

Review

Impact of HPV Infection on the Immune System in Oropharyngeal and Non-Oropharyngeal Squamous Cell Carcinoma: A Systematic Review

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Abstract: Objectives: To review the current knowledge regarding the involvement of human papilloma virus (HPV) infection and the immune system in the development of head and neck squamous cell carcinoma (HNSCC). Methods: An electronic literature search was conducted to identify articles published between 1990 and 2019 pertaining to tumor-infiltrating immune cells (TICs) in HNSCC using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Issues of clinical relevance, including tumor location, the number of tumor samples, the inclusion of additional specimens (dysplastic or normal mucosa), tumor size, methods used for HPV detection, relationship between antigen expression and patient characteristics (age, gender, smoking, alcohol consumption, etc.), and prognostic data (overall survival (OS) and recurrence-free survival (RFS)) were assessed by four blinded investigators. Results: The search identified 335 relevant studies, of which 41 met the inclusion criteria. Of these, 7 studies focused on the peripheral blood immune cell concentration in patients with HNSCC according to HPV status, and 36 studies investigated TICs in the intraepithelial and/or stromal compartment(s) according to HPV status. The immune cells studied were CD8+ T cells (N = 19), CD4+ T cells (N = 7), regulatory T cells (Tregs, N = 15), macrophages (N = 13), myeloid-derived suppressor cells (MDSCs, N = 4), and Langerhans cells (LCs, N = 2). Conclusions: Irrespective of tumor location, CD8+ and CD4+ T cells appear to play a key role in the development of HPV-related HNSCC, and their infiltration is likely associated with a significant impact on OS and RFS. To date, the roles and prognostic value of Tregs, macrophages, DCs and MDSCs remain unclear.

Keywords: HPV; cancer; head; neck; immune; cells

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in men and the eighth most common cancer in women, accounting for over 600,000 new cases per year worldwide [1,2].

The main risk factors for HNSCC are alcohol and tobacco consumption and persistent infection with human papillomavirus (HPV), which is associated with the rising incidence of oropharyngeal cancer in the United States and Europe [3,4]. HPV infection is predominantly attributable to subtypes HPV-16 and HPV-18, but geographical heterogeneity has been reported between continents [5]. Nondrinking and nonsmoking patients with HPV-induced HNSCC often present with advanced cancer, but they have a better prognosis than patients with HNSCC associated with smoking and drinking [6–11]. The better prognosis of patients with HPV-induced HNSCC could be due to the interactions between HPV antigens and the host-immune system and a better immune response against these tumors. In this respect, many studies have demonstrated significant differences in the composition of tumor-infiltrating immune cells (TICs) in HPV-induced and non-HPV-induced HNSCC over the past two decades [12,13]. Moreover, the results of some of these studies indicate that the overrepresentation of some immune cells should be considered a significant prognostic factor for HNSCC patients [14,15]. However, to date, there has been no systematic review summarizing the current knowledge of TICs and the tumor-host interaction in HPV-induced HNSCC.

The aim of this systematic review is to discuss the current knowledge of the involvement of HPV infection and the immune system in the development of HNSCC.

2. Materials and Methods

The criteria for considering studies for the systematic review were based on the population, intervention, comparison, and outcome (PICO) framework [16].

Types of studies: Prospective or retrospective clinical trials published in peer-reviewed journals were included in this review. Studies were included if they explored TICs in HNSCC, including oropharyngeal, laryngeal, hypopharyngeal and oral squamous cell carcinoma. We included studies published in English and French.

Participants and inclusion/exclusion criteria: Papers were included in the analysis if they clearly reported TICs in excised HNSCC samples or pretherapeutic biopsies from patients who were treated with conventional treatment (i.e., surgery, chemoradiation, chemotherapy, or immunotherapy). Papers that examined the recruitment of immune cells through blood analyses were also included to improve the understanding of the tumor immune microenvironment. HPV detection was required to have been performed through DNA or p16 analysis of the tumor samples. Studies focused on non-HNSCC were excluded.

Outcomes: The first outcome was the study of TICs in the intraepithelial (or intratumoral) and stromal compartments in HNSCC according to HPV status. To better understand the involvement of the immune system in the development of HPV-induced HNSCC, investigations into the expression of both cytokines and checkpoint proteins involved in tumor development were performed. Table 1 summarizes the immune cells included in this systematic review [17–25]. The second outcome was the study of tumor characteristics (tumor size, node, metastasis, and histopathology) and/or prognostic data (overall survival (OS) and recurrence-free survival (RFS)) in relation to TICs and HPV status.

Intervention and comparison: In the case of the study of the prognostic value of TIC, the authors were required to have treated their patients with conventional surgical or conservative treatments.

2.1. Search Strategy

Four authors (JRL, IS, TV, and QM) conducted searches in PubMed, Cochrane, and Scopus databases to identify articles published between January 1990 and June 2019 pertaining to the relationship between HPV infection and TICs in the development of HNSCC. Clinical studies were screened if they had database abstracts, available full texts or titles that referred to these conditions. The following keywords were used: 'HPV', 'cancer', 'carcinoma', 'head', 'neck', 'immune', and 'cell'. Final article selection was determined by the four authors, who provided a critical analysis of the publication content. The review was conducted according to the PRISMA checklist [26]. Institutional review board approval was not required.

Table 1. Immune cell roles.

Cells	Cell Marker	Theoretical Roles in Head & Neck Squamous Cell Carcinoma	Findings
CD8± T cells [9,13,18,23]	CD8	<ol style="list-style-type: none"> 1. Detection of neoplastic cells and cytotoxic effect through MHC class I binding. 2. Recruitment of Treg lymphocytes. 3. Upregulation of PD-L1 on tumor cells through an interferon (IFN)-γ-dependent Manner. 	<p>Number of CD8+ T cells increases throughout tumor progression.</p> <p>The infiltration CD8+ T cells is usually associated with a better host response to the tumor.</p> <p>Higher CD8+ T cell infiltration is associated with better OS.</p>
CD4± T cells [13,18,23]	CD4	<p>The role of CD4+ helper T cells is unclear because a wide range of CD4+ cell subsets with different functions exists.</p> <ol style="list-style-type: none"> 1. Th-1 lymphocytes may activate cytotoxic lymphocytes. 2. Th-2 lymphocytes stimulate humoral immunity and activate eosinophils. 3. Th-17 lymphocytes may have a Th-1 phenotype in the tumor microenvironment. 	<p>The high infiltration of CD4+ T cells is associated with controversial results.</p> <p>Some authors reported that in HPV–negative patient cohorts, a high level of CD4+ TILs was associated with better OS and DFS, while contradictory results were found for HPV+ patients.</p> <p>The role of Th-17 in HNSCC is still controversial.</p>
Regulatory T cells [13,15,18,23]	Foxp3	<ol style="list-style-type: none"> 1. Maintenance of the immunological tolerance to host tissues (suppressors of the anti-tumor immune response.) 2. Suppression of cytotoxic lymphocytes & other cells (MDSCs, etc.). 	<p>Tregs may promote tumor progression.</p> <p>The involvement of Tregs in HNSCC is controversial: some studies showed that an increased level of Tregs is linked with a worse prognosis.</p> <p>Others reported that high Treg counts are associated with a better prognosis.</p> <p>Immune escape is achieved by producing IL-10 and TGF-β and by consuming IL-2.</p>
Dendritic cells LC & MDSC [13,18–22,25]	<p>Immature: CD1a</p> <p>Immature: S-100</p> <p>Mature: CD83</p> <p>LC: CD207</p>	<ol style="list-style-type: none"> 1. Presentation of tumor-associated antigens to the immune system (MHC class I & II) & stimulation of T cell differentiation. 2. Immunosuppressive agents such as IL-10 and TGF-β convert immature DCs into tolerogenic DCs, inducing antigen-specific T cell tolerance through the activation of Tregs, the silencing of differentiated antigen-specific T cell tolerance and the differentiation of naïve CD4+ T cells into Tregs. 	<p>DC infiltration increases throughout tumor progression.</p> <p>The role of LC in HNSCC is controversial: some studies showed that DC infiltration decreases in HNSCC, while others observed that it was higher in fibrotic oral submucosal areas of HNSCC.</p>

Table 1. Cont.

Cells	Cell Marker	Theoretical Roles in Head & Neck Squamous Cell Carcinoma	Findings
Macrophages [13,14,18,23]	CD68 M2: CD68/CD163	<p>Presentation of tumor-associated antigens to immune system and the regulation of inflammation.</p> <p>M1: Activation of cytotoxic CD8+ T cells and Naïve CD4+ T cell differentiation into Th1 effector cells (antitumor effects).</p> <p>M2: Stimulation of Treg differentiation; the secretion of some factors (TGF-β, TNF-α, IL-10) creates a favorable environment for tumor growth and immunosuppression promotion.</p>	<p>Macrophage infiltration increases throughout tumor progression.</p> <p>Invasion, intratumoral microvessel density & angiogenic factors (VEGF) were positively associated with the level of macrophage infiltration.</p> <p>Macrophages facilitate tumor matrix generation & angiogenesis via elimination of extravascular fibrin deposits. They also stimulate HIF-1α expression, making the tumor more invasive and aggressive.</p>
Natural killers [17,18]	CD56	<ol style="list-style-type: none"> 1. Cytotoxic T lymphocyte activation 2. Direct anti-tumoral activity 	<p>NK cells are a cell therapy product capable of mediating direct and antibody-dependent cell-mediated cytotoxicity. They may directly kill HPV- & HPV+ HNSCCs. The low numbers of tumor-infiltrating CD56+ NK cells is correlated with significantly decreased OS, distant metastases-free survival and local progression-free survival (Lu).</p>

Abbreviations: DFS = disease free survival; IFN = interferon; IL = interleukin; HIF = hypoxia inducible factor; HNSCCs = head neck squamous cell carcinomas; LC = Langerhans cell; M1-2 = macrophage type 1-2; MDSCs = myeloid-derived suppressor cells; MHC = major histocompatibility complex; OS = overall survival; PD-L1 = Programmed death ligand 1; TGF = tumor growth factor; Th = T helper; TILs = tumor infiltrated lymphocytes; Treg = T regulatory cells; VEGF = vascular endothelial growth factor. This table was created based on several references.

2.2. Epidemiological Characteristics and Outcomes

An analysis of the locations of tumors, the number of tumor samples, the inclusion of additional specimens (i.e., dysplastic tissues or normal mucosa), tumor size, the relationship between antigen expression and patient characteristics (age, gender, smoking, and alcohol consumption), and prognostic data (OS and RFS) was performed. The method used for HPV detection was carefully analyzed to provide a methodologically critical analysis of the included studies.

3. Results

3.1. Study Characteristics

The initial screening identified 335 studies, of which 41 met our inclusion criteria (Figure 1). A total of 7 studies investigated the peripheral blood immune cell concentration in patients with HNSCC according to HPV status (Table 2) [27–33]. Three studies focused precisely on the evolution of the peripheral blood immune cell concentration between pre- and posttreatment (surgery or chemoradiation) [28,29,31].

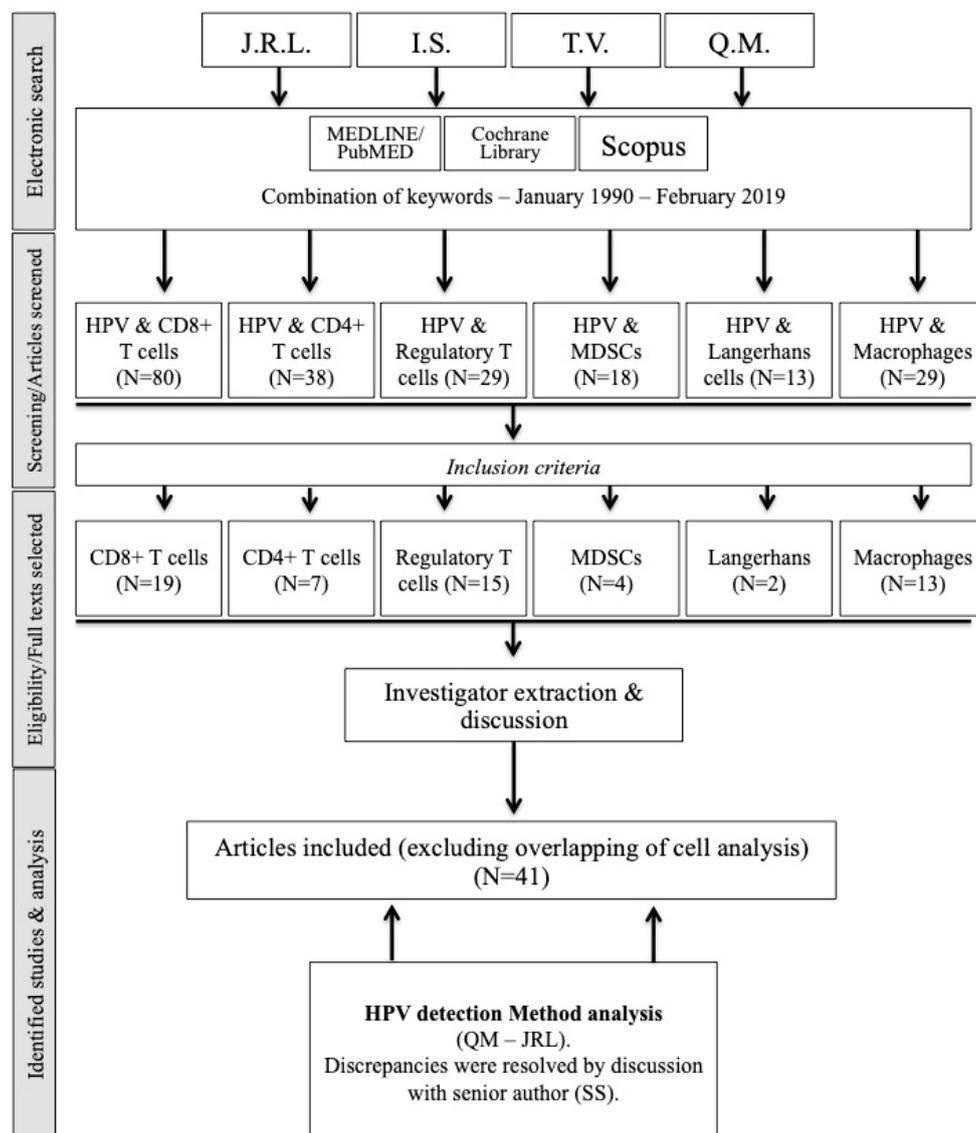


Figure 1. Flow chart. Abbreviations: HPV = human papilloma virus; MDSC = myeloid-derived suppressor cells.

Table 2. Peripheral blood immune cell concentration of patients with head and neck squamous cell carcinoma according to HPV status.

Authors	Specimens	Blood Cells & Antigens	Antigen Blood Expression	Findings
Heusinkveld 2012 [27]	Oropharynx, Oral,	Peripheral isolated blood monocytes		1. HPV16-specific T cell response comprised a broad repertoire of CD41 T-helper cells (type 1 and type 2), CD41 Tregs and CD81 T cells reactive to HPV16. 2. Stimulated CD4+ & CD8+ T cells in patients with HPV+ HNSCCs produce IFN- γ , IFN- α , IL-4, IL-5.
	Hypopharynx	T cells specific to HPV oncoproteins	N = 17/47	
	Carcinoma: 50	IFN- γ expression	N = 5/17	
	HPV+: 12-HPV-: 29	T cells specific to p53 protein IFN- γ expression Blood CD25 ^{hi} Foxp3 ^{hi} cells Cytokine production of isolated CD4+ & CD8+ T cells of HPV+ tumors	N = 7/45 N = 1/7 HPV+ = HPV- IFN- γ , IFN- α , IL-4, IL-5	
Al-Taei, 2013 [28]	Oropharynx & NT	Treg CD4+CD25 ^{hi} Foxp3+	C > NT; pre > posttreatment	1. Immunosuppression may contribute to the reduction in the HPV-specific T cell response posttreatment (i.e., surgery, chemoradiation, immunotherapy), indicating that HPV-targeted immunotherapy in oropharyngeal SCC patients posttreatment could require multiple strategies to boost T cell immunity and to overcome the influence of immunosuppressive cells.
	Carcinoma: 20	MDSC CD14-Hla- Dr-CD15hi	C > NT; pre > posttreatment	
	HPV+/p16+: 9	Memory CD3+ T Cells	Pre > posttreatment	
	HPV-/p16-: 2 HPV-/p16+: 1	Naïve T Cells, CD3+ T cells	Pre > posttreatment	
Parikh, 2014 [29]	Oropharynx	Circulating cells before CRT		1. CRT decreased circulating T cells and markedly elevated MDSCs. 2. PD-1 expression on CD4+ T cells increased after CRT as well as CD45RO+. 3. CRT suppressed circulating immune responses in patients with HPV+ tumors by unfavorably altering effector cells: suppression of immunocyte ratios and upregulation of PD-1 expression on CD4+ T cells were noted.
	Carcinoma: 22	HPV \pm : CD4+ T Cells; Treg	C = N	
	HPV+: 20-HPV-: 2	HPV \pm : CD8+ T Cells	N > C	
		HPV \pm : MDSC	C > N	
		Circulating cells after CRT HPV \pm : CD4+; CD8+ T Cells; & Treg HPV \pm : MDSC	Pre > post Post > pre	
		PD-1 expression after CRT PD-1 expression of CD4+ T Cells CD45RO+ CD4+ T cells	Post > pre Post > pre	
		PD-1 expression of Treg PD-L2 expression of AP cells	Pre = post Post > pre	

Table 2. Cont.

Authors	Specimens	Blood Cells & Antigens	Antigen Blood Expression	Findings
Lukesova 2014 [30]	Oropharynx & Oral Carcinoma: 60 <i>HPV+/p16+</i> : 27 <i>HPV+/p16-</i> : 3 <i>HPV-</i> : 30	CD3-CD56+CD16+ NK CD4+ & CD8+ T cells CD19+ cells & Tregs CD4+/CD8+ ratio Tregs elevated serum level	HPV+ > HPV- HPV+ = HPV- HPV+ = HPV- HPV+ = HPV- 37/60	1. Patients with HPV+ oral & oropharyngeal SCC have higher blood concentrations of CD3-CD56+CD16+ NK. 2. A higher Treg level & a lower CD8/Treg ratio influenced OS independently of HPV status and age. 3. Patients with HPV+ tumors had better OS and RFS than those with HPV- tumors. Among HPV+ patients, a high Treg blood concentration was associated with better OS and RFS.
Masterson 2016 [31]	Oropharynx Carcinoma: 51 <i>HPV+</i> : 41- <i>HPV-</i> : 10 <i>Normal</i> : 11	Pretreatment CD4+ against E2 Pretreatment CD8+ against E6 Pretreatment CD8+ against E7 Pretreatment CD4+CD25+ Tregs Posttreatment CD4+CD25+ Tregs	N: 8/30 N: 18/30 N: 21/30 HPV+ = HPV- HPV+: post > pretreatment	1. CD8+ T cells enhanced immunoreactivity to antigen E7 was linked to improved OS. 2. An increase in Treg level after treatment suggests that immunosuppression can contribute to a reduced HPV-specific cell-mediated response.
Ma, 2017 [32]	Oropharynx & Oral Normal/dysplasia: 30/30 Carcinoma: 196 <i>HPV+</i> : 47	HPV±: MDSC CD11b-CD33	C > D > N	1. HPV infection increases the level of blood MDSCs, promoting the recruitment of MDSCs into HNSCC areas. 2. The blood level of MDSCs is higher for cT3/T4 tumors (stages II and III) than for cT1-T2 tumors (stage I).

Table 2. Cont.

Authors	Specimens	Blood Cells & Antigens	Antigen Blood Expression	Findings
Lechner	Oropharynx, Oral,	CD45+CD3+ lymphocytes	TB > NM; TB = NB	1. The tumor microenvironment of HNSCC is characterized by strong Treg infiltration and elevated checkpoint molecule expression in T cell subsets. 2. These different cells are found in the circulation.
2017 [33]	Larynx, Hypopharynx, Sinus Carcinoma: 34 HPV-: 26-HPV+: 8 Normal: 7	CD45+CD4+ lymphocytes CD8+ T cells Naïve T cells (CD45RA+/CCR7+) Effector memory T cells Treg CD4+/CD25+/CD127low Treg CD4+/CD39+ Effector memory T cells CD45RA-/CCR7- Treg CD4+/CD25+/CD127low Checkpoint protein expression on T cells PD-1 T Cells CTLA-4+ Effector memory T cells CD45RA-/CCR7- Treg CD4+/CD39+ Treg CD4+/CD25+/CD127low	T > TB; NM > TB; TB = NB TB = T = NM = NB TB > T T > TB; NM > TB; TB = NB TB > NB; T > TB; T > NM; TB = NM TB = NB; T > TB; TB = NM HPV+ = HPV- HPV+ = HPV- T > TB; TB > NB; TB > NM TB > NB HPV+ = HPV- HPV+ = HPV- HPV+ = HPV-	

Abbreviations: C = carcinoma; CRT = chemoradiotherapy; CTLA = cytotoxic T-lymphocyte antigen; D = dysplasia; HNSCC = head neck squamous cell carcinoma; HPV = human papilloma virus; IFN = interferon; IL = interleukin; MDSC = myeloid-derived suppressor cell; N = number; NB = node blood; NK = natural killer; NM = normal mucosa; NT = normal tissue; OS = overall survival; PD-1 = programmed death-1; PD-L2 = programmed death ligand 2; RFS = recurrence-free survival; T = tumor (TNS status); TB = tumor blood; Treg = regulatory T cells.

A total of 36 studies examined TICs in the intraepithelial and/or stromal compartment(s) according to HPV status (Figure 1). Of the papers that investigated several different immune cell categories, some focused on oropharyngeal SCCs, whereas others focused on HNSCCs, including oropharyngeal and other anatomical localizations. The studies are shown in Tables 3–6 [12,15,24,30–64].

3.2. Oropharyngeal SCCs

A total of 10 and 5 studies addressed CD8+ and CD4+ T lymphocytes (Table 3) [34,36–38,40,41,43,45,47,51]. Seven studies focused on regulatory T cells (Tregs) (Table 4) [34,36,38,43,45,53,54], and 5 focused on macrophages (Table 5) [34,56,58–60]. No studies addressed myeloid-derived suppressor cells (MDSCs) or Langerhans cells (LCs).

3.3. HNSCCs

A total of 8 and 2 studies addressed CD8+ and CD4+ T lymphocytes (Table 3) [35,39,42,44,46,48–50,52]. Eight studies focused on regulatory T cells (Tregs) (Table 4) [12,15,24,33,35,39,42,49], 8 focused on macrophages (Table 5) [14,39,44,49,55,57,61,62], and 6 focused on myeloid-derived suppressor cells (MDSCs) or Langerhans cells (LCs) [32,39,44,57,63,64].

3.4. HPV and the Peripheral Blood Concentrations of Immune Cells

Because some immune cells are derived from bone marrow and are transported to tissues through the circulation, the peripheral blood number of immune cells (PBNI) was explored in 7 studies. Overall, it seems that patients with HNSCC have a higher PBNI than healthy individuals [28,29,32,33]. The role of HPV status is still unclear because only one study reported significant differences between the PBNI in patients with HPV+ and HPV– HNSCC [30]. Two authors found a high concentration of circulating CD8+ T cells against the E6 and E7 proteins in the majority of HPV+ patients with oropharyngeal SCC [29,31]. However, these observations were not supported by Heusinkveld et al., who included patients with oropharyngeal and non-oropharyngeal SCC [27]. According to Lukesova et al., natural killer cells are another type of cell that can be increased in patients with HPV+ oral or oropharyngeal SCC [30]. The other types of immune cells were less well studied, and it is difficult to pursue some lines of investigation.

According to two studies [28,29], the treatment type could have an impact on the PBNI, but the differences between these two studies (in terms of tumor location, types of treatment, and HPV detection methods) limited our comparison. The relationship between PBNI and OS was addressed in two studies [30,31]. Masterson et al. found that a high blood concentration of CD8+ T cells enhanced immunoreactivity to antigen E7, which was associated with improved OS in patients with oropharyngeal SCC [31]. In the same vein, Lukesova et al. demonstrated that patients with oral or oropharyngeal HPV+ SCC and a high Treg blood concentration had improved OS and RFS [30].

3.5. HPV and CD8+/CD4+ T Cell Tumor Infiltration

A total of 19 studies addressed CD8+ T cell infiltration (Table 3). Among the papers focusing on oropharyngeal SCC, the majority reported higher stromal or intraepithelial CD8+ T cell infiltration in HPV+ than in HPV– SCC [36–38,40,43,45–47,51]. Only Wansom et al. did not find significant differences between these two types [34]. These results are relatively similar to those of studies that investigated HNSCC. Russel et al. and Badoual et al. observed increased CD8+ T cell infiltration in the intraepithelial compartment in HPV+ HNSCC [35,39], whereas when investigating laryngeal, oral, hypo- and oropharyngeal tumor samples, Balermipas et al. did not find significant differences between HPV+ and HPV– SCC [42]. In a second study, the same authors reported the occurrence of different patterns of CD8+ T cell infiltration according to the tumor location; CD8+ T cell infiltration was higher in HPV+ than in HPV– tumor samples only in oropharyngeal SCC [46]. Other authors reported an increase in infiltration in HPV+ HNSCC irrespective of the compartment [44,48–50].

Table 3. CD8+ and CD4+ infiltration in HPV+ and HPV– Head & Neck Squamous Cell Carcinoma.

Authors	No. of Specimens	Tumor-Infiltrating Cells	Antigens/HPV	Findings
Wansom 2011 [34]	Oropharynx Carcinoma: 38 <i>HPV+:</i> 25- <i>HPV-:</i> 13	CD8+ T cells	HPV+ = HPV–	<ol style="list-style-type: none"> 1. Degree of CD8+ T cell infiltration did not differ by HPV status in oropharyngeal SCC. 2. High infiltration of CD8+ T cells was associated with better OS.
Badoual 2012 [35]	Oropharynx, Oral, Larynx, Hypopharynx Carcinoma: 64 <i>HPV+:</i> 32- <i>HPV-:</i> 32	Intratumoral CD8+ T cell infiltration Antigen expression (CD8+, CD4+ & Foxp3+) HLA-DR, CD38 & Tim-3	HPV+ > HPV– PD1+ > PD1- T cells; HPV+ = HPV–	<ol style="list-style-type: none"> 1. HPV+ HNSCC tumors had a higher number of infiltrating CD8+ T cells than did HPV– tumors. 2. PD-1+ T cells was positively correlated with a favorable clinical outcome.
Nasman 2012 [36]	Oropharynx Carcinoma: 83 <i>Gr1:</i> HPV+, <i>GP:</i> 31 <i>Gr2:</i> HPV+, <i>PP:</i> 21 <i>Gr3:</i> HPV–, <i>GP:</i> 11 <i>Gr4:</i> HPV–, <i>PP:</i> 20	CD8+ tumor infiltration	Gr1,3 > Gr2,4; HPV+ > HPV–	<ol style="list-style-type: none"> 1. HPV+ oropharyngeal SCC had a higher number of infiltrating CD8+ T cells than did HPV– SCC. 2. A high CD8+Foxp3+/tumor-infiltrating lymphocyte ratio was correlated with a better 3-year RFS. 3. A high infiltration of CD8+ in HPV+ SCC was associated with a better RFS than a low infiltration of CD8+ in HPV– SCC.
Jung 2012 [37]	Oropharynx Carcinoma: 17 <i>HPV+:</i> 10- <i>HPV-:</i> 7	CD8+ T cells (stroma) CD4+ T cells (stroma)	HPV+ > HPV–	<ol style="list-style-type: none"> 1. Stroma of HPV–positive tumors was frequently and strongly infiltrated by CD8+ and CD3+ T cells. 2. CD8+ infiltration improved OS and RFS. 3. CD3+ infiltration improved OS. 4. CD4+ staining is more important in the stroma of HPV+ SCCs.
Rittà 2013 [38]	Oropharynx Carcinoma: 22 <i>HPV+:</i> 10- <i>HPV-:</i> 12	CD3+; CD4+; CD8+ (intratumoral)	HPV+ > HPV–	<ol style="list-style-type: none"> 1. HPV16+ oropharyngeal SCC has a better prognosis than HPV– SCC. 2. No difference was found in the extent of tumor infiltration by CD25+, FoxP3+, CD3+, C68+, CD20+, CD8+, or CD4+ cells according to HPV status. 3. HPV16 load values equal or greater than 10-1 copies per cell were associated with higher densities of TIL CD3+ and Foxp3+ cells.

Table 3. Cont.

Authors	No. of Specimens	Tumor-Infiltrating Cells	Antigens/HPV	Findings
Russell 2013 [39]	Oropharynx, Oral, Sinus, Larynx, Hypopharynx Carcinoma: 34 HPV+: 9-HPV-: 26 Normal: 7	Intratumoral CD8+ T cells Stromal CD8+ T cells Expression of genes/antigens (qRT-PCR) IL-2, 4, 8, 12	HPV+ > HPV- HPV+ = HPV- C > N (IL-2: HPV+ > HPV-) Associated with upregulation of PDL1, B7H4, & FasL	1. HPV+ HNSCC exhibits a significantly increased infiltration of intratumoral CD8+ T cells compared with HPV- HNSCC. 2. qRT-PCR data demonstrated a general pattern of increased immune activation and suppression mechanisms in HPV+ samples.
Nordfors 2013 [40]	Oropharynx Carcinoma: 280 HPV+: 220-HPV-: 60	CD8+ T cells CD4+ T cells	HPV+ > HPV-	1. In HPV+ and HPV+p16a+ SCC, a higher number of CD8+ TILs was correlated with better OS and RFS. 2. In HPV- SCC, a higher number of CD8+ TILs was correlated with better OS. 3. The number of CD4+ TILs was not correlated with better OS.
Ward 2014 [41]	Oropharynx Carcinoma:270 HPV+:149-HPV-:121	CD8+ T cells CD4+T cells	HPV+(TILhigh) > HPV+(TILlow) HPV+(TILlow) = HPV-	1. HPV+ OPSCC exhibits higher levels of TILs than HPV- OPSCC. 2. HPV+ OPSCC with a high level of TILs has a better prognosis than HPV+ OPSCC with a low level of TILs. 3. HPV+ OPSCC with a low level of TILs has the same prognosis as HPV- OPSCC.
Balempas 2014 [42]	Oropharynx, Oral, Larynx, Hypopharynx Carcinoma:101	CD8+ T cells (intratumoral, stromal) CD4+ T cells (intratumoral, stromal) CD3+ (intratumoral, stromal)	HPV+ = HPV-	1. High CD3+ and CD8+ infiltration was associated with better OS, PFS, and distant metastasis -free survival. Infiltration of CD4+, CD8+ and CD3+ was similar in HPV+ & HPV- SCC. 2. CD4+ expression was not associated with a good prognosis.
Krupar 2014 [43]		Oropharynx Carcinoma: 33 HPV+: 16-HPV-: 17	CD3+ (intra and peritumoral) CD8+ T cells (intra and peritumoral) CD4+ T cells (intra and peritumoral) IL-17 (TH17 cells) (intra and peritumoral)	HPV+ > HPV- 1. In HPV+ tumors, we found significantly increased peritumoral infiltration of CD3+ T cells and trends toward higher peritumoral CD4+ and CD8+ T cell infiltration. 2. No differences between the two tumor types were observed in terms of Th17+ TILs. 3. The CD8/CD4 ratio was significantly higher in the intratumoral compartment of HPV+ tumors. 4. A significantly lower percentage of Th17+ T cells in the intratumoral compartment was noted in HPV+ cases.

Table 3. *Cont.*

Authors	No. of Specimens	Tumor-Infiltrating Cells	Antigens/HPV	Findings	
Partlova 2015 [44]		Oropharynx, Oral, Larynx, Hypopharynx, Submaxillary gland Carcinoma: 44 <i>HPV+</i> : 20- <i>HPV-</i> : 24	CD8+ T Cells; CD8+IFN-g+; % of CD8+ IFN-g+ among other T Cells CD8+IL-17+ T Cells % of CD8+IL-17+ T Cells among other T Cells In vitro cytokine production from unstimulated tumor-derived cell culture supernatants CCL-17; 21 CXCL9; 10; 12; IL-1b, 2, 17, 23; IFN-g In vitro cytokine production from stimulated tumor-derived cell culture supernatants IL-10, 17, 21; TNF-a; IFN-g mRNA expression in tumor samples Cox-2 PD-1 PD-L1 Tim-3	HPV+ > HPV- HPV+ > HPV- HPV+ > HPV- HPV+ > HPV- HPV+ = HPV- HPV+ > HPV- C > LN; C > NN; HPV- > HPV+ C = LN = NN; HPV+ > HPV- C > NN; HPV+ = HPV- C > LN > NN; HPV+ = HPV-	<ol style="list-style-type: none"> 1. HPV+ HNSCC showed significantly higher numbers of infiltrating IFN-g+ CD8+ & IL-17+ CD8+ T cells. 2. The infiltration of immune cells was associated with an increased secretion of proinflammatory cytokines. 3. The high level of CXCL12 was associated with a higher node status.
Oguejiofor 2015 [45]		Oropharynx Carcinoma: 218 <i>HPV+</i> : 139- <i>HPV-</i> : 79	Stromal & tumoral CD3+CD8+ T cells	HPV+ > HPV-	<ol style="list-style-type: none"> 1. HPV+ OPSCC has higher infiltration of CD3+CD8+ T cells in tumor & stromal areas than HPV- OPSCC. 2. CD3+CD8+ stromal infiltration was associated with increased OS.
Balermipas 2016 [46]		Oropharynx, Oral, Hypopharynx Carcinoma: 161 <i>HPV+</i> : 100 - <i>HPV-</i> : 61	Intratumoral & stromal CD8+ T cell infiltration	Oropharynx > hypopharynx & oral HPV+ > HPV- cT1-2 > cT3-4	<ol style="list-style-type: none"> 1. HPV+ HNSCC has higher infiltration of CD8 T cells than HPV- HNSCC. 2. OPSCC has higher CD8+ infiltration than non-oropharyngeal SCC. 3. High CD8+ infiltration was associated with better OS & RFS. 4. Low CD8+ infiltration was associated with a high risk of metastases.

Table 3. Cont.

Authors	No. of Specimens	Tumor-Infiltrating Cells	Antigens/HPV	Findings
Van Kempen 2016 [47]		Oropharynx Carcinoma: 262 HPV+: 44-HPV-: 218	Intratumoral CD8+	HPV+ > HPV- 1. HPV+ OPSCC have higher infiltration of CD8+ T cells than HPV- OPSCC.
Kim 2016 [48]	Oropharynx, Oral, Larynx, Hypopharynx Carcinoma: 402 HPV+: 198-HPV-: 204	CD8+ tumor oropharyngeal infiltration Checkpoint protein expression PD-1+ TILs & intratumoral LAG-3+ TILs	HPV+ > HPV - HPV+ > HPV -	1. CD8 T cell infiltration was higher in HPV+ OPSCC than in HPV- OPSCC. 2. High CD8+ infiltration was associated with better OS & RFS in HPV+ OPSCC.
Nguyen 2016 [49]	Oropharynx, Oral, Larynx, Hypopharynx Carcinoma: 278 HPV+:89-HPV-:218	CD8+ T cells (intratumoral) CD4+ T cells (intratumoral)	HPV+ > HPV-	1. Higher CD4 and CD8 TIL levels were associated with improved OS & RFS. 2. CD4 & CD8 infiltration was higher in HPV+ than in HPV- HNSCC. 3. Higher numbers of CD4 & CD8 T cells were observed in OPSCC than in HNSCC. 4. Lower numbers of CD4 & CD8 T cells were observed in HPV- OPSCC than in HPV- OPSCC.
Poropatich 2017 [50]	Oropharynx, Oral Carcinoma: 40 HPV+: 21-HPV-: 19	Intratumoral CD8+ T cells	HPV+p16+ > HPV-p16-	1. HPV+ oral & OPSCC tumors have higher infiltration of CD8 T cells than HPV- tumors. 2. High CD8+ infiltration was associated with better OS & RFS in HPV+ oral & OPSCC tumors. *
Sivars 2017 [51]	Oropharynx Carcinoma: 69 HPV+: 32-HPV-: 37	Intratumoral CD8+ T cells	HPV+DNA = HPV-DNA p16+ > p16- HPV+p16+ > HPV-DNA	1. CD8+ infiltration is higher in HPV+ tumors than in HPV- tumors. 2. High CD8+ infiltration is associated with better OS in HPV+ oropharyngeal SCC.
Kansy 2017 [52]	Not specified Carcinoma: 40 HPV+: 20-HPV-: 20 Normal: 4	Infiltration of CD8A+ Infiltration of CD8B+ PD-1 expression (qRT-PCR)	HPV+ > HPV- HPV+ > HPV- C > N; HPV+ > HPV-	1. CD8A+ & CD8B+ T cell infiltration is higher in HPV+ HNSCC than in HPV- HNSCC. 2. PD-1 expression is higher in CD8+ T cells of HPV+ HNSCC than in those of HPV- HNSCC.

Abbreviations: CCL-25 = Chemokine (C-C motif) ligand 25; C/N = carcinoma/normal tissue; CXCL = Chemokine (C-X-C motif) ligand; FasL = Fas ligand; GP - PP = good - poor prognostic; HLA-DR = Human Leukocyte Antigen - DR isotype; HPV = human papilloma virus; IFN = interferon; LAG-3 = Lymphocyte-activation gene 3 protein; LN/NM = lymph node/normal node; OPSCC = oropharyngeal SCC; OS = overall survival; IL = interleukin; PD-1 = programmed cell death 1; PD-L1 = Programmed death ligand 1; PFS = progression-free survival; qRT-PCR = Real-Time Quantitative Reverse Transcription PCR; RFS = recurrence-free survival; (HN)SCC = (Head & Neck) squamous cell carcinoma; TILs = tumor-infiltrating lymphocytes; Tim-3 = T cell immunoglobulin and mucin-domain containing-3; Th = T helper; TNF-a = Tumor Necrosis Factor alfa. * However, the expression of IL2, 4, 8, 12 was not associated with specific immune cells. These findings may or may not be associated with CD8+ T cell infiltration. * mRNA expression and cytokine production were not associated with specific immune cells. In other words, these findings may or may not be associated with CD8+ T cell infiltration. ** Statistical analyses were performed on oral & oropharyngeal tumor samples without distinction.

Table 4. Foxp3 T regulatory cell infiltration in HPV+ and HPV– head & neck squamous cell carcinoma.

Authors	No. of Specimens	Tumor-Infiltrating Cells	Antigens/HPV	Findings
Partlova 2015 [12]	Oropharynx, Oral, Larynx, Hypopharynx, Submaxillary gland Carcinoma: 44 HPV+: 20-HPV–: 24	Treg In vitro cytokine production from tumor unstimulated derived cell culture supernatants CCL-17; 21 CXCL9; 10; 12; IL-1b, 2, 17, 23; IFN-g In vitro cytokine production from tumor stimulated derived cell culture supernatants IL-10, 17, 21; TNF-a; IFN-g mRNA expression level in tumor samples Cox-2 PD-1 PD-L1 Tim-3	HPV– > HPV+ HPV+ > HPV– HPV+ = HPV– HPV+ > HPV– C > LN; C > NN; HPV– > HPV+ C = LN = NN; HPV+ > HPV– C > NN; HPV+ = HPV– C > LN > NN; HPV+ = HPV–	1. HPV+ HNSCC showed a slightly lower proportion of Tregs than HPV– HNSCC. 2. The infiltration of immune cells (i.e., Treg) is associated with increased proinflammatory cytokine secretion. 3. The high level of CXCL-12 was associated with a higher node status *
Punt 2016 [54]	Oropharynx Carcinoma: 162 HPV–: 99-HPV+: 63	CD3+Foxp3+ Treg	HPV+ > HPV–	1. Foxp3 Treg infiltration is higher in HPV+ OPSCC than in HPV– OPSCC. 2. A high number of infiltrated T cells was associated with improved DFS in the HPV+ OPSCC group.
Nguyen 2016 [49]	Oropharynx, Oral, Larynx, Hypopharynx Carcinoma: 278 HPV+:89-HPV–:218	Foxp3+ T cells (intratumoral)	HPV+ > HPV–	1. The Foxp3 intratumoral infiltration is higher in HPV+ than in HPV– HNSCC. 2. Foxp3 infiltration is higher in OPSCC than in other SCC types.
Kindt 2017 [24]	Oropharynx, Oral, Larynx, Hypopharynx Carcinoma: 110 HPV negative: 82 HPV+/p16–: 12 HPV+/p16+: 6 N/LD/SD: 10/43/45	Stromal infiltration of Foxp3+ Tregs Intraepithelial infiltration of Foxp3+ Tregs	C > SD > LD > N HPV+/p16+ = HPV+/p16– & HPV– C>N; SD, LD > N -HPV+/p16+ > HPV+/p16– & HPV–	1. Foxp3+ T cell infiltration increased with tumor progression, with the increase being more important in HPV+ patients. 2. A high Foxp3+ T cell number in the stromal compartment is associated with longer recurrence-free survival and overall survival. 3. Foxp3+ T cell number improved the prognostic value of tumor stage. 4. Stromal Foxp3+ T cell number is a strong prognostic factor independent of other risk factors i.e., tobacco, alcohol, HPV status.

Table 4. *Cont.*

Authors	No. of Specimens	Tumor-Infiltrating Cells	Antigens/HPV	Findings
Lechner 2017 [33]	Oropharynx, Oral, Sinus, Larynx, Hypopharynx, Carcinoma: 34 HPV-: 26-HPV+: 8 Normal: 7	CD4+/CD39+ Tregs CD4+/CD25+/CD127low Tregs Checkpoint protein expression on T cells PD-1 T cells CTLA-4+ CD4+/CD39+ Tregs CD4+/CD25+/CD127low Tregs	T = NM;T>TB; TB = NM HPV+ = HPV- T>TB; TB>NM T = NM HPV+ = HPV - HPV+ = HPV-	1. HNSCC is characterized by a strong infiltration of Tregs irrespective to the HPV status. 2. HNSCC is characterized by high checkpoint molecule expression in T cell subsets. These different cells are found in the circulation.
Seminerio 2019 [15]	Oropharynx, Oral, Larynx, Hypopharynx Carcinoma: 205 HPV+: 35-HPV-: 127	Foxp3+ Tregs	HPV+ = HPV -	1. No difference was found between HPV+ & HPV- SCC in terms of the infiltration of Foxp3+ Tregs. 2. A high infiltration of Foxp3+ Tregs in intratumoral & stromal compartments was associated with longer RFS & OS. 3. FoxP3+ Treg stromal infiltration, tumor stage and histological grade independently influenced patient prognosis.

Abbreviations: CCL-25 = Chemokine (C-C motif) ligand 25; C/N/LD/SD = carcinoma/normal tissue/low dysplasia/severe dysplasia; CXCL = Chemokine (C-X-C motif) ligand; FasL = Fas ligand; GP - PP = good - poor prognostic; HLA-DR = Human Leukocyte Antigen – DR isotype; HPV = human papilloma virus; IFN = interferon; LAG-3 = Lymphocyte-activation gene 3 protein; LN/NM = lymph node/normal node; OPSCC = oropharyngeal SCC; OS = overall survival; IL = interleukin; PD-1 = programmed cell death 1; PD-L1 = Programmed death ligand 1; PFS = progression-free survival; qRT-PCR = Real-Time Quantitative Reverse Transcription PCR; RFS = recurrence-free survival; (HN)SCC = (Head & Neck) squamous cell carcinoma; TIL = tumor-infiltrating lymphocytes; Tim-3 = T cell immunoglobulin and mucin-domain containing-3; Th = T helper; TNF-a = Tumor Necrosis Factor alfa. * mRNA expression and cytokine production were not associated with specific immune cells. In other words, these results may or may not be associated with Treg infiltration.

Table 5. Macrophage infiltration in HNSCC according to HPV status.

Authors	No. of Specimens	Tumor-Infiltrating Cells	Antigens/HPV	Findings
Ritta 2009 [55]	Oropharynx, Oral, Larynx Carcinoma: 59 HPV+: 27-HPV-: 32	CD68+ macrophages	HPV+ = HPV-	1. HPV+ HNSCCs do not have higher CD68+ macrophage infiltration than HPV- HNSCC. 2. A direct correlation between macrophage infiltration and tumor proliferation index was observed irrespective of the tumor subset.
Wansom 2011 [34]	Oropharynx Carcinoma: 38 HPV+:25-HPV-:13	CD68+ macrophages	HPV+ = HPV-	Degree of CD68+ macrophage infiltration does not differ by HPV status in OPSCC.

Table 5. Cont.

Authors	No. of Specimens	Tumor-Infiltrating Cells	Antigens/HPV	Findings
Lyfor-Pike 2013 [56]	Oropharynx Carcinoma: 27 HPV+: 20-HPV-:7	CD68+PD1+	C > N; HPV+ > HPV-	The tumoral infiltration of CD68+ macrophages is higher in HPV+ OPSCC than in HPV- OPSCC. CD68+ macrophages express high levels of PD-1.
Russell 2013 [39]	Oropharynx, Oral, Sinus, Larynx, Hypopharynx, Carcinoma: 34 HPV+: 9-HPV-: 26	Intratumoral CD68+ macrophages Stromal CD68+ macrophages	HPV+ = HPV- HPV+ = HPV-	HPV+ HNSCC are not characterized by higher CD68+ macrophage infiltration than HPV- HNSCC samples.
Partlova 2015 [44]	Oropharynx, Oral, Larynx, Hypopharynx, Submaxillary gland Carcinoma: 44 HPV+: 20-HPV-: 24	Monocytes & macrophages	HPV+ = HPV-	HPV+ tumor samples are not characterized by higher monocyte & macrophage infiltration than HPV- tumor samples.
Yu 2015 [57]	Oral Carcinoma: 86 HPV+: 12-HPV-: 74 Normal/dysplasia: 32/12	Stromal macrophages CD68+/CD163+	C > N; HPV+ = HPV-	The CD68+/CD163+ macrophage population increased in both HPV+ and HPV- HNSCC.
Lee 2015 [58]	Oropharynx Carcinoma: 53 HPV+: 39-HPV-: 14	High OS & DFS	High CD68+ > low CD68+ macrophages	1. A high infiltration of CD68+ macrophages is associated with low OS & DFS. 2. The number of CD68+ macrophages could be determining factors for CRT outcomes in patients with HPV+ OPSCC.
Nguyen 2016 [49]	Oropharynx, Oral, Larynx, Hypopharynx Carcinoma: 278 HPV+:89-HPV-:218	CD68+ (intratumoral)	HPV+ > HPV-	1. HPV+ HNSCC has a higher CD68+ macrophage infiltration than HPV- HNSCCs. 2. CD68+ macrophage infiltration was higher in OPSCCs than in other SCCs.
Seminerio 2017 [14]	Oropharynx, Oral, Larynx, Hypopharynx	Stromal CD68+ macrophages	C > SD > LD > N HPV+/p16+ =	1. CD68+ macrophage numbers increased during HNSCC progression in intraepithelial & stromal compartments. 2. A higher density of CD68+ macrophages was observed

Table 5. Cont.

Authors	No. of Specimens	Tumor-Infiltrating Cells	Antigens/HPV	Findings
	Carcinoma: 110 <i>HPV</i> -: 82 <i>HPV</i> +/ <i>p16</i> -: 12 <i>HPV</i> +/ <i>p16</i> +: 6 N/LD/SD: 10/43/45	Intratumoral CD68+ macrophages	<i>HPV</i> +/ <i>p16</i> - & <i>HPV</i> - C > SD > LD > N <i>HPV</i> +/ <i>p16</i> + > <i>HPV</i> +/ <i>p16</i> - & <i>HPV</i> -	in advanced stages. 3. Patients with <i>HPV</i> +/ <i>p16</i> + HNSCC had a higher CD68+ macrophage density than those with <i>HPV</i> - HNSCC. 4. High CD68+ macrophage numbers in the intratumoral compartment were associated with shorter patient OS. 5. CD68+ macrophage infiltration was a strong & independent prognostic factor of HNSCC.
Oguejiofor 2017 [59]	Oropharynx Carcinoma: 124 <i>HPV</i> +: 75- <i>HPV</i> -: 49	Intratumoral CD68+ Stromal CD68+ CD68+ PD-L1+ stromal density	<i>HPV</i> + > <i>HPV</i> - <i>HPV</i> + = <i>HPV</i> - <i>HPV</i> - > <i>HPV</i> +	1. <i>HPV</i> + OPSCC have higher CD68+ macrophage infiltration in intratumoral but not in stromal compartments than <i>HPV</i> - OPSCC. 2. High stromal infiltration of CD68+PD-L1+ is associated with better OS in patients with <i>HPV</i> - tumors.
Welters 2017 [60]	Oropharynx Carcinoma: 97 <i>HPV</i> +: 57- <i>HPV</i> -: 40	DC-like macrophages	<i>HPV</i> + > <i>HPV</i> -	1. DC-like macrophage infiltration was higher in <i>HPV</i> + OPSCC, which was correlated with better OS and a low risk of lymph node metastases.
Ou 2018 [61]	Oropharynx & non-oropharynx Carcinoma: 95 <i>HPV</i> +: 27- <i>HPV</i> -: 68	Intratumoral macrophage density	Oropharynx > non-oropharynx N2-N3 > N0, N1 <i>HPV</i> - > <i>HPV</i> +	1. <i>HPV</i> - HNSCC has a higher CD68+ macrophage intratumoral density than <i>HPV</i> - tumors. 2. There is a trend of a higher proportion of the M2 population in the CD68+ macrophage infiltration of <i>HPV</i> - tumors. 3. Low M2 macrophage density was associated with an improved 5-year disease free survival in patients treated by CRT.
Schneider 2018 [62]	Oropharynx, Oral, Larynx, Hypopharynx Carcinoma: 136 <i>p16</i> +: 24- <i>p16</i> -: 112	Oropharynx CD163+ macrophages CD163+ macrophages	<i>p16</i> + = <i>p16</i> - Oropharynx = hypopharynx = Larynx = oral: <i>p16</i> + = <i>p16</i> -	1. CD163+ macrophage infiltration did not differ between <i>p16</i> + and <i>p16</i> - HNSCC.

Abbreviations: C/N/LD/SD = carcinoma/normal tissue/low dysplasia/severe dysplasia; CRT = chemoradiotherapy; HPV = human papilloma virus; M1/M2 = macrophages 1/2; OPSCC = oropharyngeal SCC; OS = overall survival; PD-1 = programmed cell death 1; PD-L1 = Programmed death ligand 1; PFS = progression-free survival; RFS = recurrence-free survival; (HN)SCC = (Head & Neck) squamous cell carcinoma; TIL = tumor-infiltrating lymphocytes.

Table 6. Infiltration of Langerhans cells and myeloid-derived suppressor cells in HNSCC according to HPV status.

Authors	No. of Specimens	Tumor-Infiltrating Cells	Antigens	Findings
Pereira 2011 [63]	Oral Carcinoma: 27 HPV-: 18-HPV+: 9	LC (Anti-S-100 antibody) Stromal compartment infiltration	HPV- = HPV+	There was no association between the immunohistochemical labeling for LCs (S-100+) and HPV infection in oral SCC. HPV infection in oral SCC did not alter the infiltration of LCs.
Russell 2013 [39]	Oropharynx, Oral, Sinus, Larynx, Hypopharynx, Carcinoma: 34 HPV+: 9-HPV-: 26 Normal: 7	MDSC: CD11b, Arg-1, INOS, STAT3 antigen expression	C > N (HPV+ = HPV-)	1. Irrespective of HPV status, a higher expression of MDSC antigens, including CD11b, Arg-1, INOS and STAT3, were found in HNSCC.
Yu 2015 [57]	Oral Carcinoma: 86 HPV+: 12-HPV-: 74 Normal/dysplasia: 32/12	Stromal MDSC CD11b/CD33	HPV+ = HPV-	1. Irrespective of HPV status, CD11b/CD33 MDSC populations increased in oral SCC. 2. There was a positive correlation between the infiltration of MDSC & PD-L1 expression
Partlova 2015 [44]	Oropharynx, Oral, Larynx, Hypopharynx, Submaxillary gland Carcinoma: 44 HPV+: 20-HPV-: 24	MDSC In vitro cytokine production from unstimulated tumor-derived cell culture supernatants CCL-17; 21 CXCL9; 10; 12; IL-1b, 2, 17, 23; IFN-g In vitro cytokine production from stimulated tumor-derived cell culture supernatants IL-10, 17, 21; TNF-a; IFN-g mRNA expression level in tumor samples Cox-2 PD-1 PD-L1 Tim-3	HPV+ > HPV- HPV+ > HPV- HPV+ = HPV- HPV+ > HPV- C > LN; C > NN; HPV- > HPV+ C = LN = NN; HPV+ > HPV- C > NN; HPV+ = HPV- C > LN > NN; HPV+ = HPV-	1. HPV+ HNSCCs have a higher MDSC infiltration than HPV- HNSCCs. 2. The infiltration of immune cells (i.e., MDSC) is associated with increased secretion of proinflammatory cytokines. 3. HPV+ tumors had significantly lower expression of Cox-2 mRNA and higher expression of PD1 mRNA than did HPV- tumors. 4. The high level of CXCL-12 was associated with a higher node status. <i>* mRNA expression and cytokine production were not associated with specific immune cells. In other words, these results may or may not be associated with MDSC infiltration.</i>

Table 6. Cont.

Authors	No. of Specimens	Tumor-Infiltrating Cells	Antigens	Findings
Kindt 2016 [64]	Oropharynx, Oral	CD1a+ LC & MIP-3 α secretion		<ol style="list-style-type: none"> 1. LC infiltration is increased in HNSCC but decreased with HPV infection. 2. LC number is an independent prognostic factor for HNSCC. 3. Intratumoral LC infiltration is positively associated with cT in oral SCC. 4. LC infiltration is positively associated with node status. 5. Stromal infiltration of LCs is associated with better OS in HPV- but not HPV+ HNSCCs. 6. Intratumoral & stromal LC infiltration was positively associated with better RFS in HPV- tumors.
	Larynx, Hypopharynx	Stromal compartment infiltration	C > SD > LD > N	
	Carcinoma: 125		HPV+/p16+ =	
	HPV-: 82		HPV+/p16- & HPV-	
	HPV+/p16-: 21	Intraepithelial compartment	C > N; SD, LD > N	
	HPV+/p16+: 11		HPV+/p16- > HPV+/p16+	
	N/LD/HD: 25/64/54			
Ma 2017 [32]	Oropharynx & Oral	MDSC CD11b-CD33	C & D > N; HPV+: C > D > N	<ol style="list-style-type: none"> 1. MDSC infiltration is higher in HPV+ oral & OPSCC samples than in dysplastic and normal tissue samples, suggesting that HPV infection can promote MDSC aggregation into HNSCC areas. 2. There was an association between the clinical stage and pathological grade and MDSC recruitment.
	Carcinoma: 376	lncRNA expression (MDSC recruitment)		
	HPV+: 88-HPV-: 288	HOTAIR, PROM1, CCAT1, MUC19	HPV- > HPV+	
	Normal/dysplasia: 30/30			

Abbreviations: CCL = Chemokine (C-C motif) ligand; C/N/LD/SD = carcinoma/normal tissue/low dysplasia/severe dysplasia; CXCL = Chemokine (C-X-C motif) ligand; HPV = human papilloma virus; IFN = interferon; MDSC = myeloid-derived suppressor cells; OPSCC = oropharyngeal SCC; OS = overall survival; LN/NM = lymph node/normal node; IL = interleukin; PD-1 = programmed cell death 1; PD-L1 = Programmed death ligand 1; PFS = progression-free survival; RFS = recurrence-free survival; (HN)SCC = (Head & Neck) squamous cell carcinoma; Tim-3 = T cell immunoglobulin and mucin-containing domain-3; Th = TNF- α ; Necr = progression-free survival.

The high CD8+ T cell infiltration in HPV+ tumors was associated with the high expression of checkpoint proteins, including PD-1 [44,48,52], LAG-3 [48], and Tim-3 [35], in HNSCC. The expression of PD-1 by HPV+ tumors was reported in the study by Kansy et al., who found a significant association between the CD8+ antigens CD8A and CD8B and the expression of PD-1 [52]. Partlova et al. also found that HPV+ tumors had higher expression of PD-1 mRNA than did HPV– HNSCC tumors [44]. The activity of CD8+ T cells is mediated by many cytokines, leading some authors to study the expression profile of TICs. Partlova et al. found that TICs in HPV+ SCC comprised IFN-g+ and IL-17+ CD8+ T cells [44], supporting the key role of these cytokines in the inflammatory reaction related to HPV. Overall, a high number of TICs (including CD8+ T cells) in HPV+ tumors was associated with the increased secretion of the following proinflammatory cytokines: CCL-17, CCL-21, IL-2, IL-4, IL-8, IL-10, IL-12, IL-17, IL-21, TNF- α , and IFN-g [39,44]. However, neither of these two studies demonstrated that cytokine expression was directly related to the level of CD8+ T cells.

A high level of CD8+ T cell infiltration was associated with improved OS and RFS in all studies focusing on HPV+ oropharyngeal SCC [34,36,37,40,41,51]. Moreover, Ward et al. found that patients with HPV+ oropharyngeal SCC with low levels of tumor-infiltrating lymphocytes had the same prognosis as those with HPV– oropharyngeal SCC [41]. Similar overall findings were observed in HNSCC for OS [42,46,48,50] and RFS [46,48,50]. One study reported that low CD8+ infiltration was associated with a high risk of metastases [46].

3.6. HPV and CD4+ T Cell Tumor Infiltration

Seven publications have reported findings regarding CD4+ T cell infiltration in HPV–induced SCC (Table 3). Overall, CD4+ T cell stromal infiltration was reported to be higher in HPV+ than HPV– oropharyngeal SCC [37,38,40,43]. In HNSCC, Balermipas et al. reported similar levels of CD4+ T cell infiltration in HPV+ and HPV– HNSCC [42]. Regarding the prognostic value of CD4+ T cell infiltration, Nordfors et al. did not find a significant association between the level of CD4+ infiltration and OS in patients with HPV+ and HPV– oropharyngeal SCC [40]. Similar results were found in the study by Balermipas et al., in which CD4+ expression was not associated with a good prognosis [42]. Regarding the subpopulation of CD4+ T cells, Krupar et al. studied the infiltration of Th17 cells in oropharyngeal SCCs. They found that there was a significantly lower percentage of Th17+ T cells in the intratumoral compartment of HPV+ patients.

3.7. Regulatory T Cell Infiltration

Fifteen studies have examined the tumor infiltration of Foxp3 Tregs according to HPV status, including 7 that were focused on oropharyngeal SCC (Figure 1, Table 4). Overall, a few studies reported that the degree of Foxp3 Treg infiltration did not differ according to HPV status in oropharyngeal SCC [34,45], but the majority of the authors observed increased infiltration in HPV+ oropharyngeal SCC, especially in the intratumoral compartment [36,38,43,53,54]. When analyzing HNSCC studies, Kindt et al. demonstrated that Foxp3 Treg infiltration increases with tumor progression; this increase is more important in HPV+ patients [24].

Four studies reported increased infiltration of Foxp3 T cells in the intratumoral [24,35,49] or stromal [39] compartment in HPV+ compared with HPV– HNSCC. Additionally, a study by Partlova et al. reported a slightly lower proportion of Treg cells in HPV+ HNSCC. These authors also found higher levels of cytokines and chemokines in HPV+ patients than in HPV– patients, but they did not report which cells produced these cytokines [44]. In 3 studies, the authors found that the infiltration of Foxp3 T cells was similar in HPV+ and HPV– SCC [15,33,42]. Two studies aimed to study the association between Treg infiltration and checkpoint protein expression [33,35]. Irrespective of HPV status, Badoual et al. and Lechner et al. found that the infiltration of Tregs was associated with higher levels of checkpoint protein expression (i.e., PD-1) in HNSCCs [33,35].

Regarding the prognostic value of Treg infiltration, the high infiltration of Foxp3 Tregs was associated with improved OS or RFS in both oropharyngeal cancer [34,36,53,54] and

HNSCC [15,24,35,39]. Moreover, the results of the study by Kindt et al. suggested that the number of stromal Tregs is a strong prognostic factor that is independent of other risk factors, including tobacco and alcohol consumption and HPV status [24]. To date, only Balermipas et al. did not find a significant association between the degree of Treg infiltration and OS [42].

3.8. Macrophage Infiltration

From our literature search, we identified 5 and 8 publications that investigated macrophage infiltration in oropharyngeal and HNSCC according to HPV status, respectively (Figure 1 and Table 5). In 3 studies, CD68+ macrophages showed increased infiltration in HPV+ compared to HPV- oropharyngeal SCC [56–59]; only one publication did not corroborate this finding [34]. In both oropharyngeal and nonoropharyngeal SCC, CD68+ macrophage infiltration increases between dysplastic tissues and carcinoma; the macrophage infiltration density is higher in the advanced stages [14,57]. Yu et al. focused on the CD68+/CD163+ macrophage (M2) subpopulation in oral SCCs, and they did not find a significant difference between HPV+ and HPV- oral SCC according to tumor progression and HPV status [57]. However, the relationship between CD68+ macrophage infiltration and HPV status is more controversial in HNSCC. Indeed, 5 authors did not find a significant difference between HPV+ and HPV- HNSCC regarding CD68+ macrophage infiltration [39,44,55,57,62], while macrophage infiltration increased in the intratumoral compartment in HPV+ HNSCC in 3 studies [14,49,61]. Among these studies, Ou et al. reported a tendency toward a higher proportion of M2 macrophages in HPV- HNSCC [61].

The relationship between CD68+ macrophage infiltration and checkpoint protein expression has been examined in two studies [56,59]. On the one hand, Lyfor-Pike et al. reported that CD68+ macrophages expressed high levels of PD-1 [56]. On the other hand, Oguejiofor et al. found that HPV- oropharyngeal SCC had an increase in CD68+ PD-L1+ macrophages compared to HPV+ tumors; these tumors were associated with improved OS compared to HPV- oropharyngeal SCC with low CD68+ PD-L1+ infiltration [59]. Lee et al. found that the high infiltration of CD68+ macrophages was associated with poor OS and DSS [58], while Seminerio et al. demonstrated that the high intratumoral infiltration of CD68+ macrophages was linked with shorter OS in patients with HNSCC [14]. Finally, Welters et al. found that the increase in dendritic cell-like macrophage infiltration in HPV+ oropharyngeal SCC was correlated with improved OS and a low risk of lymph node metastases [60].

3.9. Dendritic Cell Infiltration

3.9.1. Myeloid-Derived Suppressor Cells

A total of 4 included studies evaluated the relationship between MDSC infiltration and HPV status in HNSCCs using different antigens, including CD11b, Arg-1, INOS, and STAT3 (Figure 1, Table 6). MDSC infiltration increased throughout tumor progression [32,39], and overall, no significant difference was found between HPV+ and HPV- HNSCC [32,39,57]. Moreover, MDSC infiltration seemed to be higher in advanced-stage than in early-stage HNSCC [32].

Regarding the expression of checkpoint proteins, Yu et al. found a positive correlation between the infiltration of MDSCs and PD-L1 expression [57]. For CD8+ T cells, Partlova et al. reported high proinflammatory cytokine expression in tumor samples, characterized by a high infiltration of MDSCs, but they did not demonstrate a potential association [44]. To date, no study has investigated the prognostic value of MDSC infiltration according to HPV status.

3.9.2. Langerhans Cells

Only 2 studies assessed LC infiltration according to HPV status (Table 6). In a cohort of 27 patients with oral SCC, Perreira et al. did not find a significant difference between HPV+ and HPV- oral SCC [63]. More recently, Kindt et al. observed that LC infiltration increased in HNSCC throughout tumor progression but decreased in the presence of HPV infection. Moreover, there was a significant

association between the LC infiltration level and cT and tumor node status [24]. The LC infiltration level was positively associated with improved OS and RFS in HPV– but not HPV+ HNSCC.

3.10. SCC Location and the Detection of HPV

The majority of studies (N = 17) were exclusively performed on oropharyngeal SCC [28,29,31,34,36–38,40,43,45,47,53,54,56,58,59]; 2 focused on oral SCC [57,62], 1 focused on nodes with unknown primary SCC [51], and the rest included upper aerodigestive tract SCC in different locations. TICs were compared with dysplastic and normal mucosa samples in 10 studies [15,24,32,33,39,44,52,56,57,64].

To detect HPV infection in tumor samples, 4 authors exclusively assessed p16 expression in samples [42,48,53,61], while 36 authors performed DNA detection using different forms of PCR (in situ hybridization, E7 RT-PCR, E6/E7 multiplex qPCR, etc.). In one study, the detection method was not specified [34].

4. Discussion and Perspectives

This systematic review emphasizes many lines of evidence and reveals uncertainties regarding the role of immune cells in the development of HPV–induced HNSCC. These findings are summarized in Table 7. Our analysis indicates that in both oropharyngeal and nonoropharyngeal SCC, HPV infection is associated with increased CD8+ T cell infiltration and PD-1 expression by CD8+ T cells and improved OS. CD8+ T cells interact with their environment through multiple cytokines, especially IFN- γ and IL-2, -4, -8, -12 and -17 [39,44]. The increased expression of IL-17 in HPV+ HNSCC cells is associated with the infiltration of Th17 lymphocytes, resulting from the differentiation of CD4+ T cells. Thus, Krupar et al. reported an increased number of Th17 lymphocytes in TICs in HPV+ oropharyngeal SCC [43]. This observation makes particular sense according to studies that demonstrated increased CD4+ T cell infiltration in HPV+ SCC [37,38,40,43]. Because recent studies have suggested that lymphocyte plasticity can occur during HNSCC development, which is characterized by Th1 phenotype expression in Th17 cells [65,66], these findings could support a potential relationship between CD8+ and CD4+ T cells in HPV–induced SCC. CD4+ T cells could be converted into Th17 cells, potentiating the cytotoxic effects of CD8+ T cells against HPV–induced SCC antigens. According to several studies, the development of TICs, including CD8+ T cells, is associated with an increase in the detectable PBNI [28,29,32,33]. In this respect, and considering the data of Parikh et al., it is probable that the detection of CD8+ T cells against E6 or E7 HPV proteins could be used in future studies to better characterize tumor immunogenicity and, as recently suggested, the response to some treatments (i.e., immunotherapy and chemoradiation) [29,31]. However, scholars should remain prudent because the usual method of assessing the PBNI (blood sampling) is performed at a single time point and does not usually consider the variation in the blood concentrations of immune cells due to other factors related to the circadian rhythm or other external causes.

Macrophages have also been extensively studied in relation to HPV status. However, only a few studies have performed coimmunostaining to identify the M1 and M2 phenotypes [61]. M2 macrophages are involved in the enhancement of immunosuppression through the stimulation of Tregs and the secretion of TGF- β , TNF- α , and IL-10, leading to the creation of a favorable tumor microenvironment. Recently, Ou et al. indicated that CD68+ macrophage infiltration in HPV–SCC may consist mostly of M2 macrophages [61]. In HPV–HNSCC, the increased proportion of M2 cells could be one factor underlying the improvement in OS.

Table 7. Summary of evidence and findings.

Findings	Supporting Studies	Non-Supporting Studies
<u>Confirmed</u>		
1. The number of peripheral blood immune cells is higher in patients with SCC than in healthy individuals.	Al-Taei, 2013; Parikh, 2014; Ma, 2017; Lechner, 2017	-
2. CD8+ T cell infiltration is higher in HPV+ than in HPV- OP & HNSCC.	Nasman, 2012; Jung, 2012; Badoual, 2012; Russel, 2013; Ritta, 2013; Nordfors, 2013; Krupar, 2014; Oguejifor, 2015; Partlova, 2015; Kim, 2016; Nguyen, 2016; Balermipas, 2016; Van Kempen, 2016; Sivars, 2017; Poropatich, 2017	Wansom, 2011; Balermipas, 2014
3. High infiltration of CD8+ T cells is associated with better OS and RFS in HPV+ OP & HNSCC.	Nasman, 2012; Jung, 2012; Nordfors, 2013; Ward, 2014; Oguejiofor, 2015; Balermipas, 2016; Poropatich, 2016; Nguyen, 2016; Kim, 2016, Sivars, 2017	-
4. CD8+ T cell infiltration is associated with the expression of checkpoint protein (PD-1) in HPV+ HNSCC.	Badoual, 2012; Partlova, 2015; Kim, 2016; Kansy, 2017	-
5. CD4+ T cell stromal infiltration is higher in HPV+ than in HPV- OPSCCs	Jung, 2012; Ritta, 2013; Nordfors, 2013; Krupar, 2014	Balermipas, 2014
6. HPV+ SCCs have higher intratumoral infiltration of Tregs than HPV- OP & HNSCCs.	Nasman, 2012; Badoual, 2012; Park, 2013; Ritta, 2013; Krupar, 2014; Partlova, 2015; Nguyen, 2016; Punt, 2016; Kindt, 2017	Wansom, 2011; Oguejiofor, 2015 Balermipas, 2014; Lechner, 2017; Seminerio, 2019
7. A high infiltration of Tregs is associated with better OS or RFS in HPV+ oropharyngeal and HNSCCs.	Wansom, 2011; Nasman, 2012; Badoual, 2012; Park, 2013; Russel, 2013; Punt, 2016; Kindt, 2017; Seminerio, 2019.	Balermipas, 2014

Table 7. Cont.

Findings	Supporting Studies	Non-Supporting Studies
<u>Highly probable</u>		
1. The number of peripheral blood CD8+ T cells (against E6-E7) is higher in patients with HPV+ SCC.	Parikh, 2014; Masterson, 2016;	Lukesova, 2014
2. HPV+ OPSCCs have a higher infiltration of CD68+ macrophages than HPV– OPSCCs.	Lyfor-Pike, 2013; Welters, 2017; Oguejiofor, 2017	Wansom, 2011
3. The level of MDSC infiltration was not associated with HPV status.	Russel, 2013; Yu, 2015; Ma, 2017	Partlova, 2015
<u>Probable</u>		
1. A high blood concentration of CD8+ T cells could enhance the immunoreactivity to antigen E7, which was associated with improved OS in patients with OPSCC	Masterson, 2016	-
2. Low CD8+ infiltration was associated with a high risk of metastases in HNSCC.	Balermipas, 2016	-
3. High CD8+ infiltration was responsible for high expression of CCL-17, CCL-21, IL-2, IL-4, IL-8, IL-10, IL-12, IL-17, IL-21, TNF-a; IFN-g.	Russel, 2013; Partlova, 2015	-
4. HPV– HNSCC have higher M2 infiltration than HPV+ SCC.	Ou, 2018	-
5. Patients with HPV– SCC and low M2 infiltration could have better 5-year PFS.	Ou, 2018	-
<u>Controversial</u>		
1. LC infiltration is influenced by HPV status.	Kindt et al., 2017	Perreira et al., 2011

Abbreviations: CCL = Chemokine (C-C motif) ligand; (HN)SCC = head & neck squamous cell carcinoma; HPV = human papilloma virus; IFN = interferon; IL = interleukin; LC = Langerhans cells; M2 = macrophage type 2; MDSC = myeloid-derived suppressor cell; OP = oropharyngeal; OS = overall survival; PD-1 = programmed death-1; PFS = progression-free survival; RFS = recurrence-free survival; TNF = tumor necrosis factor; Treg = T regulatory cells.

Because Foxp3 Tregs inhibit the activity of CD8+ T cells, these cells have been examined in many studies, and their relationship with the tumor microenvironment in HPV-induced SCC remains ambiguous. Indeed, in contrast with expectations, most studies reported that an increase in Treg infiltration, especially in HPV+ SCC, was associated with improved OS [15,24,34–36,39,53,54]. Irrespective of HPV status, some authors have reported similar findings in HNSCC [66,67]. In fact, the high infiltration of Foxp3 Tregs may inhibit the protumoral effects of inflammatory immune cells and may function as favorable prognostic markers at some tumor sites, whereas at other tumor sites, Treg infiltration may be linked to poor OS due to their conventional regulatory function [41,68,69]. The activation of Tregs may be associated with MDSC infiltration because DC subpopulations are known to stimulate Tregs in HNSCC [69]. However, we cannot advance any hypotheses about the potential role of DC in the development of HPV-induced SCC because of the low number of studies on this topic in the current literature. Future studies that aim to investigate the role of Foxp3 Tregs in HPV-induced HNSCC should examine other immune cell populations, such as MDSCs.

This systematic review permitted the identification of many factors that should be taken into consideration in the analysis of results, the comparison of studies and the future establishment of immune models.

First, many authors have combined patients with oropharyngeal and non-oropharyngeal SCC into a single group. However, HNSCC includes malignancies that arise from functionally and anatomically distinct tumor sites with different characteristics. The most blatant example concerns oropharyngeal histology, as the Waldeyer ring is composed of preexisting lymphoid tissue that is characterized by a higher sensitivity to the immune response [49]. In that respect, oropharyngeal SCC is usually heavily infiltrated by lymphocytes, in contrast to other types of HNSCC such as oral carcinoma [49,69]. Additionally, recent data have shown the heterogeneous molecular and immunological tumor profile of HNSCC at different anatomical locations [70]. Differences in the prognostic value of TIC might reflect these different biological factors, making it likely that TICs exhibit different properties depending on the tumor site and the histological and molecular subtype.

In addition to the impact of the anatomical location of tumors, the consumption of tobacco and alcohol may also have a critical impact on the significance of TICs. Indeed, because cohorts of HPV+ patients are usually small, most authors do not consider these factors to be important in the development of TICs. However, it has been suggested that tobacco and alcohol consumption are capable of stimulating the mucosal recruitment of LCs, impacting the local immune response during SCC development [18]. In the same vein, Geng et al. demonstrated that tobacco smoking and, in particular, nicotine, are known to impair the responsiveness of T cells to antigenic stimulation, while other authors found that smoking is associated with a lower number of CD8+ T cells in tissue [46,71,72].

Second, the analysis of TICs could ideally consider both the stromal and intraepithelial compartments because the level of infiltration could vary substantially between these two compartments, leading to differences in prognostic value. Some authors did not specify the compartment used for analysis, which makes an analysis of the results difficult [40,41,44].

A third important point regarding the results analysis is the method used to detect HPV infection. In clinical practice, HPV infection is detected through p16 immunostaining in many centers. Some authors have used this approach to compare data from HPV+ and HPV- SCC, while others have used PCR and other direct methods of DNA identification. However, it is well known that some tumors can be HPV+/p16- or HPV-/p16+, which can be related to the lack of specificity of p16 in identifying HPV infection. As demonstrated in two studies, the use of p16 immunostaining versus DNA detection may lead to differences in the results, biasing the conclusions of related studies [40,54].

This systematic review also showed that some immune cells are rarely studied, such as eosinophils or natural killer cells (NK cells). However, NK cells have been identified as immune cells that may directly kill both HPV+ and HPV- HNSCC tumor cells [72,73]. This anti-tumor activity of NK cells could be significantly enhanced by cetuximab or avelumab, particularly in cells with higher baseline EGFR or PD-L1 expression [73]. Clinically, irrespective of HPV status, the low number of

tumor-infiltrating CD56+ NK cells is correlated with significantly decreased OS, distant metastasis-free survival and local progression-free survival [17].

Finally, the studies that investigated the impact of HPV infection on OS included patients subject to different treatment methods, including surgery, chemoradiation, radiotherapy and immunotherapy. These different therapeutic methods may significantly impact the OS of patients irrespective of HPV status. This point must be considered in analyses of OS in future large cohort studies.

In conclusion, current knowledge regarding the immune environment of HPV-induced HNSCC is not sufficient for the establishment of a clear pathophysiological model. Although some evidence exists in terms of the roles of CD4+ and CD8+ T cells and their related impact on OS or RFS, many uncertainties persist regarding the role and prognostic value of Tregs, macrophages, DCs and other uninvestigated cells. The poor quality and the low number of available studies, the small number of HPV+ patients included in these studies and the lack of consideration of cofactors that can impact the TIC composition may explain the current inability to establish an immunological model that can better predict the prognosis of HPV-induced oropharyngeal SCC. Future studies are needed to understand the complex interaction between tumors and their immune environment. These studies should carefully consider a rigorous methodological approach for HPV detection and should include a large number of patients with well-defined tumor locations. A large panel of immune cells and the use of specific coimmunostaining should be considered in future work in order to establish a precise immunological overview of HPV+ and HPV- SCC.

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