Research &
Pharma Solutions

Tumor Mutational Burden (TMB) –
An Emerging Pan-Tumor Biomarker

Tech Note

# Tumor Mutational Burden (TMB) – An Emerging Pan-Tumor Biomarker

The immune system is complex and dynamic, with several different mechanisms the body can use to fight cancer. However, every tumor consists of an individual set of specific mutations that help the tumor to survive and overcome the immune system or therapeutic agents. So to find the best oncology treatment, a deep and accurate look into the molecular underspinnings of individual tumors is of utmost importance.

Therefore, CeGaT's tumor analyses include the determination of tumor mutational burden (TMB), a predictive biomarker that indicates if the tumor may respond more effectively to immunotherapies (immune checkpoint inhibitors).

What is TMB?

TMB describes the number of somatic mutations that are present in the patient's tumor tissue but absent from the patient's healthy tissue. In theory, tumors with a higher TMB, such as melanoma and lung cancer, show increased production of so-called neoantigens and therefore are more likely to be recognized by the immune system (Sholl et al., 2020; Strickler et al., 2021).

Neoantigens are peptides that are presented on the surface of tumor cells by major histocompatibility complex (MHC) molecules and can be recognized by neoantigen-specific T cell receptors (TCRs) (Jiang et al., 2019) (fig. 1). Besides a viral etiology, neoantigens could arise from various nonsynonymous genetic alterations, including single-nucleotide variants (SNVs), insertions and deletions (InDels), gene fusions, frameshift mutations, and structural variants (Jiang et al., 2019).
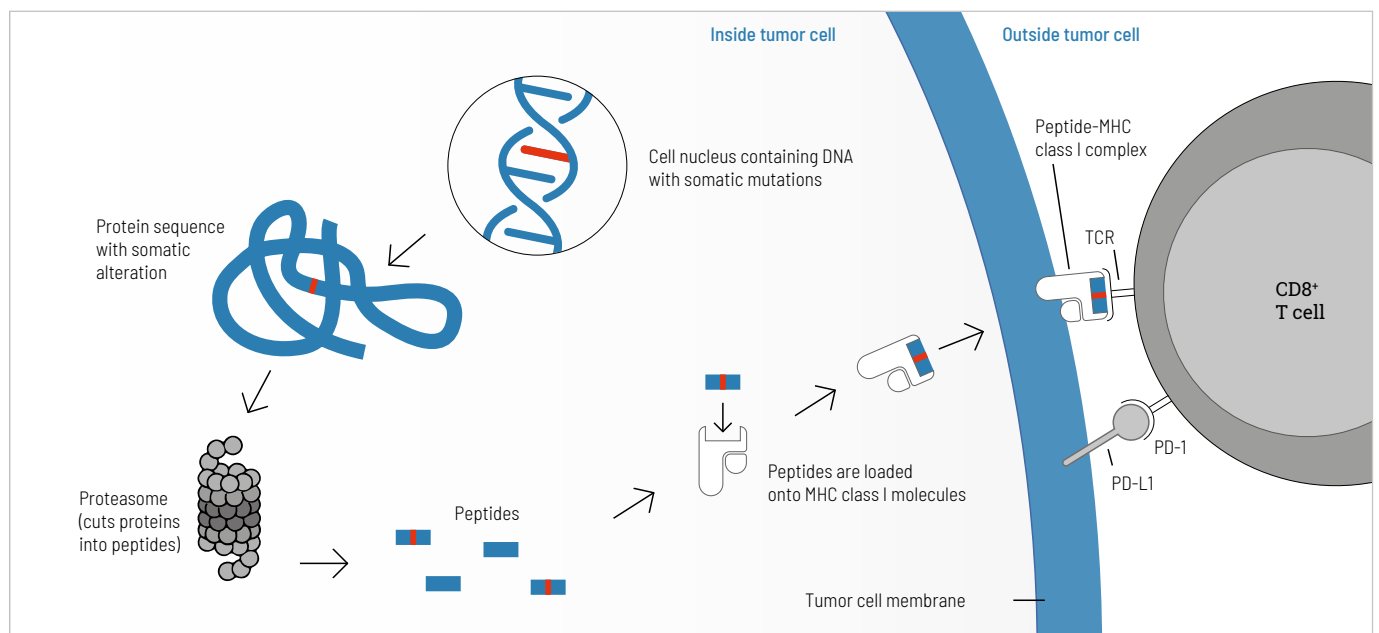
Figure 1: *Presentation of tumor cell-derived somatic peptides. Somatic mutations frequently occur in cancer and permanently alter the genomic information of the cell. These genetic changes can result in the expression of proteins with altered amino acid sequences. These proteins are processed by the proteasome into short peptides, which are subsequently loaded onto the MHC class I molecules. The peptide-MHC class I complexes then reach the tumor cell surface and are presented to effector immune cells (CD8+ T cells). Thus, peptides that carry a somatic mutation and are, as a consequence, highly immunogenic, can be presented on the tumor cell surface and cause an effective anti-tumor immune response. TCR, T cell receptor; MHC, major histocompatibility complex.*

TMB and the immune response

Tumors utilize certain immune checkpoint signaling pathways as an essential immune resistance mechanism, especially against T cells specific for tumor antigens. Therefore, blocking immune checkpoint molecules, such as programmed death-1/programmed death ligand-1 (PD-1/PD-L1) and cytotoxic T lymphocyte antigen-4 (CTLA-4), is one of the most promising approaches to activating anti-tumor T cell activity. Hence, in some tumor types, neoantigen load or TMB may be a suitable clinical biomarker to make treatment decisions for immune checkpoint inhibitors (Stenzinger et al., 2019).

Meanwhile, several clinical trials with immune checkpoint inhibitors demonstrate a correlation between a high TMB and response rates after PD-1 inhibition (Stenzinger et al., 2019; Sholl et al., 2020). Patients with high TMB reported more significant improvements in treatment outcomes and quality of life earlier in treatment (Ray et al. 2021).

For instance, in the United States the FDA has approved the PD-1 inhibitor pembrolizumab as a therapy for all TMB-high solid tumors. The approval is based on the KEYNOTE-158 study, where a high TMB was specified as equal to or greater than 10 mutations per million base pairs (Mut/Mb) (Marabelle et al., 2020).

The TMB threshold of 10 Mut/Mb has also been used in previous studies conducted in non–small-cell lung cancer (NSCLC) like CheckMate 568. The CheckMate 568 study was an open-label phase III trial that compared the efficacy of nivolumab plus ipilimumab versus chemotherapy alone in patients with stage IV or recurrent NSCLC. In patients with a high TMB (≥10 Mut/Mb), progression-free survival (PFS) was significantly longer with nivolumab plus ipilimumab than with chemotherapy alone (Ready et al., 2019). Thus, TMB with a threshold of 10 Mut/Mb can be a reasonable predictor of response to immune checkpoint inhibitors (Strickler et al., 2021).
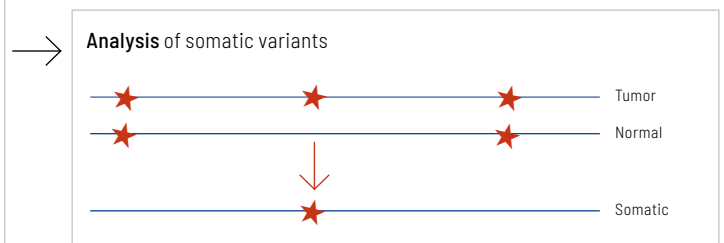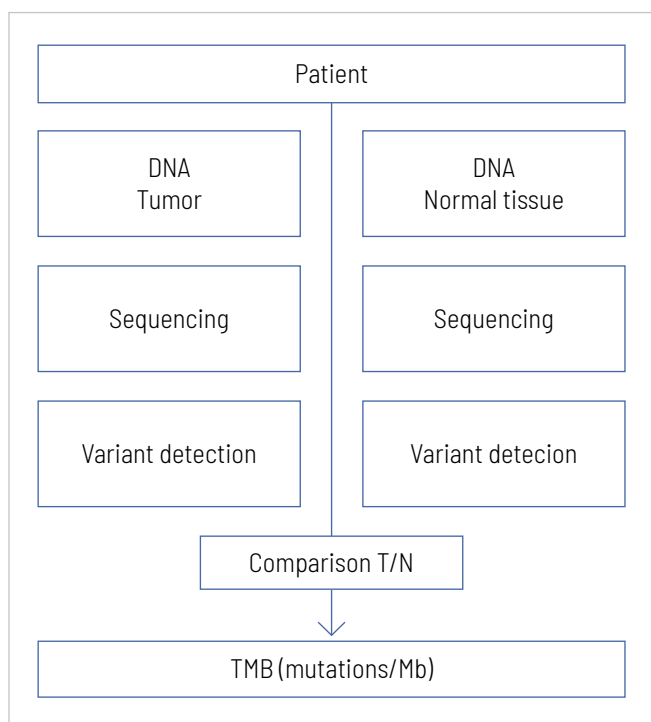

Figure 2: Analyzing tumor and healthy tissue allows for a safe detection of somatic variants.

How is TMB measured and which parameters are crucial?

To determine TMB, a tumor biopsy is performed, and DNA is extracted. The sample is sequenced, and the mutations are determined. It is crucial to analyze a sample from the patient's healthy tissue (usually blood) in parallel (Sun et al., 2018). Only by comparing the variants discovered in these two samples can the tumor-specific somatic alterations safely be detected (fig. 2).

The tumor-specific mutations evaluated for TMB are coding somatic sequence mutations (fig. 3). These detected mutations are first split into the so-called driver and passenger mutations. While driver mutations are necessary for the development and maintenance of the tumor and affect known tumor genes, passenger mutations are a consequence of the tumor's genetic instability and are evenly distributed across all genes.

A subset of a few hundreds of known and therapeutically relevant tumor genes (instead of the whole genome) is routinely sequenced in a tumor gene panel to improve sensitivity. Thus, a tumor panel will be significantly enriched for driver mutations with respect to the rest of the genome. The driver mutations must not be extrapolated to all genes, as this would lead to an overestimation of TMB. On the other hand, passenger mutations occur at the same rate in tumor genes as in all other genes and are used as the basis for extrapolation. Thus, the extrapolated TMB is the sum of the unchanged number of detected driver mutations and the number of passenger mutations extrapolated to all genes, normalized by the total size of all known genes.
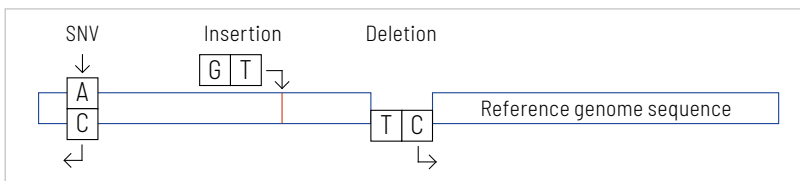

Figure 3: TMB is computed based on somatic mutations occurring in the protein-coding parts of the tumor DNA sequence (SNVs, InDels). Larger genomic changes, such as chromosome aberrations, copy number changes, and structural variants are not taken into account in the calculation of the TMB.

For the determination of TMB, the panel size, e.g., the number of genes analyzed, is a critical parameter influencing the accuracy of the results. The larger the number of analyzed genes, the more accurate the final TMB result. For best results, panel sizes greater than 1.5 Mb are recommended (Buchhalter et al., 2019) (fig. 4). CeGaT's Tumor Immuno-Oncology Analysis, at 2.2 Mb, is safely above this boundary and thus ensures a robust estimation of TMB (fig. 5).
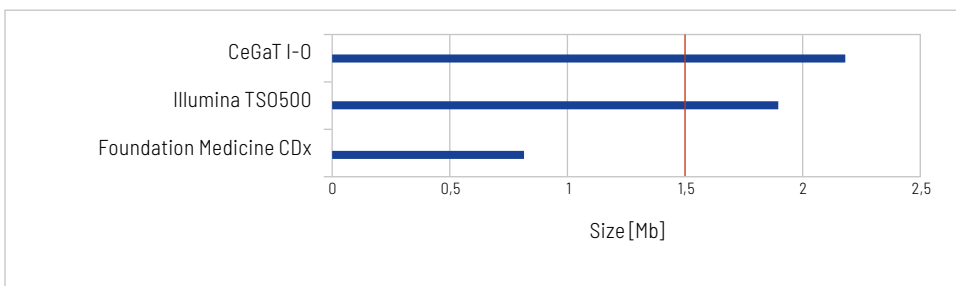
Figure 4: Comparison of panel size (Mb) of different products to determine TMB. The recommended size of 1.5 Mb (Buchalter 2018) is marked in red.
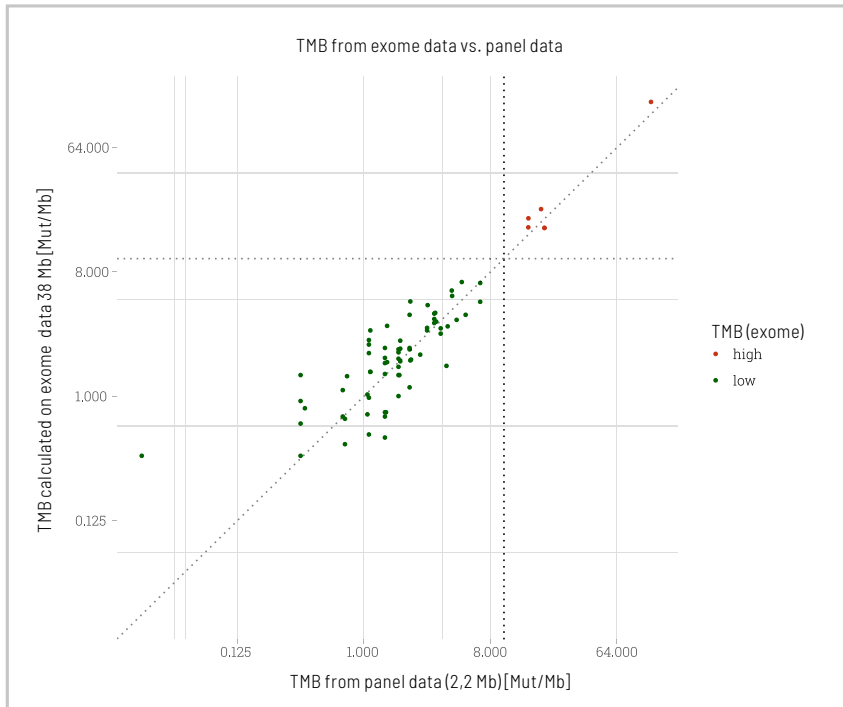
Figure 5: Extrapolation of TMB from the CeGaT tumor panel (2.2 Mb) to the complete coding region (38 Mb). Classification into low (<10 Mut/Mb) and high TMB (>=10 Mut/Mb) is shown by color.

There are also methods for determining TMB without having to analyze a normal tissue sample. These methods rely on the fact that a tumor sample almost always contains small amounts of healthy tissue as well. Thus, the sequencing of a tumor sample basically analyzes a mixture of two tissues, normal and tumor. Using statistical methods, the mixed information can be separated into two datasets and can be used for the calculation of TMB. Although the aforementioned method bears a 30% risk of error, this risk further shrinks as the content of the healthy tissue increases in the sequenced mixture (Jones et al., 2015, Sun et al., 2018, value for 50% contamination).

Best laboratory practice

The TMB value must be valid and reliably calculated to draw valuable clinical conclusions for the patient. That means the whole process must be quality assured, including the sample preparation, data generation, and bioinformatic analysis. There-fore, quality controls and standardization are essential to ensure both reproducible results and to avoid errors by taking appropriate measures (e.g., in case of a sample swapping/contamination).

CeGaT is accredited according to DIN EN ISO 15189, DIN EN ISO/IEC 17025, and the American CAP/CLIA accreditation system, and offers quality assured TMB evaluation for clinical use. We recommend that TMB analysis be based on tumor and healthy tissue in our gene panel (developed in-house), which ensures sensitive and reliable determination of TMB.

A high-quality determination of TMB is the basis for critical therapeutic decisions on implementing checkpoint inhibition. It should be considered an integral part of routine diagnostics in tumor therapy.

About Us

CeGaT is a global provider of genetic analyses for a wide range of medical, research, and pharmaceutical applications.

Founded in 2009 in Tübingen, Germany, the company combines state-of-the-art sequencing technology with medical expertise – with the aim of identifying the genetic causes of diseases and supporting patient care. For researchers and pharmaceutical companies, CeGaT offers a broad portfolio of sequencing services and tumor analyses. CeGaT generates the data basis for clinical studies and medical innovations and drives science forward with its own insights.

The owner-managed company stands for independence, comprehensive personal customer service, and outstanding quality. CeGaT's laboratory is accredited according to CAP/CLIA, DIN EN ISO 15189, and DIN EN ISO/IEC 17025 and thus meets the highest international standards. To obtain first-class results, all processes are carried out in-house under scientific supervision.

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