

Predictive biomarkers for tumor immune checkpoint blockade

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Abstract: The development of immune checkpoint inhibitors represented by PD-1 and PD-L1 has provided new hope for the treatment of advanced cancer patients. However, there are no effective predictive biomarkers, which have caused many limitations to the clinical application of immune checkpoint inhibitors. This paper combines recent domestic and international research as well as clinical trials to discuss the current status and progress of PD-L1 expression as a biomarker for tumor immunotherapy and also to discuss whether tumor mutation burden, tumor-infiltrating lymphocytes, tumor cell gene expression profiling, or peripheral blood markers would be a potential predictive biomarker for novel tumor immunotherapy. So, a brief review on this hot topic of predictive biomarkers for tumor immunotherapy is conducted.

Keywords: immunotherapy, immune checkpoint inhibitors, predictive biomarker

Background

Cancer is a serious public health problem in the world. With the development of society and aging of the population, current lifestyles, such as smoking, obesity, and lack of exercise, lead to an increasing incidence of cancer. According to the latest incomplete statistics released by the International Agency for Research on Cancer in 2017, there are approximately 16.8 million new cancer cases and 6 million cases of cancer deaths each year worldwide, and the 5-year survival rate of all tumor patients is only 67%.^{1,2}

As we know, the purpose of cancer treatment is to improve the patients' quality of life and potentially extend the patients' survival time. In the past 20 years, with the rapid development of oncology theory and technology, following the three traditional methods of cancer treatment (surgery, radiotherapy, and chemotherapy), a number of new treatments have emerged, such as targeted therapy, interventional therapy, and immunotherapy.^{3,4} It is worth mentioning the progress of tumor immunotherapy. A current review of the literature suggests that tumor immunotherapy includes active, passive, and adoptive immunotherapy. This approach induces tumor-specific effector cells and molecules in vitro and in vivo by increasing the immunogenicity of tumor antigens, stimulating and enhancing antitumor immune response, as well as increasing the sensitivity of tumors to immune effects. Among various tumor immunotherapy strategies, PD1 (also known as CD279) and PD-L1 (also known as CD274) inhibitors and CTLA-4 (also known as CD152) inhibitor are currently the focus of research.⁵

PD-1 is mainly expressed on the surface of activated T lymphocytes and B lymphocytes, and the main function is to maintain the normal auto-stable state of the immune

system by inhibiting the activation of T lymphocytes and B lymphocytes.⁶ It is also highly expressed in a large number of infiltrating T lymphocytes in the tumor microenvironment. On the other hand, PD-1 ligands include PD-L1⁷ and PD-L2,⁸ which are highly expressed on the tumor cell surface. Although the binding affinity of PD-1 to PD-L2 is significantly higher than PD-L1, the amount of PD-L1 expression on the tumor cell surface is significantly higher than that of PD-L2.⁹ For instance, PD-L1 is expressed in approximately 20%–30% of non-small-cell lung cancers (NSCLCs). CTLA-4 ligands include CD80 and CD86, which are only expressed on the surface of antigen presenting cells but not on the tumor cell surface. Therefore, CTLA-4 inhibitors play a role in inhibiting T lymphocyte activation in secondary immune organs (meaning lymph nodes). It has been found that CTLA-4 is also expressed on the surface of regulatory T lymphocytes (Tregs) which can negatively regulate cellular immunity and have antitumor immunosuppressive effects.¹⁰ At present, some studies have demonstrated that PD-1/PD-L1 inhibitors and CTLA-4 inhibitors have remarkable clinical efficacy, durable response, and low toxicity during the treatment of many advanced malignant tumors. Therefore, current research directions for immunotherapy are mainly focused on the PD-1 and PD-L1 signaling pathway⁹ as well as CTLA-4 inhibitors.

In most clinical trials, researchers have found that the expression level of PD-L1 might be an effective predictive biomarker for immunotherapy, especially in the treatment using PD-1/PD-L1 inhibitors.^{11,12} However, there remain controversies about the predictive value of this biomarker, because many clinical studies have also found that the fraction of cancer patients with low or negative PD-L1 expression can also have benefited from tumor immunotherapy of PD-1/PD-L1 inhibitors.^{13,14} Tumor mutational burden (TMB) is another potential predictive biomarker for tumor immunotherapy.^{15,16} Growing evidence has led to the consensus that TMB may be a greater predictive biomarker compared to PD-L1 expression.¹⁷ In addition, except PD-L1 expression level and TMB, many studies have also focused on the relationship between tumor-infiltrating lymphocytes (TILs) and the prognosis of malignant melanoma in CTLA-4 inhibitors, which also has been shown to have a better effect on tumor control.^{18–20}

Based on all this, tumor immunotherapy requires the selection of the most beneficial population based on highly specific therapeutically efficient predictive biomarkers. However, exploration of efficacy predictive biomarkers is a challenge for tumor immunotherapy. Here, we summarize

potential predictive biomarkers including PD-L1, TMB, gene expression profiling (GEP), TIL, and peripheral blood markers.

PD-L1 as a predictive biomarker for tumor immune checkpoint blockade

PD-L1, also known as B7-H1 or CD274, is encoded by the *PDCDL1* gene on human chromosome 9 and also the first functionally characterized ligand for PD-1. Together with its cognate ligand PD-L2, PD-L1 plays a key role in maintaining peripheral and central immune cell tolerance by binding to PD-1 receptors.⁶

Many studies have shown that there are two patterns of PD-L1 expression, namely, constitutive and inducible expression. Low levels of constitutive expression of PD-L1 can be found in resting lymphocytes, antigen presenting cells, syncytiotrophoblasts, and Langerhans cells. The primary role of constitutive PD-L1 is to maintain a stable state in proinflammatory responses of organization. In the case of inflammation or infection, an inducible PD-L1 expression, which may act as an inhibitory signal for hematopoietic, endothelial, and epithelial cells, suppresses the activation of T lymphocytes and exerts immunosuppressive effects.²¹ PD-L1 expression is mainly regulated by Toll-like receptors. $\text{INF-}\gamma$ receptors 1 and 2 can also participate in the regulation of PD-L1 expression through the Jak/STAT signaling pathway.²² Furthermore, other studies have also found that PD-L1 expression may be involved in the mutation or overexpression of carcinogenic driver genes during the development of cancer. For example, EGFR mutation in lung cancer is correlated with the expression of PD-L1 obviously, and EGFR-tyrosine kinase inhibitors can inhibit the transcription of PD-L1.²³ Other studies also found that the NPM/ALK fusion gene can upregulate PD-L1 expression through activation of STAT3 in T-cell lymphoma.²⁴ Expression of PD-L1 has been found in a number of tumor type cells including NSCLC, breast cancer, kidney cancer, malignant melanoma, gastric cancer, epithelial ovarian cancer, and other tumors.⁹ However, PD-L1 expression is significantly heterogeneous.^{25,26} Different tumors or histological types with different PD-L1 expression may require different treatment modalities. A large number of clinical studies have shown a correlation between PD-L1 expression and the efficacy of PD-1 or PD-L1 inhibitors in the treatment of advanced or metastatic tumors. We draw a conclusion from these clinical trials that patients with high expression of PD-L1 may have a higher objective response rate upon treatment with PD-1/PD-L1 inhibitors. For example, in the

CheckMate 057 study, the objective response rate for treatment with nivolumab was 37% in NSCLC patients with a PD-L1 expression $\geq 10\%$. However, the objective response rate was only 11% in patients with lower PD-L1 expression.²⁷ It was also interesting to note in the CheckMate 017 study that patients with advanced squamous lung cancer benefited from nivolumab monotherapy regardless of whether PD-L1 was expressed or not.¹³

Unfortunately, there are reports showing that sensitivity and specificity of PD-L1 can reach 100%. The reason may be as follows. First, the PD-L1 expression is not only regulated by multiple signaling pathways and molecular mechanisms but also by other immune cells in the tumor microenvironment. Second, PD-L1 expression has temporal and spatial heterogeneity.^{25,26,28} Therefore, the expression of PD-L1 at a certain time or in a tumor site does not accurately reflect the true condition of the patient's PD-L1 expression axis. Third, the heterogeneity of the PD-L1 immunohistochemistry (IHC) antibody and positive threshold was not uniform. Based on these reasons, only using the PD-L1 expression cannot yet be fully used as a treatment decision for patients who may benefit from PD-1/PD-L1 inhibitor therapy.

TMB as a predictive biomarker of efficacy for tumor immune checkpoint blockade

TMB was explored as another prediction marker in tumor immunotherapy. As is well known, TMB refers to the number of somatic mutations in the genome of tumors, excluding from germ line mutations. Current studies have suggested that TMB with many new antigens and higher immunogenicity might be more suitable and benefit from immunotherapy. Previous clinical trials in multiple tumors also have shown that there is a close positive correlation between TMB and the efficacy of PD-1/PD-L1 inhibitors. For instance, the CheckMate 026 study was designed to compare the efficacy of nivolumab against chemotherapy as first-line treatments for NSCLC patients with PD-L1 expression $>5\%$. The results showed that nivolumab, compared with chemotherapy, showed no significant increase in progression-free survival (PFS, 4.2 vs 5.9 months) and overall survival (OS, 14.4 vs 13.2 months). Compared with PD-L1, choosing TMB as a predictive biomarker can better distinguish the benefit group. Further studies also found that there were significant differences in ORR (47% vs 28%) and PFS (9.7 vs 5.8 months) for NSCLC patients with higher TMB.¹⁷ Similar results from CheckMate 052 study²⁹ and CheckMate 275 study were observed, and two clinical trials demonstrated that patients

with higher TMB had significantly increased OS upon immunotherapy, especially with PD-1/PD-L1 inhibitors.

Not surprisingly, tumor patients with higher TMB often exhibit specific DNA damage, such as microsatellite instability high (MSI-H) or mismatch repair defect (dMMR).³⁰ Currently, MSI and dMMR status are determined by polymerase chain reaction or IHC. Some studies have shown that colorectal cancer patients with MSI-H or dMMR are sensitive to PD-1/PD-L1 inhibitors.³¹ In addition, it has also been found that the treatment response of PD-1/PD-L1 inhibitors in patients with dMMR who have non-colorectal cancer is similar to patients with dMMR who suffer from colorectal cancer. Le et al³² explored the efficacy of PD-1 inhibitors in 12 different advanced tumors patients with dMMRs and drew a conclusion that dMMR status might be a predictive biomarker of anti-PD-1 therapy in all tumor patients. There were five non-controlled, multi-cohorts, multi-centers and one arm clinical trials which included a total of 149 patients with MSI-H or dMMR status, including 90 colorectal cancer and 59 patients from 14 other neoplastic species. Pembrolizumab was approved for advanced cancer patients with MSI-H or dMMR status by the America Food and Drug Administration clinical trials. This is also the first antitumor program that chooses to better benefit people based on prediction markers without relying on the source of tumor tissue.

From the current research data, TMB may be a potential predictive biomarker of PD-1/PD-L1 inhibitor therapy for advanced cancer patients. However, it is still necessary to explore the best detection method and critical value of TMB for each tumor.

GEP as a predictive biomarker of efficacy for tumor immune checkpoint blockade

GEP of tumor cells can not only guide targeted therapy but also influence tumor external microenvironment and further affect the efficacy of immunotherapy. Although the mechanism by which GEP influences the efficacy of immunotherapy is not yet clear, we can summarize four categories that influence the efficacy of immunotherapy on the basis of the results of previous studies. Negatively and positively related to the efficacy of immunotherapy, resistance, and explosive progression are associated with immunotherapy. Gainor et al³³ performed a retrospective analysis of 58 NSCLC patients treated with immunotherapy. The results showed that the objective response rate after immunotherapy was significantly lower in patients with clear mutations in EGFR or ALK (3.6% vs 23.3%, $P=0.053$).³³ Similarly, it was found that patients with

positive EGFR-T790M mutations had significantly lower PFS than patients with negative T790M in the cohort undergoing nivolumab therapy (2.1 vs 1.3 months, $P=0.099$).³⁴ Other studies have also shown that RAS gene mutation can hinder the recognition by immune cells of tumor cells due to the promotion of accumulation of PD-L1 expression, and the KARS/TP53 mutation is positively correlated with the PD-L1 expression. In addition, an article published by Dong et al in 2016 also reaffirmed that the median PFS of NSCLC patients with TP53 mutations undergoing therapy with PD-1 inhibitors was significantly longer than in those patients who had TP53 wild-type gene (14.5 vs 3.5 months, $P=0.042$).³⁵ The immunotherapy-resistance-related genes were mainly concentrated in the PTEN gene on chromosome 10, and the JAK1/2 gene and B2M were shown to have deletion mutations in malignant melanoma. There are currently few relevant studies on the genes involved in the explosive progression of immunotherapy.

Different GEPs may lead to many different effects on immunotherapy. The role of genetic testing has been more than just directing targeted therapies in the treatment of cancer. We believe more and more comprehensive genetic testing will provide further comprehensive guidance for the treatment of cancer patients in the future.

TILs as a predictive biomarker of efficacy for tumor immune checkpoint blockade

TILs refer to lymphocytes that have infiltrated into the tumor tissue from the blood circulation. When there are a large number of TILs in the tumor tissue, it indicates that the body initiates an immune response against the tumor. Previous studies have shown that the number of infiltrating lymphocytes in tumor tissues has a clear correlation with the response to chemotherapy and prognosis of breast cancer,³⁶ malignant melanoma,¹⁸ oral squamous cell carcinoma,³⁷ and other malignant tumors. How is the relationship between TILs and tumor immunotherapy? In 2016, Li et al³⁸ deduced that TILs and cancer patients' heredity change was similar by integrating 23 tumor types from The Cancer Genome Atlas database and over 10,000 tumor sample molecular sequences. It has also been found that expression of testis antigen and CD8⁺ T-cells expressing MAGEA3 are potential immunological targets for malignant melanoma. However, the infiltration of a large number of CD8⁺ T-cells may affect the clinical response of the anti-CTLA4 drug. Similarly, they also observed that Tim3 expression demonstrated the above-mentioned effects in renal cell carcinoma CD8⁺ T-cells.³⁸ In

the same year, another study found that increased TILs were associated with increased expression of tumor neoplastic antigens, and tumor neoplastic antigens might have an influence on tumor patients' survival and response to immunotherapy.³⁹ A higher number of TILs was associated with the higher predictive value of tumor response and prognosis in most clinical trials evaluating the efficacy of PD-1/PD-L1 inhibitors. Recently, Zheng et al⁴⁰ proposed that the reason the tumors outside the indications currently approved cannot benefit from immunotherapy might be related to the type of T lymphocytes in TILs. They found that there are a large number of tumor-specific clonally proliferating T-cells in the tumor tissue of patients with liver cancer, but most of these T lymphocytes are depleted.⁴⁰

Peripheral blood markers as a predictive biomarker of efficacy for tumor immune checkpoint blockade

It is well known that the inflammatory response has been shown to be closely linked to immune resistance in cancer patients. Many basic studies have also found that the inflammatory response could promote the proliferation and metastasis of tumors and activate multiple tumor signaling pathways.^{41,42} In clinical practice, there are several indicators in peripheral blood tests that can reflect the level of inflammation in cancer patients, such as white blood cell count, neutrophil count, platelet count, lactate dehydrogenase, C-reactive protein, eosinophils, as well as neutrophil-lymphocyte ratio (NLR), and platelet-lymphocyte ratio (PLR). NLR can reflect the relative balance of myeloid cells and lymphocytes in peripheral blood, including T lymphocyte subtypes CD4⁺ and CD8⁺ T-cells, B lymphocytes, and NK cells. High NLR indicates chronic inflammation and immune response. At the same time, the increase of PLR indicating the relative increase of platelets or decrease of lymphocytes can both reflect the tumor-associated inflammation and immune status. Some studies found these indicators were associated with the quality of life⁴³ and prognosis^{44–47} of cancer patients. It is important that the detection method of peripheral blood markers is convenient and noninvasive, but there is no prospective study to confirm its significance and value as a predictive biomarker.

Summary

Immunotherapy based on immune checkpoint inhibitors has completely overturned the concept of cancer treatment in the past several decades and has become one of the most

important methods for cancer treatment. Although tumor immunotherapy has achieved remarkable success in some specific advanced cancer patients, there is still a significant proportion of patients with no significant treatment effect. How to make immune checkpoint inhibitors perform better, activating TILs, is the most crucial necessity. These T lymphocytes are regarded as the main anticancer agents in the whole tumor immune cycle. Current studies have found that the process is regulated by multiple signaling pathways. For instance, cancer patients with activated Wnt/beta-catenin signaling,⁴⁸ MYC gene overexpression,⁴⁹ and loss of PTEN,⁵⁰ leading to immune escape, do not benefit from immune checkpoint blockade. There already exist some drugs which could improve the curative effects of cancer patients treated with immune checkpoint inhibitors in the clinic by fully understanding the escape mechanism of these signaling pathways, for example, PI3K inhibitor, cGAS-STING signaling activators,⁵¹ and even oncolytic virus. It is worth mentioning that cGAS-STING signaling plays an important role in the process of antitumor immunity. First, activated STING signaling pathway can induce the generation of cytokines and activate T lymphocytes to reach a targeted tumors cell. Second, the sensitivity of tumor cells to immune system treatment increases by activating STING signaling. Some scholars have found that STING agonist joint preparation can cure the PD-1 block in drug-resistant tumors in animal experiments.⁵²

Apart from the abovementioned signal pathways and lymphocytes, the further effect of tumor microenvironment on tumor cell immune escape should not be neglected. Some studies have verified that tumor-associated fibroblasts (TAFs) play an important role in tumor microenvironment and have special physiological and biochemical characteristics.⁵³ TAFs, which are different from normal fibroblasts, can inhibit the function of immune cells. An experiment observed that TAFs promoted PD-L1 expression in lung cancer cell and it also provided a new treatment strategy. We can use our own immune system to inhibit tumors by blocking TAF immunosuppression in combination with PD-L1 antibody therapy.⁵⁴

In addition, PD-1 antibody in combination with LAG-3, Tim3, OX40, Anticalin, TAA-1/2/3, Her-2, EGFR, CTLA-4, and PD-L1 inhibitors is currently in the preclinical phase of study. Although the CheckMate 143 study (NCT02017717) which explored the efficiency and safety of Opdivo (PD-1 inhibitor) plus Yervoy (CTLA-4 inhibitor) in glioblastoma patients ended in a failure with many serious adverse events,

satisfactory results can be expected by analyzing the developmental trend and clinical results of double therapy with a targeted antibody and associated drugs, for the CheckMate 064 study.⁵⁵

Therefore, in the course of the application of immunotherapy, how to maximize cancer patient's benefit, minimize the risk of toxicity, and provide accurate screening for the benefit-seeking population are urgent problems that need to be solved.

We have found that cancer patients with positive PD-L1 expression have a greater clinical benefit for immunotherapy, but the detection of PD-L1 expression alone is not sufficient for most tumor subtypes. In this review, we discussed the current status and future of PD-L1 expression as a predictive efficacy biomarker of immunotherapy, and also investigated the possibility of TMB, TILs, GEP, and peripheral blood markers as novel markers. However, simply increasing the number of predictive biomarkers of efficacy may only complicate the clinical use of PD-1/PD-L1 inhibitors and CTLA-4 inhibitors. In the future, the focus should perhaps be on effective screening of the benefit-seeking population through the standardized detection of PD-L1 expression and the combination of new immune biomarkers.

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Disclosure

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