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Overview

The Role of Biomarkers for the Prediction of Response to Checkpoint Immunotherapy and the Rationale for the Use of Checkpoint Immunotherapy in Cervical Cancer

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Abstract

Checkpoint immunotherapy has revolutionised the way that melanoma is treated and has also shown significant effectiveness in lung, bladder, renal, and head and neck cancers. At the present time, trials of checkpoint immunotherapy in cervical cancer are at early phases, but there is very good rationale for pursuing this as a treatment option, especially as cervical cancer is a virally driven cancer and therefore should be recognised by the immune system as being foreign. This review explores the biomarkers for the selection of patients for immunotherapy in other cancers, such as programmed death ligand 1 (PD-L1) expression, tumour infiltrating lymphocytes and total mutational burden, and relates these biomarkers to cervical cancer. A PubMed search was carried out for publications published in English with the terms 'immunotherapy' OR 'cervical cancer' OR 'checkpoint blockade' OR 'tumour infiltrating lymphocytes' OR 'total mutational burden'. Articles that met these criteria and were available on PubMed before 8 October 2018 were included. The results showed that PD-L1 is positive in up to 90% of cervical cancers and that the total mutational burden is moderately high, with 5–6 mutations per megabase. In addition, the tumour microenvironment in cervical cancer has an impact on prognosis, with higher ratios of CD8+ tumour infiltrating lymphocytes to CD4+ T regulatory cells being associated with improved survival. Clinical studies to date have shown the response rate of cervical cancer to checkpoint immunotherapy to be in the region to 10–25%. Cervical cancer exhibits many of the features that have been shown to be correlated with response to checkpoint immunotherapy in other tumour sites. However, response rates to date are in the region of 10–25%. Therefore, combinations of immunotherapeutic agents or checkpoint inhibitors with radiotherapy may be required to maximise the therapeutic benefit of harnessing the host immune system to fight cancer.

Key words: Cervical cancer; checkpoint inhibitors; CTLA-4; immunotherapy; PD-L1

Statement of Search Strategies Used and Sources of Information

A PubMed search was carried out for publications published in English with the terms 'immunotherapy' OR 'cervical cancer' OR 'checkpoint blockade' OR 'tumour infiltrating lymphocytes' OR 'total mutational burden'. Articles that met these criteria and were available on PubMed before 8 October 2018 were included.

Introduction

Immunotherapy harnesses the host immune system to mount an immune response against tumour cells and has become a rapidly expanding area within oncological research over recent years. The first clinical trials showing the benefit of checkpoint immunotherapy were using ipilimumab in metastatic melanoma [1]. However, combination therapy with nivolumab and ipilimumab was soon shown to be more effective than monotherapy [2]. Since then, there has been a second wave lateralisation of checkpoint immunotherapy to other cancers, such as lung, urothelial, and head and neck squamous cell carcinomas (HNSCCs) (see Table 1). Cervical cancer will hopefully be within the third wave of expansion of the use of

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immunotherapeutic agents, although clinical trials to date are still in the relatively early stages (phase I/II).

The two main pathways that are currently exploited in clinical practice are the cytotoxic T lymphocyte-associated protein 4 (CTLA-4) pathway and the programmed cell death 1 (PD-1) pathway (see Figure 1). CTLA-4 inhibits the costimulatory signals required for full activation of T cells following interaction between the T cell receptor and an antigen/major histocompatibility complex on an antigen presenting cell [15]. PD-1 is expressed on the surface of activated T cells and interacts with programmed death ligands 1 and 2 (PD-L1/2), which are on the surface of tumour cells or immune cells such as antigen presenting cells [16]. The PD-1 pathway negatively regulates the response of T cells, leading to T cell exhaustion (progressive loss of effector function due to prolonged antigen stimulation) [17]. The interaction of PD-L1 with CD80 on activated T cells also leads to negative regulation of cytoxic T cells [18].

Selection of Patients for Checkpoint Immunotherapy

Immunotherapy has the ability to produce a long-lasting response in a subset of patients (about 20%) [19]. With better patient selection and combination therapy, this proportion will hopefully increase. Unfortunately, PD-L1 status is an imperfect biomarker for the prediction of tumour response to PD-1 blockade, as the expression within tumours is heterogenous, the expression can vary temporally and techniques to assess PD-L1 expression vary [20].

In melanoma, higher expression of PD-L1 on tumour cells and tumour infiltrating lymphocytes (TILs) was associated with a higher objective response rate (ORR) and a longer progression-free survival (PFS) and overall survival with pembrolizumab [21]. However, some patients with low PD-L1 expression have durable responses and therefore anti-PD-L1 therapy should not be restricted to those with a high expression at present. Other studies in melanoma have shown improved survival with nivolumab regardless of PD-L1 status [4]. In addition, PD-1 has a second ligand, PD-L2, which is less well-characterised but has been shown to be an independent predictive marker of the response to PD-1 blockade in HNSCC patients and to be present in multiple tumour types [22]. Therefore, a combination of PD-L1 and PD-L2 expression may be more useful for patient selection.

In melanoma patients, the best predictive marker of response to PD-1 blocking agents in pretreatment biopsies was CD8+ T cell density at the invasive margin followed by CD8+ T cell density in the tumour centre, then PD-1+ cell density and PD-L1+ cell density [23]. Therefore, CD8+ cell density was more predictive of the response to treatment than PD-1 and PD-L1 expression. Secondary immune constructs, such as the immunoscore, which evaluates the density of CD3+, CD8+ (cytotoxic T cells) and CD45RO+ (memory T cells) T cells, has been shown to predict prognosis better than TNM staging in colorectal cancer [24] and is being evaluated for other tumours.

A high total mutational burden (TMB) has been shown in melanoma [25], lung [26] and colorectal [27] cancer to be associated with improved responses and survival to immunotherapy. The pathway to increased mutational burden could be due to impaired DNA repair pathways [27], uncontrolled DNA replication and a number of other mechanisms, such as loss of *TP53* DNA damage checkpoint activity or due to increased apolipoprotein B mRNA editing enzyme (APOBEC) activity [28], all of which could lead to increased production of neoantigens, which can be presented to cytotoxic T cells on major histocompatibility complex proteins on the tumour cell surface. Immunotherapy could then be used to enhance the response of these cells to the tumour.

Table 1
Clinical evidence for Food and Drug Administration approved checkpoint immunotherapeutic agents

Checkpoint	Drug	Tumour site [reference]
CTLA-4	Ipilimumab	Melanoma [1]
	Tremelimumab	Phase I/II trials [3]
PD-1/PD-L1	Nivolumab	Melanoma [4]
		Renal cell carcinoma [5]
		NSCLC [6]
		HNSCC [7]
		Urothelial carcinoma [8]
	Pembrolizumab	Melanoma [9]
		HNSCC [10]
		NSCLC [11]
		Cervical cancer [12]
	Atezolizumab	Urothelial carcinoma [13]
		NSCLC [14]

CTLA-4, cytotoxic T lymphocyte-associated protein 4; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1.

Combination Treatment with Checkpoint Immunotherapeutics

Taube *et al.* [29] identified four categories in melanoma, based on TILs and PD-L1 expression within the tumour microenvironment (see Table 2). This model could therefore be useful to determine which combination of therapies would benefit individual patients [30]. For example, chimeric antigen receptor T cells (CAR-T cells) may be required to increase the TILs within a TIL tumour and have been shown to be effective in melanoma [31]. Equally, agents that cause immunogenic cell death, such as certain chemotherapeutics (e.g. gemcitabine) and radiotherapy, could also increase T cell infiltration by facilitating the production of neoantigens [32,33]. In mouse models of colorectal and breast cancer, fractionated radiotherapy has been shown to upregulate PD-L1 expression leading to acquired resistance, which can be reversed with concomitant PD-1 pathway blockade [34]. Given that locally advanced cervical cancers are predominantly treated with chemoradiotherapy, the addition of a checkpoint inhibitor to this would be an attractive option.

Other factors to consider in TIL− patients are whether TILs are excluded from penetrating the tumour and whether

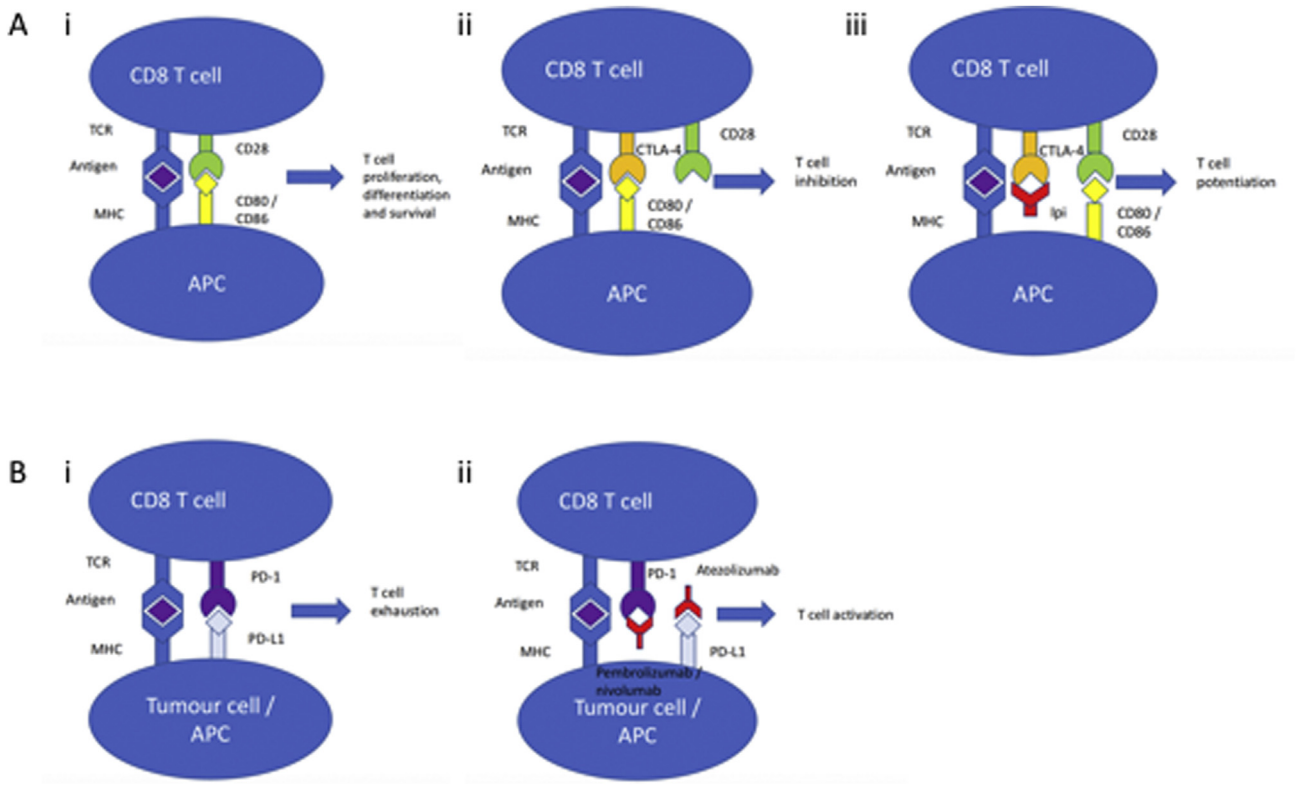

Fig 1. The main immune checkpoint pathways that are exploited in clinical use at present. (A) The cytotoxic T lymphocyte-associated protein 4 (CTLA-4) pathway. (i) Antigen is presented on the major histocompatibility complex (MHC) to the T cell receptor (TCR) by an antigen presenting cell (APC). For full activation of the T cell, costimulatory signals are required through the interaction of CD28 with CD80 or CD86. (ii) CTLA-4 is expressed on the T cell and competes with CD28 to bind CD80/CD86. Therefore, T cells cannot be fully activated. (iii) Ipilimumab (ipi) blocks CTLA-4 binding to CD80/CD86 and, therefore, potentiates T cell activity. (B) The programmed death ligand 1 (PD-L1) pathway. (i) PD-L1 on tumour cells or APC interacts with programmed cell death 1 (PD-1), which is expressed on activated T cells and leads to T cell exhaustion. (ii) Pembrolizumab and nivolumab bind to PD-1, whereas atezolizumab binds to PD-L1 to prevent the interaction between PD-1 and PD-L1, thereby promoting T cell activation.

Table 2
A summary of the proposed categories of tumour in melanoma by Taube *et al.* [29] and the possible therapeutic options

Category	TIL	PD-L1	Immunological response	Possible therapeutic options
Type I	+	+	Adaptive immune resistance	Single-agent checkpoint inhibitors
Type II	−	−	Immunological ignorance	Combination therapies, e.g. CAR-T cells and agents inducing immunogenic cell death
Type III	−	+	Constitutive PD-L1 expression	CAR-T cells Agents inducing immunogenic cell death
Type IV	+	−	Immunological tolerance − may have upregulation of other immunosuppressor molecules	May require other checkpoint inhibitors

CAR-T cells, chimeric antigen receptor T cells; PD-L1, programmed death ligand 1; TIL, tumour infiltrating lymphocytes.

other cells within the microenvironment play a role in this, such as cancer-associated fibroblasts [35]. Cancer-associated fibroblasts have been shown to be abundant in cervical cancer and alter the composition of the extracellular matrix [36]. Targeting other cells within the tumour microenvironment may be feasible in the future. Similarly, abnormal vasculature can impede the extravasation of TILs into the tumour and therefore the combination of vascular endothelial growth factor inhibition and immunotherapy could also be beneficial [37], particularly in cervical cancer, which is known to respond to bevacizumab in the metastatic setting [38].

The timing of combination approaches will be almost as critical as the combination itself and needs to be taken into consideration in the design of prospective clinical trials. Ipilimumab and nivolumab given in the neoadjuvant setting seem to be superior to adjuvant treatment in stage III melanoma, as there is a higher antigen burden at this timepoint, leading to an enhanced immune response [39]. Patients treated neoadjuvantly had a higher number of

tumour resident T cell receptors expanded in the peripheral blood compared with those treated adjuvantly. For radiotherapy, concomitant but not sequential PD-L1 blockade leads to increased survival in colorectal and breast cancer mouse models [34].

Mechanisms of resistance to immunotherapeutics are not currently well understood. However, there is evidence to suggest that the loss of Phosphatase and tensin homolog (PTEN) in melanoma cell lines contributes to resistance to immunotherapy and that phosphoinositide 3kinase (-PI3K) inhibition can overcome this [40]. This may have relevance to cervical cancer, as PI3K and PTEN mutations are some of the most frequently occurring mutations and can occur in up to 30% [41] and 6% [42] of tumours, respectively. Therefore, the combination of immunotherapy and PI3K inhibitors may be beneficial. Other possible mechanisms of resistance include Jak1/2 mutations [43] and upregulation of other immune checkpoints, such as TIM-3 [44]. Indoleamine 2,3-dioxygenase also contributes to the resistance of immunotherapies and indoleamine 2,3-dioxygenase inhibitors are in early stage clinical trials [45].

Rationale to Pursue Checkpoint Immunotherapy for Cervical Cancer

Despite initiatives to improve the prevention of cervical cancer with screening and vaccination, cervical cancer is currently the fourth most common cancer worldwide [46]. Although the prognosis for women with early stage disease is very good, the 5-year overall survival for patients with stage III disease is less than 40% [47]. Improvements in survival for locally advanced cervical cancer over recent years have mainly been through improvements in technical radiotherapy, particularly through the implementation of image-guided brachytherapy and the use of interstitial needles for patients with bulkier tumours to allow dose escalation [48]. In the recurrent or metastatic setting, the addition of bevacizumab to standard chemotherapy has significantly improved median survival from 13.3 to 16.8 months [38], but treatment options are limited thereafter. It is therefore important to explore the immunotherapy paradigm and take lessons learnt from first and second wave clinical development in other disease sites and apply them to cervical cancer.

Cervical Cancer is a Virally Driven Cancer

Almost all cases of cervical cancer are driven by high-risk human papillomavirus (HPV) infection [49]. HPV-infected cells express viral proteins E6 and E7, which interfere with p53 [50] and Rb [51], respectively, therefore allowing progression through the cell cycle. The HPV E7 gene has been shown to increase PD-L1 expression when transfected into prostate cancer cell lines and when E7 is knocked down in CaSki cells, PD-L1 expression reduced and subsequently T cell proliferation and cytotoxic activity increased [52].

As HPV is a foreign pathogen expressing foreign antigens within host cells, the host immune system should be able to recognise infected cells and eliminate them. However, the virus has evolved many ways of evading the immune system, including the downregulation of major histocompatibility complex class I proteins on the cell surface after interaction with viral protein E5 [53].

However, it is not just HPV-directed T cells that are important in the regression of cervical cancer. Stevanovic *et al.* [3] showed that in two metastatic cervical cancer patients whose disease had completely regressed after a single adoptive TIL infusion, the immunodominant T cells were directed against mutated neoantigens in one patient (35% of TILs) and a cancer germline antigen in the other (67% of TILs). HPV antigen-targeted T cells represented only 14% of TILs in both patients. This implies that both non-viral and viral antigens are important in the host immune response to cervical cancers. Both viral and non-viral antigen-directed T cells preferentially resided in the PD-1+ compartment, lending more weight to the rationale that immunotherapy should be of benefit in these patients.

Cervical Cancer Expresses PD-L1

PD-L1 is not expressed in normal cervical tissue, but is expressed in 95% of cervical intraepithelial neoplasia (CIN) 1–2 [54]. Reports of PD-L1 expression in cervical squamous cell carcinoma (SCC) vary widely from 19% [55], 22% [41], 51% [54] to 88% [56]. PD-L2 has been reported to be expressed in 29% of cervical cancers [55]. The differences in these values is probably due to the adoption of different cut-off thresholds and assays [20].

PD-L1 expression is less prevalent in cervical adenocarcinoma compared with SCC (14% versu 54%) [57]. Similar results have been seen in lung cancers, with PD-L1 expression on tumour cells being present in 52% of SCC and 17% of adenocarcinoma [58]. The reasons for this are unknown. Clinicopathological features do not appear to correlate with PD-L1 expression in cervical cancer [55,57]. Survival analyses, however, show that patients with SCC and either diffuse PD-L1 positivity or PD-L1 negativity had poorer survival than those with marginal PD-L1 positivity [57]. This may be due to constitutive activation in diffuse positivity, whereas marginal positivity may reflect a localised release of cytokines from TILs. This is in keeping with patients having a trend towards poorer PFS if their tumours show PD-L1 positivity but lack CD8+ T cells, which again is probably due to constitutive activation [56]. PD-L1 can also be expressed on other immune cells and in patients with adenocarcinoma of the cervix the presence of PD-L1+ tumour-associated macrophages was associated with a significantly poorer disease-free survival [57].

PD-1 expression on T cells increases as lesions progress from CIN 1 to CIN 3. PD-L1 expression on dendritic cells also increases [59]. In addition, the cytokine profile changes from a Th1 profile (with interferon-γ and interleukin-12) to a Th2 profile (with interleukin-10), which is more immunosuppressive [59]. The lower levels of interferon-γ as cervical lesions progress may account for the PD-L1

expression being lower in invasive SCC compared with CIN 1–2 [54].

The Tumour Microenvironment in Cervical Cancer has an Impact on Prognosis

Several studies have shown an improved survival with an increased number of TILs in stage IB [60], IIB [61] and more locally advanced tumours. A strong intraepithelial infiltration of mature M1 macrophages leads to an influx of T cells and improved disease-specific survival (DSS) and overall survival [62]. The combination of increased M1 macrophages and a high CD8+/Treg ratio improves survival further, which suggests that both factors have an additive effect on anti-tumour immunity.

CD8+ cells, CD4+ cells and Tregs are more abundant in cervical cancer than in normal cervical tissue [63,64]. Patients without lymph node metastases (which is an independent prognostic factor) have higher numbers of intraepithelial CD8+ cells than lymph node-positive patients [63]. The CD8/CD4 ratio was lower in lymph node-positive patients as was the CD8/Treg ratio. The 5-year survival rate is significantly lower in patients with a higher percentage of FoxP3+ CD4+ TILs (35.3% versus 88.9%, $P = 0.001$) [65]. In addition to looking at CD8 for TILs, CD103 is a marker of intraepithelial T cells and has been shown to be associated with improved prognosis [66].

Therefore, the composition of the tumour microenvironment in cervical cancer has an impact on survival, with a higher ratio of CD8 TILs to CD4 Tregs being associated with less extensive disease (lymph node negativity) and improved survival rates. Interestingly, numbers of Tregs and TILs are significantly increased in CIN and cervical cancer compared with colorectal cancer, lung cancer and melanoma and, therefore, cervical cancers may potentially be more immunogenic than some of the main cancers that have been shown to gain clinical benefit from immunotherapy [67].

Cervical Cancers Have an Increased Total Mutational Burden Rate

Cervical cancer has a relatively high number of somatic mutations, being ranked sixth behind melanoma, lung, bladder, oesophageal and colorectal cancers [68]. The rate of TMB in cervical cancers is about 5–6 mutations per megabase [69,70]. For a comparison with tumour sites that have been shown to respond to immunotherapeutics, the TMB rate for melanoma is 14, for bladder cancer is 8 and for HNSCC is 5 [69].

Mutational signature 2 is prevalent in cervical cancer and HNSCC, which are both associated with the APOBEC pathway [68,70]. APOBECs have roles in innate and adaptive immunity to various viruses, including HPV. APOBEC mutation signatures have also been found in HPV+ precancerous lesions of the cervix [71] and E6 has been shown to be able to upregulate APOBEC3B activity [72]. Therefore, the predominant mechanism of increased mutational burden is due to APOBEC activity, which is due to HPV infection. Therefore, the increased TMB in cervical cancers could potentially lead to the presence of more neoantigens, which could then stimulate the immune system, particularly in the presence of immunotherapeutic drugs.

Cervical Cancer is Associated with the Expression of Other Immune Inhibitory Molecules

As well as the expression of the members of the PD-1 pathway, other immunomodulatory molecules are expressed in patients with cervical cancer. Peripheral blood mononuclear cells from patients with advanced cervical cancer have been shown to have increased CTLA-4 expression and reduced CD28 expression compared with controls [73], thereby shifting the immune response to a more tolerogenic one.

A good example of another immune inhibitory molecule is TIM-3. TIM-3 is a surface receptor that is expressed on T cells (CD4 Th1, CD8 and Tregs) and innate immune cells, such as dendritic cells. It has four ligands, the main ligand being galectin-9. TIM-3 marks the most dysfunctional and immunosuppressed CD8 cells and is co-expressed with PD-1. In addition, TIM-3+ Tregs are more immunosuppressive than TIM-3− ones [74]. TIM-3 has been shown to be expressed on Treg cells and, in a small number of cervical cancer patients ($n = 3$), up to 60% of TILs were CD4+ TIM-3+, which was a significantly higher proportion than for non-infiltrating T cells [75]. These CD4+ TIM-3+ T cells were shown to significantly suppress the proliferation of autologous CD8+ T cells and expressed high levels of CD25, Fox-P3, CTLA-4 and glucocorticoid-induced tumour necrosis factor family related protein (GITR).

As well as TIM-3 being expressed on T cells, TIM-3 has also been shown to be expressed in tumour cells. TIM-3 expression is positive in 15% of biopsies of chronic cervicitis, 50% of CIN biopsies and 65% of cervical cancer biopsies [76]. Expression of TIM-3 correlated with stage, grade and presence of metastases. The 5-year survival rate for TIM-3+ cases was 46.4% compared with 80% in TIM-3 negative cases ($P = 0.006$). *In vitro*, TIM-3 was expressed throughout the cytoplasm in SiHa and Hela cervical cancer cell lines. Knockdown of TIM-3 in Hela cells leads to reduced migration and invasion of tumour cells.

Therefore, multiple immune modulatory pathways are co-opted in cervical cancer to induce an immunosuppressive tumour microenvironment. This leads one to hypothesise that immunotherapy may be beneficial in cervical cancer but probably needs to be in combination to target multiple pathways.

Head and Neck Squamous Cell Carcinoma has Shown Promising Results with Immune Checkpoint Blockade

Thirty-five per cent of oropharyngeal SCC is caused by HPV and HNSCC is also treated predominantly with chemoradiotherapy so therapeutic responses in HPV+ oropharyngeal cancer may be applicable to cervical cancer [77]. Nivolumab and pembrolizumab have been approved for use in HNSCC. Treatment with nivolumab leads to an improved overall survival in recurrent HNSCC compared

with standard chemotherapy, regardless of PD-L1 or p16 status [10]. However, patients with a PD-L1 tumour score ≥1% or who were p16 + seemed to derive a greater benefit from nivolumab. With pembrolizumab, the overall response rate was 32% in recurrent or metastatic HPV+ head and neck cancers compared with 14% in non-HPV-associated cancers [78]. The PFS at 6 months was 37% and 20% and the median overall survival was 70% and 56%, respectively, for HPV+ and HPV− cancers. Therefore, with the efficacy of PD-L1 blockade in HPV-associated cancers demonstrated in clinical trials, there are strong data to suggest that immunotherapy may be beneficial in cervical cancer.

Immune Checkpoint Blockade Results in Cervical Cancer to Date

Preliminary results from the cervical cancer cohort ($n = 24$) of KEYNOTE-028, which was a phase I study of pembrolizumab in advanced solid tumours, has shown an ORR of 17% (4/24) and a stable disease rate of 12.5% (3/24) [79]. These patients had advanced disease and PD-L1 expression ≥1%. The 6-month PFS was 13% and overall survival was 66.7%. KEYNOTE-158 is a phase II study of pembrolizumab in patients with previously treated advanced cervical cancer regardless of PD-L1 status [12]. Preliminary results from the first 47 patients have shown an ORR of 17%. PD-L1 was positive in 87% of patients (≥1%), although the ORR was independent of PD-L1 status. The Food and Drug Administration approved pembrolizumab for the treatment of recurrent or metastatic cervical cancer based on these results. Checkmate 358 has reported an ORR of 26.3% for nivolumab in cervical cancers, irrespective of PD-L1 or HPV status [80]. In addition to checkpoint inhibitors, early results from a single T cell infusion (TILs had reactivity to HPV E6 and E7) in cervical cancer patients have also shown promising results, with 3/9 patients experiencing a response (two complete responses and one partial response). The two patients with complete responses had ongoing responses at 22 and 15 months [3].

Therefore, to date, the response rates have been in the region of 10–25% (see Table 3), which is encouraging, but clearly there are improvements to be made. These improvements will probably involve combinations of drugs with or without irradiation and improved patient selection.

Toxicity from Immune Checkpoint Blockade

The toxicity from immune checkpoint blockade has been well documented from the original trials in melanoma and lung cancer patients [1]. The majority of side-effects reported are immune-related adverse events, which arise due to non-specific immunological activation [5]. Grade 3 or 4 toxicity is seen in 10–20% of patients and includes inflammation of the gastrointestinal tract, liver, skin and endocrine glands, leading to diarrhoea, hepatitis, rashes and endocrinopathies. The treatment of these side-effects is often with high-dose corticosteroids or other immunosuppressants, or in the case of endocrinopathies with hormone replacement. Colitis can occur in up to 10% of patients treated with ipilimumab and has resulted in treatment-related deaths and therefore needs to be managed aggressively with admission to hospital and intravenous corticosteroids if symptoms are severe [1].

Other Immunotherapeutic Strategies Showing Promise in Cervical Cancer

Although this review has focused on checkpoint immunotherapy in cervical cancer, there are other immunotherapeutic strategies that are currently being investigated.

Therapeutic Cancer Vaccines

Therapeutic cancer vaccines are administered to patients to stimulate a T cell immune response against tumour cells. There are various types of therapeutic cancer vaccine, including dendritic cell-based, tumour cell-based, peptide protein-based, nucleic acid-based and live vector-based vaccines (reviewed by Vici *et al.* [6]).

One example of a live vector-based vaccine is axalimogene filolisbac. It is a live-attenuated *Listeria monocytogenes* cancer vaccine that is genetically engineered to produce a fusion protein of HPV 16 E7 to stimulate an immune response against E7. To date, this is the only therapeutic vaccine for cervical cancer that has progressed to a phase III trial [7], having shown promising results in the phase II trial (GOG-0265). However, the Food and Drug Administration has recently put this trial on hold while waiting additional data regarding safety. The phase II results of GOG-0265 showed that 38% of patients were alive at 12 months after treatment with axalimogene filolisbac, which was a 52% improvement on the expected survival rate of 24.5% [8]. Adverse grade 3 events were experienced by 36% of patients, mainly symptoms of cytokine release. This vaccine has also been investigated in a randomised phase II study in combination with cisplatin or as a single agent [9]. There was no significant improvement in response rate or overall survival in the combination arm.

Adoptive Cell Transfer

In adoptive cell transfer (ACT), T cells are collected from the patient, antigen-specific T cells are activated and expanded *in vitro* and then infused back into the patient [11]. This allows much greater numbers of antigen-specific T cells to be generated than can be generated with therapeutic vaccination. The T cells can be genetically modified by introducing a CAR, which consist of an antibody-derived antigen recognition domain linked to an internal T cell signalling domain. These CAR-T cells are able to bind to a tumour-specific antigen on the surface of tumour cells and have enhanced cytotoxicity and antigen recognition capabilities and have shown good efficacy in haematological malignancies (see review in [13]).

Table 3
Results of trials using checkpoint immunotherapy in cervical cancer to date

Trial	Drug	Phase	Setting	Number of cervical cancer patients	PD-L1 threshold	Objective response rate	Stable response rate
KEYNOTE-028 [78,81] NCT02054806	Pembrolizumab	Ib	Advanced solid tumours	24	≥1%	17% (4/24)	12.5% (3/24)
KEYNOTE-158 [79] NCT02628067	Pembrolizumab	II	Previously treated advanced solid tumours	47	Not a requirement for entry but 87% were positive	17% (8/47)	Not stated
CheckMate 358 [12] NCT02488759	Nivolumab	I	Virus-associated tumours	19	Not a requirement for entry	26.3% (5/19)	Not stated
NCT01693783 [82]	Ipilimumab	I/II	Metastatic disease Second-line	42	NA	2.9% (1/34)	29.4% (10/34)
GOG9929 [83] NCT01711515	Ipilimumab	I	Sequential ipilimumab after chemoradiation for node-positive disease	34	NA	NA	NA

PD-L1, programmed death ligand 1.

The evidence for ACT in cervical cancer is currently immature but there have been some small studies published showing some early promising results. In a cohort of nine patients with metastatic cervical cancer receiving a single infusion of TILs selected for HPV E6 and E7 reactivity, two patients experienced a complete response, sustained for 22 and 15 months, and one patient experienced a partial response [3]. A phase II trial of ACT in HPV+ cancers reported an objective tumour response rate of 28% in cervical cancer patients with a complete response rate of 11% [14].

Conclusion

Cervical cancers are driven by HPV and therefore express foreign antigens specific to the tumour. However, HPV manages to evade the host immune response by various mechanisms, including upregulation of PD-L1 on tumour cells. HPV+ HNSCC has shown exciting results with PD-1 pathway blockade and, therefore, it is reasonable to assume that similar results could be seen in cervical cancer patients. The increased levels of TMB and TILs in cervical cancers give further weight to this. However, clinical trials to date have shown responses in the region of 10–25%. Even in melanoma, however, durable responses to single-agent ipilimumab are only seen in 20% of patients and, therefore, there is a real necessity to identify those patients who gain the most benefit by using a combination of biomarkers and to identify the right combination of therapies for the right group of patients. These combinations may involve multiple checkpoint inhibitors, CAR-T cell infusions, radiotherapy, chemotherapy or novel agents. For cervical cancer patients, there is the possibility to access tumour tissue throughout a patient's treatment, which presents the unique opportunity for adapting treatment based on changes in biomarkers.

Conflicts of interest

The authors have declared no conflicts of interest.

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