional findings	Actionable mutations, MSI status, and other biomarkers
Interpretation of TMB	TMB assessment regarding mutation type, minimum detection frequency, and concordance with WES
Signature and description of limitations	Explanation of limitations of TMB in clinical applications

favorable response after receiving ICIs. However, the predictive value of TMB in clinical applications varies across studies. In the KEYNOTE-021 and KEYNOTE-189 studies, TMB was not associated with ICI efficacy in combination with chemotherapy[84,85]; therefore, the predictive role of TMB may vary by treatment regimen and tumor type. In Chinese cohorts, TMB's predictive role has also been validated. A study by Tian's team[86] at Sichuan University has suggested significant improvements in ORR in patients with advanced NSCLC with TMB ≥8 mut/Mb and higher immune diversity than those with TMB <8 mut/Mb. A team led by Zhang[38] at Sun Yat-sen University has demonstrated a significant increase in PFS in patients with advanced NSCLC with TMB-H (HR: 0.43–0.45). In the future, further in-depth research is needed to optimize the clinical application of TMB and better provide guidance for the treatment of patients with cancer (**Table 3**).

bTMB

With advances in liquid biopsy technology, assessing bTMB provides a new avenue for predicting response to immunotherapy and dynamically monitoring treatment changes in patients with difficult tissue specimen access[24,112]. The use of circulating tumor DNA to detect bTMB has an advantage of high sequencing coverage. In a retrospective study in patients with NSCLC treated with atezolizumab, patients with bTMB ≥16 mut/Mb experienced prolonged PFS with this therapy[24]. In addition, combined screening with PD-L1 expression analysis enables more accurate screening of patients who have achieved prolonged PFS and OS from treatment. In a recent study of advanced NSCLC at Union Medical College Hospital, patients with high bTMB have been found to benefit from ICIs and to potentially achieve better prognostic outcomes with combined testing of other blood parameters[48]. In the TRACELib002 study conducted by Lu's team[40] at Shanghai Chest Hospital, patients with bTMB ≥16 mut/Mb had a median OS of 24.5 months after immunotherapy, which was significantly longer than the 14.6 months observed for patients with bTMB <16 mut/Mb. However, studies have also shown that although bTMB and tTMB are correlated, most of the mutations detected by each method are inconsistent. Moreover, this heterogeneity becomes more pronounced with greater intervals between tissue and blood sample collection[112]. Currently, the clinical value of bTMB has not been fully clarified, and further in-depth exploration will be necessary in future research.

Current limitations and future directions of bTMB

bTMB is a promising non-invasive surrogate for tTMB, yet its clinical implementation is hampered by substantial technical and biological hurdles. Contemporary trials and meta-analyses are actively examining its utility as a predictive biomarker for immunotherapy response. Findings have highlighted both its potential and limitations. Notable limitations include suboptimal concordance with tTMB—concordance that is highly contingent on technical variables such as cell-free DNA input, sequencing depth, and maximum somatic allele frequency[113-115]. Insufficient cell-free DNA yield or inadequate sequencing depth frequently results in poor correlation and false-negative outcomes, particularly in early-stage malignancies or tumors with low shedding propensity[114,115]. Additionally, the absence of standardized bTMB thresholds and the varying cut-off values used across investigative platforms and studies obscure clinical interpretation and impede cross-study comparability[91,114,116-118]. Biological confounders, including low tumor burden, intratumoral heterogeneity, and clonal hematopoiesis, further introduce biases leading to bTMB underestimation or overestimation, thus compromising its reliability as a biomarker[114,115,118]. Economically, the high sequencing depth required for accurate bTMB quantification elevates costs, although emerging evidence suggests that targeted panels might mitigate expenses without sacrificing analytical accuracy[91,114,119].

The MYSTIC trial, B-F1RST trial, and meta-analyses have prospectively evaluated bTMB as a predictive biomarker for immunotherapy and demonstrated its potential as well as needs for assay optimization and further validation[91,117,120-122]. Future advancements in this field are expected to involve multiple fronts, such as (1) assay optimization and harmonization, including refinement of panel design; enhancement of bioinformatics pipelines; and standardization of thresholds to increase reproducibility and clinical translatability[114,115,116,118]; (2) integration with complementary biomarkers, e.g., PD-L1 expression, genomic aberrations, and immune microenvironment signatures, to augment predictive precision for immunotherapy response; (3) implementation of dynamic monitoring *via* serial bTMB measurements, to enable real-time surveillance of treatment efficacy and disease progression[116,118]; and (4) expansion of large-scale prospective trials and meta-analyses[115,116,118,122]. In summary, bTMB has emerged as a compelling minimally invasive biomarker for immunotherapy stratification, yet its clinical integration necessitates resolution of technical, biological, and standardization challenges. Sustained efforts in trial advancement and harmonization will be critical to its successful translation into routine clinical practice.

Evidence of TMB in various cancer types

TMB has been incorporated into multiple guidelines as a predictive biomarker for immunotherapy efficacy in solid tumors (**Table 3**). In an exploratory analysis of the CheckMate 026 trial comparing nivolumab monotherapy *vs.* platinum-based chemotherapy as a first-line treatment for PD-L1-positive

Table 3 TMB thresholds and results in various clinical studies

Refs	Setting	ICI	N	TMB cut-off	Method	ORR	PFS	OS
[87]	Untreated NSCLC PD-L1 >1%	Atezolizumab	389	16 mut/1.1 Mb	NGS panel	NA	6.8 *vs.* 4.5 months	13.9 *vs.* 12.9 months
[88]	Untreated NSCLC	Nivolumab	158	<100, 100–242, >242 mut/Mb	WES	47% *vs.* 23%	9.7 *vs.* 3.6 months *vs.* 4.2 months	18.3 (TMB-high) *vs.* 12.7 months (TMB-intermediate and low)
[10]	Untreated NSCLC	Nivolumab	330	13 mut/Mb	NGS panel	45.3% (TMB-high)	7.2 *vs.* 3.2 months	NA
[89]	IC-naive NSCLC	Atezolizumab	119	16 mut/1.1 Mb	NGS panel	35.7% *vs.* 5.5%; *P* <0.0001	5.0 *vs.* 3.5 months	23.9 *vs.* 13.4 months
[90]	Advanced NSCLC	Pembrolizumab	164	175 mut	WES	23.5% *vs.* 16.9%; *P* = 0.009	4.2 *vs.* 3.7 months; *P* = 0.001	14.1 *vs.* 9.3 months; *P* = 0.006
[90]	Advanced NSCLC	Pembrolizumab	423	175 mut	WES	34.4% *vs.* 18.8%; *P* <0.001	6.3 *vs.* 4.1 months; *P* <0.001	21.9 *vs.* 12.0 months; *P* <0.001
[2]	Advanced NSCLC	Pembrolizumab	34	200 mut	WES	59% *vs.* 12%; *P* = 0.01	NR *vs.* 3.4 months	NA
[91]	Advanced NSCLC	PD-(L)1 inhibitor	50	6 mut/Mb by NGS	NGS panel	39.3% *vs.* 9.1%; *P* = 0.02	NR *vs.* 2.9 months; *P* = 0.01	NA
[92]	Advanced NSCLC	PD-(L)1 plus CTLA-4 inhibitors	852	10 mut/Mb	NGS panel	NA	5.6 *vs.* 5.1 months	
[93]	Advanced NSCLC	PD-(L)1 inhibitor	266	Continuous TMB	NGS panel	NA	HR: 0.96; *P* <0.001	HR: 0.97; *P* = 0.001
[94]	Advanced NSCLC	PD-(L)1 inhibitor	309	Median TMB	WES	Median TMB: 14.0 *vs* 7.4 (NR) mut Mb; *P* < 0.001	NA	
[95]	Advanced NSCLC	PD-(L)1 inhibitor	198	272 mut (25th percentile)	WES	Median TMB: 194 (IQR) *vs.* 131 (IQR); *P* = 0.0069	HR: 0.62; *P* = 0.004	HR: 0.62; *P* = 0.049
[96]	Advanced NSCLC	PD-(L)1 inhibitor	1,552	90th percentile	NGS panel	49.3% *vs.* 21.5%; *P* < 0.001	11.4 *vs.* 2.8 months; *P* = 0.001	36.5 *vs.* 12.4 months; *P* = 0.001
[97]	Advanced NSCLC	PD-(L)1 inhibitor	2,165	<10 *vs.* >20 mut/Mb	NGS panel		17.2 *vs.* 5.5 months; *P* = 0.001	26.9 *vs.* 10.1 months; *P* = 0.001
[95]	Advanced NSCLC with HLA-CH	PD-(L)1 inhibitor	47	HLA-corrected TMB	WES	59.4% *vs.* 20%; *P* = 0.00363	HR: 0.36; *P* = 0.007	HR: 0.29; *P* = 0.004
[8]	Pretreated SCLC	Nivolumab	133	<143, 143–247, >247 mut	WES	21.3% *vs.* 6.8% *vs.* 4.8%	1.4 *vs.* 1.3 *vs.* 1.3 months	5.4 *vs.* 3.9 *vs.* 3.1 months
[8]	Pretreated SCLC	Nivolumab plus ipilimumab	78			46.2% *vs.* 16.0% *vs.* 22.2%	7.8 *vs.* 1.3 *vs.* 1.5 months	22.0 *vs.* 3.6 *vs.* 3.4 months
[25]	Pretreated SCLC	PD-1 plus CTLA-4 inhibitor	52	9.68 mut/Mb	NGS panel	23.1% *vs.* 7.7%; *P* = 0.25	3.3 *vs.* 1.2 months; *P* = 0.01	10.4 *vs.* 2.5 months; *P* = 0.01

Table 3 Continued

Refs	Setting	ICI	N	TMB cut-off	Method	ORR	PFS	OS
[98]	Untreated melanoma	Ipilimumab	178	203.5 mut	WES	25.5% *vs.* 14.3%	HR: 0.60	HR: 0.52
[98]	Untreated melanoma	Nivolumab	176	203.5 mut	WES	62.1% *vs.* 31.5%	HR: 0.45	HR: 0.46
[98]	Untreated melanoma	Nivolumab plus ipilimumab	184	203.5 mut	WES	64.8% *vs.* 51.0%	HR: 0.55	HR: 0.53
[99]	Advanced pretreated melanoma	Ipilimumab, tremelimumab	25	100 mut	WES		TMB higher in PFS >6-months; $P = 0.010$	Longer in TMB high; $P = 0.04$
[99]	Advanced pretreated melanoma	Ipilimumab, tremelimumab	39	100 mut	WES		TMB higher in PFS >6-months; $P = 0.009$	No difference; $P = 0.10$
[100]	Advanced melanoma	Nivolumab	52	157 mut	WES	60.9% *vs.* 27.6%	HR: 0.34	HR: 0.69
[100]	Advanced melanoma	Atezolizumab	23	16 mut/Mb	NGS panel	50.0% *vs.* 0.0%	20 *vs.* 1 months; $P = 0.002$	NR *vs.* 7 months; $P = 0.0009$
[101]	BRAF V600-mutant melanoma	Atezolizumab (plus vemurafenib plus cobimetinib)	215	10 mut/Mb	NGS panel		16.6 *vs.* 11.1 months	
[102]	MMR-d tumors; MMR-p CRC	Pembrolizumab	15	Continuous TMB	WES	OR: 3.31; $P = 0.214$	HR: 0.63; $P = 0.021$	HR: 0.71; $P = 0.077$
[103]	MMR-d CRC and esophageal cancer	PD-1 plus CTLA-4 inhibitor	33	Median TMB	NGS panel	NA	NA	HR: 0.51; $P = 0.460$
[24]	MMR-d CRC	PD-1 inhibitor	22	40 mut/Mb	NGS panel	Median TMB: 54 *vs.* 29 mut/Mb; $P < 0.001$	NR *vs.* 6 months; $P = 0.001$	NR *vs.* NR; $P = 0.003$
[104]	Melanoma, NSCLC, CRC, bladder cancer, head and neck cancer, esophageal cancer, breast cancer, or RCC	ICI	995	13 mut/Mb	NGS panel		NA	HR: 0.61; $P < 0.0001$; HR: 0.69; $P = 0.042$
[81]	Pretreated urothelial carcinoma	Nivolumab	139	<85 *vs.* 85–169 *vs.* 169–569 (tertile)	WES	31.9% *vs.* 13.9% *vs.* 13.0%; OR: 2.13; $P < 0.05$	3.5 *vs.* 1.8 *vs.* 1.9 months; HR: 0.75; $P < 0.05$	11.6 *vs.* 9.7 *vs.* 5.7 months; HR: 0.73; $P < 0.05$
[16]	Pretreated urothelial carcinoma	Atezolizumab	150	Continuous TMB;	NGS panel	R: 12.4 *vs.* NR; 6.4 mut/Mb; $P < 0.001$	NA	NA
[105]	Pretreated gastric cancer	Toripalimab	54	12 mut/Mb	WES	3.3% *vs.* 7.1%; $P = 0.017$	2.5 months; $P = 0.055$	14.6 *vs.* 4.0 months; $P = 0.038$
[106]	Non-melanoma advanced solid tumors	ICI	99	20 mut/Mb by NGS	NGS panel	13% *vs.* 45%; $P = 0.027$	NA	NA

Table 3 Continued

Refs	Setting	ICI	N	TMB cut-off	Method	ORR	PFS	OS
[107]	Neoadjuvant urothelial carcinoma	Pembrolizumab	114	Continuous	NGS panel	OR: 1.06; *P* = 0.03	NA	NA
[108]	Advanced urothelial cancer	PD-(L)1 inhibitor	60	Continuous TMB	NGS panel	NA	HR: 0.95; *P* = 0.029	HR: 0.95; *P* = 0.032
[94]	Solid tumors >5% ARID1A mut	PD-(L)1 inhibitor	375	>20 mut/Mb	WES	NA	13.6 *vs.* 3.7 months; *P* <0.001	NA
[82]	Advanced solid tumors	Pembrolizumab	790	10 mut/Mb	NGS panel	29% *vs.* 6%	2.1 *vs.* 2.1 months	11.7 *vs.* 12.8 months
[109]	Advanced solid tumors	Pembrolizumab	119	102.5 mut	WES	R >NR; *P* <0.001	3.8 *vs.* 1.9 months	NA
[92]	Advanced solid tumors	PD-(L)1 and CTLA-4 inhibitors	3,775	10 mut/Mb	NGS panel		7.6 *vs.* 5.3 months	NA
[110]	Advanced solid tumors	ICI	151	20 mut/Mb	NGS panel	55% *vs.* 20%; *P* <0.001	12.8 *vs.* 3.3 months; *P* <0.001	NR *vs.* 16.3 months; *P* = 0.003
[111]	PD-L1-positive advanced solid tumors	Pembrolizumab	77	Continuous	WES	R >NR; *P* = 0.018	*P* = 0.051	NA

Only studies with at least one available outcome comparison between the TMB-high and TMB-low group or arm (by any study-specific definition) were included. In the ORR, PFS, and OS columns, all comparisons are reported as outcome in the TMB-high group *vs.* outcome in the TMB-low group.

CRC, colorectal cancer; CTLA, cytotoxic T-lymphocyte-associated protein 4; CUP, cancer of unknown primary; HLA LOH, HLA loss of heterozygosity; HR, hazard ratio; ICI, immune checkpoint inhibitors; Mb, megabase; MMR-d/p, mismatch repair deficient/proficient; Mut, mutations; NA, not available/not reported; NGS panel, next-generation sequencing-based panels for tumor mutational burden assessment; NR, non-responders; NR, not reached; NSCLC, non-small-cell lung cancer; OR, odds ratio; ORR, objective response rate; OS, overall survival; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; R, responders; RCC, renal cell carcinoma; SCLC, small cell lung cancer; TMB, tumor mutational burden; WES, whole exome sequencing.

(≥5%) NSCLC, patients with TMB-H (>243 mutations/exome) achieved the highest ORR and longest PFS with nivolumab[88]. The phase III CheckMate 227 trial prospectively evaluated nivolumab plus ipilimumab *vs.* chemotherapy in patients with TMB-H (≥10 mut/Mb), as assessed by FoundationOne CDx, and indicated significantly improved PFS under combination therapy *vs.* standard chemotherapy in patients with TMB elevation, although no OS benefit was reported[10]. In SCLC, the CheckMate 032 study confirmed that TMB-H patients (>248 mutations/exome) treated with nivolumab monotherapy or nivolumab-ipilimumab combination therapy exhibited higher ORR and longer PFS/OS than patients with TMB-L[8]. In melanoma, the phase III IMspire150 trial demonstrated that atezolizumab combined with vemurafenib and cobimetinib significantly improved PFS *vs.* placebo, vemurafenib, and cobimetinib in patients with BRAF V600-mutant TMB-H (≥10 mut/Mb) cancer[101]. Similarly, in the CheckMate 067 trial evaluating nivolumab alone or combined with ipilimumab *vs.* ipilimumab monotherapy, TMB-H (≥50th percentile) was associated with significantly improved clinical outcomes across all 3 treatment arms[98]. Growing evidence supports TMB's predictive value for immunotherapy benefits in other tumor types, including urothelial carcinoma[81,107], head and neck cancer[23], and gastrointestinal cancers (including microsatellite-stable tumors)[105,123]. On the basis of these findings, the KEYNOTE-158 study investigated the association between high tTMB (≥10 mut/Mb by F1CDx) and immunotherapy outcomes across 10 tumor types[82]. Among 790 patients, only a small subset (n = 102, 13%) met TMB-H criteria. The most common TMB-H tumor types included SCLC (33%), cervical squamous cell carcinoma (16%), and anal squamous cell carcinoma (14%). TMB-H patients showed superior ORR and PFS to TMB-low patients (ORR: 29% *vs.* 6%; 12-month PFS: 26% *vs.* 13%). These data led to FDA approval of pembrolizumab monotherapy for patients with TMB-H (≥10 mut/Mb) solid tumors that progress after prior therapies, for whom no alternative treatment options are available. Although KEYNOTE-158 supported pembrolizumab use in cancers with ≥10 mut/Mb, the therapy remains ineffective for some tumor types; therefore, a single TMB threshold cannot universally identify responders across all cancers (**Figure 1**).

Limitations of clinical applications and future exploration

Consensus 10: TMB has high heterogeneity among cancer types and has limitations in predicting immunotherapy effectiveness. We recommend combining TMB with other biomarkers to aid in judging the potential benefits of immunotherapy (recommended).

Limitations in clinical applications

TMB has many limitations in clinical applications. Although multiple studies have shown a positive correlation between TMB and ICI efficacy, in some tumor types, a low TMB may also indicate benefit from ICI therapy. For example, in patients with recurrent glioblastoma, a very low mutational burden is an important feature indicating benefit from PD-1/PD-L1 blockade therapy[124]. CD8+ T cell infiltration of tumors underpins favorable response to immunotherapy, yet in one study, CD8+ T cell counts were not found to be elevated in all cancers with TMB levels, and the ORR of patients with TMB-H tumors did not reach 20% in cancer types (e.g., breast cancer, prostate cancer, and glioma) in which CD8+ T cell levels were not significantly associated with neoantigen burden (ORR = 15.3%, 95% CI 9.2 to 23.4, P = 0.95)[19]. In addition, owing to factors such as the immunosuppressive tumor environment and poor immune cell infiltration, TMB-H might not be suitable as a predictor of efficacy in all cancer immunotherapies[125,126].

TMB values significantly differ among cancer types. Cancers associated with chronic mutagen exposure, such as lung cancer and melanoma, typically have a high TMB, whereas leukemia has a low TMB. The KEYNOTE-158 study has demonstrated a durable monotherapy benefit of pembrolizumab in patients with TMB ≥10 mut/Mb; however, cancer type representation was limited, and some common cancer types, such as colorectal cancer, were not included in the study. Moreover, a single TMB threshold is not applicable to all tumor types. TMB-H may be associated with high microsatellite instability in colorectal cancer. Schrock et al.[21] have estimated an optimal predictive threshold of 37–41 mut/Mb for TMB-H in patients with high microsatellite instability metastatic colorectal cancer treated with ICIs. Therefore, a reasonable TMB threshold should vary depending on the tumor type[127].

Moreover, the spatiotemporal heterogeneity of TMB is a major challenge in clinical applications. In a study by Kazdal[128], using a targeted sequencing panel to sequence multiple regions of lung adenocarcinoma and calculate TMB values, 30% of patients showed TMB differences among regions within the same tumor, and the maximum absolute TMB difference was 14.13 mut/Mb. Moreover, the TMB values changed over time. In an analysis of pre- and post-treatment paired data in 63 patients, TMB-L levels showed 73% concordance between primary and metastatic lesions, and only 1 of the 2 primary TMB-H lesions (50%) remained TMB-H after treatment (P = 0.32)[36].

Combined use of TMB with other biomarkers

Although TMB has emerged as an independent biomarker for immunotherapy response, combining TMB assessment with

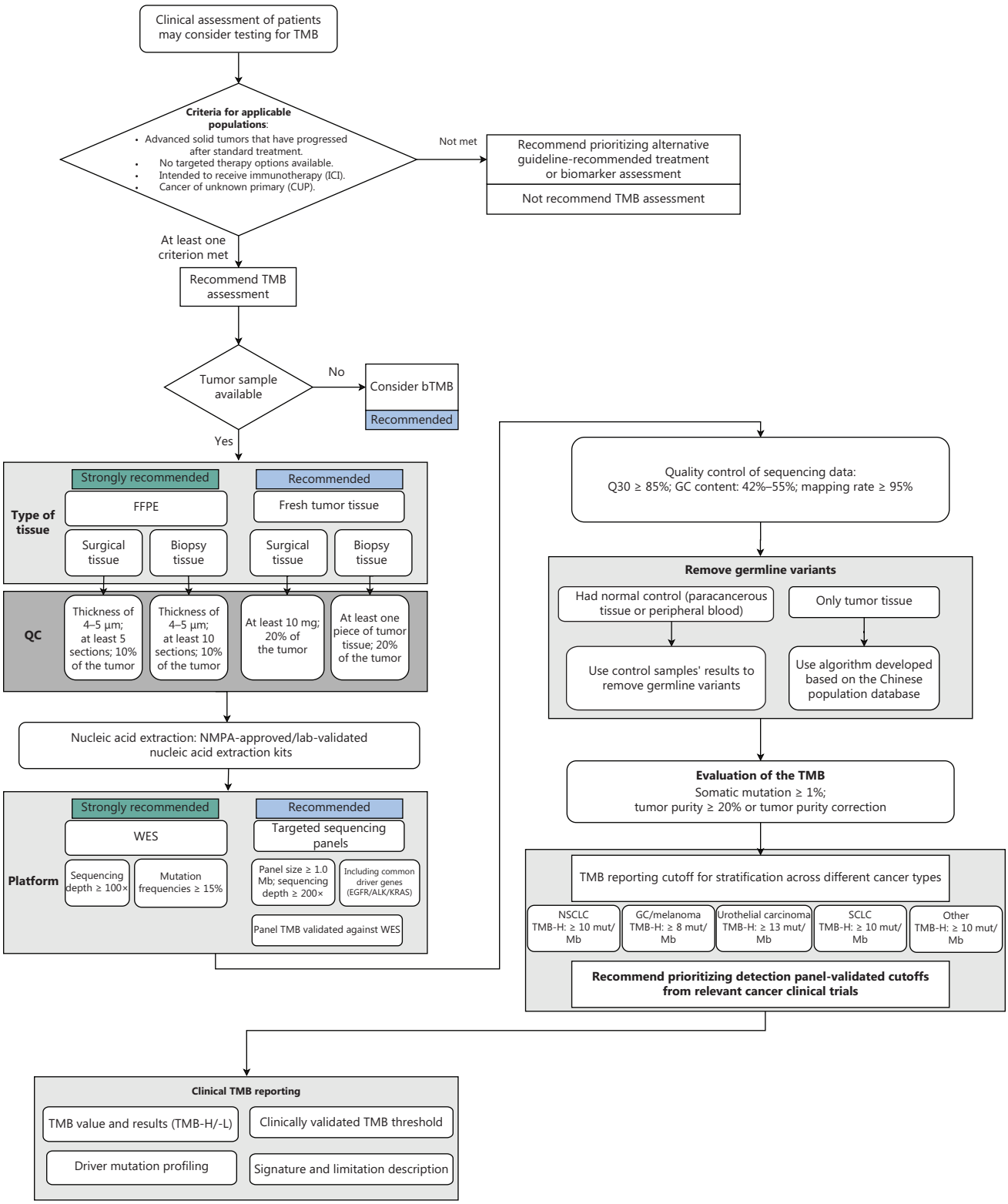


Figure 1 Flowchart of TMB testing, including sample preparation, quality control standards for sequencing data, TMB analysis principles, and key factors for TMB result reporting.

other biomarkers might be used to further stratify patients for clinical decision-making and potentially overcome several inherent limitations of using TMB alone as an ICI biomarker. Several biomarkers have been investigated alongside TMB, including PD-L1 expression, tumor-infiltrating lymphocytes, RNA gene expression profiles, blood-derived neutrophil-to-lymphocyte ratio (dNLR), and HLA variations (**Table 4**).

Generally, TMB and PD-L1 expression show weak but positive correlations[131,132]. However, this association may vary across tumor types[131,132]. Because both are potential independent predictors of immunotherapy efficacy, patients with tumors with elevated TMB and high PD-L1 expression levels might derive greater therapeutic benefits. In the CheckMate 026 study among patients with advanced NSCLC, those with TMB-H (≥243 somatic missense mutations/exome) and a PD-L1 tumor proportion score (TPS) ≥50% demonstrated the highest radiographic response probability to nivolumab, whereas patients with low/intermediate TMB and low PD-L1 expression (1–49%) showed the lowest ORR[88]. Subsequent randomized clinical trials, including KEYNOTE-010, KEYNOTE-042, IMpower 110, CheckMate 227, and MYSTIC, reported similar findings indicating improved outcomes with ICIs in patients exhibiting both TMB-H and PD-L1 expression[133,134]. However, variable definitions of TMB-H across those studies and uncertainty regarding optimal integration of these 2 biomarkers for predicting immunotherapy efficacy in NSCLC remain unresolved.

Beyond PD-L1 expression, TMB-H is often associated with elevated neoantigen burden and CD8+ tumor-infiltrating lymphocytes, and consequently, immunotherapy responsiveness[19,135]. In a recent pan-cancer analysis, in cancer types in which CD8+ T-cell levels positively correlate with neoantigen load (e.g., melanoma, lung, and bladder cancers), TMB-H correlated with a 39.8% ICI ORR, a value significantly higher than that observed for TMB-L (OR: 4.1, P <0.0001). In contrast, in cancers lacking this relationship (e.g., breast cancer, prostate cancer, and glioma), TMB-H tumors showed a lower ORR than observed in TMB-L tumors (OR: 0.46, P = 0.02)[135]. These data suggest that TMB's predictive accuracy partly depends on baseline immune cell infiltration levels, which vary across cancer types.

Emerging evidence highlights how germline and somatic HLA variations determine antigen presentation and cancer susceptibility to ICIs. Integrating HLA status with TMB might therefore enhance predictive capabilities. Somatic HLA-I loss of heterozygosity (LOH) exhibits a nonlinear relationship with TMB, thus suggesting alternative immune evasion mechanisms in TMB-H tumors[136]. In an ICI-treated non-squamous NSCLC cohort, intact HLA-I has been associated with significantly prolonged OS (HR: 0.65, P = 0.01), and outcome prediction improved in combination with TMB[137]. Studies on germline HLA polymorphisms have yielded mixed results: HLA-A*03 correlates with attenuated OS (HR: 1.48 per allele, P <0.001) across ICIs[137], whereas HLA-B44 supertypes are associated with prolonged OS, and HLA-B62 subtypes/somatic HLA-I LOH are associated with poor outcomes[138]. However, in a meta-analysis of 17 pembrolizumab trials (n >3,500), germline HLA profiles alone were insufficient to predict pembrolizumab efficacy, thus underscoring the complexity of HLA contributions to immune responses[139].

dNLR has emerged as a clinically accessible biomarker reflecting systemic inflammation-host immunity balance. A recent study has demonstrated that combining TMB with dNLR enhances predictive accuracy. Patients with high dNLR (top 20th percentile) and TMB-L (below median) showed the poorest outcomes, whereas those with low dNLR (bottom 80th percentile) and TMB-H (above median) achieved the highest ORR and survival, thus suggesting dNLR/TMB integration might optimize PD-(L)1 therapy selection[140].

Gene expression profiles (GEPs) and immune cell signatures are gaining traction as predictive tools. In a retrospective analysis of CheckMate 066/067 trials in melanoma, the highest ORR was observed with nivolumab (75.0%) and nivolumab-ipilimumab (66.7%) in patients with TMB-H and an elevated 4-gene inflammatory signature (CD274/CD8A/LAG3/STAT1), whereas 27.6% was observed with ipilimumab alone[98]. Similarly, biomarker analyses across 22 tumor types in pembrolizumab trials have indicated the highest response likelihood in tumors with TMB-H plus elevated T-cell-inflamed GEP or PD-L1 expression[110,111].

Collectively, these findings highlight the promise of combining TMB with complementary biomarkers, including PD-L1 expression, MSI status, immune infiltration, HLA variations, neoantigen landscape, and ploidy, to refine immunotherapy response prediction. Comprehensive multi-biomarker evaluation might guide treatment decisions, identify optimal beneficiaries, and advance personalized cancer care.

International *vs.* Chinese guidelines for TMB assessment

A comparative analysis of TMB assessment guidelines revealed both alignment and divergence between the Chinese consensus and major international standards, including those from ASCO, ESMO, FDA, and collaborative initiatives such as Friends of Cancer Research and QuIP (**Table 5**).

Regarding aligned consensus points, the International Consensus and the Chinese Expert Consensus are highly consistent, with core alignment in the following 5 aspects. First, both emphasize the urgent need for standardization of TMB testing, by clearly stating that TMB calculation, reporting, and analytical validation must be uniformly standardized to ensure consistency and reliability of results across laboratories and platforms[15,68,69,141]. Second, both recognize WES as the gold standard for TMB detection, while acknowledging that targeted NGS panels are more clinically practical alternative

Table 4 Studies on the combined use of TMB with other biomarkers

Refs.	Setting	Other marker	Cut-off of other marker	TMB cut-off	ORR of TMB-L	ORR of TMB-H
[97]	NSCLC	PD-L1	≥50%	≥19.00 mut/Mb	28.10%	56.50%
			1%–49%		18.70%	50.00%
			<1%		8.70%	46.70%
[88]	NSCLC	PD-L1	≥50%	≥243.00 mut/exome	34.00%	75.00%
			1%–49%		16.00%	32.00%
[129]	Pan-cancer	NLR	Low (bottom 80%)	Median or above	24.10%	38.30%
			High (top 20%)		16.80%	23.40%
[130]	Pan-cancer	CD8+ T cells	Positive	≥10.00 mut/Mb	15.30%	39.80%
			Negative		23.40%	15.30%
[98]	Melanoma	CD8+ T cell score	High	Median or above	43.80%	72.20%
			Low		16.70%	40.00%
[109]	Pan-cancer	GEP	High	≥102.50 mut/exome	11.00%	37.00%
			Low		0.00%	12.00%
[98]	Melanoma	GEP	High	Median or above	53.90%	75.00%
			Low		22.20%	54.60%

dNLR, blood-derived neutrophil-to-lymphocyte ratio; GEP, gene expression profile; NSCLC, non-small-cell lung cancer; ORR, objective response rate; PD-L1, programmed cell death-ligand 1; TMB, tumor mutational burden.

solutions. The international consensus indicates that 1–2 Mb is a common coverage range for panels, whereas the Chinese consensus further specifies that panel coverage must be ≥1.0 Mb (because coverage <1.0 Mb significantly decreases accuracy); moreover, both indicate that the gene content and coverage range of panels must be sufficient to generate reliable res ults;[15,68,69,141]. Third, both prioritize FFPE tumor tissue as the testing sample and indicate that the tumor cell content of samples must meet standards—with the threshold implicitly set at ≥20% in the international consensus and explicitly specified in the Chinese consensus—while also recommending strict quality control for DNA purity, concentration, and fragmentation degree. Fourth, both recommend detailed reporting of experimental parameters (including panel name/version, sequencing platform, and library construction method), types of variants included/excluded (e.g., whether nonsynonymous mutations or insertions/deletions are included), germline variant filtering criteria, and reference bases (e.g., reference genome version) to improve testing transparency and the comparability of the results.[15,68,69,141]. Fifth, both recognize that TMB cutoffs require clinical validation. Although both clearly state that cutoffs vary by cancer type and testing platform, both oppose the use of a unified pan-cancer TMB cutoff[15,68,69,141].

However, significant differences in key technical details and regulatory practices persist between guidelines. In terms of bioinformatics and variant filtering, the international consensus recommends relying on international public databases (e.g., gnomAD or 1000 Genomes) for germline variant filtering, and only mentions general variant calling tools such as MuTect/Mutect2, but does not address the limitations of population-specific databases or recommend tumor purity correction for TMB values. The Chinese consensus clearly indicates that international databases "are dominated by European populations and are not suitable for the Chinese population," thus emphasizing the need to use laboratory-built Chinese population databases or dedicated algorithms (e.g., the OncoTOP algorithm) to achieve high-precision germline filtering (without a need for paired tumor-normal samples). Simultaneously, the Chinese consensus recommends tumor purity correction for TMB values (which is necessary when the purity is <20%, to avoid underestimation/overestimation) and explicitly includes somatic variants with a detection frequency >5% to ensure accuracy[141]. In terms of practical clinical standards, the Chinese consensus focuses more on implementation details. First, it recommends use of nucleic acid extraction kits approved by the NMPA or verified by laboratories, and clearly specifies a DNA concentration ≥5.0 nmol/L. Second, it explicitly specifies the sequencing depth (≥200×), and it lists domestic panels approved by the NMPA or commonly used in clinical practice, including recommending panels covering

Table 5 Comparison between the international consensus and Chinese expert consensus.

Comparison dimension	Similarities	Differences: international consensus	Differences: Chinese consensus
1. TMB definition	1. Both define TMB as the average number of somatic nonsynonymous mutations per megabase (Mb) in a specific genomic region, reflecting tumor genomic mutational burden and neoantigen production.	1. Emphasizes mutation counting in coding DNA regions and does not specifically highlight population-specific considerations	1. Clearly notes that TMB assessment is affected by tumor type, sample quality, and detection platform; requires alignment with Chinese population characteristics (e.g., subsequent filtering database selection)
	2. Both confirm that TMB is associated with immune checkpoint inhibitor (ICI) efficacy and serves as a potential predictive biomarker for ICI response.	2. Provides no additional notes on factors affecting TMB assessment (e.g., tumor type or sample quality)	2. Explicitly links TMB to precise applications by clarifying its scope of use
2. Sample and nucleic acid handling	1. Both prioritize formalin-fixed paraffin-embedded (FFPE) tumor tissue as the detection sample.	1. Mentions only adherence to international regulations (CLIA, CAP, or EMA) without detailing FFPE sample parameters (e.g., fixation time or section thickness)	1. Details FFPE requirements: 10% neutral buffered formalin, 24 h fixation for surgical specimens/12 h for biopsies, 4–5 μm sections, priority for samples within 1 year
	2. Both stress that samples must meet tumor cell content requirements (to avoid low-purity interference).	2. Recommends that nucleic acid extraction meet laboratory accreditation standards without specific approval requirements for kits	2. Recommends NMPA-approved or laboratory-verified nucleic acid extraction kits
	3. Both require quality control for DNA purity, concentration, and fragmentation.		3. Specifies DNA concentration ≥5.0 nmol/L; recommends micro-nucleic acid fluorometry plus agarose gel electrophoresis for quality assessment
3. Assay and panel design	1. Both recognize whole-exome sequencing (WES) as the gold standard for TMB, with targeted panel sequencing as a clinical alternative.	1. Mentions 1–2 Mb as the most common panel coverage, without specifying a minimum threshold	1. Recommends panel coverage ≥1.0 Mb (accuracy significantly decreases at <1.0 Mb) and ideal range 1.0–3.0 Mb
	2. Both note that panel coverage affects TMB accuracy (small panels have more errors).	2. Compares hybrid capture *vs.* amplicon sequencing, but does not reference commercial panels	2. Specifies sequencing depth ≥200× (referenced from ESMO guidelines plus domestic validation)
	3. Both emphasize sequencing depth for low-frequency variant detection.	3. Does not specify a sequencing depth requirement	3. Lists domestic commercial panels (Geneseeq PRIME 425-gene, and Geneplus OncoD 1,021-gene) and requires panels to cover targeted therapy drivers plus ICI predictors
4. Algorithm and variant filtering	1. Both emphasize filtering germline variants (to avoid TMB overestimation), and recommend paired tumor-normal sequencing or population database filtering.	1. Recommends international databases (gnomAD, 1000 Genomes, or dbSNP) for germline filtering	1. Notes that international databases are Eurocentric and unsuitable for Chinese populations; recommends laboratory-built Chinese databases or the OncoTOP algorithm (no paired samples needed)
	2. Both require validation of bioinformatics pipelines (variant callers or genome alignment).	2. Mentions MuTect/Mutect2 as common variant callers but does not focus on population-specific database limitations	2. Requires inclusion of somatic variants with detection frequency >5%

Table 5 Continued

Comparison dimension	Similarities	Differences: international consensus	Differences: Chinese consensus
		3. Does not specify a requirement for tumor purity correction	3. Recommends TMB correction by tumor purity (purity ≥20% preferred; correction needed if <20%)
5. Reporting standards	1. Both require reporting assay method (panel/version, platform), TMB value (mut/Mb), variant inclusion/exclusion, and filtering criteria.	1. Focuses on methodological descriptions for cross-laboratory comparability, without providing clinical interpretation details	1. Recommends including patient clinical info (diagnosis and treatment history), sample collection details, and TMB ranking in TCGA
	2. Both emphasize reporting methodological limitations.	2. Requires tester and reviewer signatures without specifying physician qualification requirements	2. Recommends Molecular Tumor Board (MTB) for clinical decision-making
			3. Specifies that signing physicians should have a senior title/MD, pathology background, and training
			4. Provides a standardized template (95% CI, cancer-specific thresholds)
6. Clinical application recommendations	1. Both recommend TMB for advanced solid tumors progressing after standard treatment with no alternatives.	1. Cites the FDA-approved pembrolizumab threshold (TMB ≥10 mut/Mb) without cancer type differentiation	1. Proposes cancer-specific thresholds: ≥8 mut/Mb for lung cancer/melanoma/gastric cancer (according to domestic studies)
	2. Both note bTMB as a complement to tTMB (requires further validation).	2. Mentions only bTMB sample accessibility without clinical data	2. Cites domestic bTMB data (TRACELib002 trial: OS 24.5 months for bTMB ≥16 mut/Mb)
			3. Clarifies that bTMB cannot replace tTMB, yet needs prospective validation
7. Limitations and future directions	1. Both note TMB limitations: inter-cancer heterogeneity, spatiotemporal heterogeneity, and detection method variability.	1. Describes future directions focused on international harmonization (Friends of Cancer Research and QuIP project)	1. Describes future directions, emphasizing large-sample, multi-center prospective studies in Chinese populations to establish cancer-specific thresholds
	2. Both suggest combining TMB with other biomarkers (PD-L1, MSI) to improve prediction.	2. Does not include a cost-effectiveness analysis	2. Includes a cost-effectiveness analysis indicating that targeted panels are most suitable for China (lower cost and shorter turnaround than WES)
		3. Does not mention Chinese population-specific studies	3. Details combined biomarker strategies (TMB plus PD-L1 and dNLR) for precision prediction

both driver genes for targeted therapy (e.g., EGFR and ALK) and positive/negative predictors of ICI efficacy (e.g., PD-L1 and STK11). In contrast, the international consensus only mentions methodological differences between hybrid capture and amplicon sequencing, without specifying such practical standards. In terms of regulatory endorsement, in the international practice described in the international consensus, the U.S. FDA has approved specific TMB testing products (e.g., FoundationOne CDx) as companion diagnostic tools for pan-cancer ICI therapy; the corresponding regulatory practice in the Chinese consensus is that, although the NMPA has approved some domestic panels (e.g., Geneseeq PRIME) for TMB testing in specific cancer types (e.g., EGFR/ALK-negative non-squamous NSCLC), unlike the FDA, it has not yet approved pan-cancer TMB companion diagnostic products, thus resulting in differences in regulatory pathways compared with international practices[109].

Future exploration

Despite extensive discussions regarding the variability of TMB-H thresholds, primary data validating the efficacy of proposed cutoffs specifically in Chinese patient populations remain lacking. To establish reliable, clinically relevant TMB-H thresholds for specific tumor types, large-scale, prospective, multicenter studies should be conducted across China. These studies should enroll patients with diverse tumor types, use standardized TMB assessment methods (including WES and validated large gene panels), and correlate TMB values with clinical outcomes (e.g., response to immunotherapy, PFS, and OS). This approach would help identify tumor-specific TMB-H cutoffs applicable to the Chinese population that can predict treatment efficacy.

Research has indicated significant differences in the distribution of TMB and optimal cutoffs across cancer types (e.g., the median TMB in most solid tumors among Chinese patients is 4–6 mutations per megabase, and higher values are observed in colorectal cancer and lung cancer)[46]. Therefore, validation studies must be stratified by tumor type to ensure clinical relevance.

Communicating TMB results and their significance to patients

Effectively communicating TMB results is critical for patient selection, education, and informed consent in the context of immunotherapy. Given the complexity and evolving nature of TMB as a biomarker, clinicians must ensure that patients understand both the potential and limitations of TMB-guided treatment decisions. Key considerations in this communication include (1) clarifying the fundamental nature of TMB, specifically, that it quantifies the number of mutations in tumor DNA and might predict responsiveness to certain immunotherapies, and (2) elucidating its predictive utility, including that although TMB-H can indicate elevated likelihood of ICI benefit, it does not ensure a response, because outcomes are influenced by multiple additional factors[15,142]. An equally important consideration is addressing limitations and uncertainties, such as the lack of standardization in TMB testing, discrepancies between tissue- and blood-based assays, and the ongoing need for validation of cut-offs and methods. Furthermore, TMB results should be contextualized within the broader framework of other biomarkers (e.g., PD-L1 expression)[15,142], tumor type, and available treatment options. Practical aspects, including the turnaround time for TMB testing, the potential need for repeat biopsies, and implications for treatment timing, must also be discussed. Finally, supporting informed consent requires ensuring that patients understand the potential benefits, risks, and uncertainties associated with TMB-guided therapy, to facilitate shared decision-making.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

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References

1. Lawrence MS, Stojanov P, Polak P, Kryukov GV, Cibulskis K, Sivachenko A, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature. 2013; 499(7457): 214-8.
2. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015; 348(6230): 124-8.
3. Chalmers ZR, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med. 2017; 9(1): 34.
4. Xu C, Hao Y, Wang D, Zhang S, Wang W, Wang Q, et al. ECLUNG consensus/guidelines development principles and methods (2024 edition). Intelligent Pharmacy. 2025; 3(2): 141-2.
5. Vega DM, Yee LM, McShane LM, Williams PM, Chen L, Vilimas T, et al. Aligning tumor mutational burden (TMB) quantification across diagnostic platforms: phase II of the Friends of Cancer Research TMB Harmonization Project. Ann Oncol. 2021; 32(12): 1626-36.
6. Budczies J, Kazdal D, Menzel M, Beck S, Kluck K, Altbürger C, et al. Tumour mutational burden: clinical utility, challenges and emerging improvements. Nat Rev Clin Oncol. 2024; 21(10): 725-42.
7. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med. 2014; 371(23): 2189-99.
8. Hellmann MD, Callahan MK, Awad MM, Calvo E, Ascierto PA, Atmaca A, et al. Tumor mutational burden and efficacy of nivolumab monotherapy and in combination with ipilimumab in small-cell lung cancer. Cancer Cell. 2018; 33(5): 853-861.e4.
9. Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. N Engl J Med. 2017; 377(25): 2500-1.
10. Hellmann MD, Ciuleanu TE, Pluzanski A, Lee JS, Otterson GA, Audigier-Valette C, et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. N Engl J Med. 2018; 378(22): 2093-104.
11. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet. 2019; 51(2): 202-6.
12. Valero C, Lee M, Hoen D, Wang J, Nadeem Z, Patel N, et al. The association between tumor mutational burden and prognosis is dependent on treatment context. Nat Genet. 2021; 53(1): 11-5.
13. Buttner R, Longshore JW, Lopez-Rios F, Merkelbach-Bruse S, Normanno N, Rouleau E, et al. Implementing TMB measurement in clinical practice: considerations on assay requirements. ESMO Open. 2019; 4(1): e000442.
14. Vilimas T. Measuring tumor mutational burden using whole-exome sequencing. Methods Mol Biol. 2020; 2055: 63-91.
15. Stenzinger A, Allen JD, Maas J, Stewart MD, Merino DM, Wempe MM, et al. Tumor mutational burden standardization initiatives: recommendations for consistent tumor mutational burden assessment in clinical samples to guide immunotherapy treatment decisions. Genes Chromosomes Cancer. 2019; 58(8): 578-88.
16. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet. 2016; 387(10031): 1909-20.
17. Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. Lancet. 2017; 389(10064): 67-76.
18. Riaz N, Havel JJ, Makarov V, Desrichard A, Urba WJ, Sims JS, et al. Tumor and microenvironment evolution during immunotherapy with nivolumab. Cell. 2017; 171(4): 934-949.e16.
19. McGrail DJ, Pilié PG, Rashid NU, Voorwerk L, Slagter M, Kok M, et al. High tumor mutation burden fails to predict immune checkpoint blockade response across all cancer types. Ann Oncol. 2021; 32(5): 661-72.
20. Ready N, Hellmann MD, Awad MM, Otterson GA, Gutierrez M, Gainor JF, et al. First-line nivolumab plus ipilimumab in advanced non-small-cell lung cancer (CheckMate 568): outcomes by programmed death ligand 1 and tumor mutational burden as biomarkers. J Clin Oncol. 2019; 37(12): 992-1000.
21. Schrock AB, Ouyang C, Sandhu J, Sokol E, Jin D, Ross JS, et al. Tumor mutational burden is predictive of response to immune checkpoint inhibitors in MSI-high metastatic colorectal cancer. Ann Oncol. 2019; 30(7): 1096-103.
22. Ross JS, Sokol ES, Moch H, Mileshkin L, Baciarello G, Losa F, et al. Comprehensive genomic profiling of carcinoma of unknown primary origin: retrospective molecular classification considering the CUPISCO study design. Oncologist. 2021; 26(3): e394-402.
23. Hanna GJ, Lizotte P, Cavanaugh M, Kuo FC, Shivdasani P, Frieden A, et al. Frameshift events predict anti-PD-1/L1 response in head and neck cancer. JCI Insight. 2018; 3(4): e98811.
24. De Mattos-Arruda L, Siravegna G. How to use liquid biopsies to treat patients with cancer. ESMO Open. 2021; 6(2): 100060.
25. Ricciuti B, Kravets S, Dahlberg SE, Umeton R, Albayrak A, Subegdjo SJ, et al. Use of targeted next generation sequencing to characterize tumor mutational burden and efficacy of immune checkpoint inhibition in small cell lung cancer. J Immunother Cancer. 2019; 7(1): 87.
26. Pouyiourou M, Kraft BN, Wohlfromm T, Stahl M, Kubuschok B, Löffler H, et al. Nivolumab and ipilimumab in recurrent or refractory cancer of unknown primary: a phase II trial. Nat Commun. 2023; 14(1): 6761.
27. Campesato LF, Barroso-Sousa R, Jimenez L, Correa BR, Sabbaga J, Hoff PM, et al. Comprehensive cancer-gene panels can be used to estimate mutational load and predict clinical benefit to PD-1 blockade in clinical practice. Oncotarget. 2015; 6(33): 34221-7.

28. Johnson DB, Frampton GM, Rioth MJ, Yusko E, Xu Y, Guo X, et al. Targeted next generation sequencing identifies markers of response to PD-1 blockade. Cancer Immunol Res. 2016; 4(11): 959-67.
29. Roszik J, Haydu LE, Hess KR, Oba J, Joon AY, Siroy AE, et al. Novel algorithmic approach predicts tumor mutation load and correlates with immunotherapy clinical outcomes using a defined gene mutation set. BMC Med. 2016; 14(1): 168.
30. Zhuang W, Ma J, Chen X, Wang G, Lu J, Chen Y, et al. The tumor mutational burden of chinese advanced cancer patients estimated by a 381-cancer-gene panel. J Cancer. 2018; 9(13): 2302-7.
31. Li H, Meng L, Wang H, Cui L, Sheng H, Zhao P, et al. Precise identification of somatic and germline variants in the absence of matched normal samples. Brief Bioinform. 2024; 26(1): bbae677.
32. Feliubadalo L, Tonda R, Gausachs M, Trotta JR, Castellanos E, López-Doriga A, et al. Benchmarking of whole exome sequencing and Ad Hoc designed panels for genetic testing of hereditary cancer. Sci Rep. 2017; 7: 37984.
33. Garofalo A, Sholl L, Reardon B, Taylor-Weiner A, Amin-Mansour A, Miao D, et al. The impact of tumor profiling approaches and genomic data strategies for cancer precision medicine. Genome Med. 2016; 8(1): 79.
34. Budczies J, Allgauer M, Litchfield K, Rempel E, Christopoulos P, Kazdal D, et al. Optimizing panel-based tumor mutational burden (TMB) measurement. Ann Oncol. 2019; 30(9): 1496-506.
35. Endris V, Buchhalter I, Allgauer M, Rempel E, Lier A, Volckmar AL, et al. Measurement of tumor mutational burden (TMB) in routine molecular diagnostics: in silico and real-life analysis of three larger gene panels. Int J Cancer. 2019; 144(9): 2303-12.
36. Zhou KI, Peterson B, Serritella A, Thomas J, Reizine N, Moya S, et al. Spatial and temporal heterogeneity of PD-L1 expression and tumor mutational burden in gastroesophageal adenocarcinoma at baseline diagnosis and after chemotherapy. Clin Cancer Res. 2020; 26(24): 6453-63.
37. Baras AS, Stricker T, on behalf of the AACR Project GENIE Consortium. Abstract LB-105: Characterization of total mutational burden in the GENIE cohort: small and large panels can provide TMB information but to varying degrees. Cancer Res. 2017; 77(suppl 13): LB-105.
38. Fang W, Ma Y, Yin JC, Hong S, Zhou H, Wang A, et al. Comprehensive genomic profiling identifies novel genetic predictors of response to anti-PD-(L)1 therapies in non-small cell lung cancer. Clin Cancer Res. 2019; 25(16): 5015-26.
39. Buchhalter I, Rempel E, Endris V, Allgäuer M, Neumann O, Volckmar AL, et al. Size matters: dissecting key parameters for panel-based tumor mutational burden analysis. Int J Cancer. 2019; 144(4): 848-58.
40. Ai X, Jia B, He Z, Zhang J, Zhuo M, Zhao J, et al. Noninvasive early identification of durable clinical benefit from immune checkpoint inhibition: a prospective multicenter study (NCT04566432). Signal Transduct Target Ther. 2024; 9(1): 350.
41. Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol. 2013; 31(11): 1023-31.
42. Thomas SJ, Snowden JA, Zeidler MP, Danson SJ. The role of JAK/STAT signalling in the pathogenesis, prognosis and treatment of solid tumours. Br J Cancer. 2015; 113(3): 365-71.
43. Campbell BB, Light N, Fabrizio D, Zatzman M, Fuligni F, de Borja R, et al. Comprehensive analysis of hypermutation in human cancer. Cell. 2017; 171(5): 1042-1056. e10.
44. Rajendran BK, Deng CX. Characterization of potential driver mutations involved in human breast cancer by computational approaches. Oncotarget. 2017; 8(30): 50252-72.
45. Wu H, Yu Z, Liu Y, Guo L, Teng L, Guo L, et al. Genomic characterization reveals distinct mutation landscapes and therapeutic implications in neuroendocrine carcinomas of the gastrointestinal tract. Cancer Commun (Lond). 2022; 42(12): 1367-86.
46. Zhao L, Ren Y, Zhang G, Zheng K, Wang J, Sha H, et al. Single-arm study of camrelizumab plus apatinib for patients with advanced mucosal melanoma. J Immunother Cancer. 2024; 12(6): e008611.
47. Cheng DT, Mitchell TN, Zehir A, Shah RH, Benayed R, Syed A, et al. Memorial sloan kettering-integrated mutation profiling of actionable cancer targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagn. 2015; 17(3): 251-64.
48. Xu J, Wan R, Cai Y, Cai S, Wu L, Li B, et al. Circulating tumor DNA-based stratification strategy for chemotherapy plus PD-1 inhibitor in advanced non-small-cell lung cancer. Cancer Cell. 2024; 42(9): 1598-1613.
49. Mfumbilwa ZA, Simons M, Ramaekers B, Retèl VP, Mankor JM, Groen HJM, et al. Exploring the cost effectiveness of a whole-genome sequencing-based biomarker for treatment selection in patients with advanced lung cancer ineligible for targeted therapy. Pharmacoeconomics. 2024; 42(4): 419-34.
50. Li WQ, Li LY, Bai RL, Qian L, Chen NF, Cui JW. Cost-effectiveness of programmed cell death ligand 1 testing and tumor mutational burden testing of immune checkpoint inhibitors for advanced non-small cell lung cancer. Chin Med J. 2020; 133(21): 2630-2.
51. Torre-Castro J, Rodríguez M, Alonso-Alonso R, Mendoza Cembranos MD, Díaz-Alejo JF, Rebollo-González M, et al. LT and SOX9 expression are associated with gene sets that distinguish Merkel cell polyomavirus (MCPyV)-positive and MCPyV-negative Merkel cell carcinoma. Br J Dermatol. 2024; 190(6): 876-84.
52. Orvieto R, Nayshool O, Cohen L, Yung Y, Aizer A, Saar EG, et al. Impact of aging on gene expression in human oocytes: a comparative analysis of young and older patients. Reprod Biol Endocrinol. 2025; 23(1): 111.
53. An Y, Wu J, Chen Y, Li S. Comprehensive analysis of alternative splicing in Rosa roxburghii Tratt reveals its role in flavonoid synthesis. Front Plant Sci. 2025; 16: 1627126.
54. Johnson CE, Ran X, Wrobel J, Davidson NR, Greene CS, Epstein MP, et al. An analytic pipeline to obtain reliable genetic ancestry estimates from tumor-derived RNA sequencing data. Cancer Epidemiol Biomarkers Prev. 2025; 34(9): 1593-9.

55. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009; 25(14): 1754-60.
56. Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 2009; 10(3): R25.
57. Li R, Li Y, Kristiansen K, Wang J. SOAP: short oligonucleotide alignment program. Bioinformatics. 2008; 24(5): 713-4.
58. Homer N, Merriman B, Nelson SF. BFAST: an alignment tool for large scale genome resequencing. PLoS One. 2009; 4(11): e7767.
59. Bentley DR, Balasubramanian S, Swerdlow HP, Smith GP, Milton J, Brown CG, et al. Accurate whole human genome sequencing using reversible terminator chemistry. Nature. 2008; 456(7218): 53-9.
60. Li H, Ruan J, Durbin R. Mapping short DNA sequencing reads and calling variants using mapping quality scores. Genome Res. 2008; 18(11): 1851-8.
61. Rumble SM, Lacroute P, Dalca AV, Fiume M, Sidow A, Brudno M. SHRiMP: accurate mapping of short color-space reads. PLoS Comput Biol. 2009; 5(5): e1000386.
62. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L, et al. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res. 2012; 22(3): 568-76.
63. Kim S, Scheffler K, Halpern AL, Bekritsky MA, Noh E, Källberg M, et al. Strelka2: fast and accurate calling of germline and somatic variants. Nat Methods. 2018; 15(8): 591-94.
64. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010; 20(9): 1297-303.
65. Adhisantoso YG, Voges J, Rohlfing C, Tunev V, Ohm J-R, Ostermann J. GVC: efficient random access compression for gene sequence variations. BMC Bioinformatics. 2023; 24(1): 121.
66. Chan TA, Yarchoan M, Jaffee E, Swanton C, Quezada SA, Stenzinger A, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. Ann Oncol. 2019; 30(1): 44-56.
67. Wu HX, Wang ZX, Zhao Q, Wang F, Xu RH. Designing gene panels for tumor mutational burden estimation: the need to shift from 'correlation' to 'accuracy'. J Immunother Cancer. 2019; 7(1): 206.
68. Merino DM, McShane LM, Fabrizio D, Funari V, Chen SJ, White JR, et al. Establishing guidelines to harmonize tumor mutational burden (TMB): in silico assessment of variation in TMB quantification across diagnostic platforms: phase I of the Friends of Cancer Research TMB Harmonization Project. J Immunother Cancer. 2020; 8(1): e000147.
69. Stenzinger A, Endris V, Budczies J, Merkelbach-Bruse S, Kazdal D, Dietmaier W, et al. Harmonization and standardization of panel-based tumor mutational burden measurement: real-world results and recommendations of the quality in pathology study. J Thorac Oncol. 2020; 15(7): 1177-89.
70. Anagnostou V, Niknafs N, Marrone K, Bruhm DC, White JR, Naidoo J, et al. Multimodal genomic features predict outcome of immune checkpoint blockade in non-small-cell lung cancer. Nat Cancer. 2020; 1(1): 99-111.
71. Parikh K, Huether R, White K, Hoskinson D, Beaubier N, Dong H, et al. Tumor mutational burden from tumor-only sequencing compared with germline subtraction from paired tumor and normal specimens. JAMA Netw Open. 2020; 3(2): e200202.
72. Chang H, Sasson A, Srinivasan S, Golhar R, Greenawalt DM, Geese WJ, et al. Bioinformatic methods and bridging of assay results for reliable tumor mutational burden assessment in non-small-cell lung cancer. Mol Diagn Ther. 2019; 23(4): 507-20.
73. Zehir A, Benayed R, Shah RH, Syed A, Middha S, Kim HR, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med. 2017; 23(6): 703-13.
74. Ramalingam SS, Hellmann MD, Awad MM, Borghaei H, Gainor J, Brahmer J, et al. Abstract CT078: Tumor mutational burden (TMB) as a biomarker for clinical benefit from dual immune checkpoint blockade with nivolumab (nivo) + ipilimumab (ipi) in first-line (1L) non-small cell lung cancer (NSCLC): identification of TMB cutoff from CheckMate 568. Cancer Res. 2018; 78(suppl 13): CT078.
75. Lu Z, Yang S, Luo X, Shi Y, Lee JS, Deva S, et al. The combination of gene hyperamplification and PD-L1 expression as a biomarker for the clinical benefit of tislelizumab in gastric/gastroesophageal junction adenocarcinoma. Gastric Cancer. 2022; 25(5): 943-55.
76. Yu Y, Shen L, Ji W, Lu S. Standardization of pleural effusion-based tumor mutation burden (TMB) estimation using capture-based targeted sequencing. Ann Transl Med. 2021; 9(2): 140.
77. Kowanetz M, Zou W, Shames D, Cummings C, Rizvi N, Spira A, et al. OA20.01 Tumor mutation burden (TMB) is associated with improved efficacy of atezolizumab in 1L and 2L+ NSCLC patients. J Thorac Oncol. 2017; 12(1): S321-2.
78. Hellmann MD, Nathanson T, Rizvi H, Creelan BC, Sanchez-Vega F, Ahuja A, et al. Genomic features of response to combination immunotherapy in patients with advanced non-small-cell lung cancer. Cancer Cell. 2018; 33(5): 843-852.e4.
79. Paz-Ares L, Ciuleanu TE, Cobo M, Schenker M, Zurawski B, Menezes J, et al. First-line nivolumab plus ipilimumab combined with two cycles of chemotherapy in patients with non-small-cell lung cancer (CheckMate 9LA): an international, randomised, open-label, phase 3 trial. Lancet Oncol. 2021; 22(2): 198-211.
80. Barroso-Sousa R, Keenan TE, Pernas S, Exman P, Jain E, Garrido-Castro AC, et al. Tumor mutational burden and PTEN alterations as molecular correlates of response to PD-1/L1 blockade in metastatic triple-negative breast cancer. Clin Cancer Res. 2020; 26(11): 2565-72.
81. Galsky MD, Saci A, Szabo PM, Han GC, Grossfeld G, Collette S, et al. Nivolumab in patients with advanced platinum-resistant urothelial carcinoma: efficacy, safety, and biomarker analyses with extended follow-up from CheckMate 275. Clin Cancer Res. 2020; 26(19): 5120-8.
82. Marabelle A, Fakih M, Lopez J, Shah M, Shapira-Frommer R, Nakagawa K, et al. Association of tumour mutational burden with

outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. Lancet Oncol. 2020; 21(10): 1353-65.
83. Gatalica Z, Xiu J, Swensen J, Vranic S. Comprehensive analysis of cancers of unknown primary for the biomarkers of response to immune checkpoint blockade therapy. Eur J Cancer. 2018; 94: 179-86.
84. Garassino M, Rodriguez-Abreu D, Gadgeel S, Esteban E, Felip E, Speranza G, et al. OA04.06 Evaluation of TMB in KEYNOTE-189: pembrolizumab plus chemotherapy vs placebo plus chemotherapy for nonsquamous NSCLC. J Thorac Oncol. 2019; 14(10): S216-7.
85. Langer C, Gadgeel S, Borghaei H, Patnaik A, Powell S, Gentzler R, et al. OA04.05 KEYNOTE-021: TMB and outcomes for carboplatin and pemetrexed with or without pembrolizumab for nonsquamous NSCLC. J Thorac Oncol. 2019; 14(10): S216.
86. Li Y, Ji L, Zhang Y, Zhang J, Reuben A, Zeng H, et al. The combination of tumor mutational burden and T-cell receptor repertoire predicts the response to immunotherapy in patients with advanced non-small cell lung cancer. MedComm (2020). 2024; 5(6): e604.
87. Herbst RS, Giaccone G, de Marinis F, Reinmuth N, Vergnenegre A, Barrios CH, et al. Atezolizumab for first-line treatment of PD-L1-selected patients with NSCLC. N Engl J Med. 2020; 383(14): 1328-39.
88. Carbone DP, Reck M, Paz-Ares L, Creelan B, Horn L, Steins M, et al. First-line nivolumab in stage IV or recurrent non-small-cell lung cancer. N Engl J Med. 2017; 376(25): 2415-26.
89. Kim ES, Velcheti V, Mekhail T, Yun C, Shagan SM, Hu S, et al. Blood-based tumor mutational burden as a biomarker for atezolizumab in non-small cell lung cancer: the phase 2 B-F1RST trial. Nat Med. 2022; 28(5): 939-45.
90. Herbst RS, Lopes G, Kowalski DM, Nishio M, Wu YL, de Castro Junior G, et al. LBA79 – Association between tissue TMB (tTMB) and clinical outcomes with pembrolizumab monotherapy (pembro) in PD-L1-positive advanced NSCLC in the KEYNOTE-010 and -042 trials. Ann Oncol. 2019; 30: v916-7.
91. Wang Z, Duan J, Cai S, Han M, Dong H, Zhao J, et al. Assessment of blood tumor mutational burden as a potential biomarker for immunotherapy in patients with non-small cell lung cancer with use of a next-generation sequencing cancer gene panel. JAMA Oncol. 2019; 5(5): 696-702.
92. Sturgill EG, Misch A, Jones CC, Luckett D, Fu X, Schlauch D, et al. Discordance in tumor mutation burden from blood and tissue affects association with response to immune checkpoint inhibition in real-world settings. Oncologist. 2022; 27(3): 175-82.
93. Ricciuti B, Recondo G, Spurr LF, Li YY, Lamberti G, Venkatraman D, et al. Impact of DNA damage response and repair (DDR) gene mutations on efficacy of PD-(L)1 immune checkpoint inhibition in non-small cell lung cancer. Clin Cancer Res. 2020; 26(15): 4135-42.
94. Okamura R, Kato S, Lee S, Jimenez RE, Sicklick JK, Kurzrock R. ARID1A alterations function as a biomarker for longer progression-free survival after anti-PD-1/PD-L1 immunotherapy. J Immunother Cancer. 2020; 8(1): e000438.
95. Shim JH, Kim HS, Cha H, Kim S, Kim TM, Anagnostou V, et al. HLA-corrected tumor mutation burden and homologous recombination deficiency for the prediction of response to PD-(L)1 blockade in advanced non-small-cell lung cancer patients. Ann Oncol. 2020; 31(7): 902-11.
96. Ricciuti B, Wang X, Alessi JV, Rizvi H, Mahadevan NR, Li YY, et al. Association of high tumor mutation burden in non-small cell lung cancers with increased immune Infiltration and improved clinical outcomes of PD-L1 blockade across PD-L1 expression levels. JAMA Oncol. 2022; 8(8): 1160-8.
97. Huang RSP, Carbone DP, Li G, Schrock A, Graf RP, Zhang L, et al. Durable responders in advanced NSCLC with elevated TMB and treated with 1L immune checkpoint inhibitor: a real-world outcomes analysis. J Immunother Cancer. 2023; 11(1): e005801.
98. Hodi FS, Wolchok JD, Schadendorf D, Larkin J, Long GV, Qian X, et al. TMB and inflammatory gene expression associated with clinical outcomes following immunotherapy in advanced melanoma. Cancer Immunol Res. 2021; 9(10): 1202-13.
99. Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science. 2015; 350(6257): 207-11.
100. Hamid O, Bruno R, Fasso M, O'Hear C, Wu B. Safety, clinical activity, and biological correlates of response in patients with metastatic melanoma: results from a phase I trial of atezolizumab-response. Clin Cancer Res. 2020; 26(10): 2436.
101. Robert C, Lewis KD, Gutzmer R, Stroyakovskiy D, Gogas H, Protsenko S, et al. Biomarkers of treatment benefit with atezolizumab plus vemurafenib plus cobimetinib in BRAF(V600) mutation-positive melanoma. Ann Oncol. 2022; 33(5): 544-55.
102. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015; 372(26): 2509-20.
103. Mandal R, Samstein RM, Lee KW, Havel JJ, Wang H, Krishna C, et al. Genetic diversity of tumors with mismatch repair deficiency influences anti-PD-1 immunotherapy response. Science. 2019; 364(6439): 485-91.
104. Zheng M. Tumor mutation burden for predicting immune checkpoint blockade response: the more, the better. J Immunother Cancer. 2022; 10(1): e003087.
105. Wang F, Wei XL, Wang FH, Xu N, Shen L, Dai GH, et al. Safety, efficacy and tumor mutational burden as a biomarker of overall survival benefit in chemo-refractory gastric cancer treated with toripalimab, a PD-1 antibody in phase Ib/II clinical trial NCT02915432. Ann Oncol. 2019; 30(9): 1479-86.
106. Boichard A, Pham TV, Yeerna H, Goodman A, Tamayo P, Lippman S, et al. APOBEC-related mutagenesis and neo-peptide hydrophobicity: implications for response to immunotherapy. Oncoimmunology. 2019; 8(3): 1550341.
107. Necchi A, Raggi D, Gallina A, Madison R, Colecchia M, Lucianò R, et al. Updated results of PURE-01 with preliminary activity of neoadjuvant pembrolizumab in patients with muscle-invasive bladder carcinoma with variant histologies. Eur Urol. 2020; 77(4): 439-46.

108. Teo MY, Seier K, Ostrovnaya I, Regazzi AM, Kania BE, Moran MM, et al. Alterations in DNA damage response and repair genes as potential marker of clinical benefit from PD-1/PD-L1 blockade in advanced urothelial cancers. J Clin Oncol. 2018; 36(17): 1685-94.
109. Cristescu R, Mogg R, Ayers M, Albright A, Murphy E, Yearley J, et al. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. Science. 2018; 362(6411): eaar3593.
110. Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, Miller V, et al. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. Mol Cancer Ther. 2017; 16(11): 2598-608.
111. Ott PA, Bang YJ, Piha-Paul SA, Razak ARA, Bennouna J, Soria JC, et al. T-Cell-inflamed gene-expression profile, programmed death ligand 1 expression, and tumor mutational burden predict efficacy in patients treated with pembrolizumab across 20 cancers: KEYNOTE-028. J Clin Oncol. 2019; 37(4): 318-27.
112. Wang Z, Duan J, Wang G, Zhao J, Xu J, Han J, et al. Allele frequency-adjusted blood-based tumor mutational burden as a predictor of overall survival for patients with NSCLC treated with PD-(L)1 inhibitors. J Thorac Oncol. 2020; 15(4): 556-67.
113. Huang Y, Liu Q, Min X, Yang Y, Pei G, Wang S, et al. DNA Input, sequencing depth, and maximum somatic allele frequency may affect the concordance between blood TMB and tumor TMB. J Clin Oncolo. 2019; 37: e13163.
114. Schuurbiers M, Huang Z, Saelee S, Javey M, de Visser L, van den Broek D, et al. Biological and technical factors in the assessment of blood-based tumor mutational burden (bTMB) in patients with NSCLC. J Immunother Cancer. 2022; 10: e004064.
115. He J, Kalinava N, Doshi P, Pavlick DC, Albacker LA, Ebot EM, et al. Evaluation of tissue- and plasma-derived tumor mutational burden (TMB) and genomic alterations of interest in CheckMate 848, a study of nivolumab combined with ipilimumab and nivolumab alone in patients with advanced or metastatic solid tumors with high TMB. J Immunother Cancer. 2023; 11: e007339.
116. McNamara M, Jacobs T, Lamarca A, Hubner R, Valle J, Amir E. Impact of high tumor mutational burden in solid tumors and challenges for biomarker application. Cancer Treat Rev. 2020; 89: 102084.
117. Galvano A, Gristina V, Malapelle U, Pisapia P, Pepe F, Barraco N, et al. The prognostic impact of tumor mutational burden (TMB) in the first-line management of advanced non-oncogene addicted non-small-cell lung cancer (NSCLC): a systematic review and meta-analysis of randomized controlled trials. ESMO Open. 2021; 6: 100124.
118. Ahmed J, Das B, Shin S, Chen A. Challenges and future directions in the management of tumor mutational burden-high (TMB-H) advanced solid malignancies. Cancers. 2023; 15: 5841.
119. Lowe DR, Forson B, Butler M, Konigshofer Y, Huang C, Clement O, et al. Abstract 537: Development of blood TMB (bTMB) reference materials for validation of cfDNA-based targeted NGS assays that measure tumor mutational burden (TMB) in patient blood samples. Cancer Res. 2022; 82(suppl 12): 537.
120. Yang X, Zhu Z. Blood-based TMB detection and dynamic monitor in local advanced non-small cell lung cancer (NSCLC) patients. J Clin Oncol. 2019; 37: e20039.
121. Si H, Kuziora M, Quinn K, Helman E, Ye J, Liu F, et al. A blood-based assay for assessment of tumor mutational burden in first-line metastatic NSCLC treatment: results from the MYSTIC study. Clin Cancer Res. 2020; 27: 1631-40.
122. Peters S, Cho B, Reinmuth N, Lee KH, Luft A, Ahn MJ, et al. Abstract CT074: Tumor mutational burden (TMB) as a biomarker of survival in metastatic non-small cell lung cancer (mNSCLC): blood and tissue TMB analysis from MYSTIC, a phase III study of first-line durvalumab ± tremelimumab vs chemotherapy. Clinical Trials. 2019; 79(suppl 13): CT074.
123. Fabrizio DA, George TJ, Jr, Dunne RF, Frampton G, Sun J, Gowen K, et al. Beyond microsatellite testing: assessment of tumor mutational burden identifies subsets of colorectal cancer who may respond to immune checkpoint inhibition. J Gastrointest Oncol. 2018; 9(4): 610-7.
124. Gromeier M, Brown MC, Zhang G, Lin X, Chen Y, Wei Z, et al. Very low mutation burden is a feature of inflamed recurrent glioblastomas responsive to cancer immunotherapy. Nat Commun. 2021; 12(1): 352.
125. Yuan C, Xiang L, Cao K, Zhang J, Luo Y, Sun W, et al. The prognostic value of tumor mutational burden and immune cell infiltration in esophageal cancer patients with or without radiotherapy. Aging (Albany NY). 2020; 12(5): 4603-16.
126. Wang S, Xie K, Liu T. Cancer immunotherapies: from efficacy to resistance mechanisms – not only checkpoint matters. Front Immunol. 2021; 12: 690112.
127. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature. 2013; 500(7463): 415-21.
128. Kazdal D, Endris V, Allgauer M, Kriegsmann M, Leichsenring J, Volckmar AL, et al. Spatial and temporal heterogeneity of panel-based tumor mutational burden in pulmonary adenocarcinoma: separating biology from technical artifacts. J Thorac Oncol. 2019; 14(11): 1935-47.
129. Alessi JV, Wang X, Elkrief A, Ricciuti B, Li YY, Gupta H, et al. Impact of aneuploidy and chromosome 9p loss on tumor immune microenvironment and immune checkpoint inhibitor efficacy in NSCLC. J Thorac Oncol. 2023; 18(11): 1524-37.
130. Garcia EP, Minkovsky A, Jia Y, Ducar MD, Shivdasani P, Gong X, et al. Validation of oncopanel: a targeted next-generation sequencing assay for the detection of somatic variants in cancer. Arch Pathol Lab Med. 2017; 141(6): 751-8.
131. Yarchoan M, Albacker LA, Hopkins AC, Montesion M, Murugesan K, Vithayathil TT, et al. PD-L1 expression and tumor mutational burden are independent biomarkers in most cancers. JCI Insight. 2019; 4(6): e126908.
132. Chen Y, Wang Y, Luo H, Meng X, Zhu W, Wang D, et al. The frequency and inter-relationship of PD-L1 expression and tumour mutational burden across multiple types of advanced solid tumours in China. Exp Hematol Oncol. 2020; 9: 17.

133. Hellmann MD, Paz-Ares L, Bernabe Caro R, Zurawski B, Kim SW, Carcereny Costa E, et al. Nivolumab plus ipilimumab in advanced non-small-cell lung cancer. N Engl J Med. 2019; 381(21): 2020-31.
134. Rizvi NA, Cho BC, Reinmuth N, Lee KH, Luft A, Ahn MJ, et al. Durvalumab with or without tremelimumab vs standard chemotherapy in first-line treatment of metastatic non-small cell lung cancer: the MYSTIC phase 3 randomized clinical trial. JAMA Oncol. 2020; 6(5): 661-74.
135. Li F, Li C, Cai X, Xie Z, Zhou L, Cheng B, et al. The association between CD8+ tumor-infiltrating lymphocytes and the clinical outcome of cancer immunotherapy: a systematic review and meta-analysis. EClinicalMedicine. 2021; 41: 101134.
136. Montesion M, Murugesan K, Jin DX, Sharaf R, Sanchez N, Guria A, et al. Somatic HLA class i loss is a widespread mechanism of immune evasion which refines the use of tumor mutational burden as a biomarker of checkpoint inhibitor response. Cancer Discov. 2021; 11(2): 282-92.
137. Naranbhai V, Viard M, Dean M, Groha S, Braun DA, Labaki C, et al. HLA-A*03 and response to immune checkpoint blockade in cancer: an epidemiological biomarker study. Lancet Oncol. 2022; 23(1): 172-84.
138. Chowell D, Morris LGT, Grigg CM, Weber JK, Samstein RM, Makarov V, et al. Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. Science. 2018; 359(6375): 582-7.
139. Chhibber A, Huang L, Zhang H, Xu J, Cristescu R, Liu X, et al. Germline HLA landscape does not predict efficacy of pembrolizumab monotherapy across solid tumor types. Immunity. 2022; 55(1): 56-64.e4.
140. Alessi JV, Ricciuti B, Alden SL, Bertram AA, Lin JJ, Sakhi M, et al. Low peripheral blood derived neutrophil-to-lymphocyte ratio (dNLR) is associated with increased tumor T-cell infiltration and favorable outcomes to first-line pembrolizumab in non-small cell lung cancer. J Immunother Cancer. 2021; 9(11): e003536.
141. Furtado LV, Bifulco C, Dolderer D, Hsiao SJ, Kipp BR, Lindeman NI, et al. Recommendations for tumor mutational burden assay validation and reporting: a joint consensus recommendation of the association for molecular pathology, College of American Pathologists, and Society for Immunotherapy of Cancer. J Mol Diagn. 2024; 26(8): 653-68.
142. Wang X, Lamberti G, Di Federico A, Alessi J, Ferrara R, Sholl ML, et al. Tumor mutational burden for the prediction of PD-(L)1 blockade efficacy in cancer: challenges and opportunities. Ann Oncol. 2024; 35(6): 508-22.

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