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Blood Tumor Mutational Burden as a Predictive Biomarker in Patients with Advanced Non-Small Cell Lung Cancer (NSCLC)

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Abstract

The cancer-associated mortalities in the world have been linked with lung cancer, and millions of deaths are recorded every year. Non-small cell lung cancer (NSCLC) constitutes about 85 percent of the total lung cancer, and the diagnosis is usually late. The use of immunotherapy, in particular, immune checkpoint inhibitors (ICIs) that serve as programmed death-1 (PD-1) receptor and programmed death ligand-1 (PD-L1), has radically changed the treatment approach of advanced NSCLC over the last ten years. Even after the research proved survival rates of many patients have been improved with the help of immunotherapy, few of them can receive long-term clinical responses. This is why it is necessary to find the credible biomarkers acting as excellent predictors of treatment reaction to optimize therapeutic interventions, and one of them is the amount of the tumor mutational burden (TMB), which is the sum of somatic mutations per megabase of DNA in tumor cells. The traditional TMB assay technique utilizes tumor tissue using whole-exome sequencing (WES), and it can be regarded as the gold standard of the mutation detection technique. Tissue biopsies are, however, linked with the following shortcomings: they are invasive, heterogeneous, and an adequate supply of tissues is not available. To address these issues, blood-based tumor mutational burden (bTMB) has been developed as a minimally invasive biomarker, which is grounded on circulating tumor DNA (ctDNA) in plasma. Recent studies suggest that high bTMB levels are predictive of better patient clinical outcomes with immune checkpoint inhibitors. The provided review article will offer an in-depth discussion of the biological foundations of bTMB, its significance as a forecasting biomarker of immunotherapy in advanced NSCLC, the use of bTMB involving clinical implications and benefits, obstacles to its utilization, potential limitations to its administration, and the perspectives of its application to precision oncology.

1. Introduction

Lung cancer is a major health problem in the world today since it is the main cause of cancer deaths in the world. World Cancer Statistics show that millions of new cases of lung cancer are diagnosed annually, and the rate of death has not decreased, regardless of improved technologies in early diagnosis and treatment of the disease. Almost 85 percent of all types of lung cancers are non-small cell lung cancer (NSCLC), which has several histological variations, such as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma [1]. The major dilemma of the non-small cell lung cancer treatment is that most of the patients are often diagnosed with advanced or metastatic lung cancer; hence, curative surgical interventions are not usually feasible. An overview of the past treatment of advanced non-small cell lung cancer is that chemotherapy and targeted therapies have been the initial form of treatment [2].

Although these regimens enhance the survival rates, remarkable side effects and resistance to medication limit their efficiency. The treatment procedure of NSCL has been completely transformed by the emergence of immunotherapy in the past decade [3]. Immune checkpoint inhibitors (ICIs) are antibodies that target PD-1 and PD-L1 and have been shown to have considerable clinical benefits in enhancing the immune system to detect and destroy tumor cells [4]. "Immunological control points" refer to

biological mechanisms that maintain immune balance, and immunity is not formed too aggressively [5].

Nonetheless, cancer cells are capable of exploiting these processes in order to evade the immune system. By inhibiting the outcome of the interaction between the PD-1 and PD-L1, the immune checkpoint inhibitors enhance antitumor immune responses and increase the T-cell response. However, immunotherapy is effective in only a few patients. The identification of predictive biomarkers that can be utilized to identify patients with the best prognosis to respond to immune checkpoint inhibitors has thus become a relevant field of study. Some of the biomarkers that have been studied are tumor mutational burden (TMB), microsatellite instability (MSI), and expression of PD-L1 [6]. The entire number of all the somatic mutations that take place in the genetic material of a tumor is referred to as the tumor mutational load. In tumors with high-mutation load, the immune system has the capability of detecting more neoantigens. A positive immune response to immunotherapy is more likely when the neoantigen load is higher [7]. Traditionally, by using whole-exome sequencing, TMB can be measured on tumor tissue samples [8].

But the methods of invasive tissue biopsy could not get sufficient tumor tissue to do full genetic characterizations. Besides, the entire genome of the tumor may not be absolutely represented [9]. These limitations have been overcome by the methods of liquid biopsy, which have been developed to analyze the presence of circulating tumor DNA (ctDNA) in the blood samples. A new technique called the blood-based tumor mutational burden (bTMB) allows monitoring of mutations that are found in plasma and requires a less invasive methodology accompanied by fewer inconveniences of evaluating tumor genetics [10].

2. Fundamentals of the Blood Disposal Tumor Mutational Burden

The mutation load on plasma tumor DNA is referred to as blood-derived tumor mutational burden (bTMB). Apoptosis and necrosis release DNA strands of the genome into the bloodstream of tumor cells, and this genetic modification could be detected as a consequence of tumor cells [11].

2.1 Circulating Tumor DNA (ctDNA)

Circulating tumor DNA (ctDNA) is a form of cell-free DNA (cfDNA), or short fragments of genetic material identifiable in blood, that is specific to tumor cells (and is released as a consequence of tumor death by apoptosis or necrosis) or actively released (e.g., by tumor cell extracellular secretions). Identification of ctDNA can help scientists analyze tumor genomics through a liquid biopsy procedure [12].

2.2 Sequencing of the Next Generation

The third-generation sequencing of the P. aeruginosa strain will be done in week three. The analysis of ctDNA by next-generation sequencing (NGS) is an approach that is often employed in order to identify the amount of bTMB. There are hundreds of cancer-relevant gene libraries produced to produce specific gene panels utilized in the detection of somatic mutations in the tumor genome [13].

2.3 Calculation of Mutation

The observed somatic mutations are then rescaled by the number of megabases of sequenced DNA to give the bTMB value. Mutations per megabase (mut/Mb) are usually presented as the normal form of the findings [14].

2.4 Association with Tissue-Based TMB

Investigations have shown a positive correlation of tissue-derived TMB when compared to blood-derived TMB, and thus, the plasma-based testing may be employed as a dependable substitute to the tissue test in selected situations, namely in clinical settings [15].

3. Immunotherapy Response and Biological Basis of Tumor Mutational Burden

The clinical value of bTMB of advanced NSCLC can be understood based on an understanding of the biological interaction of tumor mutational burden and immunotherapy response. The mutational burden of a tumor is the sum of somatic

mutations that are contained in the tumor genome. Such mutations could be formed in different mechanisms, such as in the case of kinship errors in the DNA replication; due to the exposure to carcinogens, like tobacco smoke and ultraviolet light; and malfunctions of the DNA repair system [16].

The mutation rate in lung cancer is usually increased in the case of smokers since tobacco carcinogens lead to widespread DNA destruction. The result of these mutations may be the creation of abnormal proteins, which are referred to as neoantigens. Neoantigens are the peptides produced when proteins of tumor cells are mutated and are attached to the surface of the tumor cell by the major histocompatibility complex (MHC) molecules [17].

These neoantigens form part of foreign molecules that are recognized by the immune system. The cytotoxic T lymphocytes are thus able to recognize tumor cells with the neoantigens and launch an immune response against them [18]. As more neoantigens are generated by the tumor cells, the chances of the immune system identifying and attacking the tumor increase.

Nonetheless, cancer cells usually evolve ways of escaping immune suppression. The activation of immune checkpoint pathways is one of such mechanisms. PD-1 receptors on T cells attach to the PD-L1 receptors on tumor cells and lead to suppression of the T-cell functions. This communication inhibits the immune responses and enables the tumor cells to avoid destruction by the immune system [19].

The inhibitory interaction is inhibited by immune checkpoint inhibitors, and T-cell functions are restored. When the number of tumor cells is large (i.e., organization of high mutational burdens), the immune system would be more efficient in recognizing tumor cells after the inhibition of the checkpoint pathway. Consequently, patients who have a high tumor mutational burden are more likely to respond better to cancer immunotherapy [20].

Tumor mutational loads. Blood-based tumor mutational burden indicates the mutational profile of tumor cells by analyzing circulating tumor DNA [21]. BtMB is, therefore, an indirect neoantigen load and immunogenicity of the tumor.

4. Ways of Measuring Blood Tumor Mutational Burden

The analysis of blood tumor mutational burden level involves the use of sophisticated molecular technologies and a very attentive approach to the analysis. The process of setting the bTMB concentration of plasma samples involves a number of scientific activities.

"The collection and plasma processing of the samples were carried out as described below."

4.1 Sample Collection and Plasma Processing

The collection of the peripheral blood samples of the patients is the first process in bTMB. In the case of blood, a specific tube usually holds the blood and is designed to stabilize the circulating nucleic acid. The circulation of the tumor DNA needs to be maintained in blood samples where degradation is avoided [22]. One of the circulating cell-free DNAs in plasma is tumor-derived DNA fragments following the centrifugation of blood. The circulating tumor DNA was separated using electrophoresis on a gel and thereafter purified using gel column techniques.

4.2 Extraction of Circulating Tumor DNA

Gel electrophoresis was done to extract circulating tumor DNA, and subsequently, purification was done by using the gel column technique. The extraction of circulating tumor DNA in the plasma is done through specially designed extraction kits, which are specific to separate minute DNA fragments. Extremely delicate procedures are needed to retrieve adequate ctDNA in order to subject it to sequence examination, as it represents an incredibly small portion of the overall cell-free DNA [23].

4.3 Library Preparation for Sequencing

The library was prepared to undergo sequencing by following the steps listed

below:
Sequencing libraries are obtained after the extraction of the DNA. Sequencing adapters are then ligated to the DNA fragments, which are then amplified via polymerase chain reaction (PCR) [24]. Next-generation sequencing is then done on these libraries.

4.4 Targeted Gene Panels

A majority of the bTMB assays utilize precisely directed sequencing arrays measuring hundreds of cancer-forming-related genes [25]. With the application of these panels, the researcher can get insight into the mutations in large areas of the genome without necessarily providing whole-exome sequencing.

- FoundationOne CDx
- GuardantOMNI
- MSK-IMPACT
- Thermo Fisher Oncomine panels

These platforms enable the high-throughput and sensitive detection of mutations.

4.5 Bioinformatics Analysis

Upon the generation of sequencing data, one applies bioinformatics pipelines in the analysis of such data and identification of somatic mutations. Germline variants and sequencing artifacts are removed with the help of algorithms to ensure the detection of the mutation [26].
The total number of somatic mutations noted in the region of interest of the genome is then divided by the area or size of the sequenced segment in megabases. The value obtained is the logarithm of the blood tumor mutational burden, or mutations in thousands per megabase.

5. Clinical Studies Evaluating bTMB in Advanced NSCLC

Many clinical researches have examined the prognostic value of blood tumor mutational burden (bTMB) in patients with advanced non-small cell lung cancer treated with immunotherapy. One of the landmark studies was a study that studied the efficacy of the PD-L1 inhibitor atezolizumab in previously treated patients with NSCLC and tested the prognostic value of bTMB as a biomarker [27].

n the literature, high bTMB levels of patients increased their progression-free survival with atezolizumab compared to chemotherapy. The other relevant study was on the relationship between bTMB and clinical response to pembrolizumab. It had demonstrated that patients with high bTMB levels exhibited high objective response rates and had higher survival rates compared to those with low bTMB levels. Other than single-agent immunotherapy, bTMB has also been examined in combination therapy.

One such instance is that Checkmate clinical trials were done with the combination of nivolumab and ipilimumab to be used in patients with high tumor mutational burden in NSCLC. The results also revealed that progression-free survival was generally much better in patients undergoing combination immunotherapy who had high TMB. These findings are in line with the application of bTMB as a prognostic biomarker, which is able to characterize those patients with the greatest likelihood of responding to treatment using immune checkpoint blockades [28].

6. Comparison Between Tissue-Based TMB and Blood-Based TMB

Tissue-based TMB, as well as blood-based TMB, give good information on tumor mutation load; however, the two methods differ in a number of essential ways.

Tissue-Based TMB

The TMB of tissues is determined on biopsy samples of tumors. Whole-exome sequencing of tumor DNA can be used to identify the entire coding genome of mutations. TMB based on tissues is considered the best of all mutation analysis methods, as it covers vast genome regions [29]. TMB is a tissue-derived biomarker characterized by a simple assessment of the sample taken by a biopsy through tumor tissue. Exome sequencing of tumor DNA can be used to thoroughly identify mutations in the entire coding genome. Due to its widespread genomic coverage, tissue-based

TMB is regarded as the gold standard of mutation analyses.

Blood-Based TMB

The other methodology is referred to as blood-based TMB, in which the circulating tumor DNA is separated in plasma samples. Its advantages are that it is minimally invasive, can be repeated on a sample, and can identify mutations in more than one locus in the disease. Nevertheless, the low concentrations of ctDNA in certain cases of patients would be problematic in applying blood-based TMB.

Variability of bTMB is determined by the various sequencing platforms and sizes of panels, all of which have been found in various studies to exhibit a moderate to strong correlation with tissue TMB, which shows the clinical usefulness of plasma-based mutation analysis [30].

7. Combination of bTMB With Other Biomarkers

Even though bTMB can be used to give useful insights in regard to tumor mutation load, it might fail to address the complexity of tumor-immune interactions. Thus, bTMB concomitants may be utilized to enhance predictive bTMB performance [31].

PD-L1 Expression

PD-L1 expression is now among the most popular biomarkers for the selection of patients with NSCLC for immunotherapy. However, the PD-L1 expression is not always a true predictor of response to treatment. PD-L1 expression and bTMB may involve the use of PD-L1 expression in combination with bTMB, as they can be used for both inpatient selection because they determine the load in terms of tumor mutations and immune checkpoints [32].

Microsatellite Instability

Malfunction of the processes of repairing the mismatch in the DNA resulted in microsatellite instability (MSI). MSI-high tumors tend to have high numbers of mutations and are also immunotherapy-sensitive [33].

Tumor Microenvironment

The tumor microenvironment is highly essential in the regulation of immune responses. Tumor-infiltrating lymphocytes, cytokine, and immune cell composition occurrence can also help to make the immunotherapy successful [34].

Incorporation of genomic biomarkers with the immune microenvironment markers can provide a deeper understanding of the tumor-immune interactions.

8. Technological Advances Improving bTMB Assessment

The recent technological advances are sweeping away the inaccuracy and clinical impossibility of the blood tumor mutational burden test.

Ultra-Deep Sequencing

Ultra-deep sequencing enables one to detect mutations within ctDNA at extremely low rates. This increases sensitization in the low-tumor patients [35].

Error-Correction Sequencing

The second-generation sequencing technologies are molecular barcoding and error-correction algorithms to reduce the sequencing errors and increase the ability of mutation identification [36].

Artificial Intelligence and Machine Learning

It is at this point that machine learning algorithms are increasingly being utilized to analyze large volumes of genomic data, and they are observing patterns that are correlated with the action of immunotherapy. To improve predictive quality, the bTMB data can be integrated with clinical variables, which can be conducted using AI-based techniques [37].

9. Global Research Trends and Future Directions

Blood tumor mutational burden studies are rapidly increasing; accuracy in oncology remains in development mode. Several ongoing clinical studies assess the utility of bTMB as part of the treatment option for various cancer types, including lung

cancer, melanoma, and bladder cancer [38].

"Future research efforts will likely focus on the following:"

- Standardization of bTMB measuring processes.
- Establishing standard cutoffs of high and low bTMB.
- Implementation of bTMB in a clinical decision model
- Improvement of the anticipated reaction to immunotherapy through mixed biomarker advancement.

The sensitivity and accuracy of the liquid biopsy techniques will be improved further with the development of the new sequencing technologies and improvements in bioinformatics [39].

10. Conclusion

Tumor mutational burden. Blood-based tumor mutational burden has been identified as a useful predictor of immunotherapy response in non-small cell lung cancer (AD). Liquid biopsy enables clinicians to determine the presence of tumor mutations based on the analysis of tumor DNA fragmented by circulating cells, eliminating the risk of studying implanted tumors with invasive tissue self-detection. A high level of bTMB has been linked to high levels of neoantigen generation and response to immune checkpoint inhibitors. Clinical trials have proven that a subject who obtains a high level of bTMB is better suitably treated and has better progression-free survival. Nonetheless, there exist some concerns, such as a lack of standardization, technical variation, and biological limitations, although it has its merits. These problems will be addressed with further research and technological capabilities, whereby the clinical setting will be improved.

use of bTMB. Lastly, the combination of blood tumor mutation burden and other genomic and immunological biomarkers may enable the selection of patients more properly in order to be subjected to immunotherapy and enact a personalized cancer treatment plan.

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