



# Head and Neck Squamous Cell Carcinoma: Risk Factors, Molecular Alterations, Immunology and Peptide Vaccines

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## Abstract

Head and neck squamous cell carcinoma (HNSCC) arises from the epithelial lining of the oral cavity, hypopharynx, oropharynx, and larynx. There are several potential risk factors that cause the generation of HNSCC, including cigarette smoking, alcohol consumption, betel quid chewing, inadequate nutrition, poor oral hygiene, HPV and Epstein–Barr virus, and *Candida albicans* infections. HNSCC has causative links to both environmental factors and genetic mutations, with the latter playing a more critical role in cancer progression. These molecular changes to epithelial cells include the inactivation of cancer suppressor genes and proto-oncogenes overexpression, resulting in tumour cell proliferation and distant metastasis. HNSCC patients have impaired dendritic cell (DC) and natural killer (NK) cell functions, increased production of higher immune-suppressive molecules, loss of regulatory T cells and co-stimulatory molecules and major histocompatibility complex (MHC) class I molecules, lower number of lymphocyte subsets, and a poor response to antigen-presenting cells. At present, the standard treatment modalities for HNSCC patients include surgery, chemotherapy and radiotherapy, and combinatorial therapy. Despite advances in the development of novel treatment modalities over the last few decades, survival rates of HNSCC patients have not increased. To establish effective immunotherapies, a greater understanding of interactions between the immune system and HNSCC is required, and there is a particular need to develop novel therapeutic options. A therapeutic cancer vaccine has been proposed as a promising method to improve outcome by inducing a powerful adaptive immune response that leads to cancer cell elimination. Compared with other vaccines, peptide cancer vaccines are more robust and specific. In the past few years, there have been remarkable achievements in peptide-based vaccines for HNSCC patients. Here, we summarize the latest molecular alterations in HNSCC, explore the immune response to HNSCC, and discuss the latest developments in peptide-based cancer vaccine strategies. This review highlights areas for valuable future research focusing on peptide-based cancer vaccines.

**Keywords** Head and neck squamous cell carcinoma · Immunology · Peptide-based vaccines · Cancer immunotherapy · Tumour vaccines

## Introduction

Tumours of the head and neck are amongst the six most common cancers worldwide, with approximately 377,700 new cancer cases and 177,800 cancer deaths in 2020 (Ferlay et al. 2015; Sung et al. 2021). In the United States in 2021, it is predicted that 54,010 new cases of head and neck cancer will be diagnosed, with approximately 10,850 deaths related to these diseases (Siegel et al. 2021). More than 65% of these cancers occur in high risk geographical area include France, Eastern Europe, Brazil, Uruguay and some South Asia countries such as Taiwan, India and Pakistan (Warakulasuriya 2009).

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Although the incidence rate was slightly decreased in the United States and Canada due to reduced cigarette consumption and alcohol use, with the increased human papillomavirus (HPV) infection, the incidence and mortality rates of head and neck cancer are increasing in Taiwan and some European countries (Sturgis and Cinciripini 2007; Hwang et al. 2015; Simard et al. 2014). The incidence and mortality rates are vary from gender, with the incidence and mortality rates of males generally 2:1 higher occurrence than female in most countries (Syrjanen 2005). Despite advances in the diagnosis, surgical techniques, chemotherapy and radiotherapy, the 5-year survival rates of head and neck cancer throughout the world have not improved in the past 30 years. This is mainly owing to cancer recurrence, distant metastases, progression of second primary cancers and resistance to chemo-radiotherapy (Gilbert et al. 2013; Bonner et al. 2010; Schuler et al. 2014). These figures show that head and neck tumours continue to pose a serious threat to public health.

Head and neck squamous cell carcinoma (HNSCC), which arises from the epithelial linings of the oral cavity, hypopharynx, oropharynx and larynx, account for over 90% of head and neck cancers (Tuttle et al. 2016; Mehanna 2010; Shibata et al. 2021; Johnson et al. 2020). There are various forms of HNSCC, including oral squamous cell carcinoma (OSCC), oropharyngeal squamous cell carcinomas (OPSCC), laryngeal squamous cell carcinoma (LSCC) and hypopharyngeal squamous cell carcinoma (HSCC). These various forms of HNSCC have diverse molecular characteristics, clinical progressions, treatment methods and results (Mao et al. 2004). OSCC is one of major types of HNSCC, accounting for more than half of HNSCC, which develops in the squamous epithelium of the lip or oral cavity (Rivera 2015; Noorlag et al. 2015). Most OSCC (60%) has a poor prognosis, as they are detected at stages III and IV, and the 5-year survival rate is only 30% (Omar 2015). OPSCC occur on the crypt epithelium of the palatine, lingual tonsils and posterior pharyngeal wall (Monsjou et al. 2010). Currently, the incidence rates of OPSCC is increasing and it is estimated that these will continue to rise in many countries (Rodrigo et al. 2014; Rietbergen et al. 2013).

In this review, the risk factors, stages, molecular alterations and the immune response to HNSCC will be systematically reviewed. Additionally, the latest developments in peptide-based cancer vaccine strategies are discussed.

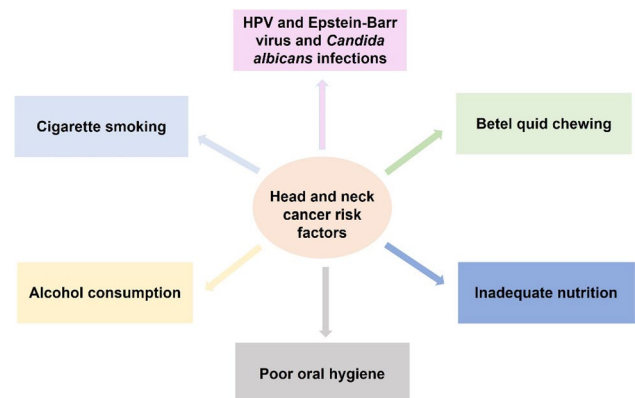
## Risk Factors Associated with HNSCC

There are several potential risk factors that are associated with the generation of HNSCC, including cigarette smoking, alcohol consumption, betel quid chewing, inadequate nutrition, poor oral hygiene, HPV and Epstein–Barr virus and

*Candida albicans* infections (Johnson et al. 2020; Canning, et al. 2019) (Fig. 1).

People with a long history of tobacco smoking and alcohol drinking frequently develop HNSCC. Approximately 80% of HNSCC patients were correlated with an increased risk due to tobacco smoking or alcohol drinking (Vigneshwaran and Williams 2014). The carcinogenic influence of cigarette smoking has been recognised as the primary cause of various types of cancers, including HNSCC. The various types of tobacco (including electronic cigarette and water pipe smoking) all have detrimental, long-term effects on health, particularly in the region of head and neck (McQueen et al. 2016; Munshi et al. 2015).

There has been an increase in the incidence of OPSCC during the last three decades, especially in younger patients, which is strongly correlated with increased risk of HPV (Nasman et al. 2015; Mehanna et al. 2013). It has been reported that the incidence of HPV-positive OPSCC increased in the USA from less than 20% to 80% in the 2000s (Fakhry and D'Souza 2013). There are over 200 serotypes of HPV and 15 of them are closely associated with HNSCC (Marur et al. 2010). In one study, scientists found that more than 90% of HPV positive OPSCC patients tested positive for HPV16 serotype infection (D'Souza et al. 2007). The mechanistic link between HPV infection and increased risk of OPSCC development is related to the E6 and E7 oncoproteins. The E6 and E7 proteins are viral oncoproteins that accelerate the degradation of the tumour suppressor protein *p53* and retinoblastoma protein (pRb), resulting in uncontrolled cancer cell growth and contributing to oncogenesis (Münger and Howley 2002; Estêvão et al. 2019). These proteins also have an important effect on cell transformation and immortalisation (Ruttkay-Nedecky et al. 2013). E7 tends to increase the expression of the Rad51 protein, which inhibits homologous recombination of DNA double strand breaks, resulting in further DNA damage and genomic



**Fig. 1** Summary of potential risk factors that cause the generation of HNSCC

instability (Park et al. 2014; Klein 2008). More recently, HPV-associated OPSCC is considered as a distinct subgroup of HNSCC entity due to its characteristic molecular alterations and therapeutic responses (Vigneswaran and Williams 2014). However, the mechanisms and disease progression of OPSCC HPV infection is still not fully understood nor is the best clinical therapy for this disease clear (Marur et al. 2010).

The role of HPV infection in OSCC development is unclear (Hubbers and Akgul 2015). In China, research has indicated that HPV infection may increase the risk of OSCC carcinogenesis (Zhou et al. 2015). High-risk HPV serotypes, especially HPV16, is positively correlated with OSCC tumorigenesis in young patients (Kaminagakura et al. 2012). Oral tongue squamous cell carcinoma (SCC) was also closely linked with HPV infection (Elango et al. 2011). In contrast, research in Japan and southern Germany did not find a relationship between HPV infection and OSCC patients (Rushatamukayanunt et al. 2014; Reuschenbach et al. 2013). Interestingly, some research found that by increasing the expression of miR-20a, a small RNA involved in cancer development and carcinogenesis, HPV-16 E7 protein may inhibit the development of OSCC (Hu et al. 2016). Further investigations are required to define the relationship between HPV infection and OSCC progression.

Infection with pathogens other than HPV is also correlated with the development of HNSCC. *Candida albicans* is the most common fungal pathogen in the oral cavity and is also closely correlated with the development of oral cancer. It has been suggested that oral *Candida* colonisation may be correlated with oral cancer development (Alnuaimi et al. 2015). Infection with Epstein–Barr virus has also been shown to be significantly associated with the carcinogenesis of nasopharyngeal carcinoma, NPC (Hettmann 2015).

Poor oral hygiene and inadequate dental care, such as irregular dental visits and not brushing teeth, play a key role in the occurrence of HNSCC, particularly when combined with cigarette smoking and alcohol consumption (Chang et al. 2013). Betel quid chewing is positively associated with oral cancer development. With or without tobacco consumption, betel quid has been shown as an independent risk factor for HNSCC (Amtha et al. 2014; Guha et al. 2014). Betel quid chewing is the main risk factor for HNSCC in an East Asian population (Lee et al. 2019). Approximately 50% of HNSCC patients are malnourished when they were diagnosed. Nutritional deficiencies, such as a lack of minerals and vitamins, might hasten the development of oral cancer. Consequently, in order to obtain optimal treatment outcomes proper nutrition should be provided to patients in addition to standard cancer treatment methodologies (Alshadwi et al. 2013). This is supported by reports that increased fruit and vegetable consumption may reduce the risk of oral cancer (Pavia et al. 2006).

## Stages of HNSCC Development

In order to assess prognosis and deliver optimal treatment, it is critical for clinicians to accurately determine the stage of tumour in HNSCC patients. From 1943 to 1952, Pierre Denoix first developed the tumour–node–metastasis (TNM) system to define the stage of malignant tumours (Rutkowski 2014). This system is dependent on three fundamental anatomical spread indicators, including the extent of primary lesion (T), regional lymph nodal spread (N) as well as distant metastasis (M) (Mao et al. 2004). It is generally accepted that HNSCC have four stages: stages I and II (early HNSCC), stages III and IV (locally advanced HNSCC) and recurrent or distant metastasis. When people are first diagnosed with head and neck cancer, more than half of them are at stage III or IV, resulting in low 5-year survival rates (Kao and Lim 2015). Although the TNM staging system is already in its 7th iteration and is widely used around the world in clinical and research settings, the current TNM system for HNSCC does not include patient prognostic factors, biological markers and molecular characteristics (Schroeff and Baatenburg de Jong 2009; Patel and Shah 2005). Therefore, a more comprehensive and detailed staging system is required.

## Molecular Alterations of HNSCC

HNSCC are caused by both environmental factors and genetic mutations, with the latter playing a more critical role in cancer progression. These molecular changes to DNA include inactivation of cancer suppressor genes and overexpression of proto-oncogenes, resulting in tumour cell proliferation and distant metastasis (Acin et al. 2011; Alshadwi et al. 2019).

### Alterations in p53 Expression

Mutations in the *p53* tumour suppressor gene can be detected in over half of HNSCC patients (Sittichai 2013). *p53* is a transcription factor that inhibits cancer growth and can arrest cell cycle and induce cell apoptosis (Perri et al. 2015a). The *p53* mutations impair the ability of the cell to regulate the cell cycle and activate cell death. Mutations in *p53* also render the cell unable to terminate DNA replication, which results in uncontrolled growth of cells and consequently the DNA mutation accumulation (Perri et al. 2015a). In addition, *p53* mutations are highly linked with worse prognosis in HNSCC patients (Acin et al. 2011). HNSCC patients with *p53* mutations respond poorly to traditional therapies, including radiotherapy and chemotherapy, mainly due to *p53*

alterations suppressing E6 and E7 protein expression (Perri et al. 2015a; Ziemann et al. 2015). A recent study by Amit showed that *p53* status was closely linked with nerve density and can promote tumour growth. Loss of *p53* stimulates neuron reprogramming in HNSCC (Amit et al. 2020). Therefore, targeting *p53* may be an effective anticancer therapy for HNSCC (Amit et al. 2020; Stransky et al. 2011).

## Alterations in Angiogenesis

Angiogenesis (blood vessel formation) is the process of vascular network growth and expansion, and is the foundation for the uncontrolled cell proliferation and metastasis of tumours (Guerra 2021). The process of angiogenesis is governed by several different growth factors including vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) (Guerra 2021; Vassilakopoulou et al. 2015; Costache et al. 2015). Impeding angiogenesis has been proposed as an efficient way to control head and neck tumours (Calixto et al. 2014). The most crucial element for blood vessel differentiation and development is the VEGF family and their receptors. More than 90% of HNSCC patients express high levels of VEGF (Costache et al. 2015). Overexpression of VEGF results in uncontrolled cell proliferation and increases radio-resistance. Consequently, the amount of VEGF expression can be considered an indicator of HNSCC, and the subsequent diagnoses, treatment, and prognoses (Costache et al. 2015; Hsu et al. 2014). VEGF significantly inhibits the maturation and differentiation of dendritic cells (DCs) from hemopoietic progenitor cells (HPC) and inhibits the growth of many other hematopoietic lineages, such as macrophages (Gabrilovich et al. 1998). This inhibition is attributed to the stimulation of nuclear factor  $\kappa$ B (NF- $\kappa$ B), which is a transcription factor that regulates many immune responses, such as cytokines and immune cell growth factors (Gabrilovich et al. 1998). Immature DCs contribute to immunosuppression through inducing differentiation of regulatory T cells (Tregs) and suppressing CD8<sup>+</sup> T cell response, resulting in inhibition of T cell function (Huang et al. 2006; Almand et al. 2001). Moreover, HNSCC highly express several VEGFs on the vascular endothelium, such as VEGF-A, VEGF-C and VEGF-D, and are also positively correlated with lymph node metastasis of OSCC (Karatzanis et al. 2012).

Another important factor that contributes to HNSCC tumourigenesis and progression is the EGFR, which is greatly expressed in most HNSCC patients (Perri et al. 2015b). Like VEGF, high levels of EGFR are strongly correlated with poor response to treatment, and a high rate of HNSCC recurrence (Rabinowits and Haddad 2012; Machiels and Schmitz 2015). Moreover, EGFR may promote angiogenesis in HNSCC patients through the hypoxia-inducing factor-1 $\alpha$  (HIF-1 $\alpha$ ) and Notch1 signalling pathways. HIF-1 $\alpha$

is a significant molecular mediator for blood vessel formation and the interaction with Notch1 can affect blood vessel formation (Wang et al. 2013). The mechanism by which EGFR influences angiogenesis in HNSCC patients through these pathways is not fully understood. As the suppression of HIF-1 $\alpha$  and Notch1 pathways may provide a promising and new therapy options for HNSCC further studies about these mechanisms are necessary (Wang 2015a).

## Alterations in Telomerase

Telomerase has a significant influence on cellular immortality and tumourigenesis, as a result, telomerase activity is a tumour diagnostic marker (Chen and Chen 2011). Normal somatic cells do not express telomerase, however, high telomerase activity is found in the majority of human carcinomas (Ruttkay-Nedecky et al. 2013; Takahashi et al. 2014). Telomerase activation is tightly associated with poor clinical outcome in locally advanced cancer (Chen and Chen 2011). The activity of telomerase is strongly correlated with human telomerase reverse transcriptase (hTERT) expression, which plays a key role in unlimited cell proliferation (Takenaka and Sato 2015). In OSCC cells, the level of hTERT was substantially higher than normal oral epithelium cells (Takenaka and Sato 2015). Therefore, increased telomerase activity may contribute to the occurrence of head and neck malignancy (Takahashi et al. 2014; Yuji et al. 2015). However, the mechanism of activation of telomerase is still unclear.

## Other Molecular Interactions

Apart from these factors, a variety of other molecular changes have been studied in the growth and development of HNSCC. High levels of signal transducers and activators of transcription (STATs) expression can be detected in the majority of HNSCC patients (Vangara and Grandis 2014). STATs, a family of cytoplasmic proteins, are responsible for signal transduction in the normal cell response to growth factors such as fibroblast growth factor and the cytokine receptor-kinase complex. Activation of STATs, especially STAT3 in cancer cells, could inhibit tumour cells apoptosis and promote cancer cell proliferation (Mali 2015). STAT3 can indirectly induce the expression of VEGF by upregulating the levels of HIF-1 $\alpha$ , which contributes to tumour angiogenesis (Vangara and Grandis 2014; Mali 2015). As a result, the levels of STAT3 provide a novel clinical outcome factor for HNSCC patients (Masuda et al. 2002).

STAT3 also plays a key role in immune system evasion through inducing increased expression of matrix metalloproteinases (MMPs) (Mali 2015). High expressions of MMPs have been observed in OSCC patients. MMPs are a family of zinc-dependent endopeptidases that degrade the basement membranes (BM) and the extracellular matrix

(ECM) (Vilen 2013). Overexpression of MMPs results in the degradation of the ECM. MMPs also release numerous substances such as cytokines from their cryptic locations. By influencing angiogenesis, proliferation, and invasion, these factors affect cell behaviour and aid cancer growth (Vilen 2013; Liu 2012).

Aberrant expression of proteins that regulate the cell cycle is commonly associated with tumourigenesis. Cyclin D1 is overexpressed during OSCC, and is a prognostic factor for OSCC patients (Hanken et al. 2014). Cyclin D1, a 45-kDa protein encoded on chromosome 11q13, is known to regulate the transition of the cell cycle from G1 phase to S phase, giving rise to cellular replication (Miyamoto et al. 2003). Increased abnormal cell proliferation is a key indicator for the aggressiveness of tumour. Overexpression of cyclin D1 in OSCC patients is closely related to worse clinical outcome and lymph node metastasis (Miyamoto et al. 2003). Similarly, overexpression of Cdk6 was also observed in more than 90% of HNSCC patients. Cdk6 is a cyclin-dependent kinase, an important regulator that drives the cell cycle transition from G1 stage to S stage, a process which plays a vital role in enhancing cell proliferation (Poomsawat et al. 2016). High expression of Cdk6 is an unfavourable factor for cancer patients, as it is indicative of an advanced tumour status (Poomsawat et al. 2016). Research has demonstrated that decreased Cdk6 expression may inhibit the development of OSCC, and that consequently Cdk6 might be regarded as a therapeutic target in this tumour (Shao et al. 2013).

Yet another cell-cycle protein that is important for tumourigenesis is *p16* (Patel et al. 2020). *p16* is a tumour suppressor gene and cyclin-dependent kinase 4 (CDK4) inhibitor, and normally functions as a checkpoint regulator of G1/S cell cycle stage (Thomas and Primeaux 2012). In normal cells it is expressed at a very low level. However, it highly expressed in HNSCC patients, indicating that a lower local recurrence and increased survival rates (Thomas and Primeaux 2012; Cai et al. 2014). Increased Cyclooxygenase-2 (COX-2) expression has been correlated to the development of many cancers, including HNSCC (Lee et al. 2002). COX-2 is one of the enzymes that participates in the synthesis of prostaglandins (PGs). COX-2 expression is generally undetectable in normal cells (Zhang et al. 2020). However, its expression can be induced by inflammatory cytokines and growth factors. Higher expression of COX-2 is correlated with the development of HNSCC invasion, metastasis, and recurrence (Eun-Young et al. 2015; Celenk et al. 2013; Morita et al. 2012). Increased expression of COX-2 promotes the synthesis of prostaglandins, enhances cancer cell proliferation, and reinforces angiogenesis (Zhu et al. 2020). Consequently, COX-2 inhibition may be a novel strategy to treat HNSCC.

## The Immune Response to HNSCC

The host immune system is closely correlated with the progression, growth, and metastasis of HNSCC (Ferris 2015). Although there have been recent advances in the development of novel cancer therapies, including anti-tumour vaccines, the results have not yet been developed into treatments that increase the survival rate of HNSCC patients. As a result, in order to establish effective immunotherapies, a better understanding of interactions between the immune system and HNSCC is required (Freiser et al. 2013). HNSCC patients have impaired DC and natural killer (NK) cell functions, higher immune-suppressive molecules, and regulatory T cells. There is also a loss of co-stimulatory molecules and MHC class I molecules, lower number of lymphocyte cell subsets and a poor response to antigen-presenting cells, APCs (Sittichai 2013; Kuss et al. 2004; Dasgupta et al. 2005; Bando et al. 2010; Strauss et al. 2007; Whiteside 2005). Immune evasion plays a key role in the progression of HNSCC, giving rise to the loss of anti-tumour immunity (Whiteside 2005).

### Dendritic Cells in HNSCC

The most robust and effective APCs are DCs, which are crucial in anti-tumour immunity (Wang et al. 2020). DCs serve as a modulator between innate and adaptive immunity, triggering T cells activation and differentiation. Through the pattern recognition receptors (PRR), immature DCs can recognise a variety of antigens which induce DCs maturation and proliferation (Wculek et al. 2020). Moreover, antigens can be captured and processed by DCs and present them to naïve T cells (as peptides bound on the MHC), which stimulates T cell response (Wang et al. 2020; Wculek et al. 2020).

Developmental defects in systemic DCs result in decreased numbers of mature DC and an increase of immature DC (Almand et al. 2000). Mature DCs present antigens to T cells, which initiates the adaptive immune response. In the peripheral circulation, the majority of DCs are immature. Immature DCs cannot express normal amounts of MHC complex and co-stimulatory molecules, such as CD86, on their surface. Consequently, they have poor capability to uptake antigen and trigger T cells activation, giving rise to the inhibition of antigen-specific immune responses (Groux et al. 2004). Immature DCs may establish immune tolerance through the up-regulation of regulatory T cells (Zhou et al. 2013). Moreover, immature DCs cannot generate some inflammatory cytokines, such as IL-6 and IL-12 (Yoo and Ha 2016). Furthermore, CD4<sup>+</sup> T cells from patients with HNSCC have low expression

of co-stimulatory signals OX40 and 4-1BB. CD8<sup>+</sup> T cells have higher production of the co-inhibitory receptor PD-1, which may significantly contribute to immune evasion (Baruah et al. 2012). This is because OX40 and 4-1BB are positive factors that prime T cell activation and PD-1 could limit T cell response (Baruah et al. 2012).

Compared with normal healthy cells, HNSCC cells do not constitutively express the human leukocyte antigen (HLA) class I molecules and lack antigen-processing machinery (APM) (Ferris 2015). HLA is one of the highly polymorphic genes located on human genome 6, encoding proteins on cell surface, which present processed antigen peptides to CD8<sup>+</sup> T lymphocytes. APM cooperates with HLA-I in processing and presenting antigenic peptides, and plays a central role in regulate immune response (Ferris et al. 2006). Normal function of HLA and APM is to mediate cellular and humoral immunity. The lack of expression of both HLA and APM result in the host immune system failing to recognise and eliminate the tumour cells (Ferris 2015; Ferris et al. 2006; Hickey et al. 2016).

### Lymphocytes in HNSCC

Another important strategy for the immune evasion of cancer cells is functional defects and apoptosis of T lymphocytes. Compared with healthy people, the level of mature CD4<sup>+</sup> and CD8<sup>+</sup> T cell subsets in circulation is greatly lower in the peripheral blood of HNSCC patients, which can lead to cancer recurrence or a secondary tumours (Kuss et al. 2004). Furthermore, T cell apoptosis may further reduce the number of T lymphocytes in HNSCC patients (Turksma et al. 2013). Immune dysfunctions of T cells have been reported to be associated with decreased expression of  $\zeta$  chain in tumour patients, a signalling molecule in T lymphocytes that correlates with the T cell receptor (TCR)-CD3 complex (Whiteside 2004).

Tumour immune evasion can also be achieved through the actions of Tregs. Tregs are CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup>, accounting for approximately 4% of all peripheral blood CD4<sup>+</sup> T cells in healthy people, playing a significant role in regulating immune system (Gasparoto, et al. xxxx). The participation of Tregs in immune responses is directed against cancer cells, pathogens and self-antigens. This may down-regulate NK cytotoxicity functions, DCs functions and cytotoxic T-lymphocyte (CTL) activity, leading to antitumour immune response (Ferris 2015). The percentage of Tregs in the peripheral circulation and cancer microenvironment is significantly higher in HNSCC patients compared with healthy humans (Schaefer et al. 2005). Nevertheless, it is still unclear that whether Tregs arise from tumour microenvironment or if they play a systemic immunity role (Elkord et al. 2010). Recent research has also shown that cancer-associated fibroblasts (CAFs) play a role in evasion from immune

surveillance by blocking T cell proliferation, and inducing apoptosis of T cells (Takahashi et al. 2015). CAFs are one of the most abundant cell types in the cancer stroma, and are involved in cancer progression and invasion (Wang 2015b). As a result, the cancer cells can evade the host immune system and grow progressively (Schreiber et al. 2011).

### Cytokines in HNSCC

HNSCC cells secrete cytokines and small molecular weight signalling molecules such as transforming growth factor- $\beta$  (TGF- $\beta$ ), prostaglandin E2 (PGE2), COX-2, VEGF, interleukin (IL)-6 and interleukin (IL)-10, all of which are closely correlated with immunosuppression (Ferris 2015).

Overexpression of immunosuppressive cytokines such as TGF- $\beta$  and IL-10 by tumour-associated macrophages (TAMs) systemically, which are commonly found in OSCC patients, significantly contributes to local immunosuppression and tumour development (Costa et al. 2013). TGF- $\beta$  may inhibit the function of the CTLs and T-helper type 1 (Th1) cells, as well as blocking the migration of DCs to lymph nodes (Weber et al. 2005; Zamarron and Chen 2011; Moutsopoulos et al. 2008). In addition, TGF- $\beta$  may affect the differentiation and maturation of B cells (Lebman and Edmiston 1999). TGF- $\beta$  can increase the production of IL-10, which has been suggested to inhibit DCs development and MHC class I expression, affecting T cell activation (Lippitz 2013). VEGF inhibit the maturation of DCs in the tumour microenvironment, leading to immune dysfunction (Johnson et al. 2007). High levels of PGE2 can be found in many epithelial cancers, which is induced by COX-2 (Whiteside 2014). PGE2 plays a critical role in tumour evasion, inhibiting the proliferation of T cells and B cells, and the cytotoxic function of NK cells (Camacho et al. 2008). IL-6 is a pleiotropic cytokine and generated by several cells such as macrophages, B and T cells and cancer cells. High expression of IL-6 has been closely related to cancer metastasis and worse prognosis (Jinno et al. 2015). The differentiation and maturation of DCs are blocked by IL-6 via STAT3 activation, which can result in immunosuppression (Park, et al. 1950).

High levels of programmed death 1 (PD-1): PD ligand 1(PD-L1) expression can be found in HPV-infected HNSCC patients, which plays a role in immune resistance (Zandberg and Strome 2014). PD-1 is an immune checkpoint receptor and a significant co-inhibitory receptor of the CD28 family. It is expressed on activated T and B cells, DCs, NK cells and monocytes, has a negative effect on activation of T lymphocytes (Lyford-Pike et al. 2013). PD-L1 can be found on the cell surface of cancer cells. The cooperation of PD-1 and its ligand PD-L1 is likely to suppress T cell activation and proliferation, leading to T cells dysfunction and an immunosuppressive microenvironment (Baruah et al. 2012; Zandberg

and Strome 2014; Lyford-Pike et al. 2013; Keir et al. 2008). Bu et al. showed that in HNSCC patients, STAT3 signaling induces immunosuppression through upregulating the expression of PD-1/PD-L1 (Bu et al. 2017). Recent studies have found that targeting PD-1/PD-L1 pathway may be an effective method in cancer immunotherapy (Constantinidou et al. 2019; Ju et al. 2020), especially when combined with other therapies (Qiao, et al. 2020). However, the efficacy of PD-1/PD-L1 monotherapy varies substantially depending on the tumour type and PD-L1 expression (Zhao et al. 2020).

## The Traditional Treatment of HNSCC

At present the standard treatment modalities for HNSCC patients include surgery, chemotherapy, and radiotherapy. Surgery and radiotherapy are considered as most common treatment methods for HNSCC patients (Argiris et al. 2008). In general, the treatment decision is not only determined by TNM stage, but also by the patient's health condition, tumour size, nutritional status, the availability of resources and prognosis following treatment. The goal of HNSCC treatment is to cure the cancer, acquire organ preservation, and improve patient quality of life (Rivera 2015; Yao et al. 2007).

### Surgery

For HNSCC patients, surgery is the primary and standard treatment (Swiecicki et al. 2016; Bessell, et al. xxxx). Nearly 30% of HNSCC patients are diagnosed at stages I or II, will be treated with surgery and/or radiotherapy, resulting in a 85% probability of survival of two years or longer (Gilbert et al. 2013). These treatments are, however, not effective for locally advanced cancer. Treatment for stages III and IV HNSCC usually use a combination of surgery, radiotherapy, and chemotherapy. Unfortunately, only 50% of stage III and IV HNSCC patients will survive for 2 years following treatment (Güneri and Epstein 2014). At present, open surgery and transoral resections are common surgical approaches used by doctors. Transoral resection patients recover quickly and the surgery is economical (Kwong et al. 2015). More recently, there is significant interest in transoral robotic surgery (TORS), which could improve visualization in the oropharyngeal and laryngeal cancer, can better retain speech and swallowing functions of patients. Nevertheless the long term effects is unclear and need to be determined (Kwong et al. 2015; Meulemans et al. 2015).

### Radiotherapy

In order to acquire organ preservation and reinforce anti-tumour effects radiotherapy is an important primary or

adjuvant approach for HNSCC patients, and is often combined with chemotherapeutic and /or targeted agents (Gilbert et al. 2013; Takahashi et al. 2014). For early stage of HNSCC, radiotherapy is often used as adjuvant treatment, with the intention to reduce side effects such as mucosal and bone necrosis (Kademani 2007). There have been some technical advances in radiotherapy during the last few decades, which may prolong the survival rate of patients and improve their quality of life (Gregoire et al. 2015). Intensity-modulated radiation therapy could substantially decrease the occurrence of grade 2–4 xerostomia, indicating this technique could be used as a standard treatment for HNSCC patients (Marta et al. 2014). Moreover, this therapy can also reduce the risk of swallowing dysfunction (Laan et al. 2013). However, this treatment has significant patient side effects such as acute and late toxicities and radio-resistance which still need to be addressed (Clement-Colmou et al. 2015). Recently, through preventing DNA repair, OBP-301 (a telomerase-specific oncolytic adenovirus) when combined with radiotherapy seems to prolong survival in HNSCC patients and overcome radio-resistance, providing a new strategy for these patients (Takahashi et al. 2014).

### Chemotherapy

Chemotherapy is classified as an adjuvant treatment, and is frequently used for patients with tumour distant metastases and local recurrence with a low efficacy (Pignon et al. 2009). Chemotherapy can be divided into induction, concomitant, or adjuvant chemotherapy, and the effect of concomitant chemotherapy was notably greater than induction and adjuvant chemotherapy. The benefit of chemotherapy decreases with age, as patients of 71 years and older did not receive any treatment benefit (Pignon et al. 2009; Pignon et al. 2007). Chemo-radiotherapy combined with induction chemotherapy can be used to support organ preservation and decrease the incidence of distant metastasis (Haddad et al. 2013). Research found neoadjuvant chemotherapy can reduce tumour size in HNSCC patients with locally unresectable cancer. Further research, including clinical trials, are required to fully investigate these treatment modalities (Vishak et al. 2015).

Despite improvements in these treatments the prognosis for HNSCC patients is still poor, and the available treatments often have serious side effects. Surgery removal of cancer from patients fails to achieve organ preservation, preventing cancer spread and recurrence, and can be disfiguring. The treatment of both surgery and radiotherapy might impair the patient's ability to speak, eat, and other regional functions (Furness, et al. xxxx). Significant swallowing dysfunction is common in patients after intensive radiotherapy and chemotherapy (Eisbruch et al. 2002). The poor prognosis of HNSCC patients is due to limited treatment modalities.

In recent years the knowledge concerning HNSCC biology and the role of immune escape has expanded significantly (Huang et al. 2021; Tinhofer et al. 2020). As a result, the development of novel and promising therapeutic immune approaches is crucial for HNSCC patients.

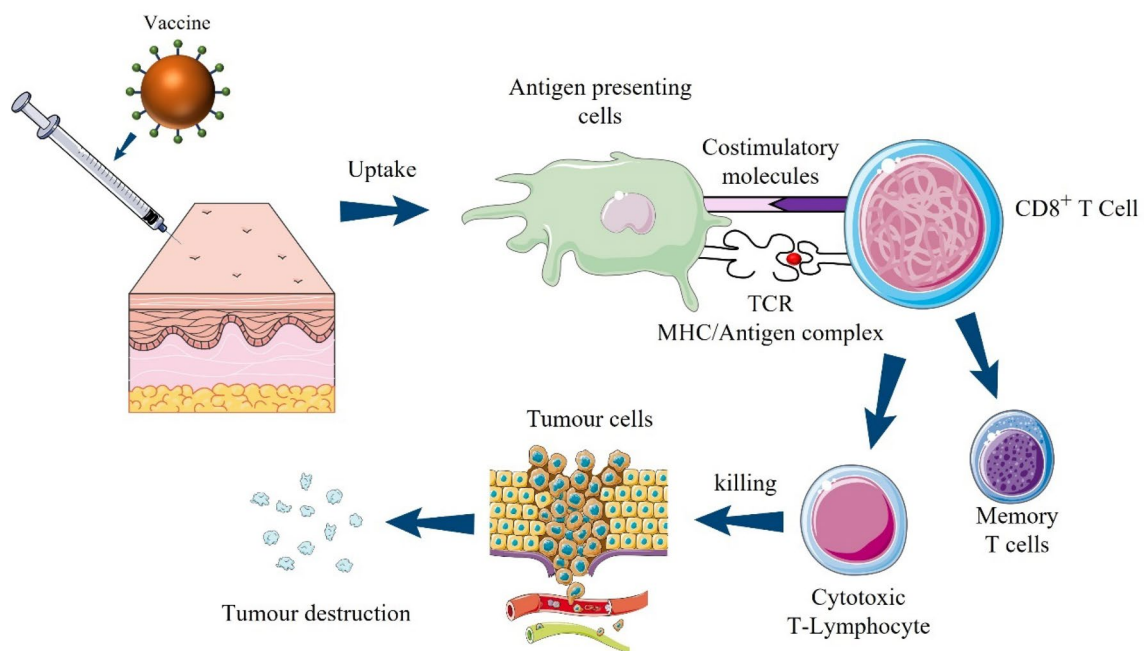
## Therapeutic Cancer Peptide Vaccines

The development of vaccines is one of the most promising and cost-effective way to protect people from infectious diseases and cancers, leading to eradication of diseases such as smallpox (Purcell et al. 2007). There are two kinds of cancer vaccines: prophylactic (disease preventative) and therapeutic (disease treatment). The former could induce humoral immune response and protect people from tumour development, while the latter (Fig. 2) attempts to treat an existing tumour by eliciting a potent cell-mediated immune response against tumour associated-antigens (TAA), and consequently reversing immune evasion (Wierzbicka et al. 2014).

A therapeutic cancer vaccine has been suggested as a promising strategy to improve outcome by eliciting a potent adaptive immune response, giving rise to the eradication of cancer cells (Wierzbicka et al. 2014). The aim of therapeutic vaccine development is to use the patient's own immune system to recognize and kill tumour cells (Wierzbicka et al. 2014). Compared with conventional treatment strategy, these

approaches have several advantages. They can specifically target cancer cells, with minimal harm to normal cells. Additionally, they could stimulate systemic anti-tumour immunity. A long-lasting memory immune response could be induced, which provides continuing protection from tumour recurrences (Krishnamachari et al. 2011).

Current research includes virus-modified tumour vaccines, DC-based vaccines, DNA vaccines, protein vaccines, peptide-based vaccines and combined strategies (Shibata et al. 2021). All of these demonstrate encouraging outcomes for immunotherapies. Peptide-based vaccines are one of the most common strategies for cancer vaccination (Shibata et al. 2021; Bezu 2018). These vaccines generally deliver the MHC class I restricted peptide epitopes, which come from shared TAAs, with the intention to activate cancer specific CD8<sup>+</sup> T cells (Calvo Tardón et al. 2019; Stephens et al. 2021). Compared with classic vaccines, there are several advantages of peptide vaccines (Skwarczynski and Toth 2016). Peptides do not have cytotoxicity to mammalian cells and are unlikely to induce allergic or autoimmune responses (Li et al. 2017; Li et al. 2017). With the development of solid phase peptide synthesis (SPPS) and SPOT synthesis, peptides are relatively simple to produce and are cost-effective, allowing production to be scaled up as required (Gaglione et al. 2019). The purity of peptide can be assessed by techniques such as mass spectrometry, and peptide stability can be demonstrated by standard



**Fig. 2** Therapeutic cancer peptide vaccines. The aim of cancer vaccines is to stimulate the body's immune system to cure cancer and prevent them from spreading. After vaccine uptake, antigens will be captured and processed by antigen presenting cells. Then, antigen

will be delivered through TCR/MHC complex to CD8<sup>+</sup> T cells. CD8<sup>+</sup> T cells could differentiate into cytotoxic T cells, which are able to directly kill cancer cells. *TCR* T cell receptor, *MHC* major histocompatibility complex



physicochemical characterisation (Li et al. 2020; Li et al. 2021a). Additionally, peptides are usually water-soluble and could be freeze-dried, stable at room temperature, and easy for storage and distribution (Wanning et al. 2020). Peptides could be designed to target unique pathogen. They can also contain multiple epitopes to target the diverse phases of life cycle or different pathogens (Fries et al. 2021). Since whole proteins cannot be used in cancer vaccines as they are much the same endogenous human protein, which could lead to autoimmune diseases, peptides may be very useful to design cancer vaccines (Malonis et al. 2020). Further, compared with DNA vaccines, peptides vaccines do not have risk of genetic recombination. Lipids, carbohydrates and phosphate groups can be added to the peptide so as to improve stability and immunogenicity (Li et al. 2021b).

## Peptide Vaccines for HNSCC Patients

Currently, several therapeutic peptide vaccines are under investigation for HNSCC (Shibata et al. 2021; Domingos-Pereira et al. 2019; Mora Roman et al. 2019; Miyazaki et al. 2011). Table 1 summarises recent peptide vaccines for HNSCC patients. For example, Trojan peptide-based vaccines were designed for advanced HNSCC patients. They consisted of HLA-I and HLA-II restricted peptide epitopes (melanoma antigen E, MAGE-A3 and HPV-16 TAA, respectively) (Voskens et al. 2012). MAGE, a cancer testis (CT) antigen, is highly expressed in HNSCC cells. HPV is strongly associated with OPSCC. MAGE and HPV were used to induce both HLA-I and HLA-II restricted immune responses. Results from clinical trials suggest that this vaccine could induce measurable systemic immune responses (Voskens et al. 2012). This vaccine was also evaluated in a phase 1 clinical trial in advanced HNSCC patients. It was found to induce an antibody response and an antigen-specific T cell response in most of patients (Zandberg et al. 2015).

More recently, a multiple peptide vaccine in a phase 2 clinical trial was demonstrated to induce a CTL response and improve the prognosis of HNSCC patients. These peptides are from three CT antigens LY6K, CDCA1, and IMP3, which are overexpressed in most of HNSCC cells rather than normal tissues. Following vaccination, the number of CD8<sup>+</sup> T cells were increased in the peripheral circulation of HNSCC patients. This indicates that the frequency of CD8<sup>+</sup> T cells was related to the overall increased survival of patients. One potential disadvantage of a peptide vaccine is that they can target one epitope of the TAA. However, this research used three peptides which together could induce stronger CTL response than one peptide (Yoshitake et al. 2015; Schlom 2012).

Targeting mutated proteins *p53* has become a promising immunotherapy area in HNSCC patients (Albers et al. 2018).

Recently, some researchers designed DCs loaded *p53* peptides as novel anti-tumour vaccines and detected in a phase 1 clinical trial. These vaccines were shown to decrease regulatory T cells, a modest vaccine-specific immune response and inhibit cancer growth (Schuler et al. 2014). In addition to *p53*, *p16* could also act as a therapy target for HNSCC. *p16*<sup>INK4a</sup>, the cyclin-dependent kinase inhibitor, is highly expressed in HNSCC cells. Recent research investigated *P16*<sup>INK4a</sup> as a vaccine target antigen for HNSCC. *p16*-based peptide vaccines are safe to these patients and may prime cellular and humoral immune responses (Reuschenbach 2016). A clinical study also showed that vaccination with *p16*<sup>INK4a</sup> could be an attractive immunotherapy for HNSCC patients. It would need more further investigations about the clinical efficacy of *p16*-based peptide vaccine (Reuschenbach 2015).

While it is now well established that the high-risk HPV is highly associated with oral cancer, research showed that HPV 16 is the major contributor for this cancer (Yete et al. 2018). The oncoproteins E6 and E7 are encoded by HPV 16, and thus immunotherapy targeting E6 and E7 may offer an effective way to prevent and treat HPV-related diseases (Yang et al. 2016; Aggarwal et al. 2019). Domingos-Pereira et al. used a synthesised HPV E7-long-peptide (E7LP) therapeutic vaccine with an encapsulated TLR9 agonist CpG, combined with carboplatin/paclitaxel (C + P) chemotherapy to target HPV-associated malignancies in a mouse model of disease. It was reported that compared to any of the dual treatments (C + P + E7LP, C + P + intravaginal CpG or E7LP + intravaginal CpG), the vaccine significantly increased survival. It was also shown that the addition of CpG greatly led to increased E7-specific CD8 T cells (Domingos-Pereira et al. 2019). Ye et al. reported that, in a OSCC model, a HPV-16mE6Delta/mE7/TBhsp70Delta fusion-protein vaccine not only suppressed tumour, but also elicited tumour death and provided protection against OSCC (Ye et al. 2013). In a recent study Yang and co-workers synthesized HPV E7 long peptide therapeutic vaccine, without using an adjuvant, aimed to against HPV-associated malignancies. Results indicated that this vaccine could generate both a local and systemic potent CD8<sup>+</sup> T cell response and anti-tumour effects. In addition, results demonstrated that this vaccine is more effective in buccal mucosal cancers than subcutaneous tumours (Yang et al. 2016). The design of this vaccine has two advantages. First, this vaccine does not have adjuvant, simplifying the design of vaccine and reducing side effects that can come from adjuvant use. Another benefit is it uses long peptides rather than short peptides. The latter may be directly loaded onto any cells which have MHC I molecules, leading to interaction between MHC I complex and TCR without co-stimulatory signals, resulting in immune tolerance (Toes et al. 1996). HNSCC is usually less immunogenic than other cancer types. Tan and

**Table 1** Summary of peptide vaccines for HNSCC patients

Vaccine composition	Targeting antigens	Patients	Clinical/preclinical	Immune responses	References
Trojan vaccines, Melanoma antigen E (MAGE)-A3 or human papillomavirus (HPV)-16, furin-cleavable linkers and a "penetrin" peptide sequence	MAGE-A3 or HPV-16	Advanced HNSCC	Clinical	Induced measurable systemic immune responses, an antibody response and an antigen-specific T cell response in most of patients	Voskens et al. 2012; Zandberg et al. 2015)
A mixture of multiple peptides derived from LY6K, CDCA1, and IMP3 and incomplete Freund's adjuvant	Three cancer-testis (CT) antigens LY6K, CDCA1, and IMP3	Advanced HNSCC	Clinical	Induced a strong CTL response and improve the prognosis of HNSCC patients; the number of CD8 <sup>+</sup> T cells were increased	Yoshitake et al. 2015)
p53 peptides adjuvant dendritic cell (DC)	p53 peptides	Advanced HNSCC	Clinical	Decreased regulatory T cells, elicited a modest vaccine-specific immune response and inhibited cancer growth	Schuler et al. 2014)
p16 <sup>INK4a</sup> derived peptide P16_37-63 Montanide® ISA-51 VG	p16 peptides	Advanced HNSCC	Clinical	Primed cellular and humoral immune responses	Reuschenbach 2016)
HPV E7-long-peptide (E7LP) therapeutic vaccine with an encapsulated TLR9 agonist CpG	HPV E7	Mouse	Preclinical	Significantly increased survival and greatly led to increased E7-specific CD8 T cells	Domingos-Pereira et al. 2019)
HPV-16mE6Delta/mE7/TBhsp-70Delta fusion-protein vaccine	HPV E6 and E7	Mouse	Preclinical	Suppressed tumour and elicited tumour death and provided protection against OSCC	Ye et al. 2013)
HPV E7 long peptide therapeutic vaccine	HPV E7	Mouse	Preclinical	Generated both a local and systemic potent CD8 <sup>+</sup> T cell response and anti-tumour effects	Yang et al. 2016)
Survivin-2B80-88 peptide	Survivin	Advanced oral cancer	Clinical	Induced an effective CTL response	Miyazaki et al. 2011)
MAGED4B peptide	MAGED4B	Newly diagnosed HNSCC	Clinical	The vaccine increased T-cell cytotoxic efficacy	Lim et al. 2014)
A dual-antigenic peptide vaccination (PV1) made composed of MAGED4B and FIX1 peptides	MAGED4B	Advanced HNSCC	Clinical	After PV1 vaccination, patients' T-cells were able to release cytotoxic cytokines	Chat et al. 2019)
Wilms' tumour 1 peptide, in combination with dendritic cell vaccination	Wilms' tumour 1 peptide	Advanced HNSCC	Clinical	After DC immunisation, five patients had long-term disease stability, while six others experienced disease progression. The median progression-free survival was 6.4 months, and the overall survival was 12.1 months, respectively	Ogasawara et al. 2019)
Placenta-specific 1 (PLAC1) peptides	PLAC1	Advanced HNSCC	Clinical	Induced potent T cell responses	Hayashi et al. 2021)

CTL cytotoxic T-lymphocyte, OSCC oral squamous cell carcinoma, HNSCC head and neck squamous cell carcinoma

co-workers found that MOC2-E6/E7 C57BL/6-syngeneic vaccine greatly increases antigen uptake and effectively prevents HNSCC immune escape (Tan et al. 2018). In addition, peptide vaccine combined with other immune therapies could provide a novel treatment option for HNSCC patients. For example, a combination of intranasal HPV peptide vaccination plus 4-1BB and CTLA-4 antibodies showed curative effectiveness and a superior safety profile against orally implanted mEER tumours (Dorta-Estremera et al. 2018). Immunotherapy Targeting HPV16/18 combined PD-1/PD-L1 inhibition may improve therapeutic outcomes in HNSCC (Aggarwal et al. 2019).

Some of these vaccines have previously been used in clinical trials. A survivin-derived peptide vaccine in phase 1 clinical trial has demonstrated the induction of an effective CTL response, and consequently has therapeutic potential for advanced or recurrent oral cancer patients (Miyazaki et al. 2011). Survivin is an inhibitor of apoptosis that reinforces cell cycle progression and enhances angiogenesis. While it is rarely detectable in normal tissues, overexpression of survivin can be found in approximately 80% of OSCC, and consequently, it is often used as a tumour antigen for oral cancer (Lo Muzio et al. 2003; Altieri 2003). Recently, the Kinesin family member 11 (KIF11) has been identified as a potential therapeutic target for oral cancer. KIF11 is a motor protein that essential to establish a bipolar spindle in cell division, and high expression of KIF11 has been closely associated with poor prognosis in oral cancer patients (Daigo et al. 2018).

MAGED4B is a melanoma antigen that promotes cell proliferation and migration that overexpresses in OSCC and high expression. Research into MAGED4B has led to the identification of immunogenic MAGED4B peptides for potential oral cancer vaccine targets. The results demonstrated that the vaccine increased T-cell cytotoxic efficacy against OSCC patients (Lim et al. 2014). The same research group later investigated the effectiveness of a dual-antigenic peptide vaccination (PV1) made composed of MAGED4B and FJX1 peptides in HNSCC patients. They showed that after PV1 vaccination, patients' T-cells were able to release cytotoxic cytokines. Additionally, patients with significant MAGED4B and FJX1 expression in their tumours were more sensitive to PV1 activation, indicating the PV1 peptide vaccine's specificity. PV1 might be a promising vaccination candidate for HNSCC patients and other malignancies that express these antigens (Chai et al. 2019).

Combination treatment provides a number of advantages, including the ability to use the synergy of various medicines to produce a satisfactory therapeutic outcome with lower medication dosages and fewer adverse effects (Karavasili et al. 2019). A clinical trial was performed with Wilms' tumour 1 peptide, in combination with DC vaccination and conventional chemotherapy, on metastatic or relapsed

HNSCC patients. The researchers observed that none of the patients experienced any serious side effects. After DC immunisation, five patients had long-term disease stability, while six others experienced disease progression. The median progression-free survival was 6.4 months, and the overall survival was 12.1 months, respectively. Therefore, this vaccine DC-based immunotherapy in combination with conventional chemotherapy appears to be safe and viable for individuals with advanced HNSCC (Ogasawara et al. 2019). In addition, patients with HPV + HNSCC may benefit from E7 immunisation coupled with surgery. Recently, a nanoparticle conjugated with E7 long-peptide and CpG in an orthotopic immunocompetent mouse model was tested. In the absence of surgery, immunisation given before or after tumour-cell injection only minimally reduced tumour development and extended survival. However, when NP-E7LP vaccination was administered before primary tumour excision, no postsurgical recurrence was detected. This indicates that the removal of the primary tumour altered the tumour microenvironment, allowing the vaccine-induced anti-tumour response to have a therapeutic impact. Patients with HPV + HNSCC may benefit from E7 immunisation combined surgery therapy (Domingos-Pereira et al. 2021). Karavasili and co-workers demonstrated that for HNSCC patients, peptide hydrogel combined with drugs (doxorubicin and curcumin) dramatically changed apoptotic/anti-apoptotic gene expression (Karavasili et al. 2019). Another new immunologic strategy to treating HNSCC patients might be a combination of *p53* peptides and chemotherapy. Ohara et al. found that *p53* can induce effective T responses, and chemotherapeutic drugs increased the responses of these CD4 T cells by phosphorylating *p53* (Ohara 2018).

Research is currently being conducted in order to identify immunotherapeutic targets for HNSCC. For example, placenta-specific 1 (PLAC1) is a cancer immunotherapy target that is expressed mainly in placental trophoblasts, but not in normal tissues. A recent study reported that PLAC1 is found in 74.5% of oropharyngeal tumours and 51.9% of oral cavity tumours, as well as many HNSCC cell lines. They also identified an HTL peptide epitope (PLAC131-50) that is able to induce potent T cell responses, suggesting PLAC1 might be a target antigen for HNSCC patients (Hayashi et al. 2021). Additionally, sperm protein (SP) 17, which is a CT antigen expressed in many types of cancers such as lung cancer and ovarian cancer. The SP17 protein was also found in HNSCC patients. This suggests that it is a promising immunotherapeutic target, as well as a possible disease biomarker for HNSCC (Schutt et al. 2017). These findings indicate that peptide-based vaccine is a promising treatment for advanced HNSCC.

Different peptide-based anti-cancer vaccines are now being tested in the clinical trials. Table 2 summarises clinical trials of peptide vaccines for HNSCC patients.

**Table 2** Clinical trials of peptide vaccines for HNSCC patients (clinicaltrials.gov)

Treatment	Study phase	Trial status	ClinicalTrials.gov identifier	Sponsor
Mutant <i>p53</i> peptide pulsed dendritic cell vaccine	Phase 1	Completed	NCT00404339	Robert Ferris
HPV therapeutic vaccine: PepCan (HPV-16 E6 peptides)	Phase 1 Phase 2	Recruiting	NCT03821272	University of Arkansas
Peptide vaccine (PANDA-VAC) administered concurrently with pembrolizumab	Phase 1	Not yet recruiting	NCT04266730	UNC Lineberger Comprehensive Cancer Center
Peptide vaccine consisting of arginase-1 (ARG1) peptides and Montanide ISA-51	Phase 1	Recruiting	NCT03689192	Herlev Hospital
CNGRC peptide-TNF alpha conjugate	Phase 1	Completed	NCT00098943	European Organisation for Research and Treatment of Cancer: EORTC
A amino acid peptide from indoleamine 2,3-dioxygenase	Phase 2	Recruiting	NCT04445064	Cliniques universitaires Saint-Luc-Université Catholique de Louvain
Combination of UCPVax vaccine and atezolizumab	Phase 2	Recruiting	NCT03946358	Centre Hospitalier Universitaire de Besancon
Human papillomavirus 16 E7 peptide	Phase 1	Completed	NCT00019110	National Cancer Institute (NCI)
S-488210/S-488211 (freeze-dried injectable formulation containing peptides)	Phase 1	Recruiting	NCT04316689	Shionogi
Ras peptide cancer vaccine containing DetoxPC adjuvant, interleukin-2 (IL-2), sargramostim (GM-CSF)	Phase 2	Completed	NCT00019331	National Cancer Institute (NCI)
Trojan peptides MAGE-A3 and HPV 16	Phase 1	Completed	NCT00257738	University of Maryland, Baltimore
<i>P16_37-63</i> peptide combined with Montanide® ISA-51 VG	Phase 1	Completed	NCT02526316	Oryx GmbH & Co. KG
Combination of pembrolizumab, HPV-16 E6/E7 and cisplatin-based chemoradiotherapy	Phase 2	Recruiting	NCT04369937	Robert Ferris
Personalized adjuvanted vaccine, GEN-009	Phase 1 Phase 2	Active, not recruiting	NCT03633110	Genocea Biosciences, Inc

HPV human papillomavirus, MAGE melanoma antigen E, TNF tumor necrosis factor

On ClinicalTrials.gov, 14 studies were discovered using the keywords “head and neck cancer” and “peptide.” As shown in Table 1, there are six completed trials, some of which have already been mentioned. Although there have been recent advances in the development of novel therapies including anti-tumour vaccines, these results to date have not yet been transferred into clinical use and thus have not increased the survival rate of OSCC patients. As a result, further investigations need to be made to establish an effective immunotherapy.

## Conclusions and Future Directions

In the past few years, there have been remarkable achievements in developing the peptide-based vaccine for HNSCC patients. However, most peptide-based anticancer vaccines researched so far are less effective, and significant challenges still need to be solved. Most of the clinical research is still at an early stage, mainly concentrating on safety,

availability, and immunogenicity (Whang et al. 2015). Although there have been recent advances in the development of novel therapies, including anti-tumour vaccines, these results to date have not yet been transferred into clinical use and thus have not increased the survival rate of HNSCC patients (Schneider et al. 2018). One of the main disadvantages of peptide vaccines is the lack of efficacy shown in clinical studies. For example, two phase 3 clinical trials (DERMA and MAGRIT) of MAGE-A3 immunotherapeutic with advanced melanoma (Dreno et al. 2018) and non-small-cell lung cancer, NSCLC (Vansteenkiste et al. 2016) patients failed. Vaccination with the MAGE-A3 immunotherapeutic elicited a strong immune response, resulting in substantial improvement in anti-MAGE-3 antibody concentrations. However, clinical effectiveness was not achieved. The reasons for the lack of clinical effectiveness might be connected to the antigen or immunostimulant used, or the failure to induce an antitumor immune response, especially cytotoxic T-lymphocyte responses (Dreno et al. 2018; Rosenberg and Restifo 2015). Another

reason might be immune evasion and suppression. Many immunosuppressive mechanisms and immunosuppressive cytokines may inhibit antitumour T-cell responses (Dreno et al. 2018). To solve these problems, the most promising way is combination therapy. Due to the complex tumour evasion routes, a combination of different strategies will be important for cancer immunotherapy. More clinical trials combined with other immune therapies will be needed to guide the next generation of HNSCC vaccine and will be valuable for future research (Aggarwal et al. 2019; Dorta-Estremera et al. 2018; Karavasili et al. 2019).

Peptide-based vaccines have poor immunogenicity, and they require a delivery system or/and adjuvant such as TCR ligands, co-stimulatory molecules, and cytokines to induce the desired immunity. The challenges of developing peptide vaccines are the selection of appropriate epitopes and adjuvants. Strong adjuvants are also critical to improving the efficacy of vaccines (Gouttefangeas and Rammensee 2018). Despite the side effects, adjuvants are needed to enhance the immune response (Leroux-Roels 2010). Another limitation of peptide vaccines is that they are MHC restricted. They must match the HLA in patients. In individuals with diverse MHC class I molecules, a specific peptide may not elicit robust cell-mediated immunity. It is difficult to design a peptide vaccine for all human population due to HLA polymorphisms. The development of long peptides that contain multiple antigenic epitopes could solve this problem (Chen et al. 2020; Slingluff 2011). However, many challenges still exist in the production of long peptides such as synthesis complexity, low yields and high costs (Isidro-Llobet et al. 2019). The future tendency in vaccination is the use of a minimal component from pathogens to induce potent and long-lasting immune responses (Skwarczynski and Toth 2014; Skwarczynski and Toth 2014; Liu et al. 2012). Additionally, personalized peptide-based vaccines are highly promising ways to achieve clinical success (Shibata et al. 2021). Future studies focusing on personalized peptide-based vaccines will be needed more. When these hurdles are addressed, peptide cancer vaccines are expected to become a strong tool for stimulating an immune response against cancer and a standard treatment in cancer immunotherapy.

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## Declarations

**Conflict of interest** There are no conflict of interest to declare.

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