Prevalence of PD-L1 expression in head and neck squamous precancerous lesions: a systematic review and meta-analysis

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Abstract
Background: Studies concerning programmed death-ligand 1 (PD-L1) expression in precancerous lesions of head and neck (HN) region have shown variable results.

Methods: We systematically reviewed the published evidence on PD-L1 expression in HN precancerous lesions.

Results: Of 1058 original articles, 14 were included in systematic review and 9 in meta-analysis. The pooled estimate of PD-L1 expression was 48.25% (confidence interval [CI] 21.07-75.98, I² 98%, tau² 0.18). PD-L1 expression appeared to be more frequent in precancerous lesions than in normal mucosa (risk ratio [RR] 1.65, CI 0.65-4.03, I² 91%, tau² 0.82) and less frequent than in invasive squamous cell carcinoma (RR 0.68, CI 0.43-1.08, I² 91%, tau² 0.22).

Conclusions: PD-L1 expression could reflect a point of balance between host immune response and cancer escape ability. High heterogeneity and moderate quality suggest that further studies with larger sample size and more rigorous case selection will allow more precise assessment of PD-L1 expression in HN precancerous lesions.

KEYWORDS
head and neck, immunohistochemistry, premalignant lesion, programmed death-ligand 1, systematic review

1 INTRODUCTION

The programmed death-ligand 1 (PD-L1) and its counterpart protein programmed death 1 (PD1) checkpoint axis has an important role in modulation of the immune response. The binding of PD1 with its ligand PD-L1 normally reduces the proliferation and activity of cytotoxic CD8 T lymphocytes against presented antigens, thus inducing tolerance. Tumors can adaptively express high levels of PD-L1 on their cell surfaces and induce host tolerance against tumor-associated antigens.1-3 In the last decade, several PD-L1/PD1 inhibitors have been developed and used with success in a variety of tumor types including lung cancer, melanoma, renal cell carcinoma, urothelial carcinoma, head and neck squamous carcinoma (HNSCC), triple-negative breast carcinoma, and mismatch
repair deficient endometrial and colorectal carcinoma. Expression of PD-L1 as a predictor of response to therapy has been mainly assessed with immunohistochemistry (IHC), but different assays exist and are approved for the predictive response to a specific drug in a specific tumor type. Furthermore, the assessment of the expression of PD-L1 with IHC can vary according to different factors including reproducibility among pathologists is variable.

Most of the prior studies on this topic concerned advanced cancer, and only a minor quota of this literature has focused on immunotherapy as a prevention therapy for preinvasive lesions. Regarding premalignant lesions, most interest has centered around checkpoint inhibitors in intraepithelial cervical neoplasia and breast ductal carcinoma in situ (DCIS), wherein the distribution of PD-L1 expression in preancancerous cells and in immune cells of the tumoral immune microenvironment (TME) could have implications for the use of checkpoint inhibitors as a preventive therapy. PD-L1/PD1 inhibitors are approved for advanced HNSCC and have shown meaningful antitumor activity both alone or in combination with chemotherapy. Patterns of toxicity of checkpoint inhibitors are different from conventional chemotherapy agents, with mainly immune-related adverse events such as endocrinopathies, but demonstrated overall improved quality of life for treated HNSCC patients. Moreover, the use of PD-1/PD-L1 inhibitors for HNSCC prior to surgery is being investigated with encouraging results. PD-L1 expression has also been shown to be highly expressed in HNSCC, but little is known about PD-L1 expression in preancancerous lesions in the head and neck region. Recently, some studies have started to investigate the expression of PD-L1 and other related TME markers in the head and neck region, and their results are highly variable.

In this review, we aimed to summarize the published evidence on the expression of PD-L1 in premalignant lesions of the head and neck region, in order to quantify the extent of PD-L1 expression and characterize the strengths, limitations, and future directions for such studies focused on preancancerous lesions.

## 2 MATERIALS AND METHODS

### 2.1 Literature search

We aimed to comply with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA). A literature search without language restrictions was carried out in the electronic databases MEDLINE-PubMed and EMBASE and the Cochrane library until December 15, 2019. Additional sources of gray literature with opengrey.

### 2.2 Article screening

Two investigators (I. G. and A. E.) independently screened all article titles and abstracts with the aid of Rayyan QCRI reference manager web application. Disagreement was resolved by consensus. Full texts of the articles that fulfilled the initial screening criteria were acquired and reviewed for subsequent inclusion, against the eligibility criteria, and disagreement was resolved by consensus. Inclusion criteria were: the presence of premalignant epithelial squamous lesions in the head and neck region with use in the study of immunohistochemical staining or immunofluorescence in situ hybridization for PD-L1 to quantify the positivity for this marker. Studies not concerning head and neck sites or dealing only with invasive squamous cell carcinomas (SCCs) were excluded, as were studies on animal models, not using morphological investigations such as cellular cultures, blot assays, or molecular techniques. The presence in the available literature of an abstract only representing a study was not considered an exclusion reason.

### 2.3 Data extraction

Two investigators (I. G. and A. E.) extracted data from the included studies. A standardized form for extraction and presentation was used. Data extracted were: author and publication year, country, type of paper if full article or abstract, design of study if prospective or retrospective, population of cases if comprising only dysplastic lesions or also invasive squamous carcinomas or hyperplastic/reactive lesions, site of lesions, number of cases, clone of immunohistochemical PD-L1 antibody with available details of staining procedure, modality of assessment of PD-L1 staining if on whole section or tissue microarray (TMA), scoring system for positivity and quantification of PD-L1 staining, number of positive and negative cases, any other outcome present in the study (e.g., correlation between PD-L1 staining and other IHC markers, odds ratios, and hazard ratios of PD-L1 staining with recurrence or malignant transformation of preancancerous lesions).
2.4 | Quality assessment

To evaluate the quality of studies included in quantitative synthesis, the Newcastle-Ottawa scale (NOS) was used. Given that studies were likely to be retrospective observational studies with performing of staining on archival cases with or without a direct comparison of expression of markers among different lesions, the NOS is an adequate tool for quality assessment of observational studies and comprises three key areas or domains: selection of participants, comparability, and outcomes. The NOS is a “star/point system” in which a study could be awarded a star/point for each item of the domains if some prespecified requirements are met. An advantage of the NOS is its adaptability to different fields, as the requirements for each item could be defined according to the specific review question. For the item of ascertainment of exposure, a star was given only when the study used whole-section IHC and the clone was provided. For the comparability/control item, studies that assessed correlation of PD-L1 expression with at least one other factor received one star, whereas studies that assessed and described PD-L1 in the tumor immune microenvironment received an additional star. Given our interest in the prevalence of staining positivity of PD-L1 in premalignant lesions as the outcome to be assessed, we applied a modified version of the NOS with removal of the two follow-up items, as the outcome of interest is not a time-event outcome. Two authors (I. G. and A. E.) completed the NOS for each study, which could be awarded up to 7 points, with ≤4 points indicating a high risk of bias.

2.5 | Quantitative synthesis and statistical analysis

All analyses were carried out using open-source software R 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria) with RStudio 1.2.5033 environment (RStudio Inc, Boston, Massachusetts) and Review Manager 5.3 (The Nordic Cochrane Center, Cochrane Collaboration, Copenhagen, 2014). Pooled proportions and their 95% confidence intervals (CIs) of cases positive to PD-L1 staining were calculated using DerSimonian-Laird random-effects model after double-arcsine transformation.20,21 When studies provided data regarding prevalence of PD-L1 positivity in both precancerous lesions and normal mucosa as control or SCC, a comparison meta-analysis was performed. Results were expressed as a risk ratio (RR) with positivity for PD-L1 as outcome event, and pooled RR with 95% CIs was calculated using DerSimonian-Laird random-effects model. Heterogeneity across studies was assessed by the $I^2$ metric and chi-square statistics. Pooled estimates were also recalculated after removal of outlier studies (ie, the studies which lie outside the bulk of other studies and could have an impact on the pooled effect size estimate) and a series of meta-regression analyses according to potential moderators at the study level were considered. Visual inspection of the funnel plot and formal Egger test for publication bias were performed.

3 | RESULTS

3.1 | Literature search

A total of 1058 articles were identified after the removal of duplicates. Of these, 18 were identified as potentially relevant to our study after initial title and abstract screening. After reading the full text, 14 studies were included in the qualitative synthesis, and data for a quantitative synthesis with meta-analysis were available in 9 studies. A detailed flow diagram of the literature screening and exclusion of all articles according to the PRISMA statement is shown in Figure 1.

3.2 | Characteristics of included studies

The studies included in this systematic review involved patients with potentially malignant squamous lesions from Asia, Europe, South America, and North America. Two of the included studies were represented by abstracts only. The type of premalignant lesions in studies varied: eight studies dealt with oral dysplasia lesions, three studies considered oral leukoplakia with or without dysplasia, two studies used the broad terminology of “oral epithelial premalignant lesion,” and one focused on actinic cheilitis. The prevalence of PD-L1 expression was one of the main aims of the study in nine publications, while in the remaining studies the main outcome was the expression of other markers of the immunological microenvironment and their correlation with PD-L1 among other molecules. Summary information on the included studies is found in Table 1.

3.3 | Quality assessment

The mean score of the included studies was 5.2, with six studies demonstrating a high risk of bias; five of these studies did not report the positivity for PD-L1 data. The mean score of studies providing data for quantitative synthesis was 5.9, with one study (represented by an abstract
only) noted to exhibit high risk of bias. The summary of the quality assessment is found in Supplementary Table S2.

3.4 | Prevalence of PD-L1 in precancerous lesions and subgroup analysis

Pooling data from nine studies,\textsuperscript{25,26,30,33-38} the pooled prevalence of PD-L1 positivity in precancerous lesions of the head and neck was 48.25\% (CI 21.07-75.98) with high heterogeneity (\(I^2\) 98\%, \(\tau^2\) 0.1797, \(P < .01\); Figure 2). The pooled prevalence of PD-L1 positivity was recalculated after removal of an outlier study (the study by Gonçalves et al\textsuperscript{35}) which was individuated with the assessment of studentized residuals, but high heterogeneity remained (pooled estimate 38.34\%, CI 19.17-59.48, \(I^2\) 95\%, \(\tau^2\) 0.0822, \(P < .01\)).

A series of meta-regression analyses was carried out based upon: whether sample size of the study was smaller or greater than the median of studies, the presence in the...
<table>
<thead>
<tr>
<th>Study author, year (Country)</th>
<th>Type of precancerous lesion (N)</th>
<th>IHC procedure and evaluation</th>
<th>Other lesions evaluated (N)</th>
<th>Other protein abnormalities evaluated</th>
<th>Proportion of dysplastic cases (%)</th>
<th>Description of other outcomes and limitations of study</th>
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<tr>
<td>Chen, 2019 (China)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Oral leukoplakia (21)</td>
<td>Whole section, clone SP142, (Abcam, Cambridge, UK, 1:100); semiquantitative scoring with cutoff 5% for positivity</td>
<td>OSCC (41), normal mucosa (25)</td>
<td>CD8 in TILs</td>
<td>28.4%</td>
<td>Higher expression of PD-L1 in OSCC and oral leukoplakia compared to controls; high correlation of PD-L1 expression with CD8 expression in TILs; higher expression of PD-L1 in OSCC not significantly associated with better survival. Small sample size; not clear whether the assessment of PD-L1 staining with image analysis tool comprises TME cells or tumor cells only.</td>
</tr>
<tr>
<td>Glass, 2017 (USA)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Oral dysplasia (12)</td>
<td>Not stated</td>
<td>OSCC (10)</td>
<td>PD-1</td>
<td>100%</td>
<td>Expression of PD-L1 in 50% of OSCC. No reporting of IHC procedure.</td>
</tr>
<tr>
<td>Gonçalves, 2017 (Brazil)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Oral leukoplakia (80)</td>
<td>Whole section, clone E1L3N, (Cell Signaling Technology, Danvers, Massachusetts, 1:400); semiquantitative scoring with 0% as negative</td>
<td>OSCC (20), normal mucosa (20)</td>
<td>Granzyme, HLA-G, HLA-E, PD-L1, IL-10, TGF-β1, -β2, and -β3 proteins</td>
<td>87.5%</td>
<td>Higher expression of HLA-G, -E, IL-10, TGF-β2, and -β3 in oral leukoplakia than in normal mucosa, similar to that of the OSCC group; density of granzyme positive immune cells in oral leukoplakia more similar to that of control group. The 100% prevalence of PD-L1 expression is not fully discussed.</td>
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<tr>
<td>Kouketsu, 2017 (Japan)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Oral epithelial precursor lesions (79)</td>
<td>Whole section, clone SP142, (Spring Bioscience, Pleasanton, California); semiquantitative scoring with 0% as negative</td>
<td>OSCC (106)</td>
<td>PD-1</td>
<td>69.6%</td>
<td>Positive correlation between PD-L1 and PD-1 in both precursor lesions and OSCC; no correlation of PD-L1 and PD-1 with clinical features in precursor lesions. No major limitations.</td>
</tr>
<tr>
<td>Lopes, 2018 (Brazil)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Actinic cheilitis (55)</td>
<td>Whole section, clone E1L3N (Cell Signaling Technology, Danvers, Massachusetts, 1:400); semiquantitative scoring with 0% as negative on scanned WSI slides</td>
<td>LSCC (40), normal mucosa (10)</td>
<td>Granzyme, HLA-G, CD8 in TILs</td>
<td>69.1%</td>
<td>Higher expression of PD-L1 and HLA-G in LSCC and actinic cheilitis than in controls; overexpression of CD8 and Granzyme B in LSCC; no correlation of PD-L1 with clinical features; correlation of PD-L1 and HLA-G in epithelial cells with stromal expression and CD8 in TILs. No major limitations.</td>
</tr>
<tr>
<td>Sieviläinen, 2018 (Finland)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Oral dysplasia (47)</td>
<td>Whole section, clone 28-8 (ab205921 Abcam, 1:400); quantitative</td>
<td>Normal mucosa (9)</td>
<td>IDO1</td>
<td>100%</td>
<td>PD-L1 expressed only in inflammatory cells of lamina propria; no correlation with clinical features.</td>
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<tr>
<td>Study author, year (Country)</td>
<td>Type of precancerous lesion (N)</td>
<td>IHC procedure and evaluation</td>
<td>Other lesions evaluated (N)</td>
<td>Other protein abnormalities evaluated</td>
<td>Proportion of dysplastic cases (%)</td>
<td>Description of other outcomes and limitations of study</td>
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<td>Stasikowska, 2018 (Poland)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Oral leukoplakia (23)</td>
<td>Selected HPF of whole section, clone not available (Abcam, 1:400); semiquantitative scoring with cutoff 5% for positivity</td>
<td>OSCC (70), normal mucosa (19)</td>
<td>CD8, CD163, and PD-L1 in immune cells</td>
<td>100%</td>
<td>The negative expression of PD-L1 in dysplastic epithelium is not fully discussed.</td>
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<tr>
<td>William, 2018 (USA)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral premalignant lesions (188)</td>
<td>Multiplex immunofluorescence, clone E1L3N NOS; 1% cutoff for positivity</td>
<td>None</td>
<td>AE1/AE3, PD-L1, CD3, CD8, CD68; LOH, and EGFR copy number gain</td>
<td>Not clearly stated</td>
<td>Lesions with high-risk LOH profiles had increased epithelial PD-L1 expression, intraepithelial CD68+/PD-L1+ cells; increased epithelial PD-L1 expression was associated with inferior OCFS together with LOH status and EGFR copy number gain. No clear reporting of proportion of histologically dysplastic cases.</td>
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<td>Wu, 2016 (China)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral dysplasia (48)</td>
<td>TMA, clone not available (Cell Signaling Technology, 1:100); automated quantification with digital image software on WSI slides</td>
<td>OSCC (204), normal mucosa (43), metastases (41)</td>
<td>B7-H4, CD11b, CD33, P13Kα p110, p-S6</td>
<td>100%</td>
<td>Prognostic role of expression of B7-H4 in OSCC, correlation of B7-H4 expression with other molecules. No reported proportion of positive cases for other molecules. TMA study; not clear whether the assessment of PD-L1 staining with image analysis tool comprises TME cells or tumor cells only.</td>
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<td>Wu, 2017 (China)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral dysplasia (48)</td>
<td>TMA, clone not available (Cell Signaling Technology, 1:100); automated quantification with digital image software on WSI slides</td>
<td>OSCC (204), normal mucosa (43), metastases (41)</td>
<td>VISTA, CTLA-4, P13Kα p110, IL13Ra2, p-STAT3, CD11b, CD33</td>
<td>100%</td>
<td>Prognostic role of expression of VISTA in OSCC, correlation of VISTA expression with other molecules. No reported proportion of positive cases for other molecules. TMA study; not clear whether the assessment of PD-L1 staining with image analysis tool comprises TME cells or tumor cells only.</td>
</tr>
<tr>
<td>Wu, 2019 (China)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral dysplasia (69)</td>
<td>TMA, clone not available (Cell Signaling Technology, 1:100); automated quantification with digital image software on WSI slides</td>
<td>OSCC (270), normal mucosa (42)</td>
<td>FAM3C, VISTA, B7-H4, Slug, SOX2, ALDH1</td>
<td>100%</td>
<td>Prognostic role of expression of FAM3C in OSCC, correlation of FAM3C expression with other molecules. No reported proportion of positive cases for other molecules. TMA study; not clear whether the assessment of PD-L1 staining with image analysis tool comprises TME cells or tumor cells only.</td>
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<tr>
<th>Study author, year (Country)</th>
<th>Type of precancerous lesion (N)</th>
<th>IHC procedure and evaluation</th>
<th>Other lesions evaluated (N)</th>
<th>Other protein abnormalities evaluated</th>
<th>Proportion of dysplastic cases (%)</th>
<th>Description of other outcomes and limitations of study</th>
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<tr>
<td>Yagyuu, 2017 (Japan)</td>
<td>Oral dysplasia (120)</td>
<td>Five HPF on whole section, clone not available (Spring Bioscience, California, 1:50), semiquantitative with 0% as negative</td>
<td>None</td>
<td>CD8, CD163</td>
<td>100%</td>
<td>Only CD163 in immune cells associated with high-grade dysplasia; epithelial and immune cells positivity for PD-L1 associate with lower malignancy free survival. Clone not reported; no other major limitations.</td>
</tr>
<tr>
<td>Yang, 2018 (China)</td>
<td>Oral dysplasia (48)</td>
<td>TMA, clone not available (Cell Signaling Technology, 1:100); automated quantification with digital image software on WSI slides</td>
<td>OSCC (204), normal mucosa (43), metastases (41)</td>
<td>CD317, B7-H3, B7-H4, CD68, CD163</td>
<td>100%</td>
<td>Prognostic role of expression of CD317 in OSCC, correlation of CD317 expression with other molecules. No reported proportion of positive cases for other molecules. TMA study; not clear whether the assessment of PD-L1 staining with image analysis tool comprises TME cells or tumor cells only.</td>
</tr>
<tr>
<td>Yang, 2019 (2019)</td>
<td>Oral dysplasia (69)</td>
<td>TMA, clone not available (Cell Signaling Technology, 1:100); automated quantification with digital image software on WSI slides</td>
<td>OSCC (270), normal mucosa (42), metastases (68)</td>
<td>LAMTOR5, p-Akt, p-S6, Galectin-9, VISTA, B7-H4, CD68, CD163</td>
<td>100%</td>
<td>Prognostic role of expression of LAMTOR5 in OSCC, correlation of LAMTOR5 expression with other molecules. No reported proportion of positive cases for other molecules. TMA study; not clear whether the assessment of PD-L1 staining with image analysis tool comprises TME cells or tumor cells only.</td>
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Note. In italics studies represented by abstracts only.

Abbreviations: ALDH1, aldehyde-dehydrogenase 1; EGFR, epidermal growth factor receptor; FAM3C, family with sequence similarity 3 member C; HPF, high power fields; IDO1, indoleamine 2,3-dioxygenase 1; IHC, immunohistochemistry; LAMTOR5, late endosome/lysosomal adapter and MAPK and mTOR activator 5; LOH, loss of heterozigosity; LSCC, lip squamous cell carcinoma; N, number; NOS, not otherwise specified; OSCC, oral squamous cell carcinoma; TILs, tumor-infiltrating lymphocytes; TMA, tissue microarray; TME, tumor microenvironment; VISTA, V-domain suppressor of T cell activation; WSI, whole-slide imaging.

aIncluded in both qualitative and quantitative synthesis.

bIncluded in qualitative synthesis only.
population of premalignant lesions of both cases with histologically defined dysplasia (e.g., leukoplakia with histological dysplasia) and cases with no histological dysplasia, country of sample if from BRICS countries (e.g., Brazil and China) vs other, and choice of cutoff 5% vs 0% for PD-L1 positivity. Neither sample size (slope 0.05 ± 0.31, \(P = .88\)), nor the mixture in the sample of dysplastic and nondysplastic premalignant lesions (slope 0.33 ± 0.29, \(P = .29\)), nor the choice of cutoff (slope 0.35 ± 0.38, \(P = .36\)) were moderators of heterogeneity. Only countries other than BRICS appeared to be a significant moderator of heterogeneity (slope −0.59 ± 0.25, \(P = .02\)), with a higher proportion of PD-L1 expression in studies from BRICS countries (85.49%, CI 57.14-99.96 vs 28.37%, CI

**FIGURE 2** Pooled estimate of programmed death-ligand 1 (PD-L1) positivity in all studies. CI, confidence interval [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 3** Pooled estimate of programmed death-ligand 1 (PD-L1) positivity in studies providing data for only dysplastic cases. CI, confidence interval [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 4** Comparison of programmed death-ligand 1 (PD-L1) expression in precancerous lesions and normal mucosa. CI, confidence interval [Color figure can be viewed at wileyonlinelibrary.com]
Pooled RR of PD-L1 positivity was 0.68 (CI 0.43–0.47, P = .02). Given that some studies were comprised of both truly dysplastic lesions and precancerous lesions with hyperplastic/reactive changes in their samples, when a separate proportion of PD-L1 expression in dysplasia lesions only and in hyperplastic/reactive lesions was reported we could specifically assess the expression of PD-L1 in dysplastic only cases. The pooled estimate was 45.04% (CI 7.39–86.30, I² 98%, tau2 0.2936) (Figure 3).

Six studies²⁵,²⁶,³³–³⁶ allowed comparison of PD-L1 expression in premalignant lesions to normal mucosa. Pooled RR of PD-L1 positivity was 1.65 (CI 0.65–4.03, P = .30) showing a tendency to higher positivity rate in precancerous lesions, but without reaching statistical significance and with high heterogeneity (I² 91%, tau2 0.82) (Figure 4). Five studies²⁵,²⁶,³³–³⁶,³⁸ allowed comparison of PD-L1 expression in premalignant lesions to invasive SCC. Pooled RR of PD-L1 positivity was 0.68 (CI 0.43–1.08, P = .10) showing a tendency to lower positivity rate in precancerous lesions compared to SCC, but without reaching statistical significance and with high heterogeneity (I² 91%, tau2 0.22) (Figure 5). Visual inspection of funnel plot and Egger’s test did not provide evidence for publication bias (P = .98).

4 DISCUSSION

Immunotherapy with PD1/PD-L1 inhibitors has been approved in the last few years for advanced HNSCC.¹²,³⁹,⁴⁰ A recent systematic review highlighted that HNSCC tumors with PD-L1 expression show a greater tumor response and better overall survival.⁴¹ Besides the approval of PD1/PD-L1 inhibitors for many invasive cancer types, the role of the PD1/PD-L1 axis has also been investigated in precancerous lesions, particularly in intraepithelial neoplasia of the cervix and in breast DCIS.⁸–¹¹ Given the role of the PD1/PD-L1 axis in the development of immune tolerance against tumor-associated antigens and the complexity of interactions in the TME, it has been speculated that intraepithelial neoplasia and precancerous lesions could reflect a situation where there is a balance between the host immune response to cancer development and the cancer’s ability to escape the host response.⁴²,⁴³ Immunotherapy with checkpoint inhibitors in this case would accordingly be considered preventive therapy which could help the host immune system to revert the induced potential tolerance against cancer cells and move the tumoral microenvironment back to a situation of immune control. Indeed, one could speculate that in clinical situations where premalignant lesions could not be fully removed by surgery, checkpoint inhibitors could be used as preventive therapy against malignant transformation, or in situations where dysplasia is present at surgical margins, given that administration of checkpoint inhibitor therapy is now being investigated in clinical settings other than advanced cancer.¹⁶ The use of immunotherapy, however, relies on the expression of PD-L1 by cancer cells and could not be as effective as expected if prevalence of expression in these precancerous lesions is low. In the head and neck region, oral leukoplakia represents the most frequent premalignant lesion and like other precancerous lesions harbors a variable risk of malignant transformation.⁴⁴ Some studies have investigated the expression of PD-L1 both in epithelial dysplastic cells and in the TME, to discover potential correlations with prognosis of premalignant lesions and with other clinical and histological features.

The reported prevalence of PD-L1 expression with various IHC assays in premalignant squamous lesions in the included studies is highly variable, and our findings confirm the high heterogeneity of studies. Indeed, of 14 included studies only 9 reported the proportion of PD-L1 positive cases, and the pooled estimate of PD-L1 positivity from these studies was 48%. The first consideration is that about half of precancerous lesions of head and neck can be positive for PD-L1, and this could be an important starting point for the use of checkpoint inhibitors as a preventive therapy. However, we believe that this estimate should be considered with caution. These studies were highly heterogeneous in terms of selection of cases, IHC assay used and sample size. The most frequently investigated lesions were premalignant lesions of the oral cavity, with only one study dealing with actinic cheilitis.³⁶ This is not surprising, as the oral cavity is considered the most common site of occurrence of premalignant lesions in the head and neck region, lesions that are easily detected and removed, as well as in which carcinogenesis factors are well established. However, the definition of these lesions was not always clear, as in most of the studies these lesions were generally defined as “oral dysplasia” or “oral premalignant lesion.” Moreover, five out of nine studies²⁵,²⁶,³³–³⁷ with available data also included in their population lesions without histologically proven dysplasia, such as leukoplakia with no dysplasia or hyperplastic/hyperkeratotic lesions, and only two²⁶,³⁵ of these provided separate data for PD-L1 expression in dysplastic and nondysplastic lesions. This could somehow hamper the reliability of the overall estimate of the prevalence of PD-L1 expression in these types of cases. Indeed, when pooling data concerning only dysplastic lesions the estimate of PD-L1 expression prevalence is 45% and high heterogeneity among studies remains. We speculated that study characteristics such as the mixture of lesions in the case population, sample size,
and choice of cutoff for PD-L1 positivity could be moderators of heterogeneity, but all of these failed to show significance in our moderator analysis. Only country other than BRICS where the study was performed showed statistical significance as a moderator. We are aware that the overall number of studies included in this meta-analysis is low which could cause the moderator analysis to have spurious associations.

The same considerations apply when we investigate the difference in prevalence of PD-L1 expression between precancerous lesions and normal mucosa or between precancerous lesions and invasive SCC. Pooling the data from six studies, the precancerous lesions were shown to have a higher level of PD-L1 expression, but importantly did demonstrate imprecision and high heterogeneity across these studies. On the other hand, precancerous lesions had a lower positivity rate than SCC, despite having the same degree of imprecision and heterogeneity. We speculate that the tendency for precancerous lesions to show a higher prevalence of PD-L1 positivity compared to normal mucosa, but lower compared to SCC, could reflect a situation where a biological alteration has already been established directed towards neoplastic transformation, but is also still under the control of the immune system, as suggested by studies on the balance of immune response.42,43 In this context, the estimated result of 48% prevalence of PD-L1 expression appears to reasonably lie between the very low expression observed in normal oral mucosa and the higher expression in SCC. Indeed, when considering tumor cell positivity only (with tumor proportion score), around 50% to 60% of HNSCC are PD-L1 positive,45 but this percentage increases to 85% when immune cells of the TME are also taken into consideration, as shown in trials that used a combine proportion score (CPS).14,46 CPS has been shown to be more predictive of response to checkpoint inhibitors in HNSCC,14,46 and this may reflect the importance of immune cells in the TME for the development and maintenance of tumor tolerance and therefore tumor progression. This was also proposed in some of the included studies,30,33 particularly the work of Yagyuu et al30 which is the only study to show a predictive value of PD-L1 expression on both tumor and immune cells for malignant transformation of dysplastic lesions. Furthermore, in some of the studies that assessed PD-L1 both in tumor and immune cells,26,30,34,36,37 a significant difference was highlighted between SCC and premalignant lesions as well as with normal controls. This trend was also highlighted in our meta-analysis, even without reaching statistical significance. This may suggest a similarity with HNSCC in terms of importance of combined expression in both tumor and immune cells to more accurately describe the biological process. Consequently, intermediate expression of PD-L1 puts premalignant lesions in the middle of a continuum from normal/hyperplastic lesions to SCC, even if available data are still insufficient to draw strong conclusions. Indeed, studies that clearly distinguished hyperplastic, low grade and high grade dysplasia lesions did not report any statistical difference among these types of lesions. However, one must also take into consideration that sample sizes may be too small to highlight a strong difference.26,33 Notwithstanding these caveats, perhaps these findings can suggest a pathway for this biological process. Further studies with a larger sample size are needed to refine the evaluation of PD-L1 expression in precancerous lesions.

Another factor to consider is the overall quality of these studies. On a modified NOS tool, the quality of studies was variable. The articles with a low quality were mainly from studies that did not provide data for quantitative synthesis. Of note, two of the included studies in our meta-analysis were represented by abstracts only.37,38 However, even fully published papers did not report a detailed description of the IHC assay used: given that high variability is known among different clones and IHC platforms, if all studies had reported the clone and the procedure it would have been possible to explore whether the IHC clone could have had a role in the observed heterogeneity in these studies and thus had an impact on the overall estimate of PD-L1 expression. At

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Precancerous lesions Events</th>
<th>Total</th>
<th>Squamous cell carcinoma Events</th>
<th>Total</th>
<th>Weight</th>
<th>Risk Ratio</th>
<th>M.H., Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen 2019</td>
<td>13</td>
<td>21</td>
<td>40</td>
<td>41</td>
<td>22.3%</td>
<td>0.63 [0.45, 0.89]</td>
<td></td>
</tr>
<tr>
<td>Glass 2017</td>
<td>2</td>
<td>12</td>
<td>5</td>
<td>10</td>
<td>7.5%</td>
<td>0.33 [0.08, 1.36]</td>
<td></td>
</tr>
<tr>
<td>Kouketsu 2017</td>
<td>21</td>
<td>79</td>
<td>72</td>
<td>106</td>
<td>21.5%</td>
<td>0.39 [0.27, 0.58]</td>
<td></td>
</tr>
<tr>
<td>Lopes 2019</td>
<td>42</td>
<td>55</td>
<td>29</td>
<td>40</td>
<td>23.8%</td>
<td>1.05 [0.82, 1.34]</td>
<td></td>
</tr>
<tr>
<td>Stasikowska 2018</td>
<td>21</td>
<td>23</td>
<td>67</td>
<td>70</td>
<td>24.9%</td>
<td>0.95 [0.83, 1.09]</td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>190</strong></td>
<td><strong>267</strong></td>
<td><strong>100.0%</strong></td>
<td><strong>267</strong></td>
<td><strong>100.0%</strong></td>
<td><strong>0.68 [0.43, 1.06]</strong></td>
<td></td>
</tr>
</tbody>
</table>

**FIGURE 5** Comparison of programmed death-ligand 1 (PD-L1) expression in precancerous lesions and squamous cell carcinoma. CI, confidence interval; OSCC, oral squamous cell carcinoma [Color figure can be viewed at wileyonlinelibrary.com]
the same time, studies not included in quantitative synthesis investigated the role of other markers of the immunological environment of precancerous lesions such as B7-H4, CD317, and V-domain suppressor of T cell activation and show variable correlation with PD-L1 expression, indicating that these other markers could be of interest in unraveling the true mechanism of the immune TME.

In summary, our work has some strengths and obviously some limitations. The strengths reside in the methodology of the systematic review, as it allowed us to objectively gather all of the evidence pertaining to the expression of PD-L1 in precancerous lesions of the head and neck region and highlight some interesting points. The limitations were related to the retrieved studies themselves. Indeed, the sample size of these studies was small in comparison with studies dealing with invasive SCC, and precancerous lesions are not always clearly defined. To establish a more reliable understanding of PD-L1 expression in premalignant lesions, we recommend that in future studies enrolled cases get selected and grading of dysplasia is handled according to standardized reporting guidelines. In addition, it also necessary to make a harmonization effort regarding both the use of the different anti-PD-L1 antibodies and the different evaluation score. Furthermore, almost all the studies included in our analysis dealt with lesions of the oral cavity, with only one study dealing with actinic cheilitis and no other premalignant lesions of other anatomic sites of the head and neck region. This accordingly makes it difficult to uncover potential differences in regional expression of PD-L1. Looking forward, we call for the contribution of larger studies with clearly defined subgroups of precancerous lesions. This will help better estimate the prevalence of PD-L1 expression in the head and neck region which could in turn help determine the potential application of immunotherapy as a preventive therapy in this setting.

CONFLICT OF INTEREST
The authors declared no potential conflicts of interest.

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REFERENCES


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