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Prognostic Significance of the Microenvironment in Human Papillomavirus Oropharyngeal Carcinoma: A Systematic Review

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Objective: The immune microenvironment of HPV-associated (HPV+) oropharyngeal squamous cell carcinomas (OPSCCs) (HPV+OPSCCs) differs from that of HPV-independent oropharyngeal cancers (HPV-independent OPSCCs). The literature on the subject is very abundant, demanding an organized synthesis of this wealth of information to evaluate the hypothesis associating the favorable prognosis of HPV+OPSCC patients with a different immune microenvironment. A systematic review of the literature was conducted regarding the microenvironment of HPV+OPSCCs.

Data Source: MEDLINE/PubMed, Embase, and Cochrane Library databases.

Review Methods: A literature search was performed following PRISMA guidelines (Moher D. PLoS Med. 2009). The PEO (Population, Exposure, and Outcome) framework is detailed as follows: P: patients with oropharyngeal squamous cell carcinomas, E: human papillomavirus (HPV), and O: histological and immunological composition of the tumoral microenvironment (TME). No meta-analysis was performed.

Results: From 1,202 studies that were screened, 58 studies were included ($n = 6{,}474$ patients; $n = 3{,}581$ (55%) HPV+OPSCCs and $n = 2{,}861$(45%) HPV-independent OPSCCs). The presence of tumor-infiltrating lymphocytes (TIL), CD3+ in 1,733 patients, CD4+ in 520 patients, and CD8+ (cytotoxic T lymphocytes (CTL)) in 3,104 patients, and high levels of PD-L1 expression in 1,222 patients is strongly correlated with an improved clinical outcome in HPV+OPSCCs.

Conclusion: This systematic review provides the most comprehensive information on the immune microenvironment of HPV+OPSCCs to date. Tumor-infiltrating lymphocytes and PD-L1 expression are associated with a favorable prognosis. B, CD8+ and resident memory cells densities are higher in HPV+OPSCCs. The importance of myeloid lineages is still a matter of debate and research.

Key Words: HPV, oropharyngeal tumor, squamous cell carcinoma, immunology.

Level of Evidence: N/A

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INTRODUCTION

The prevalence of oropharyngeal squamous cell carcinomas (OPSCCs) in developed countries has been increasing continuously for over 20 years[2] due to the rising incidence of HPV-associated OPSCCs (HPV+OPSCCs).[3] Given that HPV+ and HPV− OPSCCs display important clinical differences, in 2017, the 8th TNM classification[4] elaborated staging, taking into account the HPV-associated or HPV-independent character of OPSCCs.

HPV+OPSCCs patients possess unique characteristics: the patients are younger and of higher socioeconomic status, with no precancerous lesions, unlike alcohol- and tobacco-associated cancers or HPV-associated genital cancers.[2] HPV-associated OPSCCs have also been shown to respond better to radiation and chemotherapy. Their prognosis and overall survival (OS) are better. However, not all patients respond equally to these radical therapies.[5,6] Some explanations, such as smoking, have been suggested, but these appear to be insufficient.[7]

Prognostic stratification and prediction of therapeutic response can be sought in the composition of the tumor microenvironment (TME). TME is the location where the tumor is surrounded by normal cells, immune cells, and supporting cells. According to Hanahan and Weinberg,[8,9] the imbalance between immune control and tumor growth, diverting the mechanisms of cellular homeostasis, is at the origin of the expansion and progression of tumors.

The microenvironment of HPV+OPSCCs differs from that of HPV-independent OPSCCs. This is reflected in a large number of publications. Cytotoxic CD8+ T cells have long been known to correlate with prognosis in HPV+OPSCCs,[10,11] after having been described in other solid cancers and in head and neck cancers.[12]

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Conversely, CD4+FoxP3+ T cells (regulator T cells: Treg) appear to have had a more controversial role, sometimes correlated with an altered[13] or improved prognosis.[10,14]

In 2016, Saber et al.[15] conducted the first systematic review on the subject, followed by an update in 2018 by Lechien et al.[16] Between 2019 and 2022, 458 publications appeared on the subject, including nearly 30 narrative reviews,[17,18] none of them systematically conducted. Fundamental discoveries have been made that were overlooked in previous reviews, such as the roles of memory resident T cells (T_{RM}),[19–21] M1-M2 macrophage polarization[22] and neutrophils and NK cells in cancer.[23]

The abundance of publications has resulted in a major need: the prioritization and synthesis of this information. The narrative reviews give a potentially author-biased view of the state of the art and obscure the discrepancies found in the literature.

The goal of the present study is to conduct a systematic review on the tumor microenvironment of HPV +OPSCCs, as compared with HPV-independent OPSCCs to evaluate the hypothesis associating the favorable prognosis of HPV+OPSCC patients with a different immune microenvironment.

MATERIALS AND METHODS

PRISMA guidelines were followed for selection of the studies.[1] The PEO (Population, Exposure, Outcome) framework is detailed as follows: P: patients with oropharyngeal squamous cell carcinomas, E: human papillomavirus (HPV), and O: histological and immunological composition of the tumoral microenvironment (TME).

The following search algorithm was applied to MEDLINE/PubMed, Embays, and the Cochrane Library in March 2023: *(HPV OR papillomavirus) AND (immunology OR immune OR immunological OR CD4 OR CD8 OR TRM OR CD103 OR Granzyme B OR neutrophils OR Polymorphonuclear Myeloid-Derived Suppressor Cells OR macrophages OR CD68 OR CD163) AND (oropharynx OR oropharyngeal OR tonsil OR tongue base) AND (Squamous cell carcinoma OR carcinoma OR tumor)*. Neither time nor language constraints were applied. Inclusion criteria were articles including HPV+OPSCCs patients and results reporting on microenvironment or immune parameters. Exclusion criteria were strictly in vitro analysis; strictly genomic study; immunological studies dedicated to vaccine development; strictly animal study; OPSCCs data non distinguishable from other Head and Neck cancers; HPV-associated patients not separated from HPV-independent patients; case reports or cases series <5 cases; review and meta-analysis. Studies dealing with primary tumor or nodes were reviewed separately. The search was conducted independently in December 2022 by four authors (R.B., B.M., Y.A., J.M.).

The following data were extracted: details of the included studies (reference as first author, year of publication, and study design); patient characteristics (number of total oropharyngeal cases, number of HPV+OPSCCs and HPV-independent OPSCCs); methods of the immune analyses, that is, techniques as RNA sequencing (RNAseq), Transcriptomic data from the Cancer Genome Atlas (TCGA), multiplex immunofluorescence (mIF), immunohistochemistry (IHC), hematoxylin–eosin-safran stain (HES), flow cytometry (FC), mass cytometry (MC), tissue microarray (TMA) and molecular biomarkers assessed; outcomes (parameters associated with a favorable/longer or a unfavorable/shorter outcome, that is, loco-regional control (LRC), progression-free survival (PFS), recurrence-free survival (RFS), disease-specific survival (DSS), overall survival (OS), parameters significantly higher or lower in the HPV+OPSCCs group; and impact of therapeutic intervention if applicable.

Cofactors that may interfere with the outcomes of the included studies were screened using the Tool to Assess Risk of Bias in Cohort Studies developed by the Clarity Group (McMaster University, Ontario, Canada) and the Agency for Healthcare Research and Quality (US).[24] Age, comorbidities (including immunologic diseases or treatments), exclusion of recurrent cases, HPV confirmation by PCR, deviations from initial protocol (including uncertainty about possible chemotherapeutic or radiation prior to the microenvironment evaluation), or missing data (HPV status, inadequate follow-up or insufficient characterization of cellular populations) were assessed. Due to the expected heterogeneity in the reported data, no meta-analysis was performed.

RESULTS

The flow chart is shown in Figure 1. Overall, 1,202 studies were screened, and 58 studies were included ($n = 6{,}474$ patients; 3,581 (55%) HPV+OPSCCs and 2,861 (45%) HPV-independent OPSCCs). A retrospective design was found in 50 studies ($n = 6{,}016$), prospective in 4 studies ($n = 319$), and observational or cross-sectional in 4 studies ($n = 139$). Two studies examined peripheral blood (PB) with the tumor microenvironment ($n = 86$); all the others examined histological specimens ($n = 6{,}388$). Sixteen studies relied on p16 immunochemistry detection to determine the HPV status, and 42 confirmed this with PCR to detect HPV DNA. One study reported analyses performed on nodes.[25] All the others were conducted on primary tumors. Immunochemistry (IHC) was used in 41 studies, multiplex immunofluorescence (mIF) was performed in 11 studies, flow cytometry (FC) in 8 studies, tissue microarrays (TMA) in 4, and TCGA RNAseq data were analyzed in 3 studies.

Studies reporting an association between microenvironment biomarkers or cells with a significant favorable or unfavorable clinical outcome are detailed in Table I for lymphoid cells, Table II for myeloid cells, and Table III for molecular biomarkers. Biomarkers significantly associated with a favorable or an unfavorable clinical outcome according to the sizes of studied populations are summarized in Figure 2.

Parameters found to be significantly higher in HPV +OPSCCs vs HPV-independent OPSCCs, regardless of clinical outcome, were B cells, T cells, CD8+ T cells,[26] CD8+CD103+resident memory T cells (TRM),[27,28] PD1+ TRM, CD8+CTLA-4+ T cells, CD27−CD45RO+CD45RA− (effector memory T cells (Tem)),[29] CD4+FoxP3+ regulator T cells (Treg), CD3+CD4+PD1+ T cells, FoxP3, PD1, PD-L1, LAG-3, TIM-3, VISTA molecular biomarkers,[30] TLR2, 4 and 7,[31,32] CD45-ARNm,[33] and CD69.[28]

Parameters found to be significantly lower in HPV +OPSCCs vs HPV-independent OPSCCs, regardless of clinical outcome, were CD8+:CD4+FoxP3+ (Treg) ratio,[27] CD4:CD8 ratio,[10] PD-L1 and CD8+PD-L1+ in one study,[34] CD45RA+CD45RO− (Naïve T cells),[29] IL-17, CD3-IL-17+,[35] TLR5, TLR9,[32] and FoxP3 in one study.[36]

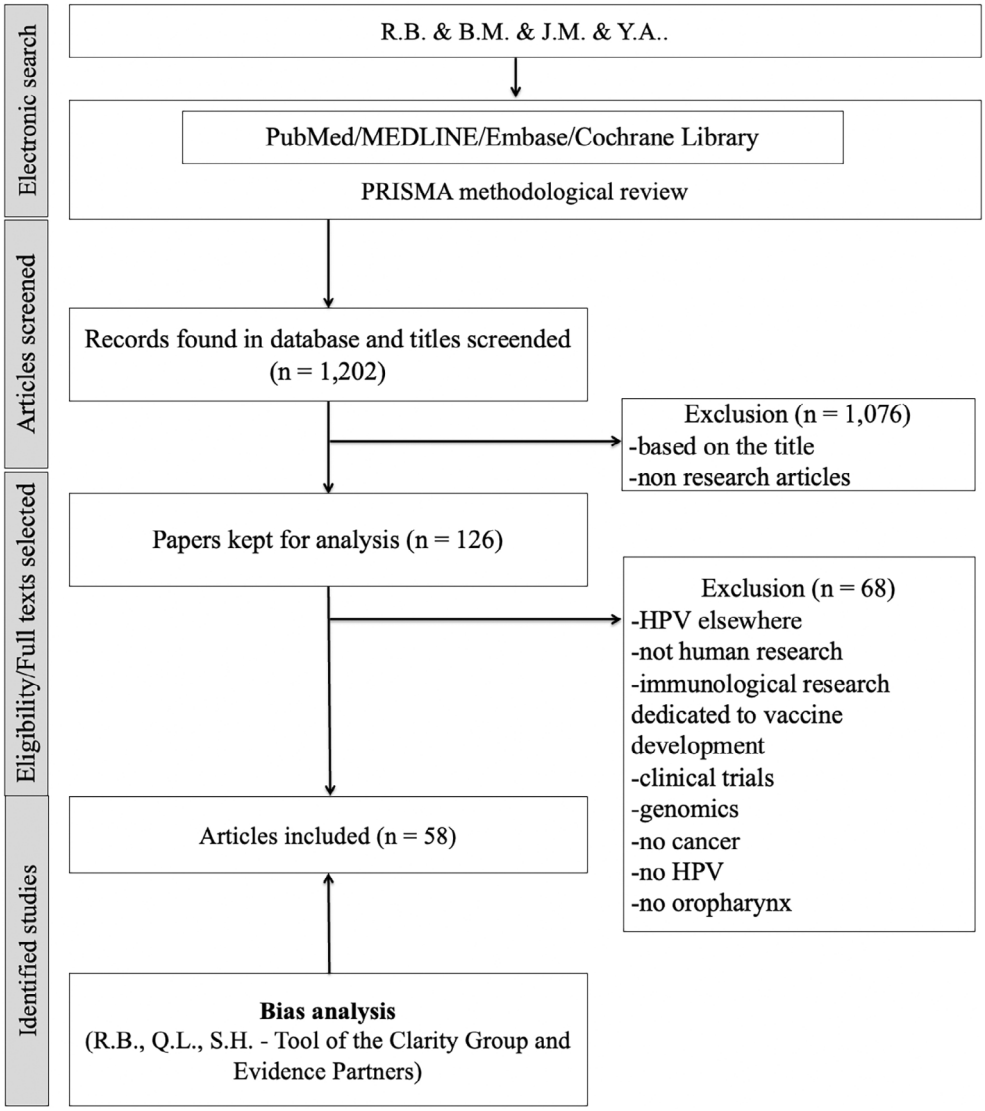

Fig. 1. Flow Chart. Systematic review of microenvironment composition and prognostic impact in HPV-associated oropharyngeal squamous cell carcinomas, conducted with PRISMA methodology. PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analysis.

One study found lower levels of M2-TAM,[37] which is described to be protumoral.[22]

Six studies provided data regarding the significance of tissue architecture and showed better clinical outcomes (either OS, DSS, LRC, or RFS) for stromal localization of CTL,[34,38–40] stromal PD-L1[34] and intratumoral high densities in CTL, PD-L1, TAM and TAM expressing PD-L1 in HPV+OPSCCs only.[34,41]

Four studies described the impact of a treatment (surgical excision and/or chemoradiation) on the immune microenvironment of HPV+OPSCCs.[42–45] Kim et al.[42] distinguished three types of TME: -immune-rich (exhausted T cells CD8+PD1+ and M1 macrophages CD68+CD163− high); −mesenchymal CD8+ T-cell exclusion from the tumor and high mesenchymal cells and TGFβ and -xenobiotic (CD8+ T cells low and CD73+ high) with a decreasing prevalence of HPV+OPSCCs (i.e., immune-rich microenvironment consisted in 100% HPV-positive cancers and xenobiotic microenvironment consisted in a majority of HPV-negative cancers, mesenchymal microenvironment being mixed between HPV-positive and HPV-negative cancers).

The two first TME subtypes have a better outcome, either after surgery or PD1-PD-L1 blockers treatment. Masterson et al.[43] found a decrease in CD4:CD8 T-cell ratio and an increase in CD4+CD25+ T cells (Treg) 3 months after a chemoradiation in HPV+OPSCCs only. Al-Taei et al.[45] found a decrease in HPV-specific T-cell responses after chemoradiation or surgery and radiation. Lee et al.[44] found no impact of therapy on the TME either in HPV+OPSCCs or in HPV-independent OPSCCs.

Descriptions of the methods and results of all the included studies are shown in Supplementary Table 1. The results of the bias assessment tool are detailed in Supplementary Table 2.

TABLE I.
Studies Demonstrating an Association Between Microenvironment Parameters and Clinical Outcomes in HPV+OPSCCs—Lymphoid Cells.

	Studies Documenting a Favorable Clinical Outcome		Studies Documenting an Unfavorable Clinical Outcome	
Parameters	References	No of Patients (No. of HPV+/No. of HPV−)	References	No of Patients (No. of HPV+/No. of HPV−)
CD3	Haave 2022,[47] Almangush 2022,[48] Kemnade 2020,[46] De Meulenaere 2017,[40] Van Kempen 2016,[49] Punt 2016,[35] Oguejiofor 2015,[50] Ward 2014,[51] Krupar 2014,[36] Jung 2013,[52] Rajjoub 2007[53]	1,733 (753/976)	-	
Non-Treg (CD3+FoxP3−)	Punt 2016[35]	162 (63/99)	-	
CD3+FoxP3+	Ljokjel 2022,[101] Haave 2022,[47] Spector 2019,[54] Punt 2016[35]	809 (427/380)	-	
CD4 T cells	Spector 2019,[54] Lee 2015,[44] Ward 2014,[51] Wansom 2012[10]	520 (330/178)	-	
Treg (CD4+FoxP3+)	Liu 2022,[55] Hur 2022,[66] Cioni 2019[59]	528 (383/151)	-	
Th1 Treg (CD4+FoxP3+Tbet+)	Santegoets 2019[102]	50 (50/0)	Tosi 2022[27]	39 (24/14)
Th	Liu 2022[55]	315 (270/51)	-	
Tfh	Liu 2022[55]	315 (270/51)	-	
CD4+PD1+	Badoual 2013[11]	64 (32/32)	-	
CD8 T cells	Tosi 2022,[27] Liu 2022,[55] Young 2020,[39] Wuerdemann 2020,[63] Kemnade 2020,[46] Gurin 2020,[38] Spector 2019,[54] Hladíková 2019,[56] Cioni 2019,[59] Solomon 2018,[41] Schoenfeld 2018,[103] Oguejiofor 2017,[34] De Meulenaere 2017,[40] De Meulenaere 2017,[104] Masterson 2016,[43] Oguejiofor 2015,[50] Lee 2015,[44] Ward 2014,[51] Turksma 2013,[29] Nordfors 2013,[57] Jung 2013,[52] Wansom 2012[10]	3,104 (1,873/1,209)	-	
T_{RM} (CD8+CD103+)	Hewavisenti 2020,[28] Salomon 2019,[105] Welters 2018[62]	336 (269/67)	-	
Tem (CD8+CD161+)	Welters 2018[62]	97 (57/40)	-	
CD8+PD1+	Kim 2020,[42] Badoual 2013[11]	110 (58/51)	-	
CD8+PD-L1+	Wuerdemann 2020,[63] De Meulenaere 2017[40]	269 (51/218)	-	
CD20 (B cells)	Hladíková 2019[56]	72 (63/9)	-	
CD56 (NK cells)	Wagner 2016[70]	140 (34/106)	Tosi 2022[27]	39 (24/14)
CD4:CD8 ratio	-		Ward 2014,[51] Krupar 2014[36]	307 (165/138)
FoxP3:CD8 ratio	-		Ward 2014,[51] Wansom 2012[10]	320 (174/134)

Abbreviations: Th = helper T cells; Treg = regulatory T cells; Tfh = T follicular helper cells; Tem = T effector memory; T_{RM} = resident memory T cells; NK = natural killer cells.

DISCUSSION

This study represents the most updated systematic review regarding the immune microenvironment of the tumors in HPV+OPSCCs compared with HPV-independent OPSCCs, also taking into account clinical outcomes. As main findings, this review established that a number of parameters are strongly associated with a better clinical outcome in HPV+OPSCCs. The presence of CD3+ tumor-infiltrating lymphocytes (TIL) among 1 733 patients,[35,36,40,46–53] CD4+ among 520 patients,[10,44,51,54] and CD8+ (cytotoxic T lymphocytes (CTL)) among 3,104 patients,[10,27,29,30,34,38–40,43,44,46,50–52,54–57] and high levels of PD-L1 expression among 1,222 patients[30,38–41,58,59] seems to be predictive of better LRC and longer RFS, DSS, and OS. All these findings were based on large sample sizes, ranging from 520 up to more than 3,000. These correlations have also been described in HPV-independent OPSCCs patients.[14,60,61]

Other cellular populations, more recently described and based on smaller sample sizes, appear to be associated with longer survival in HPV+OPSCCs patients, such as T_{RM},[28,41,62] Tem,[62] CD8+PD1 T cells,[11,42] CD8 + PD-L1+[40,63] and B cells.[56] Results in other solid tumors support these findings.[20,64,65]

However, conflicting results are reported for some elements, mainly due to insufficient characterization of cell profiles. For instance, the significance of Treg requires further discussion. A high density of CD4+ FoxP3+ Treg has been reported to be correlated with a favorable outcome in HPV+OPSCCs[55,59,66] as well as in all types of Head and Neck Squamous Cell Carcinoma.[14] Th1 Treg (CD4+FoxP3+Tbet+) have been reported to be

TABLE II.
Studies Demonstrating an Association Between Microenvironment Parameters and Clinical Outcomes in HPV+OPSCCs–Myeloid Cells.

Parameters	Studies Documenting a Favorable Clinical Outcome		Studies Documenting an Unfavorable Clinical Outcome	
	References	No of Patients (No. of HPV+/No. of HPV−)	References	No of Patients (No. of HPV+/No. of HPV−)
CD68 (TAM)	Haave 2022[47]	168 (92/76)	Azzimonti 2021,[71] Ou 2019,[72] Snietura 2020,[37] Lee 2015[44]	228 (138/90)
CD68+PD-L1+	-		-	
CD68+TREM-1+	-		Azzimonti 2021[71]	27 (27/0)
CD163	-		Snietura 2020[37]	85 (45/40)
M1-TAM (CD68+CD163−)	Tosi 2022,[27] Kim 2020[42]	85 (50/33)	-	
M2-TAM (CD68+CD163+)	-		-	
CD68+CD169+	Topf 2019[25]	21 (4/17)	-	
Mast cells	-		Tosi 2022[27]	39 (24/14)
Monocytes (CD14+)	Santegoets 2020[67]	27 (27/0)	-	
MDSC	-		-	
DC (CD1c+)	-		-	

Abbreviations: DC = dendritic cells; M1 (M1) and M2 (M2) = polarized macrophages; MDSC = myeloid-derived suppressor cells; TAM = tumor-associated macrophages.

associated both with a favorable[67] and an unfavorable clinical outcome in HPV+OPSCCs.[27] FoxP3 is also induced after transient T-cell activation, which makes interpretation of this marker difficult.[68,69] The correlation with a favorable clinical outcome could therefore be biased. Some authors have investigated the importance of Treg infiltration by studying the imbalance between tumor-specific cytotoxic activity and regulation using the CD4:CD8 ratio or the FoxP3:CD8 ratio. Using this method, FoxP3+ Treg cells are more likely to be associated with a poor clinical outcome.[10,36,51]

Otherwise, conflicting data are due to insufficient analysis of small populations such as NK cells,[70] the significance of tumor-associated macrophages (TAM),[37,44,47,71,72] mast cell infiltration,[27] and monocyte infiltration.[67] All of these markers were investigated in sample sizes ranging from 27 to 168 patients. The antitumor significance of M1 macrophages was found in two studies,[27,42] and, to date, M2 macrophages have not been correlated with an unfavorable prognosis in HPV+OPSCCs. However, one study found lower levels of M2-TAM,[37] described to be protumoral, in the HPV+OPSCCs microenvironment.[22]

The favorable prognosis of CD8+PD1+ cells and T_{RM} could be explained by the higher density of these T-cell populations in tumor-specific cells.[21,73,74] This specificity towards the tumor characterizes an immune-activated and antitumor environment. In the case of HPV+OSCC, an anti-HPV T-cell immune response could increase the overall antitumor T-cell response and could contribute to a better clinical outcome. The anti-HPV T-cell specific response has been reported to be associated with the presence of T_{RM} and with a better

TABLE III.
Studies Demonstrating an Association Between Microenvironment Parameters and Clinical Outcomes in HPV+OPSCCs–Molecular Biomarkers.

Parameters	Studies Documenting a Favorable Clinical Outcome		Studies Documenting an Unfavorable Clinical Outcome	
	References	No of Patients (No. of HPV+/No. of HPV−)	References	No of Patients (No. of HPV+/No. of HPV−)
PD1	Schoenfeld 2018[103]	81 (64/9)	—	
PD-L1	Young 2020,[39] Wuerdemann 2020,[63] Gurin 2020,[38] Hong 2019,[58] Cioni 2019,[59] Solomon 2018,[41] De Meulenaere 2017[40]	1,222 (597/624)	—	
PD-L2	—		—	
CTLA-4	—		—	
TIM-3	—		—	
LAG-3	—		—	
VISTA	—		—	
HLA-DP/DQ/DR	Cioni 2019[59]	142 (60/82)	—	
HLA-DRA	Cioni 2019[59]	142 (60/82)	—	

Abbreviations: CAP = capillary endothelial cells; LAG-3 = lymphocyte-activation-protein 3; TIM-3 = T-cell immunoglobulin and mucin-domain containing-3; TLR = toll-like-receptor; VISTA = V-domain Ig suppressor of T-cell activation.

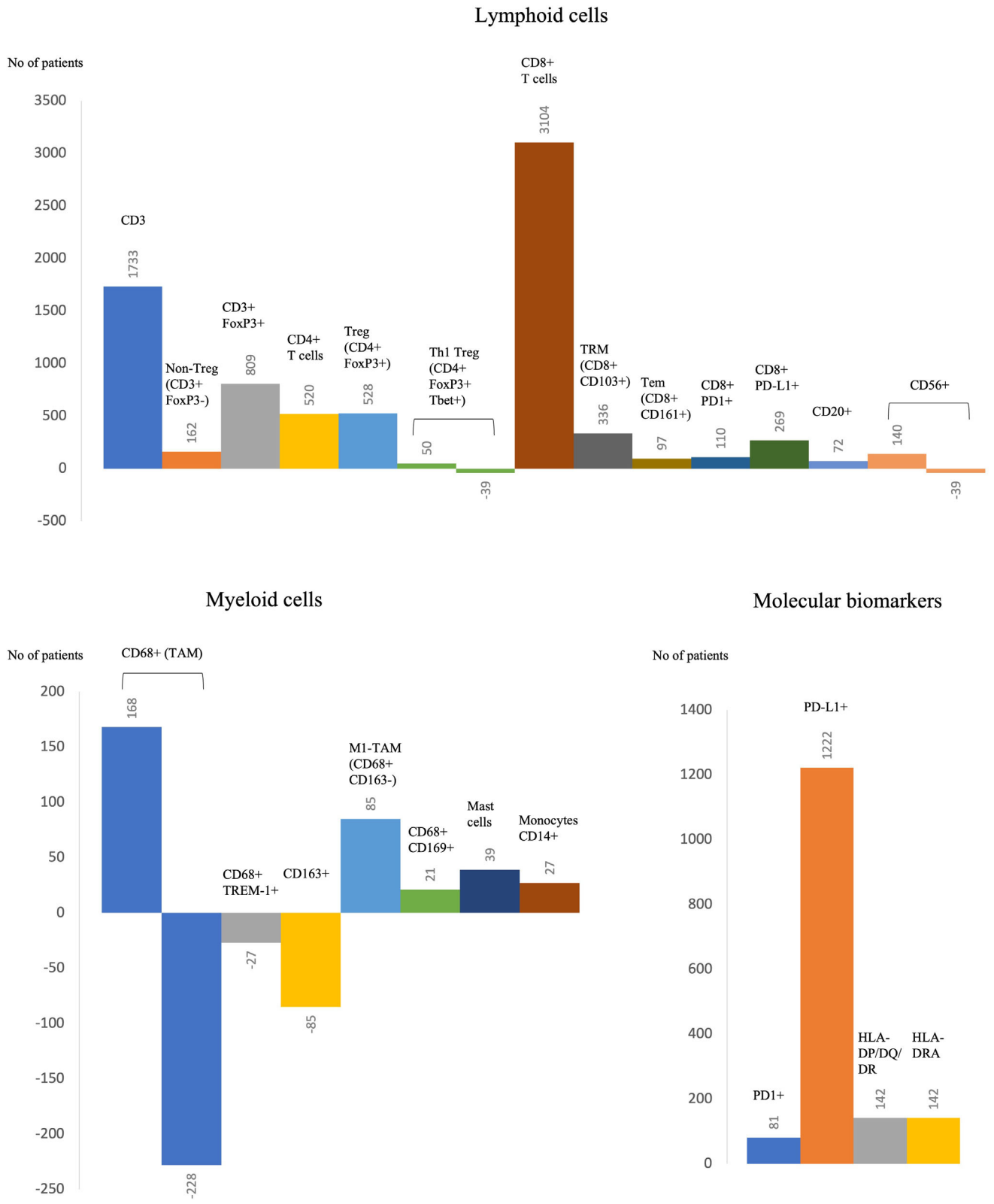

Fig. 2. Sizes of population associated with favorable or unfavorable clinical outcomes for lymphoid, myeloid, or molecular parameters in the microenvironment of HPV+OPSCCs in published studies in MEDLINE/Pubmed, Cochrane, and Embase libraries. positive numbers of patients correspond to favorable clinical outcome, negative numbers of patients correspond to favorable clinical outcome. Treg = regulatory T cells; Th = helper T cells; TRM = resident memory T cells; Tem = effector memory T cells; and TAM = tumor-associated macrophages. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

prognosis.[62] This element has also been described in peripheral blood, and the prognosis might be possible to determine from circulating HPV-specific T cells.[75]

A trend towards greater characterization of cell subpopulations is evident in recent years. The specificity of action and phenotypes of TIL subtypes, memory resident or effector memory CD8+ T cells or CD4+ T cells,[76] has been applied or should be extended to the analysis of the microenvironment of HPV+OPSCCs. Similarly, the evaluation of myeloid lineages, primarily macrophages, and polarized M1 (CD163−) or M2 (CD163+) TAM must be conducted among wider cohorts to definitively prove or refute an association with HPV+OPSCCs or clinical outcome.

Some immune parameters are clearly different between HPV+OPSCCs and HPV-independent OPSCCs: densities of infiltrating lymphocytes (CTL, T_{RM}, and Treg),[26,27,55,66] TAM,[26] and PD-L1[58] are more prominent in HPV+OPSCCs. PD-L1 density is correlated with high TIL infiltration, tumor stage, and lymph node invasion[58] in HPV+OPSCCs only. However, PD-1/PD-L1 blockers therapies have not proved to be undeniably more efficient in HPV+OPSCCs: some studies advocating the pros[77,78] and others the cons.[79,80] This element echoes with the implication of other molecules alongside immune checkpoints as CD137 (4-1BB), CD226 or OX-40.[81,82] These surrounding elements able to regulate, amplify or control the activation of CD8+ T cells, and Treg or generate T_{RM} may even angle FoxP3+ CD4+ T cells (Treg) *via* T-box transcription factor eomesodermin (Eomes) in an antitumoral cytotoxic activity as demonstrated by Akhmetzyanova et al. in a murine model of virus-driven carcinoma.[83] Agonistic antibodies are known for some of these molecules.[84,85]

The understanding of the full range of signaling and activation players leading to tumor destruction is only emerging. Prognosis and treatment will become personalized, especially in HPV+OPSCCs which is an immunologically specific entity as this study pointed out. A comprehensive examination of the HPV+OPSCC microenvironment offers paths to explore new molecules and therapeutic options for HPV-positive patients. In the same time, the development of new therapies, based on the immunological properties of cancers, opens up the possibility of de-escalation trials sparing radiotherapy and chemotherapy. The link between research into the microenvironment and immunological treatment is a perpetual quest towards practical clinical applications of the knowledge presented here.

No single immune parameter at the level of the tumor microenvironment is currently able to explain the favorable prognosis of HPV+OPSCCs and their better response to both chemotherapy and radiation.[5] Differences in the immune composition of the HPV+OPSCCs microenvironment offer hope for a differential response to immunotherapies (anti-PD-L1) or the development of new immune molecules, as opposed to HPV-independent OPSCCs. In 2017, Ferris et al. demonstrated the efficacy of Nivolumab (Opdivo®, Bristol-Myer Squibb, US) in recurrent or metastatic squamous cell carcinomas of the head and neck[86] and results regarding better responses of HPV+OPSCCs to anti-PD-1/L1 are conflicting.[77–80] Thinner and deeper characterization of immune cells subpopulation appear again crucial in the light of immunotherapy. Experimental data suggest that anti-PD-1/L1 therapy sow the seeds of resistance to immune checkpoints blokade (ICB) in sparing some immunosuppressive Treg clones.[87] Adjunct immune therapies may provide a clinical impact as important as the ICB therapies emergence based on the comprehensive analysis of Head and Neck tumors microenvironment. This review advocate for not solely defining cell populations by their most obvious regulator or effector characteristics, but to disseminate to the medical community the widest possible knowledge of the properties of the immune cells individually.

Nonetheless, the included studies are of a low level of evidence, as they are retrospective and insufficiently specific regarding characterization of the studied cells, with relatively small populations. Particular attention should be paid to further investigation of the most specific subpopulations dedicated to antitumor action, such as T_{RM}, or the most specific for HPV+OPSCCs. Single-cell transcriptomic studies, possibly spatial transcriptomic analyses to take into account the architecture of the microenvironment and the interactions of the cells between them, are necessary. Analysis of the whole transcriptome will be able to differentiate characteristics of CTL subpopulations of HPV+OPSCCs, or analysis of TCR V(D)J sequence and repertoire will provide information on tumor specificity and cytotoxic response.[88–90] The influence of treatment on the microenvironment, insufficiently studied until now, must also be taken into account.[91] Few studies have focused on lymph node involvement, making it a *terra incognita* for HPV+OPSCCs research, although HPV+OPSCCs is likely to develop lymph node invasion. These studies have not addressed the emerging concept of the formation of pre-metastatic niches in lymph nodes.[92]

Head and Neck cancers benefit from detailed translational research in Onco-Immunology. Advances in characterization, prognosis definition, and clinical therapeutics achieved in other organs or related to histologically different cancers, could be sought in OPSCCs, as immune populations and functions are ubiquitous. Therapeutic breakthroughs, such as the ICB discovery that won Honjo[93] and Allison[94] the Nobel Prize in Physiology or Medicine in 2018,[95] are now benefiting head and neck cancers. To date, some biomarkers of antitumor activation are promising but have been insufficiently explored in Head and Neck cancers, such as CD161, associated with rapid effector memory T-cell activity and favorable prognosis,[62,96] or CD137, a cytotoxic T-cell coactivation molecule and potential therapeutic target.[73,97,98] Myeloid cells and tumor-associated macrophages impact on clinics and therapeutics in a major "hot spot" in immunology,[99,100] under-explored in OPSCCs. Gathering information about the specificity of the HPV+OPSCCs immune microenvironment is critical to determining new prognostic biomarkers and therapeutic targets[60] that can benefit all patients with head and neck cancers.

CONCLUSION

In conclusion, this systematic review represents the most up-to-date synthesis of the literature on the microenvironment in HPV+OPSCCs with respect to clinical outcome and differences from HPV-independent OPSCCs. The favorable prognostic significance of TIL, CTL, and PD-L1 has been established in large samples, but the prognostic significance of Treg remains debated. The influence of TRM, NK cells, TAM on clinical outcome needs to be evaluated in larger series.

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