Front. Med. 2019, 13(4): 438–450
https://doi.org/10.1007/s11684-018-0674-4

REVIEW

PD-1/PD-L1 blockade in cervical cancer: current studies and perspectives

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Abstract **Cervical cancer (CC) is the fourth most commonly diagnosed female malignancy and a leading cause of cancer-related mortality worldwide, especially in developing countries. Despite the use of advanced screening and preventive vaccines, more than half of all CC cases are diagnosed at advanced stages, when therapeutic options are extremely limited and side effects are severe. Given these circumstances, new and effective treatments are needed. In recent years, exciting progress has been made in immunotherapies, including the rapid development of immune checkpoint inhibitors. Checkpoint blockades targeting the PD-1/PD-L1 axis have achieved effective clinical responses with acceptable toxicity by suppressing tumor progression and improving survival in several tumor types. In this review, we summarize recent advances in our understanding of the PD-1/PD-L1 signaling pathway, including the expression patterns of PD-1/PD-L1 and potential PD-1/PD-L1-related therapeutic strategies for CC.**

Keywords PD-1; PD-L1; immune checkpoint blockade antibody; immunotherapy; cervical cancer

Introduction

Antitumor immunity can be divided into innate and adaptive components. The adaptive immune response, in which cytotoxic T lymphocytes (CTLs) play a vital role, is tumor antigen-specific [1]. However, tumor cells can acquire various ways to escape from host immune surveillance and clearance. These mechanisms fall into three phases, namely, elimination, equilibrium, and escape, and they are collectively termed cancer immunoediting [2]. During the escape phase, defective antigen presentation results in suppressed T cell activation, immunosuppressive immune cell infiltration, and immunosuppressive cytokine secretion, causing an immunosuppressive environment to form [3]. Under normal conditions, T cells are activated by two signals. The first signal is antigen-specific and initiated by combining an antigen and T cell receptor (TCR), whereas the second signal is antigen-independent and initiated by binding co-signaling receptors expressed on the T cell surface and their ligands. These co-signaling receptors can be divided into two types, namely, co-stimulatory and co-inhibitory receptors, which deliver positive and negative signals, respectively, to fully activate naïve T cells [4]. Immune checkpoints, which serve as the inhibitory signals of T cell activation, help maintain self-tolerance, prevent autoimmunity, and protect normal cells during pathological processes [5].

PD-1 is an immune checkpoint that belongs to the CD28/CTLA-4 family [6] and is present on the surface of diverse immune cells, especially T and B lymphocytes, monocytes, dendritic cells (DCs), and natural killer cells [7]. PD-L1, a ligand of PD-1, is widely expressed by various cells. The binding of PD-1 to PD-L1 transmits an inhibitory signal that downregulates T cell activation, proliferation, cytotoxic activity, and cytokine production [8,9]. In addition to PD-L1, the ligand PD-L2 (B7-DC, CD273) also interacts with PD-1, which shares similar affinity, to deliver a potentially suppressive signal. PD-L2 is expressed only on macrophages and DCs and may interact with a potentially stimulatory receptor, the repulsive guidance molecule b (RGMb) [10,11]. In various tumor types, aberrant high PD-L1 or PD-1 is expressed by tumor cells, tumor-infiltrated immune cells, and stromal cells [12]. The upregulation of the PD-L1 and PD-1 interaction induces T cell anergy, functional exhaustion, apoptosis, and the induction of immune suppressor cells. This interaction also increases the secretion of inhibitory

Received July 19, 2018; accepted October 27, 2018
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cytokines (such as IL-10 and TGF-β) and favors the conversion of T cells into Tregs [13–17]. Tumors exploit these changes to suppress anti-tumor T cell activity and evade host immunity, thereby facilitating immune evasion and tumor progression [15,18]. However, increases in the levels of inhibitory molecules in tumors provide a target for immunotherapy aimed at restoring T cell-mediated immunity. Ongoing clinical trials of several tumor types have reported that checkpoint blockades that target the PD-1/PD-L1 axis evoke effective clinical responses, suppress tumor progression, and improve survival with acceptable toxicity [19–22] (Fig. 1).

Cervical cancer (CC) remains the fourth most commonly diagnosed female malignancy and one of the leading causes of cancer-related mortality worldwide, especially in developing countries [23,24]. Given the availability of advanced screening measures, HPV prophylactic vaccines, and removal of precancerous lesions, the incidence and mortality of CC is anticipated to decrease in the future. However, more than half of all CC cases are diagnosed at advanced stages [25]. Comprehensive therapy has been applied to CCs. For early CCs, radical hysterectomy is the preferred modality through the surgical removal of cervical tumor tissues and some surrounding structures [26]. For locally advanced cancers, concurrent chemoradiation consisting of external radiotherapy and intravenous systemic chemotherapy based on platinum is the standard treatment [27]. For patients with recurrent and metastatic cancers, therapeutic options are extremely limited. Chemotherapy is the primary therapy but has limited efficacy and severe toxicity; thus, the outcome of patients remains poor [28]. Under these circumstances, new effective treatments are needed. In the last few years, a deeper understanding of the HPV-mediated immunologic response and the ability of immune checkpoint inhibitors to initiate effective antitumor activity in various tumor types have driven further exploration of this new, targeted therapy in CCs. In this review, we focus mainly on the role of PD-1/PD-L1 and its usefulness as a therapeutic blockade in CCs.

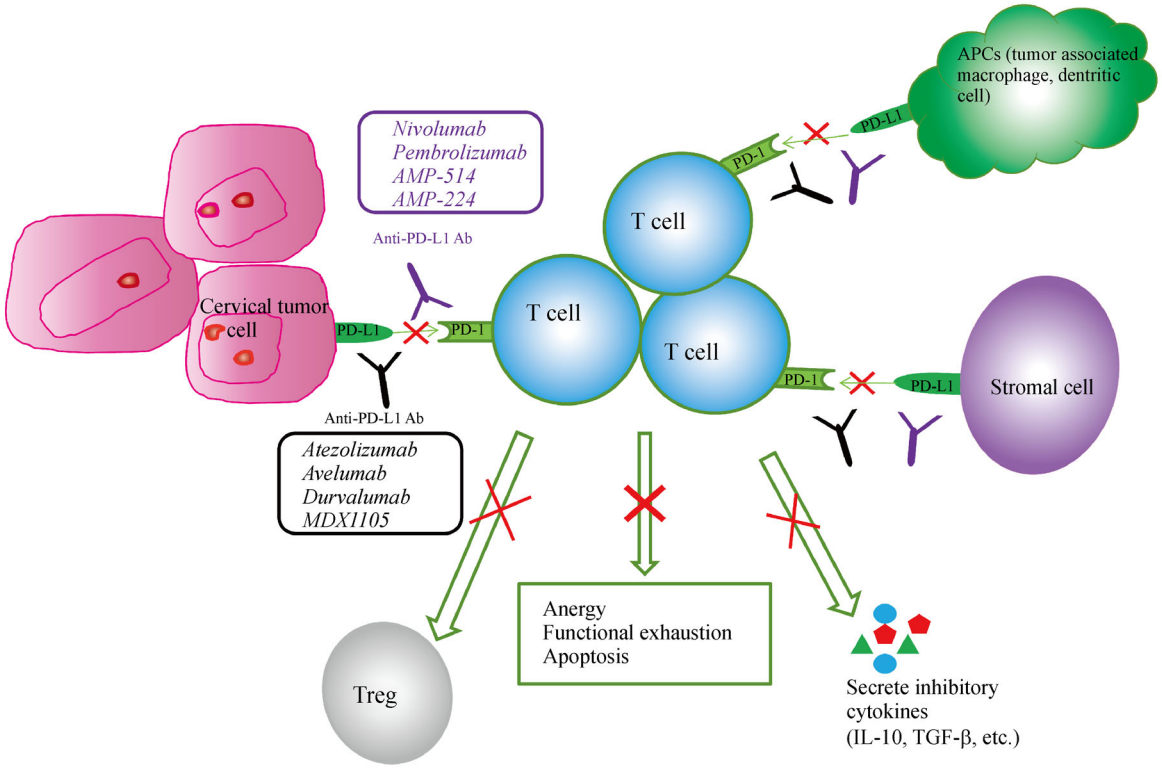

Fig. 1 In cervical tumor microenvironment, elevated PD-L1 is expressed by tumor cells, tumor-infiltrated immune cells, and stromal cells. The upregulation of the PD-L1 and PD-1 interaction induces T cell anergy, functional exhaustion, and apoptosis. The interaction also increases inhibitory cytokine secretion (such as IL-10 and TGF-β) and favors the conversion of T cells into Tregs. In system immune status, overexpression and activation of the PD1/PD-L1 pathway also exist. Tumors exploit these mechanisms to suppress anti-tumor T cell activity and evade host immunity, facilitating immune evasion and tumor progression. Antibodies targeting the PD-1 pathway, including PD-1 antibodies and PD-L1 antibodies that inhibit the PD-1/PD-L1 or PD-1/PD-L2 interaction, provide antitumor therapy by restoring T cell-mediated immunity.

Potential role of PD-1/PD-L1 in cervical malignancy

Despite the promising therapeutic effects of PD-1/PD-L1 blockades, not all patients treated in a spectrum of clinical trials for solid tumors have responded to them. Ongoing efforts are aimed at identifying predictive biomarkers of PD pathway blockades to allow the improved selection of optimal patients in whom the greatest returns can be obtained. Several biomarkers, including tumor-derived and immune cell-derived markers, have been reported in recent studies. The former includes the expression of PD-L1 on tumor cells, neoantigens, and high tumor mutational load, whereas the latter includes an increase in the expression of PD-L1 on immune cells, presence of tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment, and ratio between effector $CD8^+$ T cells and $FoxP3^+$ regulatory T cells in tumors [29–31]. Considering the important role of biomarkers, a series of correlative studies was constructed to determine the expression pattern of PD pathway components and further understand the potential mechanisms underlying its activities in patients with cervical malignancies. The results of these experiments should support further applications of PD-1/PD-L1 blockades (Table 1).

PD-L1 expression is upregulated in tumor cells in multiple cancer types, and this phenotype is associated with poor clinical outcomes. Thus, IHC is currently commonly applied to test for the expression of PD-L1 as a biomarker on tumor specimens [49–51]. Various studies of CC cases have been conducted to explore the diverse pathological types and stages of CC, and some of the resulting data have been consistent. In Reddy's study, PD-L1 levels were measured in patients with squamous cell carcinoma (SCC), adenosquamous carcinoma (ASC), and adenocarcinoma (AC). Their results showed that 34.4% of the cases showed positive PD-L1 expression, whereas benign cervical tissues were negative. Patients with SCC were more PD-L1 positive than those with ASC and AC; however, these three pathological types were not significantly different [40]. Notably, the PD-L1 scoring scheme used in this study was adopted from Garon *et al*. In this scheme, tumor biopsies in which at least 50% of the cells were positive for PD-L1 were evaluated as "PD-L1 positive." This platform was established in a clinical trial of non-small-cell lung cancer (NSCLC) in which patients with PD-L1-positive tissues (defined as $\geqslant 50\%$) promisingly showed positive reactions to a targeted immune checkpoint inhibitor. However, whether this cutoff point is also suitable for the predictive biomarkers of reactions to PD1/PD-L1 blockade therapy in CCs remains unknown. In another study by Heeren, PD-L1 positivity ($> 5\%$ of the tumor cells used as a cutoff point) was found in 54% of SCCs and 14% of ACs. The rate of PD-L1 positivity in patients with AC was significantly lower than that in patients with SCC ($P < 0.001$), contrary to the data reported by Reddy [35]. Considering the different cutoff points utilized in the two trials, we cannot conclusively state that PD-L1 positivity varies among different pathological types of CCs. Additionally, in Enwere's trials, 120 patients with stages IB to IVA were tested and analyzed for PD-L1 expression. All patient samples (95.7%) exhibited different degrees of PD-L1 expression, and approximately 73.3% and 39.7% of samples were PD-L1 positive on the basis of $> 5\%$ and $> 50\%$ criteria for positivity, respectively [33]. Another study produced results consistent with those of Heeren in that no significant correlation was found between PD-L1 positivity and clinicopathological characteristics, including tumor size, vaginal and parametrial infiltration, and lymphatic metastasis [33,35]. The PD-L1 expression levels in patients with recurrent CCs were also detected by Ring, whose results suggested that PD-L1 expression is increased in tumor cells of all patients [42]. Overall, PD-L1 expression is significantly elevated on tumor cells of patients with CCs irrespective of the pathological type or

Table 1 Expression of PD-1/PD-L1 in different cervical neoplastic subtypes

Conditions	Subtypes	PD1/PD-L1 expression	Reference
HPV infection	Negative	−	[32–48]
	Positive	+/−	
Histological abnormality	Negative	−	
	Positive	+/−	
CIN grade	I	−/+	
	II	+ /++	
	III	++/+++	
Pathological type	SCC	AC	
	++/+++	−/+	
Metastasis	Negative	Positive	
	++	+++	

−/+/++/+++, low or negative expression/low expression/moderate expression/high expression.
CIN, cervical intraepithelial neoplasia; SCC, squamous cell carcinoma; AC, adenocarcinoma.

disease stage. Several reports from clinical trials targeting PD-1 inhibitors in various tumors have shown that PD-L1 expression levels in tumors are modestly correlated with the therapeutic efficacy of PD-1 blockades [21,52–56]. The high expression of PD-L1 in CC supports the rationale for using PD-1/PD-L1 antagonist therapy in affected patients. Finally, a recent meta-analyses reported that a high PD-L1 expression is correlated with poor survival [19,57,58].

Tumor-derived biomarkers, in addition to some immune cell-related indexes, are highly correlated with clinical responses and outcomes, including increased PD-L1 expression on immune cells, presence of TILs in tumor microenvironments, and ratio of effector $CD8^+$ T cells to $FoxP3^+$ regulatory T cells in tumors [29,59,60]. During neoplasia, changes in immune cell activation and differentiation profiles are detected in cervical intratumoral and peritumoral environments, characterized by $CD4^+$ T and $CD8^+$ T cell inactivation, Treg cell proliferation, DC immaturation, and M2 cell formation [61,62]. In cervical lesions, TILs and TAMs express significantly high levels of PD-L1 and PD-1 [33,37,39,42]. Similarly, tumor-positive lymph nodes contain significantly higher PD-L1-positive APCs and Tregs than tumor-negative draining lymph nodes in patients with CC [34]. Moreover, the peripheral blood samples of patients with CC contain $CD8^+$ T cells and DCs with elevated PD-1/PD-L1 levels, and high levels of immunosuppressive cytokines (TGF-β and IL-10) [32]. In conclusion, the overexpression and activation of the PD-1/PD-L1 pathway have been noted in the immune system and *in situ* tumor microenvironments of patients with CC, leading to the inactivation and apoptosis of tumor-specific T cells and the formation of local and systemic immunosuppressive environments and contributing to immune evasion and tumor progression. In another study by Liu *et al*., cervical tumor cells were blockaded with soluble PD-1 and then cocultured with PBMCs; PBMC proliferation and CTL activity significantly increased. Inhibiting the PD-1/PD-L1 interaction restores the T cell-mediated immune response and enhances anti-tumor immunity [38]. These various studies collectively provide a strong rationale supporting the notion that using therapeutic modalities based on biomarkers, which are derived from immune or tumors cells that target the PD-1/PD-L1 pathway, should demonstrate favorable efficacy in CCs.

Some trials have investigated PD-L1 expression at the genetic level to predict therapeutic efficacy and clinical outcomes [63–65]. CD274 and PDCD1LG2 are genes that encode two PD-1 ligands, namely, PD-L1 and PD-L2, respectively. Howitt investigated the status of CD274 and PDCD1LG2 and found that they are co-amplified in a considerable number of cervical SCCs. These results provide a genetic basis for the differences in PD-L1 expression observed in a portion of SCCs. The status of encoding genes may contribute to the identification of a subset of patients who are candidates for PD-1-trageted therapies [36]. Another study by Rieke showed that the hypermethylation of homologous recombination DNA repair genes (RAD51B and XRCC3) is linked to an immune-evasive phenotype in SCCs [41]. These results based on epigenetic data may provide novel insights into the predictive biomarkers of immune checkpoint inhibition.

Relationship of HPV infection and PD-1/PD-L1 expression in cervical malignancy

Human papillomavirus (HPV) is closely linked to cervical carcinogenesis, including cervical intraepithelial neoplasia (CIN) and CC [66]. A total of 100 HPV genotypes have been described, and over 20 of these types are carcinogenic to the cervix. HPV16 and HPV18 are the main types that drive cervical malignant progression and account for over 70% of all cases [67,68]. HPV is composed of capsid proteins that cover non-enveloped circular double-stranded DNA. The HPV genome consists of 8 kilo base pairs that are divided into five early genes encoding the E1, E2, E4, E5, and E7 proteins and two late genes encoding the L1 and L2 structural proteins [69,70]. Notably, E6 and E7, which promote the degradation of p53 and pRB, respectively, play a causative role in generating and maintaining HPV-associated malignancies [71,72]. Viral infection is initiated in the basal epithelial cells of the transformation zone, where cells actively replicate and differentiate, and the virus remains episomal for multiplication. When the epithelial cells differentiate into terminal cells, late genes are expressed, and the viral capsid is assembled. The virion is then packaged for release from the superficial epithelial layers [67,73]. Most of the infection and some initial lesions are spontaneously cleared by the immune response. During persistent infection, viral DNA can be integrated into the host cell genome. During this process, some genes (E1, E2, E4, E5, L1, and L2) are deleted. The deletion of E2, a negative transcriptional regulator of E6 and E7, leads to an increase in the expression of E6 and E7, ultimately resulting in malignant transformation in the cervix [74,75].

PD-L1 is expressed at significantly higher levels in dysplastic/neoplastic cells in HPV-associated CIN and SCC than in normal cervical tissues and other gynecologic malignancies [38,39,46–48]. HPV-infected cervical epithelial cells that do not display histological abnormalities do not also express PD-L1; thus, productive viral infection likely plays a critical role in inducing PD-L1 expression [39,46,48]. We speculate that PD-L1 expression is both a promising biomarker for differentiating productive HPV infection in the cervix and a therapeutic target for clearing

HPV infection. More investigations are needed to explore these potential strategies. The mechanisms underlying HPV infection-induced overexpression of PD-L1 on CC cells are continually explored. During the process of HPV infection, the expression of viral E6 or E7 genes may be crucial for driving PD-L1 expression [76,77]. Liu's studies showed that the increases in PD-L1 expression in CC cells and CINs are mediated by HPV16 E7 [38]. As speculated in Mezache's study, certain early open reading frames (ORFs) play a role in PD-L1 expression on cervical malignant cells [39]. Moreover, the integration of HPV into the PD-L1 locus leading to amplification of the PD-L1 allele drives PD-L1 expression [45].

PD-1/PD-L1 expression levels on immune cells in tumor microenvironments have also been tested and analyzed in several other studies. Intratumoral HPV antigen induces high CTL infiltration and causes CTL dysfunction [78]. Yang *et al*. reported that HPV-positive patients exhibit elevated levels of PD-1 and PD-L1 on cervical T cells and DCs, respectively, and these increases are positively correlated with the CIN grade. Additionally, the opposite expression patterns were observed for B7-1 and B7-2 (two co-stimulatory molecules) on DCs [43,47]. DCs are the most powerful antigen-presenting cells that can deliver both co-stimulatory and co-inhibitory signals to T cells [79]. Enhanced PD-1/PD-L1 interactions suppress the antigen-delivering function of DCs, resulting in the downregulation of first signals and consequential inhibition of T cell activation. A decrease in the expression of co-stimulatory molecules on DCs or an increase in the levels of co-inhibitory molecules of PD-1/PD-L1 on T cells and DCs negatively regulate the second signals of T cell activation. All of these molecular patterns are responsible for the induction of T cell tolerance and the depression of the CMI response, resulting in a persistent HPV infection and CIN progression (Fig. 2). Consistent results were obtained by Mezache, who reported significantly more PD-L1-positive mononuclear cells in CIN and SCC tissues than in normal cervical epithelia, which were rarely observed. PD-1 expression showed a similar pattern. Co-expression analysis of inflammatory cells revealed a preponderance of $CD8^+$ cytotoxic T cells in PD-L1-positive areas, but they remain in a quiescent state. Moreover, the activity of the PD axis may negatively regulate Th1 cytokines (interferon-γ and IL-12) and positively regulate Th2 cytokines (IL-10 and TGF-β) in the cervix, contributing to localized immunosuppression [39]. These diverse studies show that the inhibitory PD-1/

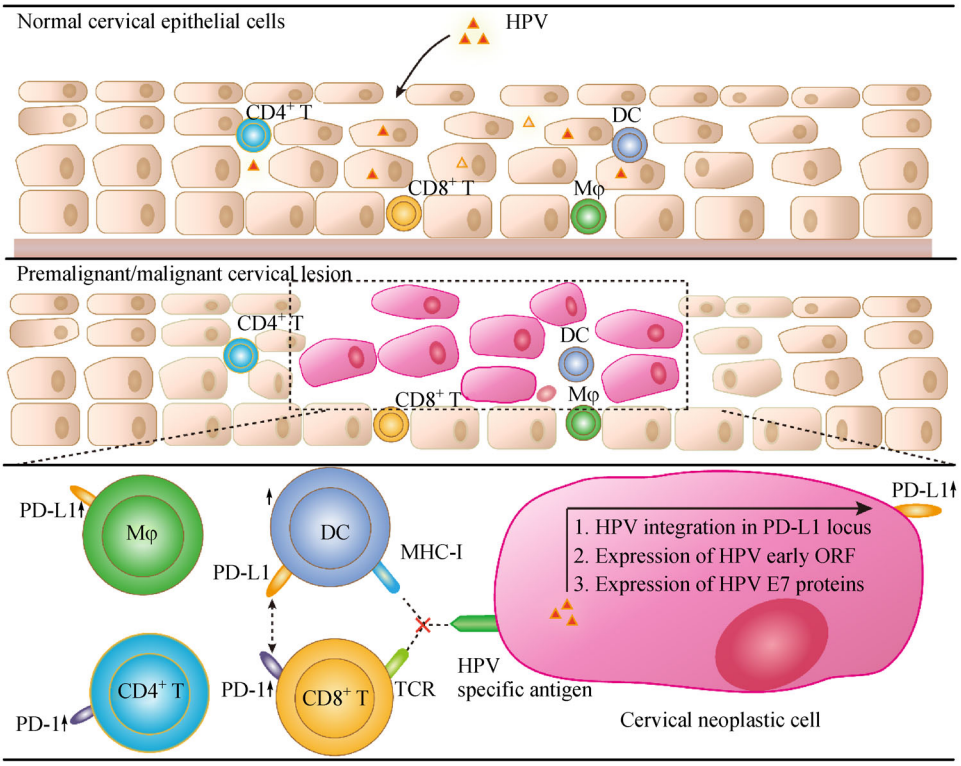

Fig. 2 Schematic outline showing the interplay of HPV and PD pathway. Persistent HPV infections upregulate PD-1 and PD-L1 expression levels on cervical cells and infiltrated immune cells. Activation and interaction of the PD pathway facilitate CTL dysfunction and exhaustion, negatively influencing HPV clearance. Malignant cervical lesions are formed, and progress with PD-1/PD-L1 expression is further elevated.

PD-L1 pathway is upregulated in HPV-associated CINs, and this change negatively regulates cervical cell-mediated immunity to HPV and contributes to HPV-related CIN progression. Thus, immunotherapies that target the blockade of PD-1/PD-L1 ligations may be especially useful in patients infected with cervical HPV. However, in contrast to the uniformly high PD-1/PD-L1 expression and comparable therapeutic response rates of HPV-positive and -negative cases reported in head and neck squamous cell carcinoma (HNSCC) [78,80], PD-1/PD-L1 expression and clinical response to PD pathway blockade displays a lower pattern for HPV-negative CINs and CCs [47,81]. Additional investigations are necessary in the application of PD pathway blockade in HPV-negative cervical malignancies, which are poorly studied. PTEN or ARID1A proteins may potentially serve as therapeutic targets for those cases without HPV infection [81].

The potential of PD pathway inhibitors and their underlying mechanisms has gained enormous interest as an antitumor therapy, but few investigations have explored their effects in CCs. As reflected by existing studies, the lack of a uniform standard for patient classification based on PD-1/PD-L1 staining is an important shortcoming. First, consistent guidelines are needed for the use of staining methods and antibodies, so that comparable results can be achieved. Second, discrepancies exist on how both automated and manual scoring methods are used for image analysis, and assay validation tools are needed for these techniques as well. Third, a comprehensive analysis of the percentages, staining intensity, and region and range of PD-L1/PD-1 expressing cells is needed to define a specific cutoff point for expression. The development of a standard scoring system would promote systematic consistencies across different studies and provide additional evidence for clinical applications.

Clinical development of PD-1 and PD-L1 blockades in CCs

The significant role the PD-1 pathway plays in tumor immune evasion indicates the huge potential of applications that use antibodies to induce its blockade to treat tumors. Antibodies that target the PD-1 pathway include the PD-1 antibodies and PD-L1 antibodies, which inhibit PD-1/PD-L1 or PD-1/PD-L2 interactions. To date, nine antibodies have been tested in hundreds of clinical trials aimed at examining over 20 types of solid and hematological tumors, and some of these antibodies have been approved for clinical application (Table 2). Among these nine antibodies, five are being used in clinical trials that include subjects with CC (Table 3).

Nivolumab

Nivolumab is a genetically engineered, fully human immunoglobulin G4 (IgG4) monoclonal anti PD-1 protein antibody. It is the first generation of PD-1 antibodies, and its clinical activity and pharmacological characteristics were first clarified in a Phase I study (NCT00441337) of 39 patients with various advanced malignancies [82]. Among the 39 subjects, 12 demonstrated stable disease or tumor regression, and one achieved a durable complete response. Subsequently, nivolumab was included into a spectrum of clinical trials for multiple tumor types; acceptable side effects, tumor response rates, and durability of responses were widely observed. Currently, nivolumab has been approved for use in various tumors, including melanoma, NSCLC, renal cell cancer (RCC), HNSCC, classic Hodgkin's lymphoma (cHL), colorectal cancer, and urothelial carcinoma [83–88].

A phase II clinical trial (NCT02257528) is currently

Table 2 PD-1- and PD-L1-blocking agents in clinical development

Target	Agent	Antibody class	Company	Approval
PD-1	Nivolumab (BMS-9336558, MSX1106, ONO-4538, Opdivo®)	Human IgG4	Bristol–Meyers Squibb	FDA approved for melanoma, NSCLC[a], RCC[b], HNSCC[c], cHL[d], colorectal cancer, urothelial carcinoma
	Pembrolizumab (MK3475, Keytruda®)	Humanized IgG4	Merck	FDA approved for melanoma, NSCLC, RCC, cHL, HNSCC, colorectal carcinoma, urothelial carcinoma
	AMP-514 (MEDI0680)	Humanized IgG4	MedImmune	–
	AMP-224	PD-L2-IgG2a fusion protein	Amplimmune	–
PD-L1	Atezolizumab (MDPL-3280A,Tecentrip®)	Human IgG1	Genentech	FDA approved for urothelial carcinoma, NSCLC
	Avelumab (MSB0010718C, BAVENCIO®)	Fully Human IgG1	Merck Serono	Urothelial carcinoma, MCC[e]
	Durvalumab (MEDI4736, IMFINZI®)	Human IgG1	AstraZeneca	Urothelial carcinoma
	MDX1105(BMS-936559)	Human IgG4	Bristol–Myers Squibb	–

Data sources: https://druginfo.nlm.nih.gov/ and https://www.fda.gov/Drugs/.
[a] non-small-cell lung cancer. [b] renal cell cancer. [c] head and neck squamous cell carcinoma. [d] classic Hodgkin's lymphoma. [e] Merkel cell carcinoma.

Table 3 Ongoing PD pathway-targeted clinical trials of subjects with CC

Target	Agent	Clinical indication and ongoing evaluation	Stage of development
PD-1	Nivolumab (BMS-9336558, MSX1106, ONO-4538, Opdivo®)	*NCT02257528: treating persistent, recurrent, and metastatic CC	Phase II
		*NCT02465060: treating patients with mismatch repair deficiency (loss of MLH1 or MSH2 by IHC) in advanced refractory solid tumors	Phase II
		*NCT02379520: HPVST cells alone or in combination with nivolumab in HPV-related carcinoma	Phase I
		*NCT03126110:INCAGN01876 + nivolumab or/and ipilimumab treating advanced or metastatic malignancies	Phase I/II
		*NCT03241173:INCAGN01949 + nivolumab or/and ipilimumab treating advanced or metastatic malignancies	Phase I/II
		*NCT03298893: in combination with radiotherapy and cisplatin in locally advanced CC	Phase I
		*NCT02628064: treating advanced solid tumors including CC	Phase II
	Pembrolizumab (MK3475, Keytruda®)	*NCT02628067: treating advanced solid tumors including CC	Phase II
		*NCT02635360: in combination with chemoradiation for the treatment of advanced CC	Phase II
		*NCT03144466: in combination with radiotherapy and cisplatin treating advanced CC	Phase I
		*NCT03192059: in combination with radiation and an immune modulatory cocktail treating advanced and/refractory CC endometrial carcinoma or uterine sarcoma	Phase II
		*NCT02858310: TCR gene therapy targeting HPV-16 E7 with or without pembrolizumab for HPV-associated cancers	Phase I
		*NCT03444376: in combination of GX-188E vaccination treating advanced, nonresectable HPV16 and/or 18 + CC	Phase Ib-II
		*NCT03635567: in combination with chemotherapy treating persistent, recurrent, or metastatic CC	Phase III
		*NCT03367871: in combination with chemotherapy and bevacizumab treating CC	Phase II
PD-L1	Atezolizumab (MDPL-3280A, Tecentrip®)	*NCT02921269: in combination with bevacizumab treating recurrent, persistent, or metastatic CC	Phase II
		*NCT03074513: in combination with bevacizumab treating rare solid tumors including CC	Phase II
		*NCT03073525: in combination with Vigil treating advanced gynecological cancers	Phase II
		*NCT02914470: in combination with carboplatin-cyclophosphamide treating advanced breast cancer and gynecologic cancer	Phase I
		*NCT03614949: in combination with stereotactic body radiation therapy treating recurrent, persistent, or metastatic CC	Phase II
		*NCT03340376: in combination with doxorubicin treating recurrent CC	Phase II
	Avelumab (MSB0010718C, BAVENCIO®)	*NCT03260023: in combination with TG4001 treating HPV-16 + recurrent or metastatic malignancies	Phase I/II
		*NCT03217747: in combination with or without radiation, or radiation and cisplatin treating limited, locally advanced or metastatic solid tumors including CC	Phase I/II
	Durvalumab (MEDI4736)	*NCT01975831: in combination with tremelimumab treating advanced solid tumors	Phase I
		*NCT02725489:in combination with Vigil and durvalumab treating advanced women's cancers	Phase II
		*NCT02291055: in combination with ADXS11-001 in previously treated locally advanced or metastatic cervical or HPV + head and neck cancer	Phase I/II
		*NCT03452332: in combination with stereotactic ablative radiotherapy and tremelimumab treating cervical, vaginal, or vulvar cancer	Phase I

Data sources: www.clinicaltrials.gov.

evaluating the ability of nivolumab to treat persistent, recurrent, and metastatic CC. This study will measure the frequency of objective tumor responses, incidence of adverse events, overall survival (OS), and progression-free survival (PFS) to assess antitumor activity. A laboratory biomarker analysis including immune cells and tumor cell-related biomarkers will be performed to further explore biomarker effectiveness.

Another phase II trial (NCT02465060) is studying treatments that target particular genetic abnormalities (such as mutations, amplifications, or translocations) in tumors. In this study, patients with various tumors, including CCs, and loss of MLH1 or MSH2 (a mismatch repair deficiency) receive nivolumab. Furthermore, three ongoing trial studies are currently examining the effectiveness of combining nivolumab with standard chemoradiotherapy and other therapies, including HPVST cells, INCAGN01876, and INCAGN01949 (NCT03298893, NCT03527265, NCT02379520, NCT03126110, and NCT03241173).

Pembrolizumab

Pembrolizumab is another humanized monoclonal immunoglobulin (Ig) G4 antibody that targets PD-1. Similar to nivolumab, pembrolizumab has produced an effective and durable response in many tumor types in completed clinical trials and has received approval for treating multiple tumors [89–93]. In June 2018, FDA approved pembrolizumab for patients with recurrent or metastatic CC with disease progression on or after chemotherapy and whose tumors express PD-L1 (CPS $\geqslant$ 1). The broad application of pembrolizumab in CCs is being evaluated in several ongoing studies. A trial involving patients with multiple types of advanced solid tumors (NCT02628067), including CCs, will evaluate the objective response rate (ORR) of subjects and explore predictive biomarkers. The efficacy of using pembrolizumab in combination with other therapies, including chemotherapy, radiation, vaccination in addition to immune modulatory cocktails, and E7 TCR cells, is being studied to treat CCs and explore efficient therapies for affected patients (NCT03367871, NCT03635567, NCT3444376, NCT02635360, NCT03144466, and NCT03192059).

Atezolizumab/MDPL-3280A

Atezolizumab, a human IgG1 that targets PD-L1, can block PD-L1 from binding PD-1. It was the first anti-PD-L1 drug to be approved by the FDA [94] and is currently used to treat urothelial carcinoma and NSCLC [95,96]. As a promisingly effective PD-L1 blockade antibody, atezolizumab has been tested in clinical trials to evaluate its efficacy and safety in subjects with CCs. In these trials, atezolizumab is being assessed in combination with chemotherapy, radiation, bevacizumab, and Vigil (NCT03614949, NCT03340376, NCT02921269, NCT03073525, and NCT02914470).

Avelumab/MSB0010718C

Avelumab is a fully human, IgG1 lambda, PD-L1-blocking monoclonal antibody that has been approved by the FDA to treat patients with Merkel cell carcinoma since March 23, 2017 [97]. This drug is the first FDA-approved product that treats this type of cancer. On May 9, 2017, avelumab was also approved for locally advanced or metastatic urothelial carcinoma [98]. Two trials, each including a cohort of patients with CC, are ongoing to reveal the efficacy of avelumab for treating this type of tumor (NCT03260023 and NCT03217747).

Durvalumab/MEDI4736

Durvalumab is an antibody that blocks PD-1 from binding to PD-L1 and B8-1 and is being tested in many clinical trials involving multiple tumor types. The newest development related to this treatment was announced on May 1, 2017, when the FDA granted accelerated approval to durvalumab, considering its confirmed ORR for treating patients with locally advanced or metastatic urothelial carcinoma [99]. In clinical trials involving subjects with CCs, the effectiveness and safety of durvalumab were assessed in combination with radiotherapy, ADXS11-001, tremelimumab, and Vigil (NCT03452332, NCT02291055, NCT01975831, and NCT02725489).

Future perspectives

Considering the immunological indicators that have been identified in CCs and the promising efficacy of blockade agents, we anticipate that immune therapies targeting the PD pathway will play an important role in patients with CCs. However, more investigations are still needed. Although the PD-1/PD-L1 pathway has been studied for nearly two decades, the mechanisms underlying its participation in the regulation of immunity remain relatively unknown. PD-1-recruited Shp2 phosphatase promotes the dephosphorylation of CD28 rather than TCR, indicating that the therapeutic efficacy of PD-1 pathway blockades acts by reactivating co-stimulatory molecule signaling rather than TCR signaling [100,101]. This result changes which factors should be targeted in the PD-1/PD-L1 signaling pathway, suggesting that co-stimulatory pathways play crucial roles in regulating effector T cell function and anti-PD-L1/PD-1 therapeutic responses. More studies are urgently needed to increase our understanding of PD1 pathway mechanisms. Additionally, ongoing clinical trials of several host- and tumor-

derived biomarkers of the PD pathway blockade response have been proposed, as well as trials of biomarkers associated with adaptive resistance to PD-1 blockade [102]. Whether these biomarkers have a separate or conjoined predictive value and how laboratory indexes can be uniformly detected should be examined in future studies. Moreover, methods for achieving maximal therapeutic effects while minimizing tolerable side effects when antibodies are used alone or in combination with other therapies will also be evaluated in future clinical trials.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 81373867).

Compliance with ethics guidelines

Yumeng Wang and Guiling Li declare that they have no conflicts of interest. This manuscript is a review article and does not involve a research protocol that requires the approval of the relevant institutional review board or ethics committee.

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