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Review Article

Multimodal detection of PD-L1: reasonable biomarkers for immune checkpoint inhibitor

Qiao Yang, Zihan Xu, Linpeng Zheng, Luping Zhang, Qiai You, Jianguo Sun

Cancer Institute of The People's Liberation Army, Xinqiao Hospital, Army Medical University, Chongqing 400037, China

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Abstract: Immune checkpoint inhibitor (ICI) therapy had achieved significant clinical benefit in multiple malignant solid tumors, such as non-small cell lung cancer, melanoma and urothelial cancer. ICI therapy not only revolutionarily altered the treatment strategy of malignant solid tumors, but also dramatically prolonged overall survival. However, the objective response rate (ORR) of ICI therapy in second line treatment remains 20% or less. How to find patients eligible for ICI therapy by effective biomarkers became hot nowadays. High expression of PD-L1 protein in tumor cells or tumor microenvironment (TME) had been identified to be a logical biomarker for predicting efficacy of ICI therapy and approved by the U.S. Food and Drug Administration to be an indicator of initiating treatment for some solid tumors. Controversially, patients with low PD-L1 protein expression might also show clinical benefit. In this sense, tissue PD-L1 protein expression might not be a precise biomarker. Multimodal detection of PD-L1, such as PD-L1 protein, PD-L1 mRNA, and circulating PD-L1, might provide a comprehensive tumor profile and could find the patients who are more suitable for ICI therapy. Besides, dynamic monitoring of PD-L1 expression could shed light on efficacy assessment and drug resistance. ICI-based combination strategy had demonstrated better outcome than ICI alone. Single biomarker might not be efficient to precisely find advantage patients. Combined biomarkers could better instruct the consideration of therapeutic regimen. In addition, nomogram and artificial intelligence platform could integrate multiparameter information of biomarkers which might shed light on tumor profile and give a hint to treatment decision.

Keywords: Immune checkpoint inhibitor, biomarker, PD-L1, liquid biopsy, multimodal

Introduction

In recent years, immune checkpoint inhibitor (ICI) therapy leads the new direction of cancer treatment. Due to their definite curative effects and low side effects, multiple ICI therapies have been approved for clinical application. Although ICI therapy has been proven to provide a long-term survival benefits, about 16% patients experienced 5-year overall survival [1], the efficacy of ICI therapy remains low. The objective response rate (ORR) of programmed cell death-1 protein receptor (PD-1)/programmed cell death-1 protein ligand (PD-L1) monoclonal antibody (mAb) therapy was only about 10-40%, and that of single cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) blockade was lower, most patients did not respond to ICI therapy [2]. How to optimally pick out suitable patients for ICI therapy is one of the research hotspots currently.

So far, many biomarkers have demonstrated the ability to effectively predict tumor response, for instance, PD-L1, tumor mutation burden (TMB), microsatellite instability (MSI), mismatch repair deficient (dMMR), tumor neoantigen burden (TNB), tumor-infiltrating lymphocytes (TILs), effector T-cell (Teff) gene signature, T-cell receptor clonality, intestinal microbiota, genetic feature, etc. [3-5]. Tissue biopsy is the main method for obtaining biomarker signature. Liquid biopsy, an emerging way to reveal tumor-related molecular information by analyzing peripheral blood or other liquid samples, also plays an important role in selecting patients, as well as highlights the significance of dynamic monitoring during treatment.

The PD-1/PD-L1 pathway is the key mechanism for tumor immune escape. The increasing PD-L1 protein expression on the surface of tumor cells (or antigen presenting cells, dendritic cells) could directly bind to PD-1, which expresses on T cells. This process limits the activation and proliferation of T cells, and weakens their cytotoxicity against tumor cells [2, 6]. Both PD-1 and PD-L1 blockade could reverse the immunosuppressive effect. Hence, PD-L1 expression in the tumor microenvironment (TME) might be a reasonable biomarker to predict efficacy of ICI therapy for malignant solid tumors.

In this mini-review, we investigated the multimodal PD-L1 detection of malignant solid tumors, highlighted the significance of circulating PD-L1 expression and combined predictive models.

Tissue PD-L1 expression

PD-L1 is a cell surface protein encoded by the CD274 gene. Not only will tumor cells up-regulate the expression of PD-L1 after exposure to interferon-γ and other cytokines, but also some immune cells in the TME have increased PD-L1 expression (such as antigen presenting cells, dendritic cells, macrophage and T cells, etc.) [7]. On October 24, 2016, Pembrolizumab had been approved by U.S. Food and Drug Administration (FDA) for first-line treatment of metastatic non-small cell lung cancer (NSCLC) patients whose tumor proportion score (TPS) of PD-L1 protein expression ≥ 50%, or for metastatic NSCLC patients who failed platinum-based chemotherapy and whose tumor PD-L1 expression level ≥ 1%. It's the first time that FDA approved ICI for lung cancer with PD-L1 as a selection indicator.

In clinic work, pre-treatment positive PD-L1 expression on tumor cells or immune cells, which was evaluated by immunohistochemistry (IHC), had demonstrated a logical biomarker for predicting favorable prognosis of ICI therapy in various cancer types. The OAK trial compared atezolizumab with docetaxel in advanced NSCLC [8]. Patients with high PD-L1 expression in the TME (defined as PD-L1 expression ≥ 50% on tumor cells or PD-L1 expression ≥ 10% on tumor-infiltrating immune cells) had significantly prolonged median overall survival (mOS) than those with low PD-L1 expression (defined as PD-L1 expression < 1%) when treated with atezolizumab. On the contrary, for patients treated with docetaxel, the mOS remained similar irrespective of PD-L1 expression. The CheckMate 057 trial demonstrated similar findings in advanced nonsquamous NSCLC patients whom were treated with nivolumab [9]. In melanoma patients treated with ICI therapy, those with PD-L1 expression ≥ 5% had better ORR than others [10]. In the phase II CheckMate 275 trial, the ORR was 28.4% in metastatic urothelial carcinoma patients with high PD-L1 expression (≥ 5%) compared to 16.1% in those with low PD-L1 expression (< 5%) [11]. In addition, the efficacy of ICI therapy in other malignant tumors, such as bladder cancer, head and neck squamous cell carcinoma, gastric and gastroesophageal junction cancer, hepatocellular carcinoma, was also associated with PD-L1 expression [12-15]. However, partial patients with negative PD-L1 expression (TPS < 1%) could also benefit from ICI treatment, which makes PD-L1 protein expression a controversial biomarker [16].

The expression of PD-L1 mRNA in tumor could be an alternative form of PD-L1 protein to select suitable patients for ICI therapy, since PD-L1 mRNA amplification was found to be associated with PD-L1 protein expression in tumor [17, 18]. The advantages of assessing PD-L1 mRNA expression lie in its specificity, reproducibility, and interpretative objectivity. Except for amplification, a rare PD-L1 gene rearrangement could lead to obviously increasing of PD-L1 transcripts. The aberrant PD-L1 transcripts overexpression was caused by 3'-UTR disruption of PD-L1 gene and speculated to be more susceptible to ICI therapy [19]. This structural variation could be exploited as a novel genetic marker for selecting patients.

Circulating PD-L1 expression

Analysis of circulating tumor cells (CTCs) is one of the classical liquid biopsy to unmask real-time profile of tumor. CTCs are derived from primary tumor site, and exist in peripheral blood [20]. Mazel M [21] et al, identified the presence of PD-L1 protein in CTCs for the first time. Eleven out of sixteen breast cancer patients had PD-L1 positive CTCs, whereas the proportion of PD-L1$^+$ CTC in individuals varied from 0.2% to 100%. Consistent with this, the existence of PD-L1$^+$ CTC was also identified in

advanced bladder cancer as well as NSCLC [22, 23]. In theory, PD-L1$^+$ CTC could also evade attack from the immune system because of the interaction of PD-1 and PD-L1. In consequence, patients with PD-L1$^+$ CTC might have a higher likelihood of formation of metastases.

Tumor-derived exosomes play a key role in mediating tumor immune escape, invasion, migration and drug-resistance. Studies have uncovered that exosomes contain abundant of cargo, for instance, protein, mRNA, miRNA, DNA, etc. Hence tumor-derived exosomes are regarded as the biomarker for early diagnosis, monitoring, and prognostic evaluation [24-26]. Recent estimate confirmed the existence of PD-L1 protein in exosomes in vivo an in vitro. The PD-L1$^+$ exosome could directly bind to PD-1 or deliver PD-L1 to PD-L1 negative tumor cells, then led to exhaustion of T cell. This suppression function could be reversed by ICI or exosome inhibitor [27]. Same finding was performed in clinic, Whiteside and colleagues [28] used beads to capture tumor-derived exosomes in plasma, then identified PD-L1$^+$ exosome/bead complexes by flow cytometry. The PD-L1$^+$ exosomes were correlated with disease stage and lymph node status in head and neck squamous cell carcinomas patients. PD-L1^{high} exosomes could suppress effector T cells activation, but also could be reversed by anti-PD-1 antibody.

In vitro, the tumor-derived soluble PD-L1 (sPD-L1) remains the necessary domain bounded to PD-1. sPD-L1 might contribute to systemic damage of host immunity by producing immunosuppressive signal, then promote apoptosis of activated T cells and tumor progression [29]. sPD-L1 elevated in NSCLC patients before treatment compared with normal control [30]. Further study found that pretreatment plasma sPD-L1 concentration could predict prognosis of nivolumab therapy against NSCLC. Patients with high sPD-L1 expression had shorter OS than those with low sPD-L1 expression (the cut-off point was 3.357 ng/mL). The ORR was 59% in low sPD-L1 patients, but only 25% in high sPD-L1 patients [31].

Taken together, the detection of PD-L1 expression through liquid biopsy has garnered attention so far. The different circulating PD-L1 forms, as mentioned above, might serve as surrogate markers for evaluating prognosis and efficacy of ICI treatment. However, there was no evidence about whether circulating PD-L1 expression was consistent with tissue PD-L1 expression, and whether circulating PD-L1 had the similar value to predict tumor response as tissue PD-L1, either. More clinical trials are urgently needed to verify the role of circulating PD-L1.

Dynamic change of PD-L1 expression

As we know, most cancer patients received epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), chemotherapy, radiotherapy (RT), or concurrent radiochemotherapy as the first-line treatment before initiating ICI therapy. Emerging studies suggested that traditional therapeutic regimen could affect PD-L1 expression in the TME [32, 33]. In vivo, radiation could induce the upregulation of PD-L1 on tumor cells and myeloid cells as well as reprogram the TME and recruit immune cells infiltrating into the TME. In a clinical study on 12 esophageal cancer patients with paired tissue samples before and after RT, an upregulation of PD-L1 was detected and associated with a favorable prognosis [34]. On the contrary, EGFR-TKIs could down-regulate PD-L1 expression in vitro [35]. Therefore, reassessment of PD-L1 expression is necessary prior to initiate ICI therapy.

On the other hand, the PD-L1 expression might alter during the period of ICI therapy. A report used a 12-marker IHC panel to interrogate the immune profiling in the TME of melanoma patients treated with ICI [36]. No difference of PD-L1 expression was found between responders and non-responders before treatment. However, after 2-3 cycles of ICI therapy, a second biopsy shed light on higher PD-L1 expression in responders versus non-responders. Another clinical trial also reported that PD-L1 expression significantly increased in responders than in non-responders within 2 months of commencing ICI treatment [37]. It follows that the dynamic observation of PD-L1 expression in the TME is conducive to assess therapeutic efficacy of ICI and subsequent treatment, but in clinical practice, multiple tissue biopsies are difficult to achieve. On the contrary, liquid biopsy needs only blood or urine samples. It's feasible to obtain multiple real-time circulating PD-L1 expression during the disease progression. A research reported that

Table 1. References summary of different forms of PD-L1 expression

Tissue PD-L1	PD-L1 protein	[8-15]
	PD-L1 mRNA	[17-19]
Circulating PD-L1	CTC PD-L1	[21-23]
	Exosome PD-L1	[27, 28]
	Soluble PD-L1	[30, 31]
	Dynamic PD-L1 change	[22, 37-39]

PD-L1, programmed cell death-1 protein ligand; CTC, circulating tumor cell.

patients with continuous PD-L1 expression on CTCs from baseline to 6 months who were treated with nivolumab experienced poor outcome. Whereas, patients with positive PD-L1 CTCs at first but turned to negative at 6 months achieved a clinical benefit [22]. Combined with previous literatures [36, 37], we speculate that tumor PD-L1 may temporarily elevate in the early on-treatment of ICI therapy because ICI could hamper the combination of PD-1 and PD-L1. But eventually, after a long-term response to ICI therapy, tumor PD-L1 expression may gradually decrease.

Plasma exosomal PD-L1 mRNA level alteration was reported to be associated with response to ICI therapy in melanoma and NSCLC [38]. Patients responded to treatment had decreased exosomal PD-L1 mRNA expression at 2 months compared with baseline. The exosomal PD-L1 mRNA expression did not alter too much in patients with stable disease. Not surprisingly, in progressive disease, the exosomal PD-L1 mRNA levels elevated.

It thus appears that each treatment method may affect PD-L1 expression in different ways, to dynamically assess PD-L1 expression might timely discover resistance to current therapeutic regimen and instruct following treatment.

The limitations of PD-L1 as a biomarker

Multimodal detection of PD-L1 expression (summary in **Table 1**) had demonstrated powerful ability to instruct ICI therapy, especially for evaluating efficacy by dynamical assessment. However, there are some limitations of using PD-L1 as a biomarker. First, different clinical trials used different immunohistochemistry antibodies and testing platforms. A review [39] showed Ventana SP142 had poor concordance with other IHC antibodies. The most sensitive antibody for assessing PD-L1 protein expression was Ventana SP263. In addition, discrepancies among laboratories also affected the results. Multiple detection approaches of circulating PD-L1 expression had been proposed, but which one is more sensitive and precise remains unknown. Similarly, there are no standard detection methods for different forms of circulating PD-L1. Secondly, heterogeneity exists inter- and intra-tumor. A single tissue biopsy may not comprehensively reveal tumor features [40]. Next, no accepted cutoff value has been set for neither tissue PD-L1 expression nor circulating PD-L1 expression. In clinical trials, the prespecified tissue PD-L1 cutoff values varied from 1% to 50%. The optimal cutoff point of circulating PD-L1 expression was calculated by ROC curve analysis. Obviously, uncertain cutoff values affected survival analysis. Moreover, not all patients with high PD-L1 expression experienced clinical benefit, partial PD-L1 negative patients obtained survival benefit. All these limitations make PD-L1 a controversial biomarker.

Combined strategies

In addition to PD-L1, TMB and MSI-high/dMMR had been approved by FDA as biomarkers to stratify patients. Especially MSI-high/dMMR were identified as a pan-cancer marker, any unresectable or metastatic solid tumors with MSI-high/dMMR could be treated with pembrolizumab [41]. Even so, current known biomarkers have limited predictive values. In addition, different ICI-based combination therapies nowadays have demonstrated obvious survival benefits, posing a challenge for searching more reasonable biomarkers to maximize therapeutic benefit.

Strategies of combined biomarkers which could comprehensively describe immune status of TME might optimally guide patient selection and exert an influence on the treatment strategy. A recent study showed that, in NSCLC patients treated with PD-1/PD-L1 blockade, the rate of durable clinical benefit (DCB) was similar when using PD-L1 or tumor mutation burden (TMB) alone as a predictor (35.3% and 29.4%, respectively). But it increased to 50.0% when considered PD-L1 and TMB as a combined predictor [42]. Base on the PD-L1 expres-

sion and tumor-infiltrating lymphocytes (TILs) status, tumor could be divided into four subtypes [43]. One of them comprised high PD-L1 expression and vast pre-existing T cells. This cancer subtype is more suitable for ICI therapy. Other three subtypes are absence of PD-L1 or TILs or both, these subtypes may need combined treatment strategy to enhance antitumor effects [44].

Nomogram and deep learning in biomarker screening

So far, plenty of biomarkers have emerged. The effective integration of these predictive parameters might help to precisely screen eligible patients for ICI therapy. Nomogram is a tool that can integrate multiple variables based on mathematical models [45]. In clinical practice, using nomogram to integrate biologic and clinical information and explore the correlation between these variables and clinical outcome had demonstrated advantages than conventional prognosis markers [46]. Hence, we propose that using nomogram to set up an immune score model might offer insight into treatment decision making. This immune score model should entirely or partly include the following variables: PD-L1 expression, TMB, MSI/dMMR, TNB, Teff gene signature, TILs, genetic feature in TME and/or blood, as well as intestinal microbiota, serum markers, clinicopathological parameter. The optimized nomogram model for predicting prognosis of ICI therapy warrants further investigation.

Last decade, the theory and technology of artificial intelligence (AI) have become increasingly mature. The method of deep learning exhibited powerful function in exploring the laws of large-scale biological databases, especially in Omics data, such as genomics, gene transcription. A study demonstrated that the target gene expression profile can be inferred by a model which was trained by deep learning of known landmark genes expression. And the prediction accuracy of deep learning significantly exceeded linear regression model [47]. In addition to genomics, the AI platform could also integrate proteomics, metabolomics, lipidomics derived from tissue samples, and all blood tests information, image omics, as well as clinical records such as age, gender, history and so on. Then all the components could be applied to high order mathematic algorithm by using machine learning to discover associations and correlations. Finally, all the biomarkers and their weight coefficient could be presented. Even new biomarkers might be inferred. Not only that, AI platform can be exploited for monitoring drug-resistance and predicting recurrence [48]. Thus, designing the individualized treatment regimen through AI platform might be an effective way to improve clinical benefit.

Conclusions

Thus far, each ICI therapy has a wide range of indications, including different tumor types. It is a challenge to find molecules, genes, or markers which could accurately and sensitively predict response to ICI therapy. As discussed above, pre-treatment tissue PD-L1 protein expression may not serve as a perfect biomarker. Although tumor PD-L1 mRNA expression was verified to be consistent with tissue PD-L1 protein expression, no clinical trials supported its predictive value. Circulating PD-L1 expression had emerged as alternative biomarkers to predict prognosis of ICI therapy. Compared with tissue biopsy, the sample source of liquid biopsy are much easier to obtain. Moreover, the dynamic observation of peripheral PD-L1 expression (e.g. PD-L1$^+$ CTC, PD-L1$^+$ exosome, sPD-L1) changes during ICI therapy course has significant implications for efficacy assessment and drug resistance monitoring. Even some limitations make PD-L1 a controversial biomarker, a comprehensive PD-L1 information obtained by multimodal detection may better select eligible patients for ICI therapy.

Currently, many biomarkers had been proposed to predict prognosis in different situations, however single biomarker had limited indications and predictive power. The predictive value was improved when combining two or more biomarkers. In addition, by using nomogram model or AI platform, we could integrate massive parameters, not only including immune-related biomarkers, to obtain comprehensive information for patient selection or management decision making. But it must be pointed out that the advantages of nomogram model and AI platform might not equal to good performance in prospective clinical trials. Hence, to investigate

the correlation between ICI therapy strategies and biomarkers, further prospective studies are warranted.

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Disclosure of conflict of interest

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Address correspondence to: Jianguo Sun, Cancer Institute of The People's Liberation Army, Xinqiao Hospital, Army Medical University, Xinqiao Street, Shapingba District, Chongqing 400037, China. E-mail: sunjg09@aliyun.com

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