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Predictive biomarkers in PD-1/PD-L1 checkpoint blockade immunotherapy

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ABSTRACT

Checkpoint blockades turn on a new paradigm shift in immunotherapy for cancer. Remarkable clinical efficacy, durable response and low toxicity of programmed death 1 (PD-1)/programmed death ligand-1 (PD-L1) checkpoint blockades have been observed in various malignancies. However, a lot of cancer patients failed to respond to the PD-1/PD-L1 checkpoint blockades. It is crucial to identify a biomarker to predict the response to checkpoint blockades. The overexpression of PD-L1 is an important and widely-explored predictive biomarker for the response to PD-1/PD-L1 antibodies. However PD-L1 staining cannot be used to accurately select patients for PD-1/PD-L1 pathway blockade due to the low prediction accuracy and dynamic changes. Tumor-infiltrating immune cells and molecules in the tumor microenvironment, or along with PD-L1 expression, may be important in predicting clinical benefits of PD-1/PD-L1 checkpoint blockades. Gene analysis has proven to be new approach for judging the potential clinical benefit of immune checkpoint inhibitors, such as mutational landscape and mismatch-repair deficiency. Further preclinical and clinical studies are necessary to carry out before its application in clinical practice. Challenges should be overcome to identify patients accurately who will benefit from PD-1/PD-L1 checkpoint blockades. In this review, we focus on the predictive biomarkers for checkpoint blockades of PD-1/PD-L1 pathway.

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Introduction

The immune system plays an important role in eradicating abnormal or cancer cells. Multiple mechanisms may prevent anti-tumor immunity in the generation process of tumors. Overexpression of inhibitory checkpoints by tumors or immune cells can dampen autoimmunity, form immunosuppressive microenvironment, cause immune tolerance and immune escape. Checkpoint blockades turn on a new paradigm shift in immunotherapy for cancer, which focuses on the disinhibition of native anti-tumor immune responses [1].

Although amazing results are observed in checkpoint immunotherapy, a lot of cancer patients failed to respond to the programmed death 1 (PD-1)/programmed death ligand-1 (PD-L1) checkpoint blockades. In the new era of precision medicine, searching a predictive biomarker to select real patients who would benefit from checkpoint blockades is crucial to prevent them from autoimmune adverse effects and high cost of such agents. This review is focused on the predictive biomarkers for the response to PD-1/PD-L1 pathway checkpoint blockades.

Materials and methods

All published papers were obtained from the PubMed database, using the subsequent MeSH (Medical Subject Heading) terms: "checkpoint blockade", "immunotherapy", "PD-1", "PD-L1", "PD-1/PD-L1", "prediction or predictive", "response", "gene analysis", "tumor environment". The reports from annual meeting of American Society of Clinical Oncology (ASCO), European Society for Medical Oncology (ESMO, European Cancer Congress) and the International Association for the Study of Lung Cancer (IASCL) were searched out on the official website http://meetinglibrary.asco.org/, http://www.europeancancercongress.org and https://www.iaslc.org/.

Mechanism of PD-1/PD-L1 blockades

Interaction of PD-1 with its ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC), contribute to the suppression of T-cell function and the

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restriction of tumor cell killing (Fig. 1) [2,3]. PD-1 protein is T-cell coinhibitory receptor with ligand specificity. PD-L1 is expressed in various types of cells, including placenta, pancreatic islet cells, mesenchymal stem cells and immune cells [2]. The overexpression of PD-L1 in tumor cells can avoid T cell cytolysis and facilitate cancer formation [4–6]. High PD-L1 expression was significantly associated with poor differentiation of tumor ($P = 0.001$) and poor prognosis ($P < 0.001$) in non-small cell lung cancer (NSCLC) and malignant melanoma [7–9].

Inhibiting the interaction of PD-1 and its ligands can significantly enhance T cell function, resulting in anti-tumor activity [10,11] as shown in Fig. 1. Several antibodies blocking either PD-1 or PD-L1 have been developed for clinical application. These agents are generally classified into two groups: anti-PD-1 antibodies, such as nivolumab (BMS-936558), and pembrolizumab (MK-3475, also known as lambrolizumab) and anti-PD-L1 antibodies, such as atezolizumab (MPDL3280A) and avelumab [11–17].

Clinical efficacy of PD-1/PD-L1 checkpoint blockades

Prominent clinical benefits of PD-1/PD-L1 checkpoint blockades were observed in melanoma [11–13,18], lung cancer [14,15], bladder cancer [16], renal cancer [17], and others. Both anti-PD-1 and anti-PD-L1 showed promising efficacy in melanoma [11–13,18], which is the first cancer approved by Food and Drug Administration (FDA) for treatment with PD-1/PD-L1 checkpoint blockades. For NSCLC patients receiving pembrolizumab (KEYNOTE001 trial), the objective response rate (ORR) was 19.4% with the median duration of response of 12.5 months, and 9.5% of grade 3 or higher treatment-related adverse events (AEs) [14]. Anti-PD-L1 therapy provided considerable antitumor activity in bladder carcinoma [16]. Meanwhile, in renal cell carcinoma (RCC) patients treated by nivolumab, the ORR was above 20% while 11% of patients experienced grade 3 or higher AEs [17]. The ORR of nivolumab was as high as 87% in treating patients with relapsed or refractory Hodgkin's lymphoma [19].

Owing to prominent clinical efficacy, durable responses and low toxicity, multicenter randomized comparative trials are carried on to compare the efficacy of PD-1/PD-L1 checkpoint blockades with chemotherapy or ipilimumab (anti-cytotoxic T-lymphocyte-associated protein 4, anti-CTLA-4) (Table 1). In the phase III trial of nivolumab vs docetaxel in previous treatment of advanced or metastatic squamous NSCLC (Checkmate 017) and non-squamous NSCLC (Checkmate 057), nivolumab treatments resulted into better ORR (20% vs 9%, $P = 0.0083$; 19% vs 12%, $P = 0.0246$, respectively) and longer median overall survival (mOS) (9.2 months vs 6.0 months, $P = 0.00025$; 12.2 months vs 9.4 months, $P = 0.0015$, respectively) than the docetaxel chemotherapy [15,20]. Improved progression-free survival (PFS) were observed in squamous NSCLC [15], but not in non-squamous NSCLC [20]. In Checkmate 066 trial for patients who have metastatic melanoma without BRAF mutation, first-line treatments with nivolumab led to significant improvements in ORR, PFS and overall survival (OS) in comparison with dacarbazine [11]. In RCC, patients treated by nivolumab showed higher ORR and longer median OS than those treated by everolimus [22]. Checkmate 037 has confirmed better clinical benefits of nivolumab treatment than chemotherapy in melanoma [21]. In ipilimumab-refractory melanoma (KEYNOTE-002), patients treated by pembrolizumab have better ORR and PFS than those treated by investigator-choice chemotherapy (paclitaxel plus carboplatin, paclitaxel, carboplatin, dacarbazine or oral temozolomide) [23]. In a recent released trial (KEYNOTE006), 834 patients with advanced melanoma were assigned randomly to pembrolizumab or ipilimumab, it was demonstrated that the pembrolizumab had better ORR, longer PFS and OS than ipilimumab ($P < 0.001$) [24]. In addition, grade 3–5 AEs occurred much less in PD-1/PD-L1 checkpoint blockades than did in compared agents (chemotherapy or ipilimumab) in above trials [11,15,20,21,23,24].

PD-1/PD-L1 checkpoint blockades show a clinical benefit with safety profile over current standard care, which may become the new standard treatment for advanced NSCLC and melanoma. Phase III randomized trials in other solid cancers are undergoing, which might offer more clinical evidences.

The predictive role of PD-L1 expression

PD-L1 is up-regulated in selected solid tumors and it can be detected by immunohistochemistry (IHC) on tumor cells (TCs)

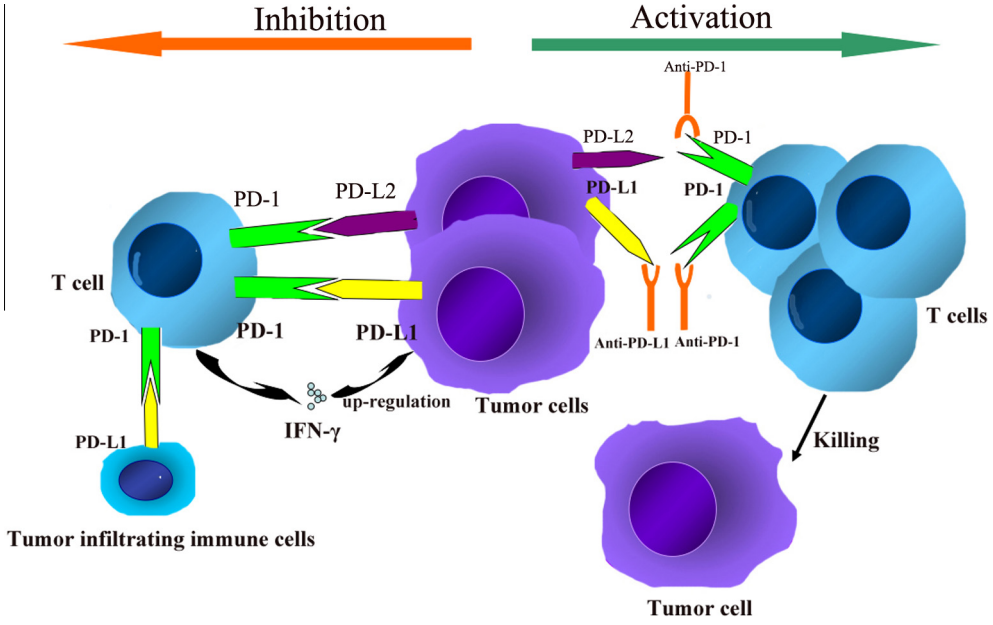

Fig. 1. The mechanism of anti-PD-1 and anti-PD-L1 checkpoint blockades. PD-1 is expressed by T cells. PD-L1 is expressed in tumor cells and tumor infiltrating immune cells. Combination of PD-1 and PD-L1/PD-L2 contribute to the suppression of T-cell function. Inhibiting the interaction of PD-1 and its ligands can significantly enhance T cell function, resulting in antitumor activity.

Table 1
The efficacy of PD-1/PD-L1 checkpoint blockades comparing with chemotherapy or ipilimumab.

Trials	Patients	Tumors	PD-1/PD-L1 blockades	Compared agents	PD-1/PD-L1 blockades vs compared agents				Refs.
					ORR	PFS	OS	AE 3–5	
CheckMate 066	418	Melanoma without BRAF mutation	Nivolumab	Dacarbazine	40.0%:13.9% $P < 0.001$	mPFS 5.1:2.2 mons $P < 0.001$	1-yr-OS 72.9%:42.1% $P < 0.001$	11.7%:17.6%	[11]
CheckMate 017	272	NSCLC squamous	Nivolumab	Docetaxel	20%:9% $P = 0.0083$	mPFS 3.5:2.8 mons 1-yr-PFS 21%:6.4% $P = 0.0004$	mOS 9.2:6.0 mons 1-yr-OS 42%:24% $P = 0.00025$	7%:57%	[15]
CheckMate 057	582	NSCLC non-squamous	Nivolumab	Docetaxel	19%:12% $P = 0.02$	mPFS 2.3:4.2 mons 1-yr-PFS 19%:8% $P = 0.3932$	mOS 12.2:9.4 mons 1-yr-OS 51%:35% $P = 0.002$	10%:54%	[20]
CheckMate 037	405	Advanced melanoma	Nivolumab	Dacarbazine paclitaxel + carboplatin Everolimus	31.7%:10.6%	mPFS 4.7:4.2 mons	NR	9%:32%	[21]
CheckMate 025	821	Advanced or metastatic RCC	Nivolumab		25%:5% $P < 0.001$	mPFS 4.6:4.4 mons $P = 0.11$	mOS 25:19.6 mons $P = 0.002$	19%:37%	[22]
KEYNOTE 002	540	Ipilimumab-refractory melanoma	Pembrolizumab (10 mg/kg)	Chemotherapy 1	25%:4% $P < 0.001$	6-mon-PFS 38%:16% $P < 0.001$	NR	14%:26%	[23]
KEYNOTE 006	834	Advanced melanoma	Pembrolizumab	Ipilimumab	33.7%:11.9% $P < 0.001$	6-mon-PFS 47.3%:26.5% $P < 0.001$	1-yr-OS 74.1%:58.2% $P < 0.001$	13.3%:19.9%	[24]

PD-1, programmed death-1; PD-L1, programmed death-ligand 1; ORR, objective response rate; PFS, progression free survival; OS, overall survival; AE, adverse effect; NR, nor reported; NSCLC, non-small cell lung cancer; chemotherapy 1, paclitaxel plus carboplatin, paclitaxel, carboplatin, dacarbazine, or oral temozolomide.

and immune cells (ICs). The expression level of PD-1 in tumor-infiltrating T cells is less predictive for the response to nivolumab than PD-L1 expression in solid cells [19,25]. These properties make PD-L1 be a potentially promising biomarker to predict the response to PD-1/PD-L1 checkpoint blockades. The association of PD-L1 expression and the efficacy of PD-1/PD-L1 checkpoint blockades are investigated in a lot of studies (Table 2).

Wide variability of PD-L1 expression is observed in different tumor types. The overexpression of PD-L1 is significantly associated with better response to PD-1/PD-L1 blockade in melanoma [21,25–27], NSCLC [25,26], RCC [25], ovarian cancer [28] and others. In non-squamous NSCLC, the clinical efficacy of nivolumab is much better in PD-L1 positive patients than in PD-L1 negative patients at any predefined cut-off values. The ORR and mOS are 31% and 17.2 months in PD-L1 positive ($\geqslant$1%) patients while only 9% and 10.4 months in PD-L1 negative (<1%) patients [20].

Some studies have shown the efficacy of PD-1/PD-L1 antibodies independent of PD-L1 expression [11,15,18]. In advanced RCC treated by nivolumab, the median OS was 21.8 months among patients with 1% or greater PD-L1 expression while 27.4 months among patients with less than 1% PD-L1 expression [22]. In squamous NSCLC, the clinical benefits are independent of PD-L1 expression at the cut-off value of 1%, 5%, or 10% [15]. In order to explore the potential predictive role of PD-L1 expression, a meta-analysis including twenty trials (1475 patients) was conducted [31]. In the overall sample, the ORR of patients with positive PD-L1 expression is significantly higher than those with negative PD-L1 expression (34.1 vs 19.9%, $P < 0.0001$) [31]. However, it was noting that a significant part of PD-L1 negative patients also respond to anti-PD-1/PD-L1 antibody.

The challenge of PD-L1 expression as the predictive biomarker

PD-L1 expression is controversial in predicting which tumor subtypes are responsive to anti-PD-1/PD-L1 immunotherapy, and in determining which individual patient may benefit from therapy. The following information may provide the explanation, at least partly for this problem.

PD-L1 expression in tumor cells and/or immune cells

PD-L1 can be expressed on TCs and/or ICs (Fig. 1). Multiple studies have demonstrated that PD-L1 expression on TCs and ICs may predict the response to PD-1/PD-L1 checkpoint blockades (Table 3). However, not all tumors show simultaneous PD-L1 positive both on TCs and ICs. As in bladder cancer, only one of 68 patients has PD-L1 IHC score of 2/3 for both ICs and TCs [16]. So, which is more important in predicting the response? PD-L1 expression by TCs was correlated significantly with ORR and clinical benefit to anti-PD-1 therapy, while the correlation of PD-L1 expression by ICs with ORR does not reach the statistical significance in multiple solid cancers [25]. However, in metastatic bladder cancer, PD-L1 in ICs is the most predictive for the response to an anti-PD-L1 antibody [16]. In microsatellite instable colon cancer, PD-L1 is expressed predominantly in ICs rather than TCs [32]. The association of PD-L1 expression on ICs with treatment response to atezolizumab can reach the statistical significance, while the association with PD-L1 expression on TCs is not observed in several solid cancers [18]. In genitourinary cancer (no prostate), only PD-L1 expression on ICs were detected, and the ORR was 46% and 16% for PD-L1 IHC 2/3 and 0/1 patients, respectively [33]. In NSCLC, PD-L1 expression on both TCs and ICs can identify patients with the improved OS, PFS and ORR from atezolizumab treatments [31].

Table 2
Association of PDL1 expression on tumor cells with immune response of anti-PD-1 antibody.

Refs.	Agents	Tumors	Cutoff values	PD-L1+ (%)	Anti-body	Objective response (ORR)			Conclusions
						PD L1 positive	PD L1 negative	*P*-value	
[11]	Nivolumab anti-PD-1	Melanoma	5%	35.4	Dako	52.7%	33.1%	NR	PD-L1 alone does not seem to be useful in the selection of patients
[15]		NSCLC squamous	1%	83	Dako	18%	17%	0.94	Survival benefit was independent of PD-L1 expression
			5%			21%	15%	0.29	
			10%			19%	16%	0.64	
[17]		RCC	5%	27	28-8	31%	18%	NR	PD-L1 negative patients also respond to nivolumab
[20]		NSCLC non-squamous	1%	78	Dako	31%	9%	0.0019	PD-L1 expression is predictive of benefit with nivolumab
			5%			36%	10%	0.0020	
			10%			37%	11%	0.0021	
[21]		Melanoma	5%	46	Dako	43.6%	20.3%	NR	PD-L1 is a potential predictor
[25]		Melanoma	5%	53	5H1	39%	6%	0.025	TC PD-L1 expression correlated with ORR to anti-PD-1 therapy
		NSCLC		53					
		RCC		89					
		CRC		13					
[26]		Melanoma, RCC,NSCLC, CRC, Prostate	5%	60	5H1	36%	0%	0.006	A relationship was suggested between PD-L1 expression on TCs and ORR
[29]		Melanoma	1%	52	28-8	39%	23%	0.004	PD-L1 staining associated significantly with response
			5%	27		67%	19%		
[14]	Pembrolizumab anti-PD-1	NSCLC	50%	23.2	22C3	45.2%	NR	<0.01	PD-L1 expression in at least 50% of tumor cells correlated with improved efficacy
[27]		Melanoma	1%	77	NR	51%	6%	0.0012	Tumor PD-L1 positivity was associated with improved ORR
[30]		Gastric cancer	1%	40	22C3	NR	NR	0.1	PD-L1 may be important to enrich patients
[16]	Atezolizumab anti-PD-L1	Bladder cancer	1%	29	NR	43%	11%	NR	Tumors expressing PD-L1-positive had high response rates
			5%	11		($\geqslant$5%)	(<5%)		
			10%	7					
[18]		Multiple cancer	5%	12–36%	SP142	TC0 21% TC1 18%	TC2 0% TC 3 46%	0.079	No association between response and the PD-L1 expression on TCs
[28]	Avelumab anti-PD-L1	Ovarian cancer	1%	NR	NR	12%	5.9%	NR	PD-L1+ tumors expression shows a trend towards better response

PD-1, programmed death-1; PD-L1, programmed death-ligand 1; RCC, renal cell cancer; NSCLC, non-small cell lung cancer; CRC, colonrectal cancer; NR, not reported; TC, tumor cells.

Table 3
PD-L1 expression on tumor cells and immune cells.

Refs.	Tumors	Agents	PD-L1 expression definition	PD-L1 positivity		ORR(PD-L1+: PD L1−)		Conclusion
				TC	IC	TC	IC	
[16]	Bladder Cancer	Atezolizumab	IHC0 < 1%	TC0 71%	IC0 30%	TC0/1 11%	IC0 8.3%	Tumors expressing PD-L1-positive tumor-infiltrating IC had particularly high response rates
			IHC1 ⩾ 1% but <5%	TC1 18%	IC1 43%	TC2/3 43%	IC1 13%	
			IHC2 ⩾ 5% but <10%	TC2 4%	IC2 18%		IC2 40%	
			IHC3 ⩾ 10%	TC3 7%	IC3 9%		IC3 50%	
[18]	NSCLC	Atezolizumab	⩾5% as positivity	24%	26%	TC0 21%	IC0 13%	There appears to be an association between response and the PD-L1 expression, especially in tumor infiltrating immune cells in pretreatment samples
	RCC		IHC0 < 1%	5%	25%	TC1 18%	IC1 21%	
	Melanoma		IHC1 ⩾ 1% but <5%	5%	36%	TC2 0%	IC2 17%	
	HNSCC		IHC2 ⩾ 5% but <10%	19%	28%	TC3 46%	IC3 46%	
	Gastric cancer		IHC3 ⩾ 10%	5%	18%			
	CRC			1%	35%			
	Pancreatic cancer			4%	12%			
[24]	Melanoma	Nivolumab	5%	53%	50%	39%:6%	35%:11%	TC PD-L1 expression correlated with ORR to anti-PD-1 therapy
	NSCLC			53%	53%	$P = 0.025$	$P = 0.142$	
	RCC			89%	100%			IC PD-L1 expression did not correlate with ORR to anti-PD-1 therapy
	CRC			13%	50%			
	Prostate cancer			0%	0%			
[33]	Genitourinary (non prostate)	Atezolizumab	IHC0 < 1%	NR	IC0 17%	NR	IC0 13%	Response is promising for IHC 2/3 urothelial bladder cancer
			IHC1 ⩾ 1% but <5%		IC1 30%		IC1 19%	
			IHC2 ⩾ 5% but <10%		IC2 39%		IC2 44%	
			IHC3 ⩾ 10%		IC3 14%		IC3 67%	
[34]	NSCLC	Atezolizumab	TC0 or IC0 < 1%	TC/IC0	32%	TC/IC0	8%	PD-L1 on both TC and IC can be a predictive marker to anti-PD-L1 therapy
			TC1/2/3 or IC1/2/3 ⩾ 1%	TC/IC 1/2/3	68%	TC/IC 1/2/3	18%	
			TC2/3 or IC2/3 ⩾ 5%	TC/IC 2/3	37%	TC/IC 2/3	22%	
			TC3 ⩾ 50% IC3 ⩾ 10%	TC/IC 3	16%	TC/IC 3	38%	

PD-1, programmed death-1; PD L1, programmed death-ligand 1; TC, tumor cells; IC, immune cells; NR, not reported; NSCLC, non-small cell lung cancer; RCC, renal cell cancer; HNSCC, head and neck squamous cell cancer; CRC, colonrectal cancer; NR, not reported; TC, tumor cells; IHC, immunohistochemistry.

Dynamic PD-L1 expression

The expression of PD-L1 can be induced by activated tumor antigen-specific T cells [35]. Thus, the expression of PD-L1 can be considered as a dynamic process during the recognition of effective T-cell antigen. Microsatellite instability high (MSI-H) colorectal cancer can attract tumor-infiltrating lymphocytes (TILs) and up-regulate PD-L1 expression in tumor epithelial cells [36]. An increased PD-L1 expression in the serial tumor biopsy during the atezolizumab therapy exhibited accompanied decrease in the tumor longest diameter [18]. The expression of PD-L1 is also dynamic during the tyrosine kinase inhibitors (TKI)-targeted therapy [37]. In order to evaluate whether targeted therapy can affect PD-L1 expression, its expression was compared in pre- and post-TKI biopsies in epidermal growth factor receptor (EGFR)-mutant and anaplastic lymphoma kinase (ALK)-positive metastatic NSCLC. The expression levels of PD-L1 in biopsies changed due to TKI therapy at 13 (22%) EGFR-mutant patients and 2 (25%) ALK-positive patients [37]. Considering the dynamic changes of PD-L1 expression, the evaluation at a single time point may not reflect an evolving immune response or predict the response to PD-1/PD-L1 pathway blockades.

Heterogeneity of PD-L1 expression in the same patient

To determine the degree of intra-patient concordance, PD-L1 status was evaluated in different lesions for 58 melanoma patients. It was found that PD-L1 expression was frequently discordant between the primary melanoma and metastasis, and between locoregional disease and distant metastasis. Various expression levels can also be found in different melanoma metastases originating from the same patients [38]. In RCCs, PD-L1 positivity on tumor cells was 32% in the primary tumors and 23% in the matching metastases. Totally 11 of 53 cases showed discordant PD-L1 staining on TCs between primary tumors and metastasis [39]. In NSCLC, discordance of PD-L1 expression was seen in 11% cases with negative primary and positive metastasis and in 12% cases with positive primary and negative metastasis [40]. Regarding PD-L1 intra-patient expression heterogeneity, detection in the one tumor may not accurately reflect the biology of other lesions.

Reliability of detection methods

PD-L1 expression in human cancers is usually investigated using the anti-PD-L1 antibody by IHC staining in formalin-fixed paraffin-embedded tissue samples. It is important to note that different anti-PD-L1 mAbs (Table 2) and staining techniques (manual versus automated techniques) may result in different positive rates for TCs or ICs. PD-L1 detection in NSCLC was performed using three different primary antibody and protocols. The PD-L1 positivity is 36% by SP142, 24% by E1L3N and 34% by the 28-8 clone, respectively [41]. Furthermore, PD-L1 contains two small hydrophilic regions for antibody binding during IHC detection, thus resulting in the low efficacy of IHC approach [42,43]. Due to the limited binding sites in PD-L1 protein for IHC detection, IHC antibodies typically bind PD-L1 at structurally unique sites when compared with therapeutic PD-L1 antibodies [6].

For the inherent problem of IHC, fluorescence in situ hybridization (FISH) is adored in patients with relapsed or refractory Hodgkin's lymphoma. The genes encoding PD-L1 and PD-L2 are key targets of chromosome 9p24.1 amplification. Copy number in chromosome 9p24.1 is assessed using FISH in tissue sections. The frequent amplification of chromosome 9p24.1 was observed in lymph nodes, which provides a compelling rationale for evaluating the efficacy of PD-1 blockade [19].

Although there are limitations for IHC assay, it is cheap and easy to conduct. The status of human epidermal growth factor receptor-2 (HER-2), as the biomarker for trastuzumab treatment, is defined as positive if tumor samples are scored as IHC3+ or IHC2+ and gene amplification in gastric and breast cancers [44,45]. Therefore, it may be the solution to detect PD-L1 status by combining IHC and gene amplification.

What is the reasonable cut-off value?

The cut-off values of 1%, 5% or 10% are frequently used to define the positive rate of PD-L1 staining. Different cut-off values may lead to the difference in predicting function. In melanoma, the ORR of the samples with PD-L1 staining above 5% is 67%, which is much higher than 39% in the sample with PD-L1 staining above 1%. Significant association between PD-L1 staining and response is observed at the membranous staining level of 5%, but not at 1% [29]. Some researchers chose all three values to explore the best cut-off value [15,20]. When compared with docetaxel, patients with the PD-L1 positive rate above 1% show better OS and ORR in non-squamous NSCLC [20], while patients with PD-L1 positive rate above 10% have no difference in OS or ORR in squamous NSCLC [15]. Was 10% not high enough? In KEYNOTE001 trial, the ORR to pembrolizumab was as high as 45.2% when 50% was adopted as the cut-off value for PD-L1 positivity in NSCLC [14]. The lack of the clear definition for PD-L1 positive rate limits the validation of PD-L1 as a predictor to PD-1/PD-L1 checkpoint blockades. It may not be reasonable to make consistent cut-off value for all tumors. However, it is difficult to decide the cut-off value according to different cancers because there are so many kinds of cancers and various biological properties in the same cancer, especially for NSCLC [15,20].

Candidate biomarkers in the tumor microenvironment

Since PD-L1 staining cannot be used routinely to accurately select patients for PD-1/PD-L1 pathway blockade, exploring molecules or cells in the tumor microenvironment related to the immune response may provide new insights into the molecular characteristics associated with clinical response of PD-1/PD-L1 blockades.

Tumor infiltrating immune cells

Recent studies have shown that PD-L1 expression is correlated with the presence of TILs [25]. TILs possess the possibility to predict the response of checkpoint blockades. By detecting tumor biopsies before and during pembrolizumab treatments for melanoma, a predictive model based on CD8 expression is established [46]. Compared to the samples from patients with tumor progression, pre-treatment samples from patients in response group show higher CD8+ cell density at the invasive margin. CD8+ cell density in biopsy samples during serial treatments exhibits a parallel increase at both the invasive margin and tumor center in the response group ($r = 0.71$, $P = 0.001$), but not in the progression group. The response group is significantly associated with higher number of CD8+, PD-1+ and PD-L1+ cells when compared to the progression group (CD8, $P = 0.0001$; PD-1, $P = 0.0002$; PD-L1, $P = 0.006$). However, pre-treatment CD8+ T cells in tumors failed to predict the response of atezolizumab [18].

The activation of indoleamine 2,3-dioxygenase (IDO), tryptophan catabolic enzyme, plays important roles in the suppression of T cell activity, induction of T regulatory cells and engenders immune tolerance to tumor antigens [47]. Several studies reported combined expression of markers for active immune response (CD8)

and immunosuppressive mechanisms (IDO and PD-L1) in melanoma [48,49]. IDO expression is of interest as a predictive marker to immunotherapy response and was firstly found to be associated with clinical activity of ipilimumab in advanced melanoma ($P = 0.012$). IDO was detected in 37.5% in the benefit group and 11.1% in the non-benefit group [50]. Herbst et al. assessed a series markers to identify predictors for PD-L1 inhibition. The results demonstrated elevated IDO1 expression in pre-treatment tumors in the responding group and a generalized activation of T help 1 (Th1) cell-response in the regressing lesions [18]. Further studies are needed to establish if IDO can be an independent predictor for response to checkpoint blockades in clinical practice.

BCL-2-interacting mediator of cell death-Bim

Bim is up-regulated following PD-1 engagement with PD-L1. Then, the overexpression of Bim leads to more apoptosis of T cells [11]. Bim expression in peripheral blood tumor-reactive CD11a high PD-1+CD8+ cytotoxic T lymphocytes was evaluated as a marker to predict response to pembrolizumab for patients with metastatic melanoma [11]. Compared to the patients with radiographic progression disease, the responders after 4 cycles had higher frequency of Bim+/PD-1+CD8 T cells at baseline ($P = 0.04$). The examination of serial peripheral blood samples has confirmed that the frequency of Bim in PD-1+CD8 T cells decreased after the first 3 months of treatments in 9/9 responders, but increased or no change in all 5/5 non-responders ($P = 0.003$). It suggests that an active PD-1 and PD-L1 blocking can result in a larger T cell population rescued from cell death. The measurement of Bim frequency and level in tumor-reactive PD-1+CD8 T cells from peripheral blood may help to select patients for the benefit from anti-PD-1 therapy, and provide a new non-invasive way to monitor the response to anti-PD-1 blockade in metastatic melanoma [51]. However, the results should be validated in a larger prospective cohort in metastatic melanoma and other solid tumors.

Interferon-γ

Interferon-γ (IFN-γ) is an important regulator of immunity in tumor microenvironment, which is released by activated T cells and up-regulate PD-L1 in both TCs and ICs (Fig. 1). In melanoma, responding patients treated by anti-PD-L1 antibody had the elevated pre-treatment IFN-γ in blood as well as IFN-γ-inducible genes [18]. Immune-related gene expression pattern was evaluated in melanoma from 19 patients enrolled in KEYNOTE-001 trial. Both IFN-γ-10 gene and the expanded-immune-28 gene were significantly associated with ORR ($P = 0.047$ and 0.027) and PFS ($P = 0.016$ and 0.015). These results are consistent with the clinical response to checkpoint blockades in patients with a preexisting IFN-mediated adaptive immune response [52]. However, such correlation is weak in patients with RCC or NSCLC [18]. Further confirmation of these new signatures is required in predictive role to PD-1/PD-L1 checkpoint blockades.

Somatic mutations

Somatic mutations have the potential to encode immunogenic neoantigens, which is important to cancer immunity, so there is the possibility to predict the response of immune checkpoint blockade according to the somatic mutation level.

Janus kinase 3 (JAK3)

JAK3 signaling regulates PD-L1 expression in Hodgkin's lymphoma [19]. In a case report, a patient with chemo-refractory advanced lung cancer achieved extraordinary and repeated response to anti-PD-L1 antibody [53]. Comprehensive genomic profiling is performed in the tumor samples and only identifies a variant of $JAK3^{V722I}$, which increased the expression of PD-L1 and might contribute to PD-L1 mediated immune checkpoint blockades evasion in NSCLC [53]. However, it is unclear whether JAK3 alteration in the case report could be generalizable to other patients or other cancers responding to PD-1/PD-L1 blockades. Further studies are needed to establish the definitive role of JAK alteration as a mechanism-based predictive marker of the response to PD-1/PD-L1 blockades.

Mismatch-repair deficiency

Colorectal cancer (CRC) appears to be refractory to checkpoint blockades and only 1/33 shows the response to PD-1 blockade [26]. Mismatch-repair (MMR) deficiency is also observed in only a small fraction of CRCs [54,55]. Thus, there is possibility that MMR-deficient tumors may be more responsive to PD-1 blockade than MMR-proficient tumors. Le et al. initiated a phase II study to evaluate the clinical activity of pembrolizumab in 41 patients with or without MMR deficiency [56]. The immune-related ORR is 40% for MMR-deficient CRCs and 0% for MMR-proficient CRCs. Immune-related PFS rate is 78% and 11% for MMR-deficient and proficient CRC, respectively, which suggests that MMR status can predict clinical efficacy of pembrolizumab. There are several possible reasons that MMR-deficient CRC show better response to PD-1 blockade. Different signaling pathways in two types of tumors may result in difference in the secretion of soluble factors in the tumor microenvironment, which can result in differential activation of PD-1 pathway. MMR-deficient CRC tumors have 10–100 times more somatic mutations than MMR-proficient colorectal tumors [54,57,58], which lead to more neoantigen formation. Moreover, MMR-deficient cancers contain prominent lymphocyte infiltrates [59,60].

The immune-related ORR and PFS in patients with MMR-deficient non-colorectal cancers are similar to those in patients with MMR-deficient colorectal cancers [56]. The results suggest the possibility of MMR status applied for predicting the response to checkpoint blockades in other cancers. Most importantly, gene analysis might be a new approach for judging the potential application of immune checkpoint inhibitors. However, the sample size in this study is small and MMR deficiency occurs only in a small fraction of patients. Further preclinical and clinical studies are necessary before its application in clinical practice.

Mutational landscape

Whole-exome sequencing (WES) has enabled the comprehensive characterization of somatic mutations in tumor samples [61]. Melanomas and lung cancers display high nonsynchronous mutations per tumor [62]. An ongoing effort is to employ mutational landscape to identify candidate patients who will benefit from checkpoint blockade immunotherapy.

In melanoma, WES was performed on tumors from 64 patients treated with CTLA-4 blockade. The results indicated that a high mutational burden correlated with a sustained clinical benefit [63]. A signature defined by mutation-derived neoepitopes could predict durable clinical benefit (DCB) from CTLA-4 blockade in melanoma [63,64]. WES was also used to unveil the genomic determinants of the response to pembrolizumab in NSCLC [65]. Higher somatic non-synonymous mutation burden is found to be associated with improved ORR, DCB and PFS. The sensitivity and specificity using non-synonymous mutation burden larger than 178 for DCB are 100% and 77% in the discovery cohort and 86% and 75% in the validation cohort. The results suggest the mutational landscape shapes the response to anti-PD-1 therapy in NSCLC [65].

The possible explanation for association between mutation burden and efficacy of checkpoint blockade is that tumor antigens as a consequence of somatic mutations, functions as the target of T cells activated by checkpoint blockade immunotherapy [65,66]. Gubin et al. used genomics and bioinformatics approaches to rapidly and accurately identify tumor-specific mutation antigens (TSMA). They confirmed TSMA were the targets of anti-PD-1 and anti-CTLA-4 in mice [66].

The mutation landscape have an important impact on the understanding of response to PD-1/PD-L1 blockades. However, there are limitations for using mutation landscape to identify potential patients. Firstly, there were tumors with higher nonsynonymous mutations that did not respond to checkpoint blockades [63,65]. Secondly, the WES is expensive, time-consuming technique, which is unavailable in clinical practice now. Thirdly, the mutation frequency is various in diverse cancers [67] and even in one cancer type it is influenced by the exposure degree to the environment mutagens [61], such as smoking [68]. The large variability of somatic mutation makes it difficult to set a same cut point for mutation burden to predict the response to checkpoint blockade immunotherapy. Fourthly, WES analysis yields too many candidate mutant peptides. Combination of WES with mass spectrometry may provide an approach to identify neo-epitopes [62] .

Clinical trial design in checkpoint blockades

The research of potential predictive biomarkers is a key aspect of all anti-tumor treatment strategies [69]. To improve the proportion of patients benefiting from therapy, the identification of predictive biomarkers should be addressed in the clinical trials. Despite the challenges for PD-L1 as a biomarker to predict response to PD-1/PD-L1 checkpoint blockades, the FDA granted accelerated approval for pembrolizumab for treating patients with refractory metastatic NSCLC tumors that express PD-L1 in October 2015. Meanwhile, the diagnostic PD-L1 HIS 22C3 pharmDx test was also approved to detect PD-L1 expression in NSCLC tumors. However, PD-L1 alone may be not sufficient to predict the response to PD-1/PD-L1 blockades immunotherapy. PD-L1 expression, along with cells or molecules in tumor microenvironment, should be further explored as the potential markers in future clinical trials. For high costs and time-consuming of WES, the mutation burden or MMR of the tumor and/or peripheral blood compartment may be explored to predict and monitor response in some best-funding clinical trials. The fully understanding the complex network of interactions among checkpoint blockades, molecules or cells in immune system and tumor response may foster identification of ideal marker in this field.

Conclusion

IHC-based PD-L1 expression on tumor cells or immune cells is an important, but not a definitive predictive biomarker for the response to PD-1/PD-L1 blockade. First, in some cancers, the response to PD-1/PD-L1 blockades is independent of PD-L1 expression. Second, PD-L1 positive patients show higher response and some PD-L1 negative patients also reveal the response to PD-1/PD-L1 checkpoint blockades. Third, variability in methods and antibodies may lead to different results. Fourth, the clear definition for PD-L1 positivity is still not achieved. Standardization of staining and scoring methods should be warranted before PD-L1 can be widely used to predict response. Furthermore, the expression is dynamic and heterogeneous due to changes of microenvironment or therapy. The evaluation of PD-L1 at a single time point or single tumor may not predict the response to PD-1/PD-L1 pathway blockades.

Tumor infiltrating immune cells and molecules in the tumor microenvironment, or along with PD-L1, may be important in predicting clinical benefits of PD-1/PD-L1 checkpoint blockades. Mismatch-repair status has shown quite valuable prediction of clinical efficacy of anti-PD-1 immune checkpoint inhibitor. Although the sample size in the study is small and mismatch-repair deficiency is only observed in a small fraction of patients, those results suggest that gene analysis may be a new approach for judging the potential clinical benefit of immune checkpoint inhibitors. In summary, a lot of challenge has to be overcome to accurately identify patients who will benefit from PD-1/PD-L1 checkpoint blockades.

Conflict of interest

The authors declare to have no conflicts of interest.

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