Review

Evaluating Tumour Mutational Burden as a Key Biomarker in Personalized Cancer Immunotherapy: A Pan-Cancer Approach

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Evaluating Tumour Mutational Burden as a Key Biomarker in Personalized Cancer Immunotherapy: A Pan-Cancer Approach

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Abstract: Background: Tumour mutational burden (TMB) is increasingly recognized as a vital biomarker for predicting the efficacy of immune checkpoint inhibitors (ICIs) in cancer treatment. Despite its growing importance, the effectiveness of TMB as a predictive marker is well established in lung cancer and melanoma but remain ambiguous for breast and prostate cancers. **Objective**: This study aims to evaluate the role of TMB in predicting response to ICIs across four major cancer types—lung, melanoma, breast, and prostate—and to address the variability in TMB's predictive value. **Methods**: A comprehensive review of the current literature was performed, analysing studies that investigated TMB and its association with ICI therapy outcomes in the 4 specified cancer types. **Results**: The analysis reveals a strong consensus on the predictive value of TMB in lung cancer and melanoma, where high TMB levels are associated with improved clinical outcomes and better responses to ICIs. In contrast, the evidence for breast and prostate cancers is less conclusive, with variability in results highlighting the need for further research. Specifically, high TMB in these cancers does not consistently predict better responses to ICIs, suggesting that additional biomarkers or refined criteria might be necessary. **Conclusion**: TMB is a promising biomarker for predicting responses to ICI therapy, particularly in lung cancer and melanoma. However, its predictive value in breast and prostate cancers remains uncertain, underscoring the need for more extensive studies. Future research should focus on standardizing TMB evaluation methods and exploring additional biomarkers to improve treatment personalization and outcomes in these cancer types.

Keywords: tumour mutational burden (TMB); immune checkpoint inhibitors (ICIs); biomarkers; cancer immunotherapy; cancer treatment outcomes; personalized medicine

1. Introduction

Immunotherapy has revolutionized cancer treatment, offering more effective therapeutic options with a reduced toxicity profile compared to conventional chemotherapy. Immune checkpoint inhibitors (ICIs), such as antibodies against PD-1/PD-L1 and CTLA-4 [1], play a crucial role in reactivating the immune system to combat tumour cells. However, not all patients respond to these therapies, underscoring the need to identify reliable biomarkers to predict treatment response.

Tumour Mutational Burden (TMB), defined as the total count of mutations per megabase in tumour DNA [1], has been suggested as a promising biomarker to predict the effectiveness of immunotherapy. A high mutation load may produce neoantigens that trigger a strong immune response, indicating that tumours with elevated TMB are more likely to respond well to immune

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checkpoint inhibitors. Although TMB's predictive value is well-established in non-small cell lung cancer and melanoma, its role in other cancers, such as breast and prostate cancer, is still uncertain.

This study, therefore, seeks to provide a thorough analysis of TMB's role in four major cancer types—lung, melanoma, breast, and prostate—and to examine the variability in its predictive value across these tumour types. Furthermore, we emphasize the need for standardizing TMB assessment methods and explore its potential as a biomarker for optimizing personalized immunotherapy approaches.

1.1. What is TMB?

TMB can be defined as tumour's total number of mutations. TMB value can vary depending on the technique used to measure it [1]. Currently, the measurement is performed using NSG (next generation sequencing). Several NGS approaches are developed, and the target region ranges from genome-wide analysis (WGS- whole genome sequencing) to whole-exome analysis (WES- whole exome sequencing). Recently, the FDA approved the gene panel assay FoundationOne CDx and authorised MSK-IMPACT for testing solid tumours for genetic alterations [2,3]

1.2. Testing Method: WES

Whole exome sequencing allows the exploration of all the protein-coding regions of the human genome. This technology facilitates the examination of genetic mutations associated with cancer, abnormalities that are mainly located in the exome regions. In WES the focus is on specific regions of the genome, the protein-coding fragment. This allows us to identify genetic abnormalities that will impact protein function [4]. WES is a powerful tool for the detection of various genomic changes both in coding and noncoding DNA that are influential in cancer development [5]. Changes in the exome can lead to different amino acids substitutions in protein. This event can lead to weakened activity of multiple tumour suppressors, such as APC in colorectal cancer, VHL in renal cell carcinoma or BRCA in breast cancer [6–8]. There are also modifications in cell cycle regulators, such as TP53 or RB1, and in repair mechanisms, which will predispose to cancer development. By measuring the TMB (mutational burden) we can monitor the activity of those systems [9,10].

Currently, there are 2 primary types of NGS methods: DNA amplification-based sequencing (Illumina, Ion Torrent) and single molecule real-time sequencing (Pacific Biosciences, Oxford Nanopore. The tissue samples analysed can be liquid-based (blood sample), freshly frozen, formalin-fixed or paraffin-embedded (FFPE). Each type of sample requires its own specific isolation kits [4].

The first step of WES is an adequate examination of the sample, made by a pathologist. The used sample should contain a proper amount of tumour cells to make a difference between germline and somatic mutations. It is important to keep in mind that the DNA quality deteriorates with time and after FFPE conservation [4]. After the examinations of the samples, the data processing starts, usually with a quality control. The low-quality reads are eliminated. The next step is to align the reads to a reference genome, then a second quality control and finally, the removal of the duplicated reads.

The main advantage of the WES technique is that it can scan the entire genome of a sample and provide information about the low-frequency mutations, that collectively can determine a phenotypic appearance [11].

1.3. Corelation Between TMB and Immunotherapy

Nowadays, the immune checkpoint inhibitors have become the standard therapy for various solid tumours, such as melanoma, NSCLC or renal cell carcinoma [12]. The response to this therapy can be measured by a reliable biomarker, TMB, which represents the total number of mutations per coding sequence in the tumour genome. Currently, using WES to detect TMB is a widely accepted method [4]. Despite the potential of TMB as a predictive biomarker, there remains a lack of consensus regarding its definition, determination method and adequate cut-off values. For example, the Foundation Medicine has divided the TMB into 3 categories: high TMB (>20 mut/MB), intermediate

 doi:10.20944/preprints202411.2060.v1

TMB (6-19 mut/Mb) and low TMB (less then 5 mut/Mb) [13]. Regarding this issue, the Friends of Cancer Research have established a team whose primary goal is to standardize the use of TMB [14].

1.4. The Interpretation and Reporting the TMB Value:

The TMB quantification is influenced by 4 main factors [1]:

1. Tumour purity: this represents the overall percentage of cancerous cell within a tumour sample. This measurement is analyst-dependent and can lead to errors since the used sample may not represent the tumour's region which will be analysed.
2. Library construction and sequencing: this is represented by DNA fragments with a defined length which will be analysed using various bioinformatics programs.
3. The pipeline used to call mutations: represents the algorithm used to remove germline variants. This is a vital step in the identification of different somatic mutations which are responsible for producing tumour neo-antigens. These antigens will be eventually recognised as non-self by the immune system.
4. The capacity to extrapolate TMB values from the restricted genomic space sampled by gene panels: this step is based on the in silico analysis performed on samples to determine the concordance between WE- based TMB and panel-based TMB [1].

1.5. Can We Use TMB as a Predictive Biomarker?

Typically, T cells recognize different neo-antigens produced by various mutational mechanisms and presented by MHC molecules from the cancerous cell' surface and target those cells for destruction. To evade T cells and to supress the immune system, a tumour has the capacity to produce proteins that, normally, function as checkpoints that attenuate immune responsiveness. The main reason for using immune checkpoints is to block the interaction between T cells and tumour proteins, with the aim to reactivate the immune system. Once the immune system is reactivated, T cells can differentiate normal cells from cancerous ones. This process is facilitated by the presence on the cell' surface of immunogenic antigens. Since these molecules arise from mutations, the more neo-antigens that are present, the higher the TMB. This is the hypothesis that supports the idea the higher the TMB, the greater the chances of responding to a treatment based on ICIs [1]. However, there is also evidence that approximately 60% of patients with high-TMB do not have a malignancy that responds to ICIs [15].

To determine whether TMB can be used as a predictive biomarker or not, several studies were conducted. The most informative study is that conducted by Hao-Xiang Wu and his team [16]. In this study 20 primary solid cancers from 6035 patients were analysed and for each type, the impact of TMB on the overall survival (OS) was evaluated using the Kaplan-Meier method. Survival analysis showed in the end that TMB has a significant impact in OS in 14 cases out of 20. According to this, the impact of the TMB was classified into 3 categories: TMB-worse group (it includes 8 types of malignancies- the patients with a high-TMB has a poorer prognostic compared with those with a lower value), TMB-better group (6 types of cancers- the patients with a higher-TMB has a better prognosis and a decreased mortality rate and the TMB-similar group where the value of TMB didn't have an impact on OS [16].

2. Methodology

2.1. Literature Search and Selection

To evaluate the role of **Tumour Mutational Burden (TMB)** in predicting the response to immune checkpoint inhibitors (ICIs) in four major cancer types (non-small cell lung cancer, melanoma, breast cancer, and prostate cancer), we conducted a systematic review of the scientific literature. Key databases, including **PubMed, Scopus, and Web of Science**, were searched for articles published between **2010 and 2023** using keywords such as "Tumor Mutational Burden," "immune checkpoint inhibitors," "NSCLC," "melanoma," "breast cancer," "prostate cancer," and "biomarkers."

 doi:10.20944/preprints202411.2060.v1

2.2. Inclusion and Exclusion Criteria

Inclusion criteria:

- Published in English;
- Focused on the relationship between TMB and response to ICIs in at least one of the four analyzed cancer types;
- Used standardized methods for measuring TMB (whole-exome sequencing or approved gene panels);
- Reported clinical data on the efficacy of immunotherapy based on TMB levels.
- Exclusion criteria:
- Did not include clinical data related to patients treated with immunotherapy;
- Were narrative reviews or commentary articles without empirical data;
- Did not use validated methods for TMB assessment.

2.3. Data Extraction and Analysis

Data from the selected studies were extracted and organized by cancer type and reported **TMB levels** (low, intermediate, high). Key outcomes included response rates to immunotherapy, **progression-free survival (PFS)**, and **overall survival (OS)**, correlated with TMB levels. The methods used to assess TMB, such as whole-exome sequencing (WES) and gene panel tests (e.g., FoundationOne CDx), were also compared.

2.4. Quality Assessment of Studies

The quality of the included studies was assessed using criteria such as sample size, methods for TMB assessment, and the robustness of statistical analyses. Studies were rated according to the **Newcastle-Ottawa Scale**, and studies with lower scores were excluded from the final analysis to ensure scientific rigor.

2.5. Statistical Analysis

To evaluate the association between **TMB** and clinical outcomes, Cox regression models were used to calculate **hazard ratios (HRs)** for survival and response to immunotherapy. Meta-analyses were conducted using **Review Manager (RevMan 5.4)**, and heterogeneity across studies was assessed using the $\mathbf{I^2}$ statistic. A significance level of $\mathbf{p < 0.05}$ was considered statistically significant.

3. Lung Cancer & TMB

Pulmonary cancer is the most common cause of death from cancer, worldwide [17]. It includes different histologically subtypes, of which non-small cell lung cancer (for short NSCLC) represents approximately 85% [18]. Only a small percentage (20-25%) of patients with NSCLC have an early-stage diagnosis. In this case, elective resection is done with curative intent [17]. Over the last years, a better understanding of NSCLC' biology led to the identification of various predictive biomarkers, for example EGFR, BRAF mutations or ALK and ROS1 rearrangements. Due to these findings, several target therapies have arisen. In NSCLS a targetable alteration is found in approximately 50% of patients, so for the other half it is imperative to find a biomarker to improve the clinical outcomes [19].

Nowadays, the only biomarker approved is PD-L1 expression, assed by immunohistochemistry (IHC), an inexpensive technique and performed using standard histopathology equipment. The PD-L1 expression is an important criterion during the process of splitting patients in those who will receive treatment with Pembrolizumab in monotherapy (in this case the PD-L1 cut-off is >=50%) [20] and those who will receive a dual-immunotherapy Nivolumab plus Ipilimumab (PD-L1 cut-off of 1%) [20]. The cut-off's values are explained by the multitude of assays used by investigators in clinical trials. By knowing that we can sustain the affirmation that PD-L1 is a heterogeneous variable and that is important to standardize it. For this reason, there is a need for identifications of other biomarkers, including TMB [19].

 doi:10.20944/preprints202411.2060.v1

Given the variety of treatment options available, biomarkers are essential to determine which patient subgroup is most likely to benefit from a particular therapy. Although not ideal, high PD-L1 expression levels can help select patients who are likely to benefit from monotherapy with pembrolizumab. TMB is a promising biomarker, but assays and criteria for defining high TMB need standardization and obtaining sufficient tissue samples can be challenging.

In current literature, there are 3 main studies that suggest the possibility of TMB utilisation, as a biomarker in NSCLC.

Table 1. Comparison between the 3 main studies regarding TMB use in NSCLS.

Study	Studied treatment	Primary endpoint/s	Secondary endpoint/s
CheckMate 227	Nivolumab + Ipilimumab vs. Platinum-based chemotherapy	PFS based on TMB OS based on PD-L1	PFS based on TMB OS based on TMB
CheckMate 568	Nivolumab + low dose Ipilimumab	ORR based on PD-L1	ORR/ PFS/OS/ efficacy by TMB & PD-L1
CheckMate 026	Nivolumab vs. Platinum-based chemotherapy	PFS based on PD-L1	PFS/OS/ORR based on PD-L1

All three studies included a large number of patients, evenly distributed between the study arms. They presented similar inclusion and exclusion criteria. The results of these studies concluded that immunotherapy is a better therapeutic option than chemotherapy in NSCLC, and dual immunotherapy is superior to monotherapy compared to chemotherapy (median PFS 4.2 m in Nivolumab group vs. 5.9m in chemotherapy; median OS 14.4m for Nivolumab vs. 13.2m for chemotherapy) [21]. Additionally, the use of TMB as a potential marker for predicting treatment response was also discussed in these studies. The TMB cut-off was universally accepted to be 10 mutations/Mb, with higher values not being associated with improved outcomes [22]. For Nivolumab + Ipilimumab combination, the 1-year PFS was longer than in chemotherapy arm (42.6% vs. 13.2%) and the median PFS was 7.2m, for those patients with TMB ≥ 10 mut/Mb. For those with less than 10 mut/Mb the median PFS was 3.2m in Nivolumab + Ipilimumab arm [23]. CheckMate 568 [22] had an interesting finding, regarding the biomarkers. The medical team observed that, regardless of PD-L1's value, ORR, as a secondary endpoint, was higher in patients with an increased TMB (≥ 10 mut/Mb). As a primary endpoint, PD-L1 ≥1% had a better outcome, with an ORR 41% vs. 15% (<1%). Based on these results, we can conclude that, in addition to PD-L1 we can use TMB to guide our treatment choices and to predict our patients' outcomes.

Despite these positive results, there are also studies that suggest the opposite. These inconsistencies highlight the importance of large-scale analyses to universally establish the predictive value of TMB as a predictive biomarker.

To support the idea of a negative correlation, we will present the conclusions of a retrospective study performed on 136 NSCLC patients [24]. Chae et al. underwent ctDNA testing in one institution, with an additional validation cohort analysed in another institution. The ctDNA TMB was measured by counting all detected mutations of the sequencing length. After the analysis, the conclusion was that a higher ctDNA TMB was significantly linked with the history of smoking ($p < .05$, chi-squared test). Among patients treated with immune checkpoint inhibitors (n=20), increased TMB was correlated to shorter progression-free survival (PFS) and overall survival (OS; 45 vs. 355 days; hazard ratio [HR], 5.6; 95% confidence interval [CI], 1.3–24.6; $p < .01$, and OS 106 days vs. not reached; HR, 6.0; 95% CI, 1.3–27.1; $p < .01$, respectively). There was also a small number of patients (n=12) in whom there was nonsignificant correlation. In conclusion, this study suggested that a higher TMB can be associated with a negative clinical outcome. Additionally, the authors of the study emphasize the importance of large-scale studies, given that their analysis had several limitations, such as the insufficient length of the tested DNA and the use of a single type of commercially available kit for this testing.

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4. Melanoma & TMB

Apparently encouraging, TMB can be used as a predictive biomarker for ICIs and is likely to be included in the future treatment protocols for various types of malignancies. Based on the genetics of melanoma, we will be able to predict the response to treatment (based on CTLA-4 blockade). Although, a high TMB is associated with a benefit from ICIs, is not the only factor. Further research is needed, to understand better the influence of the somatic neoepitops shared by patients with a prolonged benefit from ICIs [25]. For understanding the role of TMB, Dousset *et al.* [26] included in their study 102 patients with advanced malignant melanoma. Their clinic-pathological characteristics, as well as tumour genomic outcomes were collected. An important aspect of this study was the patients' distribution by sun-exposure pattern in 3 different groups: chronically exposure (head and neck), intermittently exposure (arms, trunk, legs) and protected regions (feet, toes, soles, genitals, mucosal and uveal region). For these patients, the TMB was analysed on the recently metastatic sample prior to administration of PD-L1 inhibitors therapy.

The analysed group was primarily composed of men (57%) and the median age at diagnosis was 59.3 years. The majority of melanomas were superficial (36%), followed by unknown primary site (19.6%) and nodular type melanoma (16,8). The rarest locations were identified as: acral lentiginous (12 cases out of 102, 11,8%), mucosal melanoma (9 cases, 8,8%), uveal melanoma (5 cases, 5) and naevocytoid and desmoplastic, 1 case for each type. The BRAF mutation was evaluated as well, and a positive result was received in 34 cases. Out of 102 patients, 80 were treated with anti-PD-L1 monotherapy. TMB was assessed in 94 cases and the median value was 12,4 mut/Mb.

The primary endpoint of this study was to determine if the sun exposure may influence the TMB and consequently the ICIs treatment response. The TMB was significantly higher in chronically exposed areas (*37,2 mut/Mb vs 13,6 mut/Mb and 4 mut/Mb respectively*). The results were according to with the initial hypothesis, TMB is linked to the sun exposure and UV signature. It was stated that, in cutaneous melanoma, TMB is higher than in other cutaneous, nonmelanoma tumours, due to the mutagenic effects of UV exposure. Both the UV signature of the primary site and the metastatic ones are associated with an increased TMB, suggesting that melanoma metastases carry the exact UV signature present in the primary site of origin [26].

5. Breast Cancer & TMB

One of the most common malignancies seen in women is breast cancer (*BC)* and various biomarkers, such as *ER, PR* and *HER2*, are currently used for therapeutic decision-making processes [27]. Although, there are several treatments protocols for BC, almost 30% of patients will eventually develop advance disease which requires another treatment approach. The reduced efficacy of targeted therapies and the relatively poor prognosis of advanced BC patients, have underscored the need to explore new treatment approaches, including immunotherapy [28,29]. Recent studies have shown that Pembrolizumab and Atezolizumab plus Nab-Paclitaxel offer promising clinical benefits for patients with advanced *triple-negative BC*. As an initial step, PD-L1 was proposed as a biomarker for evaluating immunotherapy efficacy, but there were some concerns about its accuracy, since PD-L1 testing has its own challenges (variability among assays or lack of standardization) [29].

In BC patients, TMB has not been well characterized, but we will discuss further the conclusions of the initial analysis for its role in BC. Mei *et al.l* [27] analysed 62 advanced BC cases between January 2014 and June 2018. The samples were tested by Foundation Medicine and TMB by FoundationOne CDx next generation sequencing (NGS). The TMB values were classified into 3 main groups: low (1-5 mut/Mb), intermediate (6-19 mut/Mb) and high (>=20 mut/Mb). The demographic features of the study included median age (53.8 years old, range 30–78), the majority were metastatic cases (49, 79.0%). Fifty-two (83.9%) cases were invasive ductal carcinoma (IDC), 6 cases (9,7%) were invasive lobular carcinoma, 2 cases (3,2%) were metaplastic carcinoma and another 2 cases were neuroendocrine carcinomas [27].

From a biomarker perspective, we note that 36 cases (58,1%) were ER positive, 38 cases (61,3%) were PR positive, 5 cases (8.1%) were HER2 positive and 22 cases (35,5%) were triple negative. Among 62 cases, 3 of them (4,8%) had high TMB, 27 (43,6%) had intermediate TMB and the majority

 doi:10.20944/preprints202411.2060.v1

(32 cases, 51,6%) had low TMB. Since cases with the high and intermediate groups were not so numerous, the team decided to combine these two and compared them with the low TMB group.

As a first conclusion of this study, the team have represented by the evidence of the associations between increased TILs and the group of cases with intermediate/ high TMB compared with the association between TILs level and low TMB group ($p = 0.0018$). There were no other correlations between TMB and another clinicopathologic characteristics. There were several gene mutations evaluated in this study. The second most important conclusion was linked with the commonly seen genetic mutation. The most common mutation identified among the 62 cases was TP53 (59.7%), followed by PIK3CA (33.9%). Interestingly, out of the 6 BC cases with BRCA (1/2) mutations analysed, 5 had intermediate or high TMB, while only one case exhibited low TMB ($p = 0.0002$). 34 DNA damage repair (DDR) genes were included in the NGS panel of this study. 13 cases exhibited at least one DDR gene mutation, while the remaining 49 cases did not show any DDR gene mutations. Clinicopathologic features and TMB, as well, were compared between cases with and without DDR mutations. BCs with DDR mutations had a higher TMB compared to those without DDR mutations (12.08 vs. 6.57 average mutations; $p = 0.043$). There were no differences observed for other clinicopathologic characteristics and the two groups [27].

Although is one of the first studies regarding the impact of TMB in breast cancer, the authors sustain that more analysis should be made on this topic, since immunotherapy is a relatively new option for BC treatment and no major data are not available in the literature. The limited sample size ($n = 62$) restricted the significance of this analysis. Future studies with larger cohorts are necessary to confirm these results.

6. Prostate Cancer & TMB

Prostate cancer is the second most common type of malignancy seen in males [30]. For this type of cancer, challenges regarding the treatment persist, particularly for castration-resistant prostate cancer (*CRPC*). Metastatic CRPC is associated with a poor prognosis, with a median survival period of less than 2 years [31]. Recently, immune checkpoint inhibitors targeting Programmed cell death 1 and its ligand (PD-1/PD-L1) and Cytotoxic T-lymphocyte antigen-4 (CTLA-4) have shown promising preliminary results in various tumours. However, the effectiveness of immunotherapy remains limited by its low efficacy. Potential predictive biomarkers, such as tumour mutational burden (TMB), are currently evaluated.

Graf et al. [32] performed a study aimed at assessing the outcomes of patients treated with immune checkpoint inhibitors (ICIs) versus taxane chemotherapy, with a focus on tumour mutational burden (TMB) role. Immune checkpoint inhibitors (ICIs) can induce significant responses and provide long-term benefits in some patients with metastatic cancer who have undergone numerous prior treatments. However, the rate of clinical benefit varies significantly by tumour type. Unfortunately, for patients with metastatic castration-resistant prostate cancer (mCRPC), the objective response rate to ICIs treatments is reported to be around 3% for those without programmed cell death ligand 1 (PD-L1) expression and 5% for those with PD-L1-expressing tumours [33]. For this reason, there has been increasing interest in identifying other biomarkers that could pinpoint mCRPC patients who are more likely to achieve greater clinical benefits from ICIs compared to alternative treatments.

In this study, a total of 741 men were evaluated between January 2011 and April 2021. They were subjected to genomic testing through the comprehensive genomic profiling (CGP) assays provided by Foundation Medicine. Patients were included in this study if they received either single-agent anti–PD-1 axis therapy or single-agent taxane in the mCRPC setting and had their TMB assessed through tissue biopsy. The main clinopathological characteristics were represented by the median age, 70 (ranges between 64-76 years) and the baseline median pretreatment PSA levels of 79.4 ng/ml. A total of 108 patients (18.8%) had ECOG scores of 2 or greater, and 644 patients (86.9%) had received prior systemic treatments for mCRPC. A total of 45 patients (6.1%) received ICIs, while 696 patients (93.9%) received taxanes. Patients who received ICIs and those who received taxanes showed no significant differences in age, pretherapy PSA levels, ECOG scores, prior NHT use, prior prescribed

 doi:10.20944/preprints202411.2060.v1

opioid use, and biopsy site. However, it is important to note that patients who received ICIs had higher TMB compared to those receiving taxanes (3.5 mut/Mb vs. 2.5 mut/Mb; P < .001).

PSA levels were evaluated in 607 patients. Among them, 14 patients had a TMB above 10 mut/Mb and were treated with ICIs. Of these, 4 exhibited a reduction in PSA levels by approximately 50%. In contrast, none of the patients with a TMB below 10 mut/Mb showed a reduction in PSA levels greater than 50%. For patients treated with taxanes, no relationship between TMB value and PSA level was demonstrated.

The FDA approved cut-off for TMB was 10 mut/Mb. As conclusions, the study showed that both TTNT (*time to next treatment*) and OS (*overall survival*) were adjusted by received drug class (ICIs vs. Taxanes). The patients' group with TMB <10 mut/ Mb and ICIs therapy had a worse average TTNT then those treated with taxanes (2.4 m vs 4.1m). The reverse pattern for TTNT was observed for those with TMB> 10 mut/Mb (8.0 m vs 2.4 m). The OS evaluation does not present major differences between those with TMB< 10 mut/ Mb despite the treatment choice (median OS 4.2m vs 6.0m). However, in those cases with TMB greater then 10 muts/Mb the OS has an elevated value when ICIs were administered, compared to taxanes (median OS 19.9 m vs 4.2 m) [32].

Despite the evidence of improved clinical outcome for the group with high TMB and ICIs therapy, the study has some limitations: it is not a randomized study, the treatment was chosen by the clinician and the number of patients that received ICIs was reduced compared to the taxane group. Also, the biopsy timing was not considered. This leaves room for future studies to demonstrate the effectiveness of using TMB in a larger cohort of patients undergoing immunotherapy treatment.

7. Discussion

Representing some of the most severe forms of cancer globally, lung, breast, prostate cancer and melanoma continue to pose challenges for clinicians when it comes to selecting the optimal management protocol. The key to a successful treatment lies in the individualization of the protocol, based on the clinical and pathological characteristics of each patient. From the perspective of evaluating treatment efficacy, there is an increasing need to identify a marker that can highlight the category of patients who are most likely to have a better therapeutic respond.

Immunotherapy marks a significant advancement in cancer treatment. The mechanism is based on the reactivation of the tumour immune cycle, which will restore the body's natural anti-tumour immune response. Currently, there are at least 4 types of immunotherapy strategies: immune checkpoint inhibitors (ICIs) such as Programmed Cell Death Protein-1 (PD-1) and Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4), chimeric antigen receptor T-cell therapy, tumour vaccines, and lastly, peripatetic immunotherapy. Although these therapies have significantly improved clinical oncology outcomes, not all patients have experienced the benefits. Therefore, it is essential to determine which patients are most likely to respond favourably to immunotherapy [34].

To date, PD-L1 is the only biomarker used to predict the response to ICIs. However, since its evaluation varies depending on the kit used by each medical teams, there is a massive need for the detection of a reliable biomarker for predicting the therapeutic responses. Lastly, several studies have detected another biomarker that can be used for predicting the therapeutic outcomes. Tumour mutational burden (TMB) represents the total number of mutations (substitutions, insertions or deletions) that occurs in a tumour sample [35]. TMB can be divided into 3 categories: low, intermediate, and high, depending on the cut-off value considered by each medical team. As observed in the section dedicated to NSCLC, the universally accepted cut-off value for TMB at this time is 10 mut/Mb, with higher values not being correlated with improved outcomes. This cut-off value itself represents a limitation of any study on this topic, as there is currently no consensus on this value.

Various research has demonstrated that TMB offers some advantages over other biomarkers. Firstly, TMB can be measured in the blood, which is beneficial in cases where tumour tissue specimens are unavailable. Non-invasive blood-based TMB (bTMB) has emerged as a promising method for assessing TMB in clinical settings for immune checkpoint inhibitor (ICI) treatment.

 doi:10.20944/preprints202411.2060.v1

However, the reliability of bTMB measurements can vary due to several factors, including the technical aspects of the ctDNA assay, such as the sensitivity of variant detection and the accuracy of bTMB assessment. Additionally, biological factors like the tumor fraction in plasma cell-free DNA (cfDNA) and the heterogeneity within circulating tumor DNA (ctDNA) can impact results [36].

Consequently, the agreement between bTMB and tissue-based TMB (tTMB) may be low, which can complicate the interpretation of bTMB results and limit its effectiveness as a biomarker for predicting response to ICIs [36]. Using paired testing of both tumour and blood-based TMB assessments may help mitigate variations caused by tumour heterogeneity [37]. The clinical application of blood-based TMB (bTMB) using ctDNA necessitates further standardization to establish it as a dynamic biomarker. This is especially important when monitoring cancers over time, particularly in situations where repeated tumour biopsies are difficult to obtain. Efforts to harmonize genomic profiling and predict responses to immune checkpoint inhibitors (ICIs) will need to focus on evaluating biomarkers from circulating tumour cells (CTCs), ctDNA, and extracellular vesicles (EVs) [36]. Secondly, unlike PD-L1, which can only predict the response to PD-1/PD-L1 inhibitors, TMB can forecast the response to various immunotherapies, including PD-1/PD-L1 inhibitors, anti-CTLA4 antibodies (such as ipilimumab) [38].

In addition to PD-L1, TMB can also be correlated with other biomarkers, such as GEP, to predict patients' responses to immunotherapy. In this context, the study led by Cristescu, and his team [39] showed that TMB and GEP had a modest correlation with each other, and both were independently able to predict patient responses across the KEYNOTE clinical trials. Their analysis revealed that ORRs were higher in patients with both high GEP and high TMB (37% to 57%), moderate in those with high GEP and low TMB (12% to 35%) or low GEP and high TMB (11% to 42%). The lowest or no response were observed in patients with both low GEP and low TMB (0% to 9%). Furthermore, patients with elevated levels of both TMB and GEP had longer PFSs. The results were consistent when evaluating TMB alongside PD-L1 expression. This analysis demonstrates that TMB, along with inflammatory biomarkers such as T cell–inflamed GEP and PD-L1 expression, can categorize human cancers into distinct groups with varying clinical responses to immunotherapy. It also helps identify underlying, targetable biological patterns associated with these groups. Both TMB and inflammatory biomarkers independently predict therapeutic response, potentially reflecting different aspects of neo- antigenicity and T cell activation. This method could offer a framework for future precision medicine, aiding in the rational design and assessment of combination therapies involving anti–PD-1 and/or anti–PD-L1 treatments [38].

The study focused on highlighting the response to pembrolizumab monotherapy based on TMB and GEP. Therefore, patient groups categorized by TMB and GEP status exhibit significant variations in their clinical response to Pembrolizumab. Specifically, the groups with only one positive biomarker—*either high TMB and low GEP or low TMB and high GEP*—demonstrate considerably lower response rates compared to the group with both high TMB and high GEP. This suggests the possible presence of resistance mechanisms to Pembrolizumab. To identify potential resistance mechanisms, several molecular analyses were conducted [38].

The incidence of somatic mutations varies across different tumour types, with NSCLC exhibiting the highest mutation frequency, ranging from 0.1 to 100 mut/Mb [36]. A retrospective analysis performed Rizvi, and his team [40] demonstrated that the efficacy of ICIs therapy in NSCLC patients is related to the TMB value. Patients with high TMB demonstrated better efficacy and higher survival rates compared to those with a lower TMB. Klempner [41] additionally observed that a TMB cut-off value of 10 mut/Mb could predict the efficacy of ICIs in NSCLC patients, with higher TMB thresholds correlating with longer progression-free survival. In most of the NSCLC studies, targeted Next Generation Sequencing (NGS) suggest a TMB cut-off value around 10 mut/Mb. While many studies have shown the predictive value of TMB for ICIs therapy in NSCLC patients, some have reported negative outcomes, particularly concerning long-term survival. This discrepancy may be due to the limited attention and research on TMB in this context [37].

In addition to the studies related to lung cancer, the specialized literature also includes studies analysing cases of malignant melanoma treated with ICIs. In Eckardt's study [42], the effectiveness

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of using TMB as a biomarker for patients treated with ICIs was demonstrated. Furthermore, the study's approach to the BRAF mutation, identified as a potential predictive biomarker for cases treated with targeted therapies, such as Dabrafenib, was also noteworthy. Those 2 markers were classified as independent viable predictive biomarkers for relapse-free survival period (ranges between 21%-100%) in patients diagnosed with malignant melanoma. Melanomas with BRAF mutations associated with high TMB are likely to benefit from adjuvant anti-PD-1 therapy. Conversely, patients with low TMB may gain more from adjuvant BRAF and MEK inhibitors, assuming their tumour tissue is less heterogeneous. However, the lack of direct comparative studies on this topic means that both adjuvant treatment options should be discussed with patients who have BRAF mutations [41].

Lastly, prostate and breast malignancies have a poor response to ICIs treatment, but in the last years, important improvements have been made in this direction. Because of that, several studies have started to investigate the importance of TMB value in these types of cancers. There is one preliminary study [43], that analysed 48 patients diagnosed with metastatic prostate/breast cancer and demonstrated that TMB, in these cases, is not directly associated with a better treatment response to ICIs. Instead, it was associated with a significantly higher number of genomic alterations and more pronounced MSI. These findings suggest that, with further research, TMB and MSI could potentially be correlated with a favourable response to ICIs in the future. Although minimal activity was observed in the group of patients with blood TMB evaluation/MSI-not detected, clinical benefit was noted in patients with notable MSI defects [43].

8. Conclusions

As a conclusion, in our comprehensive analysis, TMB holds significant promise as a predictive biomarker for the response to immune checkpoint inhibitors across various cancer types. Our analysis of current literature highlights a more established consensus regarding the importance of TMB in NSCLC and melanoma, where high TMB has been associated with better clinical outcomes. However, the role of TMB in breast and prostate cancers remains less clear, indicating a need for further research in these areas.

Despite the variability in findings, the potential of TMB to guide tailored immunotherapy treatment is undeniable. As we continue to explore and refine our understanding of TMB, especially in less well-defined cancers, it becomes essential to conduct large-scale, randomized studies. These studies will aid in standardizing TMB evaluation and confirming its predictive efficacy, ultimately advancing the precision of cancer treatment and enhancing patient prognoses.

In addition to PD-L1 and TMB, there are several other parameters associated with immune checkpoint inhibitor outcomes, such as prognostic scores like LIPI and dNLR, tumour-infiltrating lymphocytes (TILs), T-effector/IFN-γ signatures, general immune fitness, soluble inhibitors, tumour metabolism, and the microbiome. Combining these parameters could provide valuable insights. However, a challenge with integrating multiple biomarkers is the increasing number of potential patients' subgroups. This complexity makes it harder to establish cutoffs and understand interdependencies. Most clinical trial reports have only combined PD-L1 and TMB. Additionally, obtaining sufficient tissue for comprehensive analysis—such as defining histological subtypes, testing molecular drivers, and evaluating PD-L1 and TMB, can be challenging. Moreover, many predictive biomarker tests are costly for routine use. Exceptions include predictive scores based on routinely collected laboratory values, such as the LIPI score, which uses only pretreatment neutrophil, lymphocyte, and LDH levels. The performance of these scores should be assessed and compared with PD-L1 and TMB testing [44].

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