# Analysis and Clinical Relevance of Tumor Mutational Burden

*Tumor mutational burden (TMB) is a genomic biomarker. In contrast to other biomarkers that are detected as a genomic alteration in a specific gene or via methods like immunohistochemistry, TMB is calculated. The most common approach defines TMB as all protein coding somatic mutations detected in a tumor sample normalized to the number of bases sequenced (mut/Mb) [1]. However, disagreements about calculation method, clinical indication for immunotherapy, and other factors have hindered establishment of TMB as a standardized biomarker.*

## The clinical relevance of TMB

A high tumor mutational burden (TMB-H) was initially discussed as a biomarker for immune checkpoint inhibitor therapy [2,3,4]. Mid 2020, the FDA approved pembrolizumab for solid tumors with a TMB-H defined as more than 10 mutations/megabase (mut/Mb) [5]. However, the clinical relevance, a pan-cancer threshold, and the methodology of TMB determination are still heavily disputed. In the same year that the FDA approved TMB as a pan-cancer biomarker, the National Comprehensive Cancer Networks (NCCN) removed TMB as an emerging immune biomarker from the NCCN Panel in their guidelines for non-small cell lung cancer. The organization does not recommend basing treatment decisions on TMB status [6]. The underlying reasons for removing TMB from the NCCN guidelines were an update of the CHECKMATE 227 trial, a missing cut-off definition for TMB-H, and the unstandardized TMB measurement [6,7,8].

The threshold and clinical relevance of TMB are equally debated in other cancer types. Studies use different threshold criteria, most commonly a fixed number (e.g., 10 mut/Mb) or a percentage for a cancer type or cohort [8]. The calculation methodology also varies in studies, depending on cancer type and detection assay. A meta-analysis of cohort data from various NSCLC trials highlights these differences in detection method, defined threshold and sample source among trials (Table 1) [2].

| Study | TMB-evaluable patients, n (%) | Detection method | Threshold defined | Sample type |
|---|---|---|---|---|
| KEYNOTE-042 | 793/1274 (62.2) | WES | 175 mut/exome | Tissue |
| CheckMate 227 part 1-2 | 679/1166 (58.2) | NGS (FoundationOne®CDx) | 10 mut/Mb | Tissue |
| CheckMate 026 | 312/541 (57.6) | WES | 243 missense mut | Tissue |
| MYSTIC | 315/744 (42.3) | NGS (FoundationOne®CDx) | 10 mut/Mb | Tissue |
| MYSTIC | 296/746 (39.6) | NGS (FoundationOne®CDx) | 10 mut/Mb | Tissue |
| MYSTIC | 523/744 (70.2) | NGS (Guardant OMNI®) | 20 mut/Mb | Blood |
| MYSTIC | 541/746 (72.5) | NGS (Guardant OMNI®) | 20 mut/Mb | Blood |
| IMpower110 | 389/554 (70.2) | NGS (FoundationOne®CDx) | 16 mut/Mb | Blood |

Table 1: Excerpt from baseline characteristics of TMB cohorts in a meta-analysis of NSCLC trials. WES: whole exome sequencing; NGS: next generation sequencing [2]

Moreover, an analysis of whole exome sequencing (WES) data from 4000 samples of primary tumors in The Cancer Genome Atlas or of Weill Cornell Medicine Advanced metastatic tumors revealed broad variability in TMB range and in the calculated cut-off [1]. The calculated cutoffs ranged between 0.91 mut/Mb for thyroid carcinoma to 55.06 for uterine corpus endometrial carcinoma [1]. Only few were near the pan-cancer TMB threshold of 10 mut/Mb.

Thus, it is recommended to use a TMB threshold specific to each cancer type instead of a universal pan-cancer threshold [1,9]. Moreover, the metastatic and disease stage should be considered [1].

### Measuring TMB

The need to standardize TMB measurement affects laboratories as well [2]. Lab-to-lab variation in TMB calculation, normalization, assays, and mutation detection limits is a major hinderance to informative assay and study comparison.

Listing the parameters to consider in the TMB methodology helps to understand its complexity.

First, the sample itself can cause discrepancies due to low quality or FFPE fixation artifacts. Moreover, the tumor cellularity in tissue samples should exceed a predefined value (e.g., 20% tumor cells for WES [10,9]) since low-content samples cannot be assessed, but the minimum tumor fraction required is not standardized. That value also differs between WES and targeted panels [11]. Finally, discussions are on-going about whether TMB values derived from blood and tissue samples differ or correlate in their predictive information [4].

Regarding assays, a WES approach is considered the gold standard [8]. The large target region size of a WES assay in combination with a normal sample (i.e., a control sample from healthy tissue or blood) allows for the most precise calculation of TMB and omits potential issues with prediction of variant lineage. However, turn-around-time and costs hinder a broad application of WES in clinical routine [8]. Thus, comprehensive genomic profiling with panels evaluated by next-generation sequencing (NGS) is often utilized [3]. Medium sized and large panels are typically sequenced at high coverages (>500x) and have good sensitivity for variant detection. In combination with sophisticated counting and lineage prediction algorithms, panels with coding regions of at least ~1.1 Mb in size can be used to accurately assess TMB when compared with TMB data from WES [10]. However, some studies have reported overprediction of TMB calculated from a panel compared to WES [12].

TMB calculation can also differ because the variants considered are not standardized. The most prominent example are synonymous mutations and whether they should be included or excluded [1]. SNVs and InDels are typically counted. Moreover, the normalization method is not standardized. Some providers normalize to the protein-coding target region size covered by the assay, whereas others normalize to the full target region size of the assay. Since the fraction of protein-coding sequences strongly varies between different assays, normalization to the full target region size limits TMB comparisons among patients analyzed with the same assay. Conversely, however, normalization to the coding fraction of the target region results in higher TMB values compared to normalization to the full target region size.

These limitations and issues must be addressed when using TMB as a biomarker.

## TMB as part of reporting

As described previously, TMB can be calculated based on WES or panel sequencing. Thus, bioinformatic tools support TMB calculation and integrating the result with other genomic alterations found in a patient sample. MH Guide supports the annotation of variants and displays a TMB value based on parameters defined by the user. Ideally, the full spectrum of results from a patient's genomic profile, including TMB, helps matching a patient to a targeted therapy or an eligible clinical trial [13].

Because TMB calculation and thresholds are not yet standardized in the scientific community, MH Guide enables researchers and pathologists to define their own threshold according to their expertise and the lab test in use. That is, the default ruleset in MH Guide can be customized by the user. Parameters of this ruleset cover the configuration of the TMB calculation within MH Guide, and allow users to change counting, normalization, and TMB thresholds based on their clinical expertise. Besides calculation of TMB, MH Guide enables reporting of TMB-high, TMB-low or as a score (mut/Mb) deriving from a VCF input file. Alternatively, TMB biomarkers can be manually added based on calculated TMB and patient disease (figure 1). MH strongly recommends that customers configure TMB counting and thresholds based on their own clinical expertise.

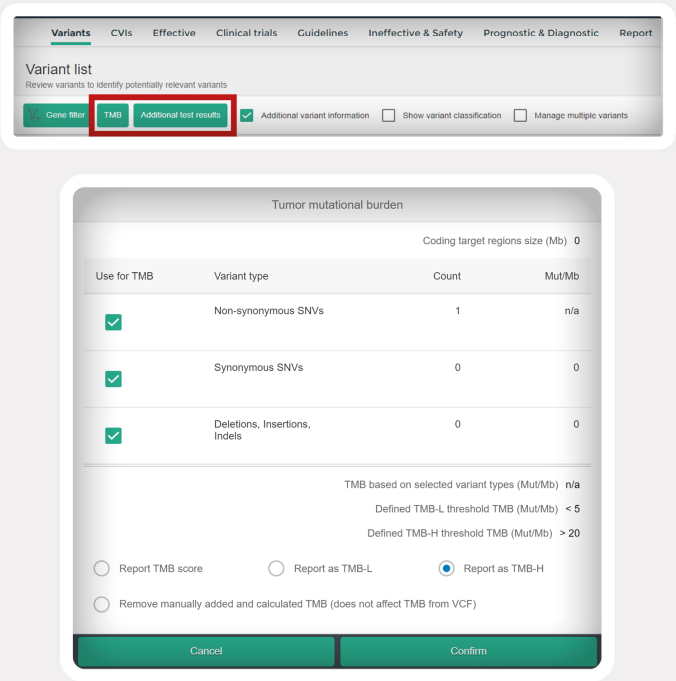
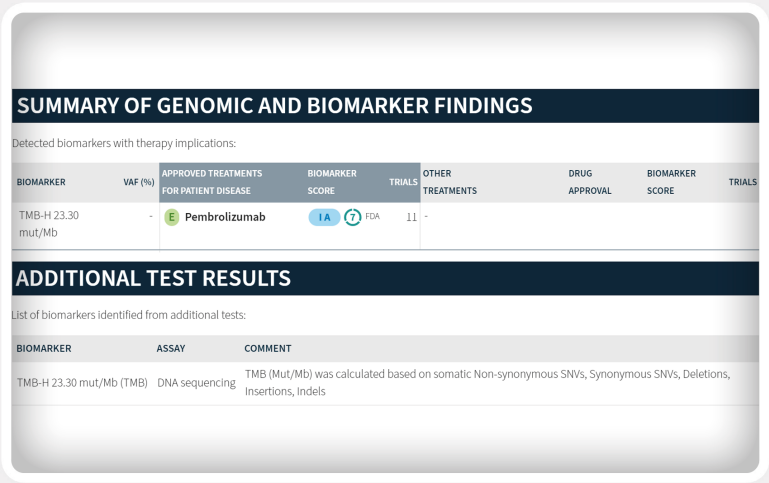



Fig. 1:
Manually adding a TMB biomarker in MH Guide through the "TMB" button or via the "additional test results" button

Lastly, the TMB is displayed in the generated report as part of the patient's molecular profile (figure 2). Thus, MH Guide supports the treating physician and members of the molecular tumor board in making a therapy decision for each patient based on up-to-date and clinically relevant genomic data.



SUMMARY OF GENOMIC AND BIOMARKER FINDINGS

Detected biomarkers with therapy implications:

| BIOMARKER | VAF (%) | APPROVED TREATMENTS FOR PATIENT DISEASE | BIOMARKER SCORE | TRIALS | OTHER TREATMENTS | DRUG APPROVAL | BIOMARKER SCORE | TRIALS |
|---|---|---|---|---|---|---|---|---|
| TMB-H 23.30 mut/Mb | - | E Pembrolizumab | I A | 11 | - | | | |

ADDITIONAL TEST RESULTS

List of biomarkers identified from additional tests:

| BIOMARKER | ASSAY | COMMENT |
|---|---|---|
| TMB-H 23.30 mut/Mb (TMB) | DNA sequencing | TMB (Mut/Mb) was calculated based on somatic Non-synonymous SNVs, Synonymous SNVs, Deletions, Insertions, Indels |

Fig. 2:
Display of TMB in the summary and additional test result section of the MH Guide report

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