Prognostic Role of PD-L1 Expression in Oral Squamous Cell Carcinoma Varies by Oral Compartments and Immunostaining Patterns: A Meta-analysis

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January 24, 2023

Abstract

Blocking the PD-L1/PD-1 pathway efficiently enhanced antitumor immunity that reduced tumour growth and improved survival, whereas the prognostic roles of PD-L1 positivity in oral squamous cell carcinoma (OSCC) were controversial. This study aimed to determine the clinicopathological and prognostic significance of PD-L1 expression in tumor cells (TCs) or tumor-infiltrating lymphocytes (TILs) of OSCC. The systematic retrieve was performed for seeking suitable studies through PubMed, Web of Science, the Cochrane Library and Scopus. 46 studies were ultimately included in the meta-analysis. This study showed that the levels of PD-L1 expression in TCs were relatively high in femal patients (P <0.001), non-smoker (P <0.001), non-drinkers (P =0.037), advanced stage (P =0.028), N+ status (P =0.027), and tumours with high levels of PD-1 (P =0.024), and CD8+ (P =0.022). High PD-L1 expression in TCs had significant effect on worse LRFS (P=0.004), and also was more likely to have worse OS in Asia (P =0.018). Both DSS (P =0.035) and DFS (P =0.003), at a 5% cut-off of PPC, had positive association with high PD-L1 expression. The results of OS showed that a worse prognosis for 5H1 (P =0.032), and a favourable prognosis for 22C3(P =0.001). E1L3N was shown to be associated with an worse DSS (P =0.014). High expressions of PD-L1 in TCs had a worse OS (P =0.023) and DFS (P =0.003) in OSCC of the tongue. Consequently, future study should especially consider oral compartments and methods for PD-L1 immunostaining as confounding factors when observing PD-L1 of response to anti-PD1 therapy.

1 Introduction

Oral squamous cell carcinoma (OSCC) is the most commonly diagnosed oral cancer, which accounts for approximately 90% of all malignant oral neoplasm[1]. Programmed cell death ligand-1 (PD-L1) is a surface glycoprotein of cancer cells, which is regarded as the inhibitory receptor programmed cell death-protein 1 (PD-1) on T lymphocytes[2]. PD-L1 involves in the immune response evasion by binding to PD-1 that downregulates T-cell responses.

As one of the most common immunologic checkpoints, the axis PD-1/PD-L1 has showed prognostic significance that mediates immune tolerance. Accompanied by the increasing number of researches finding that activation of immune checkpoint blockade is effective in tumour growth control and survival extension, controversy has also been persistent due to non-homogeneous conclusions. Several studies approved of either positive[3,4][4, 5], or negative association of PD-L1 overexpression with prognosis[5,6], while other studies showed no prognostic significance of PD-L1 expression[7,8].

Inconsistent results were mainly presumed to be related to the various methods for immunohistochemical staining, stage of diagnosis, tumor location, and the feasibility of radical surgical resection[9]. The previous

meta-analyses reached a consensus that PD-L1expression in OSCC patients was not significantly correlated with overall survival (OS), but major limitation of them did not consider oral subsites or immunostaining patterns as confounding factors.

To date, the role of PD-L1 expression in OSCC of tongue has attracted attention of the increasing number of studies. It was reported that high PD-L1 expression was associated with worse OS that was exclusively found in OSCC of the tongue. However, the prognostic significance of PD-L1 expression was not observed in oropharyngeal carcinomas[10]. To find the discrepancy complicated by the use of different immunostaining patterns, comparison was conducted stratified by various scoring systems, antibodies clone. One study indicated that no relationship between PD-L1 expression and survival was found by using either combined positive score (CPS) or tumour proportion score (TPS)[9]. In contrast, other reports have found high PD-L1 expression was linking to better prognosis when cut off of [?] 5% of TPS and [?] 1 of CPS[11]. A recent approach assessed PD-L1 expression measured by two different anti-PD-L1 antibodies (22C3, E1LN3), and revealed that high PD-L1 expression detected by 22C3 presented a worse prognosis in terms of disease-specific survival (DSS)[12].

Therefore, it was appropriate for performing a meta-analysis to determine whether the prognostic significances of PD-L1 expression in OSCC differed by oral compartments and methods for PD-L1 immunostaining.

2 Materials and methods

2.1 Search strategy

Searches strategy were carried out in PubMed, Web of Science, the Cochrane Library and Scopus for all studies, and search terms were developed as follows: ((oral OR mouth OR mouth [mesh] OR mouth neoplasms [mesh] OR tongue OR tongue neoplasms [mesh] OR alveolar OR gingiva* OR lip OR gum OR buccal OR palate* OR retromolar OR head and neck OR head and neck neoplasms[Mesh]) AND (squamous cell carcinoma OR squamous cell carcinoma [mesh] OR cancer)) AND (PD-L1 OR PDL1 OR Programmed Death Ligand 1 OR B7-H1 OR B7H1 OR CD274 OR PDCD1L1).

2.2 Study selection and inclusion criteria

In this systematic review, original studies were selected when conformed to the established inclusion criteria, as further stated subsequently: (1) Publications using English language. (2) Data not received on animal samples. (3) Observational studies. (4) Reports focusing on the evaluation of PD-L1 in clinicopathological parameters and survival outcomes. (5) Studies supplying existing odds ratio (OR) or hazard ratio (HR) along with 95% CI , or else, adequate data to calculated. (6) Participants not undergoing any radiotherapy/chemotherapy before surgical resection.

Two researchers were responsible for independently screening titles and abstracts, and then deeply evaluated the retrieved articles in the primal search. The articles were discarded that were performed in nonhuman subjects, editorials, duplicates, expert comments, review in various forms, meta-analysis, clinical trials.

2.3 Data extraction and risk of bias assessment

For all enrolled studies, the following data were extracted by two reviewers in regards of authors, publication years, county, number of patients, anti-PD-L1 antibody, definitions of PD-L1 positivity, IHC cutoffs, anatomic location, clinicopathological and prognostic variables. The risk of bias in the individual studies was appraised using the Reporting Recommendations for Tumour Marker Prognostic (REMARK) guidelines[13],which were composed of six dimensions as summarised in **Supplemental Fig.S2.**

2.4 Statistical analysis

Meta-analysis of this study included two aspects. In the primary aspects, pooled estimates of the OR were estimated for the associations of PD-L1 expression in clinical parameters, which were computed by natural logarithm OR, and its standard errors (SEs). In the second aspects, pooled estimates of HR of PD-L1 related to prognostic factors were performed by a random-effects model, in which natural logarithm HR and its SEs

were calculated. The estimated HR of individual study was preferred to entered to this analysis if they was reported in the case of various adjusted factors. Several studies did not provided direct HR, but its information were available for synthesizing the estimated HR, and then methods described in Tierney et al.[14] were performed to impute the estimated HR. We assumed clinicopathological and prognostic significance of PD-L1 expression was confounded by methods for PD-L1 immunostaining such as antibody, definition of PD-L1 positivity, IHC cutoffs. Studies reporting more than one type of them were separated as different data sets, correspondingly.

The heterogeneity across studies was investigated through Cochran's Q test ($p < 0.1$) and Higgins I^2, of which values 25%, 50% and 75% respectively indicated low, mild and high heterogeneity[15]. In order to identify the sources of heterogeneity, subgroup analyses were performed on the basis of grouping by assumed confounding factors. Pooled estimates synthesized by at least three studies were considered to be robust that no obvious fluctuation happened when removing one study at a time. Publication bias was determined by Begg's and Egger's tests[16].

3 Results

3.1 Characteristics and quality analysis of enrolled studies

Literature search was performed using the priori determined search strategy. A total of 2658 records were retrieved through searching databases of PubMed, Web of Science, the Cochrane Library and Scopus. After exhaustive screening and careful assessment, 46 studies including 4484 patients were ultimately included in the meta-analysis[1-9,11,12,17-51]. The detailed process and the reason of excluded studies were demonstrated as flow chart, **Fig.1** .

The characteristics of entered studies were expatiated in **Table 1** . Among them, two studies {de Vicente, 2019 #4;Foy, 2017 #7}were splitted as two separate data sets on account of different antibodies[12,39]{de Vicente, 2019 #4;!!! INVALID CITATION !!!, #0;Quan, 2020 #20}. One study was stratified as two separate data sets for prognosis due to two different tissue sources, and also, was tread as three separate data sets for clinical parameters based on three various assay cutoffs[9]. A study was converted to two separate data sets as a result of two different assay cutoffs. There were two separate data sets for clinical parameters and three separate data sets for prognosis in study reported by Pena-Cardelles et al.[11] Six studies focused on PD-L1 expression in TC and IC that were calculated separately as two independent datasets[1,23,34,35,47,49]. 46 enrolled studies were judged whether to provide clinical variables or prognostic variables, of which results were marked as "Y" or "N". The classification and assessment for variables suitable for synthesis were detailed in **Table S1** .

Quality analysis of enrolled studies was performed according to the evaluation criteria adapted from REMARK guidelines, of which results were summarized in **Supplemental Fig. S1.**

3.2 Comparison of high and low PD-L1 expression associated with clinical parameters

This study showed that high expression of PD-L1 in TCs was correlated with femal patients (OR,0.68; 95% CI: [0.56, 0.82]; P <0.001). Meta-analysis of twenty-three studies contrasting the smokers to non-smokers revealed that high PD-L1 expression in TCs was associated with non-smoker (OR, 0.62; 95% CI: [0.48, 0.79]; P <0.001). High expression of PD-L1 in TCs was observed in non-drinkers (OR, 0.69; 95% CI: [0.49, 0.98]; P =0.037) (**Fig.2**). However, the consequent meta-analysis suggested that the statistically significant association was disappeared between the expression of PD-L1 in TILs involving gender, smoking and drinking (**Supplemental File 2**).

There were no significant differences between the expression of PD-L1 in TCs and age, T status, M status, grade, or anatomical location. (**Supplemental Fig.S3-S7)** . Thirty studies were selected to identify whether the N status was associated with PD-L1 expression. The results showed that N+ patients were more likely to have high expression of PD-L1 when compared to N0 patients (OR, 1.34; 95% CI: [1.03, 1.74]; P =0.027)(**Supplemental Fig.S8)** .Tumor stages were grouped as stage I/II and stage III/IV that were used to assess the discrepancy in comparison of high/low expression. Seventeen studies were ultimately

enrolled in meta-analysis. The result showed that patients with stage III/IV had high PD-L1 expression (OR, 1.41; 95% CI: [1.04, 1.90]; P =0.028)(**Supplemental Fig.S9)** . The significance was also observed in the association of high PD-L1 expression in TCs with high levels of PD-1 (OR,33.57; 95% CI: [2.08, 542.52]; P =0.024), and CD8+ (OR,5.03; 95% CI: [1.23, 20.50]; P =0.022)(**Supplemental Fig.S10)** . No significant association was found between PD-L1 expression on TILs and any clinicopathological variables (**Supplemental File 2**).

3.3 Synthesis of results and subgroup analysis for association of high/low PD-L1 expression with prognostic factors

Meta-analysis of studies for OS indcated that PD-L1expression on TCs had no significant effect on OS (HR, 1.10; 95% CI: [0.86, 1.40]; P =0.461), and statistical heterogeneity (I^2 = 80.7%, p < 0.001) emerged(**Supplemental Fig.S11)** . Also for DSS (**Supplemental Fig.S12)** , fifteen studies reported ready-made HR or provided sufficient data to calculate the estimated HR that was synthesised that yield a no statistically significant result (HR, 1.22; 95% CI: [0.86, 1.74]; P =0.258) with significant heterogeneity (I^2 = 62.6%, p < 0.001). Likewise, the meta-analysis of studies for disease-free survival (DFS), progression-free survival (PFS) and local-regional progression-free survival (LRFS) were respectively performed, of which pooled estimated HR were achieved correspondingly. Only the result for LRFS showed significant finding (HR, 1.77; 95% CI: [1.20, 2.62]; P =0.004) without significant heterogeneity (I^2 = 0.0%, p = 0.590) (**Supplemental Fig.S13)** . Five studies assessed association between the expression of PD-L1 on TILs and OS, of which the pooled result showed no statistical significance (**Supplemental File 2**). The estimated HR for DSS, DFS, PFS and LRFS were unattainable to synthesize due to only one study reporting prognostic values.

Sources of heterogeneity might be attributed in part to geographic region, scoring systems, antibody type, tumour anatomic location. Due to the a lack of study on the relationship between PD-L1 expression on TILS and prognosis, subgroup analysis was performed only on studies with prognosis associated with TCs.

Geographic region was stratified for Asia and non-Asia with comparison for prognostic value and showed no significant differences for OS, DSS. Nevertheless, DFS showed high expression of PD-L1 in TCs was more likely to have worse prognosis (HR, 1.65; 95% CI: [1.09, 2.49]; P =0.018) (**Supplemental Fig.S14)** . Various scoring systems including semiquantitative evaluation (SE), percentage of positive cell (PPC), CPS , TPS, H score (combination of the staining distribution and intensity scoring systems), were used to recombine these studies for OS, DSS and DFS. There were no statistically significant results of prognosis showed in TPS or CPS. Both DSS (HR, 1.65; 95% CI: [1.04, 2.64]; P =0.035) and DFS (HR, 1.50; 95% CI: [1.15, 1.96]; P =0.003), at a 5% cut-off of PPC, had positive association with high PD-L1 expression(**Supplemental Fig.S15)** .

Subgroup analysis decided by antibody type was carried out for OS, DSS and DFS when two or more studies providing prognostic values for one type of antibody. The results of OS showed that a worse prognosis for 5H1(HR, 2.50; 95% CI: [1.08, 5.76]; P =0.032), and a favourable prognosis for 22C3(HR, 0.43; 95% CI: [0.27, 0.69]; P =0.001). For DSS, high PD-L1 expression detecting by E1L3N was shown to be associated with an worse prognosis(HR, 1.78; 95% CI: [1.13, 2.80]; P =0.014). There was no evidence suggested the association of DFS with PD-L1 expression was determined by antibody type(**Supplemental Fig.S16)** . Significant association of PD-L1 expressions in TCs with staining location was not detected(**Supplemental Fig.S17)** . Seven studies were viable for synthesising that reported OS in terms of location of tongue. It was shown that high expressions of PD-L1 in TCs had a worse prognosis in OSCC of the tongue (HR, 1.24; 95% CI: [1.03, 1.49]; P =0.023). Four studies assessed the DFS of PD-L1 expressions and results showed that high PD-L1 expression in TCs was associated with worse prognosis (HR, 2.03; 95% CI: [1.28, 3.22]; P =0.003)(**Fig.3**). Only one study was available for PFS, LRFS that was insufficient to combine.

Publication bias of studies in terms of the role of PD-L1 expressions in clinicopathological and prognostic was determined by Egger's tests and was shown by funnel plots, of which results suggested absence of proof (**Supplemental File 3**).

4 Discussion

The interactions of PD-L1 binding to its inhibitory receptor PD-1 inactivate the T cells recognizing the antigen of tumour cells, and consequently, the generation of population of cytotoxic T lymphocytes is reduced that provide an opportunity to cancer cells for escaping immune surveillance. This finding has triggered research to shift treatment from targeting molecules directly on the surface of cancer cells to non-contact methods for blocking the PD-L1/PD-1 pathway for better activation of the immune system[4]. An increasing number of studies have confirmed that the application of PD-1/PD-L1 treatment efficiently enhance anti-tumor immunity that reduces tumour growth and improves survival, whereas the prognostic roles of PD-L1 positivity in OSCC are controversial.

Our results revealed a significant association between PD-L1 expression levels and clinicopathological factors. The levels of PD-L1 expression in TCs were relatively high in female patients, non-smoker, non-drinkers, which in line with previous findings of meta-analysis reported by Lenouvel et al[52]. Consumption of alcohol and tobacco have been proved to be positively associated with OSCC recurrence and poor prognosis, but have no significant association with PD-L1 expression on TCs[4]. Previous study using PD-L1 antibody to detect the PD-L1 expression found that non-smokers or non-alcohol consumers express higher frequency of PD-L1 expression[12]. Interestingly, the protective effect of PD-L1 expression was observed in females for OS and DSS, which did not weaken with smoking, or drinking[1]. It has been reported that PD-L1 is overexpressed in never-smokers and never-drinkers (NSND) who undergo the immunosuppressive therapy with pembrozulimab. The enrichment of T-cell activation, interferon-γ(IFN-γ) and PD-1 signalling were observed in OSCC from NSND. Overexpression of PD-L1 induced by IFN-c, the unique feature in NSND, was associated with rate of TILs. It could be inferred that PD1/PD-L1 blockade enhanced the immunity response that provided potential benefit of PD1/PD-L1 inhibition in NSND[39]. In addition, our meta-analysis also revealed that PD-L1 upregulation in TC was associated with clinicopathological features involving advanced stage, N+ status, in favour of the findings of previous reports[19,24,28,30].

To date, the prognostic value of PD-L1 in OSCC cells has been identified in several meta-analysis. Consensus of them indicated that expressions of PD-L1 in TCs had protective effects on OS with no significance[52-56], which was different from our study identifying prognostic role of PD-L1 expression in TCs was insignificant and unfavorable. As for DFS, four meta-analysis suggested that expression of PD-L1 was associated with better survival regardless of no statistical significance[53-56]. However, a previous study hold the opposite perspective that expression of PD-L1 probably lead to reduced survival[52]. In terms of DSS, our results revealed that PD-L1 expression exhibited a trend towards worse survival on DSS, which in line with the results reported by Troiano et al and Lenouvel et al.[52,56] There was a significant association for worse LRFS in tumors characterized by high PD-L1 expression on TCs in our study. Geum et al. suggested locoregional recurrence had a significantly lower survival rate, and simultaneously, was significantly correlated with PD-L1 expression[3]. The remarkable advantages of this study was not only the abundant studies that was already enrolled in previous meta-analysis, but also in the first exclusively comprehensive evaluation of the oral compartments and immunostaining patterns as confounding factors for prognostic role of PD-L1 expression in OSCC.

The role of PD-L1 in the prognosis of OSCC have been undetermined due to expectable disparities in tissue of origin, location of staining, variations in immunostaining patterns (ie, assay cutoffs, antibody clones)[9]. In our study, high PD-L1 expression in TCs was associated with an disoperative effect on prognosis in DFS when only Asian patients of OSCC were analyzed. In OS and DSS of Asian patients, high PD-L1 expression on TC also had a tendency to increase the risk of poor prognosis. This might be attributed to the discrepancies in diet and lifestyle between the Asia and the non-Asia.

Of the selected studies, it was not difficult to find that tongue as the subsite of OSCC was more common than other subsite, suggesting that tongue might be an important site affecting the prognosis and clinicopathological factor of OSCC. In this study, we firstly compared the PD-L1 expression in TCs between tongue and other sites of OSCC patients, and the results showed no significant difference of any clinicopathological factors between the two groups. Next, we pooled the studies restricted only to tongue of OSCC with respect

to prognosis and, found a significant relationship between the high expression of PD-L1 in TCs and worse DSS and OS, and the heterogeneity simultaneously decreased from 81% to 0%. Previous study have demonstrated that PD-L1 overexpression in TCs of the tongue and the floor of the oral cavity was associated with with a worse OS[1]. It was reasonable to speculate that discrepancies of the results in regard to the role of PD-L1 expressions in prognosis was attributed to skewed distribution of cancer location.

The use of different antibodies clones for immunohistochemistry resulted in discrepant results of association between PD-L1 expression and prognosis in OSCC. A trial in lung cancer comparing four anti-PD-L1 antibodies (22C3, 28-8, SP142, E1L3N) found that the patients detected as PD-L1-positive cases was similar for all antibodies except for SP142 which was only 50% as much as the other three of them[57]. Interestingly, SP142 showed more sensitive detection efficiency than 22C3 in another trail lung cancer[58]. This study extracted antibody of PD-L1 including 22C3, 28-8, 5H1, E1L3N, SP142 as different subgroups. The results indicated that OS was worse in 5H1, but greater in 22C3 in OSCC patients with high PD-L1 expression in TCs. A study using two different anti-PD-L1 antibodies (clones E1L3N and 22C3) to evaluate the prognostic significance of tumor PD-L1 expression in OSCC. Consequently, significant results of DSS only appeared in 22C3 but not in E1L3N[12], in contrast to our findings demonstrating DSS was more likely to get worse with high PD-L1 expression detecting by E1L3N. It was not ignored that the positive expression ratio of tumor PD-L1 expression in OSCC tested by E1L3N was lower than 22C3 whether the cut off is [?] 1% or [?] 10%[12]. Subgroup analysis of our study revealed that almost no intra-heterogeneity was found in group of E1L3N and 22C3 in aspects of OS, DFS. Combined with our results, in OSCC, it can be believed that 22C3 seemed to be more delicate than E1L3N, and the PD-L1 expression in TCs appeared to have the protective role in OS via 22C3 as well as a damaging effect on DSS via E1L3N. Therefore, using different antibodies to evaluate the relationship between PD-L1 expression and prognosis probably lead to various results. Due to the lack of studies comparing the various antibodies specific to OSCC, our results needed to be interpreted with caution and were expected to be validated by more high-quality studies.

We evaluated the influence of PD-L1 expression on prognosis by using separate scoring methods. In this study, both DSS and DFS showed a significant result at a 5% cut-off of PPC. As for OS, 5% cut-off of 4 scores cut-off of H score presented a reduced survival. The cut-off value of 5% was frequently chosen in many clinical trials focusing on targeted anti-PD-L1 therapies. PD-L1 expression was associated with worse prognosis for OS and LRFS when a 5% cut-off of positive cells was applied[28]. Previous study aimed to explore the effects of expression of PD-L1 on survival rates and showed that PD-L1 expression were unrelated to in DFS and OS with TPS using different cutoffs of 1%, 5% and 10%[2], in support of the data reported by Wirsing et. al[22]. One study[9], using cutoffs of 5% of TPS, PD-L1 positivity was significantly associated with a better prognosis in DSS and OS, but this association was disappeared when confounded other factors[8]. PPC is defined as PD-L1-staining cells (TCs or TILs) divided by the total number of each type of cells, of which difference from TPS or CPS is that the denominator of the calculation formula of them is the total number of viable TCs. Therefore, a lower PPC score than TPS or CPS did not accurately represent the effect of immune checkpoints on TCs under the same conditions. Unlike with TPS, CPS reflecting an aggregate score of TC and IC evaluated expression of PD-L1in TC, as well as the impact of different IC on the tumor microenvironment. A randomized three-arm phase III KEYNOTE-048 trial was conducted in head and neck cancers (HNSCC) immunotherapy[59,60], PFS and OS were tested in three groups including the CPS [?]20, the CPS [?]1, and the total population. In comparison with EXTREME regimen, patients undergoing the treatment of pembrolizumab plus chemotherapy or pembrolizumab monotherapy significantly improved OS and PFS, and the protective effect was progressively declining in the three groups. The percentage of HNSCC tumor cells expressing PD-L1 was 85% of when CPS was [?]1[59], which decreased to 50% when measured using TPS[61]. The response rate of pembrolizumab seemed to be increased in higher levels of PD-L1 expression, and CPS was more excellent in ability to predict response to anti-PD1 therapy comparing with TPS[62]. This prompted the FDA to approve the use of pembrolizumab monotherapy against HNSCC only in patients with a CPS score [?]1, which drew interest from two studies focusing on the prognostic value of PD-L1 expression recorded by TPS or CPS in OSCC. One study[11] revealed the protective effect of PD-L1 expression was found on DSS and OS when positivity based on TPS (>5%) was used, and was observed

on OS with the positivity defined as CPS >1. In the other study[9], no statistical significance was found in the association between PD-L1 and survival in either CPS or TPS. Patients with CPS>1 showed a trend towards improved survival, while TPS>1% seemingly represented opposite trend. It need to be emphasized that tumours were classified into into four groups based on the presence of PD-L1 positivity in TC and IC, and the total prevalence of PD-L1 expression in both TC and IC or only in TC account for 72% in OSCC and showed good response to immunotherapy. Consequently, it should consider the combination of PD-L1 expressing in both TC and IC when observing PD-L1 of response to anti-PD1 therapy.

Immune cells as components of the immune environment, including CD4+ ,CD8+ TILs, have shown to be correlated with PD-L1 expression and play an important role in the mechanisms of immune response evasion of OSCC[63]. Several studies reported PD-L1-expressing tumor cells correlated positively to increased infiltration of CD4+ and CD8+TILs[2,8,22,40], whereas other studies did not show any correlation[11,25]. Our study indicated that the PD-L1 high expression group was significantly associated with high infiltration by PD-1, CD8+ TILs, strengthening that the activity of CD8+TILs was reduced through interaction of PD-L1 expression in TCs activating expression of PD-1 on CD8+ TILs. In recent years, TILs were gradually considered as a predictor for the response of solid tumors to anti-PD1 therapy[64,65]. However, the prognostic value of PD-L1 in TILs was not fully understood. Our study on prognosis showed that the PD-L1 expression on TILs did not reveal a relationship with survival, which was consistent with a previous study[11]. Inversely, one study reported that longer OS and LRFS were appeared in the group of overexpression of PD-L1 in TILs[35]. Subramaniam et al.[49]reported that low TIL PD-L1 expression was significantly correlated with reduced LRFS, but this association was disappeared confounded other factors. This was consistent with what was reported previously in a study that high expression of PD-L1 in TILs was associated with better OS of OSCC without the confounding factors of nodal metastases[1]. It has been called adaptive immune resistance that a high TIL infiltration appeared simultaneously with PD-L1 positive TCs. The strong correlation between TILs and tumor PD-L1 staining implied PD-L1 may have been induced via enhanced T cell production of IFN-γin the same way as in tumour cells[7].

Our study has several limitations. First, the prognostic values of several studies were estimated rather than provided directly. The actual association between PD-L1 expression and prognosis was masked on account of potential confounding factors. Second, the evaluation of the role of PD-L1expression in TILs on DSS, DFS, PFS and LRFS were not available to performed as a result of the lack of relevant data.

Conclusions

Current study indicated the expression of PD-L1 in TCs was relatively high in female patients, non-smoker, non-drinkers. The PD-L1 upregulation in TCs in cancers of advanced stage, N+ status was observed. The significant association of high PD-L1 expression in TCs in prognosis was only seen in LRFS. Although high PD-L1 expression in TCs had a trend toward significance of worse prognosis in OS, DSS and DFS in the initial analysis, the associations became significant based on stratified analysis of hypothesized confounders. It was worth noting that high expression of PD-L1 in TCs of tongue of OSCC was associated with worse DFS, OS. Future research on the ability to predict response to anti-PD1 therapy should focus on methods for PD-L1 immunostaining and various compartments of the oral cavity. In addition, TILs were shown to be independent of clinicopathological factors and prognosis, and relevant studies were expected to further confirmed.

Acknowledgements

This manuscript was supported by Scientific Research Promotion Plan of Anhui Medical University (2021xkjT013), and Projects of Natural Science Research of Anhui Provincial Department of Education (KJ2020A0163).

Conflict of interest

The authors declare that they have no conflict of interest.

Reference

1. Adamski Ł J, Starzyńska A. High PD-L1 Expression on Tumor Cells Indicates Worse Overall Survival in Advanced Oral Squamous Cell Carcinomas of the Tongue and the Floor of the Mouth but Not in Other Oral Compartments. *Biomedicines* . Sep 1 2021;9(9)doi:10.3390/biomedicines9091132

2. Cho YA, Yoon HJ, Lee JI, Hong SP, Hong SD. Relationship between the expressions of PD-L1 and tumor-infiltrating lymphocytes in oral squamous cell carcinoma.*Oral oncology* . Dec 2011;47(12):1148-53. doi:10.1016/j.oraloncology.2011.08.007

3. Geum DH, Hwang DS. PD-L1 Expression Correlated with Clinicopathological Factors and Akt/Stat3 Pathway in Oral SCC. *Life (Basel, Switzerland)* . Feb 4 2022;12(2)doi:10.3390/life12020238

4. Lin YM, Sung WW, Hsieh MJ, et al. High PD-L1 Expression Correlates with Metastasis and Poor Prognosis in Oral Squamous Cell Carcinoma. *PloS one* . 2015;10(11):e0142656. doi:10.1371/journal.pone.0142656

5. Ahn H, Yang JM, Kim H, et al. Clinicopathologic implications of the miR-197/PD-L1 axis in oral squamous cell carcinoma. *Oncotarget* . Sep 12 2017;8(39):66178-66194. doi:10.18632/oncotarget.19842

6. Kogashiwa Y, Yasuda M, Sakurai H, et al. PD-L1 Expression Confers Better Prognosis in Locally Advanced Oral Squamous Cell Carcinoma. *Anticancer research* . Mar 2017;37(3):1417-1424. doi:10.21873/anticanres.11465

7. Lenouvel D, González-Moles M. Clinicopathological and prognostic significance of PD-L1 in oral cancer: A preliminary retrospective immunohistochemistry study. *Oral diseases* . Mar 2021;27(2):173-182. doi:10.1111/odi.13509

8. Satgunaseelan L, Gupta R, Madore J, et al. Programmed cell death-ligand 1 expression in oral squamous cell carcinoma is associated with an inflammatory phenotype.*Pathology* . Oct 2016;48(6):574-80. doi:10.1016/j.pathol.2016.07.003

9. Miranda-Galvis M, Rumayor Piña A, Sales de Sá R, et al. PD-L1 expression patterns in oral cancer as an integrated approach for further prognostic classification. *Oral diseases* . Oct 2021;27(7):1699-1710. doi:10.1111/odi.13714

10. Ukpo OC, Thorstad WL, Lewis JS, Jr. B7-H1 expression model for immune evasion in human papillomavirus-related oropharyngeal squamous cell carcinoma. *Head and neck pathology* . Jun 2013;7(2):113-21. doi:10.1007/s12105-012-0406-z

11. Peña-Cardelles JF, Pozo-Kreilinger JJ, Roncador G, Esteban-Hernández J, Moro-Rodríguez JE, Sastre-Perona A. Prognosis Value of Immunoregulatory Molecules in Oral Cancer Microenvironment: An Immunohistochemical Study.*Biomedicines* . Mar 19 2022;10(3)doi:10.3390/biomedicines10030710

12. de Vicente JC, Rodríguez-Santamarta T, Rodrigo JP. PD-L1 Expression in Tumor Cells Is an Independent Unfavorable Prognostic Factor in Oral Squamous Cell Carcinoma. Mar 2019;28(3):546-554. doi:10.1158/1055-9965.epi-18-0779

13. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. Reporting recommendations for tumor marker prognostic studies (REMARK). *Journal of the National Cancer Institute* . Aug 17 2005;97(16):1180-4. doi:10.1093/jnci/dji237

14. Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials* . Jun 7 2007;8:16. doi:10.1186/1745-6215-8-16

15. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Statistics in medicine* . Jun 15 2002;21(11):1539-58. doi:10.1002/sim.1186

16. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test.*BMJ (Clinical research ed)* . Sep 13 1997;315(7109):629-34. doi:10.1136/bmj.315.7109.629

17. Yoshida S, Nagatsuka H, Nakano K, et al. Significance of PD-L1 Expression in Tongue Cancer Development.*International journal of medical sciences* . 2018;15(14):1723-1730. doi:10.7150/ijms.27860

18. Hirai M, Kitahara H, Kobayashi Y, et al. Regulation of PD-L1 expression in a high-grade invasive human oral squamous cell carcinoma microenvironment. *International journal of oncology* . Jan 2017;50(1):41-48. doi:10.3892/ijo.2016.3785

19. Troeltzsch M, Woodlock T, Pianka A, et al. Is There Evidence for the Presence and Relevance of the PD-1/PD-L1 Pathway in Oral Squamous Cell Carcinoma? Hints From an Immunohistochemical Study. *Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons* . May 2017;75(5):969-977. doi:10.1016/j.joms.2016.11.006

20. Quan H, Shan Z, Liu Z, et al. The repertoire of tumor-infiltrating lymphocytes within the microenvironment of oral squamous cell carcinoma reveals immune dysfunction. *Cancer immunology, immunotherapy : CII* . Mar 2020;69(3):465-476. doi:10.1007/s00262-020-02479-x

21. Togo M, Yokobori T, Shimizu K, et al. Diagnostic value of (18)F-FDG-PET to predict the tumour immune status defined by tumoural PD-L1 and CD8(+)tumour-infiltrating lymphocytes in oral squamous cell carcinoma. *British journal of cancer* . May 2020;122(11):1686-1694. doi:10.1038/s41416-020-0820-z

22. Wirsing AM, Ervik IK, Seppola M, Uhlin-Hansen L, Steigen SE, Hadler-Olsen E. Presence of high-endothelial venules correlates with a favorable immune microenvironment in oral squamous cell carcinoma. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* . Jun 2018;31(6):910-922. doi:10.1038/s41379-018-0019-5

23. Tojyo I, Shintani Y, Nakanishi T, et al. PD-L1 expression correlated with p53 expression in oral squamous cell carcinoma. *Maxillofacial plastic and reconstructive surgery* . Dec 2019;41(1):56. doi:10.1186/s40902-019-0239-8

24. Akisada N, Nishimoto K, Takao S, et al. PD-L1 expression in tongue squamous cell carcinoma. *Medical molecular morphology* . Mar 2021;54(1):52-59. doi:10.1007/s00795-020-00261-7

25. Kikuchi M, Yamashita D, Hara S, et al. Clinical significance of tumor-associated immune cells in patients with oral squamous cell carcinoma. *Head & neck* . Feb 2021;43(2):534-543. doi:10.1002/hed.26498

26. Wang H, Mao L, Zhang T, et al. Altered expression of TIM-3, LAG-3, IDO, PD-L1, and CTLA-4 during nimotuzumab therapy correlates with responses and prognosis of oral squamous cell carcinoma patients. *Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology* . Sep 2019;48(8):669-676. doi:10.1111/jop.12883

27. Kouketsu A, Sato I, Oikawa M, et al. Expression of immunoregulatory molecules PD-L1 and PD-1 in oral cancer and precancerous lesions: A cohort study of Japanese patients.*Journal of cranio-maxillo-facial surgery : official publication of the European Association for Cranio-Maxillo-Facial Surgery* . Jan 2019;47(1):33-40. doi:10.1016/j.jcms.2017.04.013

28. Straub M, Drecoll E, Pfarr N, et al. CD274/PD-L1 gene amplification and PD-L1 protein expression are common events in squamous cell carcinoma of the oral cavity.*Oncotarget* . Mar 15 2016;7(11):12024-34. doi:10.18632/oncotarget.7593

29. Chen TC, Wu CT, Wang CP, et al. Associations among pretreatment tumor necrosis and the expression of HIF-1α and PD-L1 in advanced oral squamous cell carcinoma and the prognostic impact thereof. *Oral oncology* . Nov 2015;51(11):1004-1010. doi:10.1016/j.oraloncology.2015.08.011

30. Takamaru N, Fukuda N, Akita K, Kudoh K, Miyamoto Y. Association of PD-L1 and ZEB-1 expression patterns with clinicopathological characteristics and prognosis in oral squamous cell carcinoma. *Oncology letters* . Mar 2022;23(3):75. doi:10.3892/ol.2022.13195

31. Klein M, Wermker K, Hallermann C, Pannier F, Hölzle F, Modabber A. Immune checkpoint analysis in lip cancer. *Journal of cranio-maxillo-facial surgery : official publication of the European Association for Cranio-Maxillo-Facial Surgery* . Oct 2021;49(10):950-958. doi:10.1016/j.jcms.2021.05.007

32. Takahashi H, Sakakura K, Arisaka Y, et al. Clinical and Biological Significance of PD-L1 Expression Within the Tumor Microenvironment of Oral Squamous Cell Carcinoma.*Anticancer research* . Jun 2019;39(6):3039-3046. doi:10.21873/anticanres.13437

33. Sales de Sá R, Miranda Galvis M, Mariz B, et al. Increased Tumor Immune Microenvironment CD3+ and CD20+ Lymphocytes Predict a Better Prognosis in Oral Tongue Squamous Cell Carcinoma. *Frontiers in cell and developmental biology* . 2020;8:622161. doi:10.3389/fcell.2020.622161

34. Wilms T, Gu X. PD-L1 in squamous cell carcinoma of the oral tongue shows gender-specific association with prognosis. *Oral diseases* . Oct 2020;26(7):1414-1423. doi:10.1111/odi.13414

35. Huang W, Zhou X, Liao Q, et al. Clinicopathological and prognostic significance of PD-1/PD-L1 axis expression in patients with tongue squamous cell carcinoma.*Journal of cellular physiology* . Oct 2020;235(10):6942-6953. doi:10.1002/jcp.29590

36. Hanna GJ, Woo SB, Li YY, Barletta JA, Hammerman PS, Lorch JH. Tumor PD-L1 expression is associated with improved survival and lower recurrence risk in young women with oral cavity squamous cell carcinoma. *International journal of oral and maxillofacial surgery* . May 2018;47(5):568-577. doi:10.1016/j.ijom.2017.09.006

37. Maruse Y, Kawano S, Jinno T, et al. Significant association of increased PD-L1 and PD-1 expression with nodal metastasis and a poor prognosis in oral squamous cell carcinoma.*International journal of oral and maxillofacial surgery* . Jul 2018;47(7):836-845. doi:10.1016/j.ijom.2018.01.004

38. Balermpas P, Rödel F, Krause M, et al. The PD-1/PD-L1 axis and human papilloma virus in patients with head and neck cancer after adjuvant chemoradiotherapy: A multicentre study of the German Cancer Consortium Radiation Oncology Group (DKTK-ROG). *Int J Cancer* . Aug 1 2017;141(3):594-603. doi:10.1002/ijc.30770

39. Foy JP, Bertolus C, Michallet MC, et al. The immune microenvironment of HPV-negative oral squamous cell carcinoma from never-smokers and never-drinkers patients suggests higher clinical benefit of IDO1 and PD1/PD-L1 blockade. *Annals of oncology : official journal of the European Society for Medical Oncology* . Aug 1 2017;28(8):1934-1941. doi:10.1093/annonc/mdx210

40. Mattox AK, Lee J, Westra WH, et al. PD-1 Expression in Head and Neck Squamous Cell Carcinomas Derives Primarily from Functionally Anergic CD4(+) TILs in the Presence of PD-L1(+) TAMs. *Cancer research* . Nov 15 2017;77(22):6365-6374. doi:10.1158/0008-5472.can-16-3453

41. Moratin J, Metzger K, Safaltin A, et al. Upregulation of PD-L1 and PD-L2 in neck node metastases of head and neck squamous cell carcinoma. *Head & neck* . Aug 2019;41(8):2484-2491. doi:10.1002/hed.25713

42. Naruse T, Yanamoto S, Okuyama K, et al. Immunohistochemical Study of PD-1/PD-L1 Axis Expression in Oral Tongue Squamous Cell Carcinomas: Effect of Neoadjuvant Chemotherapy on Local Recurrence. *Pathology oncology research : POR* . Apr 2020;26(2):735-742. doi:10.1007/s12253-019-00606-3

43. Oliveira-Costa JP, de Carvalho AF, da Silveira da GG, et al. Gene expression patterns through oral squamous cell carcinoma development: PD-L1 expression in primary tumor and circulating tumor cells. *Oncotarget* . Aug 28 2015;6(25):20902-20. doi:10.18632/oncotarget.3939

44. Schneider S, Kadletz L, Wiebringhaus R, et al. PD-1 and PD-L1 expression in HNSCC primary cancer and related lymph node metastasis - impact on clinical outcome.*Histopathology* . Oct 2018;73(4):573-584. doi:10.1111/his.13646

45. Tsai MS, Chen WC, Lu CH, Chen MF. The prognosis of head and neck squamous cell carcinoma related to immunosuppressive tumor microenvironment regulated by IL-6 signaling. *Oral oncology* . Apr 2019;91:47-55. doi:10.1016/j.oraloncology.2019.02.027

46. Udeabor SE, Adisa AO, Orlowska A, Sader RA, S G. Assessment of Programmed Cell Death Proteins in Oral Squamous Cell Carcinoma. *Afr J Biomed Res* 2018;21:11-13.

47. Manikhas GM, Kutukova SI, Beliak NP, Ivaskova JV, Popova NV ea. Has the PDL1 expression in tumor and immune cells the prognostic role in oral cavity squamous cell carcinoma? *J Clin Oncol* 2018;36: e18025–e18025.

48. Ahmadi N, Gao K, Chia N, et al. Association of PD-L1 expression in oral squamous cell carcinoma with smoking, sex, and p53 expression. *Oral surgery, oral medicine, oral pathology and oral radiology* . Dec 2019;128(6):631-638. doi:10.1016/j.oooo.2019.07.008

49. Subramaniam N, Nambiar A, Dhar S, et al. Low PDL1 Expression in Tumour Infiltrating Lymphocytes Predicts Local Recurrence in Oral Squamous Cell Carcinoma. *Indian journal of surgical oncology* . Jun 2021;12(2):408-414. doi:10.1007/s13193-021-01333-5

50. Chen XJ, Tan YQ, Zhang N, He MJ, Zhou G. Expression of programmed cell death-ligand 1 in oral squamous cell carcinoma and oral leukoplakia is associated with disease progress and CD8+ tumor-infiltrating lymphocytes. *Pathology, research and practice* . Jun 2019;215(6):152418. doi:10.1016/j.prp.2019.04.010

51. Zhao L, Li P, Zhao L, et al. Expression and clinical value of PD-L1 which is regulated by BRD4 in tongue squamous cell carcinoma. *Journal of cellular biochemistry* . Feb 2020;121(2):1855-1869. doi:10.1002/jcb.29420

52. Lenouvel D, González-Moles M, Ruiz-Ávila I, Gonzalez-Ruiz L, Gonzalez-Ruiz I, Ramos-García P. Prognostic and clinicopathological significance of PD-L1 overexpression in oral squamous cell carcinoma: A systematic review and comprehensive meta-analysis. *Oral oncology* . Jul 2020;106:104722. doi:10.1016/j.oraloncology.2020.104722

53. Yang WF, Wong MCM, Thomson PJ, Li KY, Su YX. The prognostic role of PD-L1 expression for survival in head and neck squamous cell carcinoma: A systematic review and meta-analysis. *Oral oncology* . Nov 2018;86:81-90. doi:10.1016/j.oraloncology.2018.09.016

54. Jia YQ, Yang B, Wen LL, Mu WX, Wang Z, Cheng B. Prognostic value of immune checkpoint molecules in head and neck cancer: a meta-analysis. *Aging* . Jan 22 2019;11(2):501-522. doi:10.18632/aging.101756

55. Tang H, Zhou X, Ye Y, Zhou Y, Wu C, Xu Y. The different role of PD-L1 in head and neck squamous cell carcinomas: A meta-analysis. *Pathology, research and practice* . Jan 2020;216(1):152768. doi:10.1016/j.prp.2019.152768

56. Troiano G, Caponio VCA, Zhurakivska K, et al. High PD-L1 expression in the tumour cells did not correlate with poor prognosis of patients suffering for oral squamous cells carcinoma: A meta-analysis of the literature. *Cell proliferation* . Mar 2019;52(2):e12537. doi:10.1111/cpr.12537

57. Rimm DL, Han G, Taube JM, et al. A Prospective, Multi-institutional, Pathologist-Based Assessment of 4 Immunohistochemistry Assays for PD-L1 Expression in Non-Small Cell Lung Cancer. *JAMA oncology* . Aug 1 2017;3(8):1051-1058. doi:10.1001/jamaoncol.2017.0013

58. Xu H, Lin G. Assessment of Concordance between 22C3 and SP142 Immunohistochemistry Assays regarding PD-L1 Expression in Non-Small Cell Lung Cancer. *Sci Rep* . Dec 5 2017;7(1):16956. doi:10.1038/s41598-017-17034-5

59. Rischin D HK, Greil R, et al. Protocol-specified final analysis of the phase 3 KEYNOTE-048 trial of pembrolizumab (pembro) as first-line therapy for recurrent/metastatic head and neck squamous cell carcinoma (R/M HNSCC). *J Clin Oncol*2019;37:6000-6000.

60. Burtness B HK, Greil R, et al. KEYNOTE-048: Phase III study of first-line pembrolizumab (P) for recurrent/metastatic head and neck squamous cell carcinoma (R/M HNSCC). *Germany: European Society of Medical Oncology Munich* . 2018;

61. Concha-Benavente F SR, Trivedi S, et al. Identification of the cell-intrinsic and -extrinsic pathways downstream of EGFR and IFNgamma that induce PD-L1 expression in head and neck cancer. *Cancer research* . 2016;76:1031-1043.

62. Seiwert TY BB, Mehra R, et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. *Lancet Oncol* . 2016;17:956-965.

63. Zhu Q, Cai MY, Weng DS, et al. PD-L1 expression patterns in tumour cells and their association with CD8(+) tumour infiltrating lymphocytes in clear cell renal cell carcinoma. *Journal of Cancer* . 2019;10(5):1154-1161. doi:10.7150/jca.29052

64. Ock CY, Keam B, Kim S, et al. Pan-Cancer Immunogenomic Perspective on the Tumor Microenvironment Based on PD-L1 and CD8 T-Cell Infiltration. *Clinical cancer research : an official journal of the American Association for Cancer Research* . May 1 2016;22(9):2261-70. doi:10.1158/1078-0432.ccr-15-2834

65. Teng MW, Ngiow SF, Ribas A, Smyth MJ. Classifying Cancers Based on T-cell Infiltration and PD-L1. *Cancer research* . Jun 1 2015;75(11):2139-45. doi:10.1158/0008-5472.can-15-0255

Figure legends

Fig 1. Flow chart presented the detailed process of study selection

Fig 2. Forest plot showing association between PD-L1 expression in TCs and gender(A), smoking(B), drinking(C).

Fig 3. Forest plot showing association between PD-L1 expression in tumor cells and prognosis in TCs of tongue of OSCC

Table 1. Characteristics of the enrolled studies. Study	**Table 1.** Characteristics of the enrolled studies. Year	**Table 1.** Characteristics of the enrolled studies. Country/Number of patients	**Table 1.** Characteristics of the enrolled studies. Anti-PD-L1 antibody	**Table 1.** Characteristics of the enrolled studies. Definition of PD-L1 expression in stained cells	**Table 1.** Characteristics of the enrolled studies. Assessment for IHC stained cells	**Table 1.** Characteristics of the enrolled studies. Anatomic location	**Table 1.** Characteristics of the enrolled studies. Clinicopathological variables assessment	**Table 1.** Characteristics of the enrolled studies. Prognos variables assessm
Yoshida et al.	2018	Japan/135	28-8 Abcam	TC:M	Semiquantitative evaluation([?]50%)	Only tongue	Y	Y
Adamski et al.	2021	Poland/109	E1L3N,Cell Signaling	TC:M TILs:M&C	Percentage of positive cells (TC:>10%; TILs:>20%)	Tongue,floor of the oral cavity,others.	Y	Y

Hirai et al.	2017	Japan/24	Abcam	TC:M	Percentage of positive cells(>10%)	NE	Y	N
Cho et al.	2011	Korea/45	Abcam(clone ab82059)	TC:M &C	H-score(ranging 0-5,[?]3)	Tongue,floor of mouth, buccal mucosa, retro-molar trigone, hard palate.	Y	Y
Lenouve et al.	2020	Spain/55	22C3 clone,Dako	TC:M	Tumour propor-tion score (TPS)[?]5%	NE	Y	Y
Troeltzsch et al.	2016	Germany/88	E1L3N, Cell signaling	TC: NS	Semiquantitative evaluation([?]5%)	Tongue,alveolar process maxilla and hard palate,soft palate, floor of the mouth, alveolar process mandible.	Y	N
Quan et al.	2020	China/83	Clone E1L3N, CST	IC: NS	Density of cells(>10%)	NE	Y	Y
Togo et al.	2020	Japan/59	28-8 Abcam	IC:M	Semiquantitative evaluation([?]1%)	Tongue,Gingiva,Buccal mu-cosa,Oral floor,Lip.	Y	Y
Wirsing et al.	2017	Norway/75	Clone SP263, Ventana	TC: M&C	Percentage of positive cells(>10%)	NE	Y	Y
Tojyo et al.	2019	Japan/49	790-4905 rabbit mono-clonal, Ventana	TC:M TILs:M	Percentage of positive cells (TC:[?]5%; TILs:[?]1%)	NE	Y	Y

Geum et al.	2022	Korea/81	Abcam	TC:N&M&C	H-score([?] mean value)	maxilla,mandible,floor of mouth,Buccal mucosa +etc.	Y	Y
Lin et al.	2015	Taiwan, China/305	GTX104763	TC:M&C	Intensity of staining (ranging 0-3,[?]2)	NE	Y	Y
Akisada et al.	2020	Japan/121	E1L3N,Cell Signaling	TC:NS	Percentage of positive TCs([?]50%)	Only tongue	Y	Y
Pena-Cardelles et al.	2022	Spain/65	Clone 22C3, Dako	TC:M	CPS[?]1,TPS[?]1%/10%	Tongue,mouth	Y	Y
Miranda-Galvis et al.	2021	Brazil/123	Abcam(clone 73-10, ab228415)	TC, IC: M	TPS[?]1%/5%/10%	NE	Y	Y
Kikuchi et al.	2019	Japan/103	Clone 28-8; Abcam	TC:M	CPS[?]1	NE	N	Y
Wang et al.	2019	China/36	Abcam, ab213524	TC:M	H-score([?]4)	NE	N	Y
de Vicente et al.	2018	Spain/125	Clone E1L3N, clone 22C3, Dako	TC:NS	Percentage of positive TCs([?]10%)	Tongue,floor of the mouth,gum,buccal,retromolar,palate	Y	Y
Kouketsu et al.	2017	Japan/106	Clone SP142,Ventana	TC:M&C	H-score([?]1)	Tongue,Upper gingiva, Lower gingiva, Hard palate,Buccal mucosa, Floor of mouth,Lower lip.	Y	Y
Straub et al.	2016	Germany/80	Clone E1L3N	TC:M	Percentage of positive cells ([?]5%)	NE	Y	Y
Chen T-C et al.	2015	Taiwan, China/218	Proteintech Group	TC:M	Percentage of positive cells ([?]5%)	NE	N	Y

Takamaru et al.	2022	Japan/169	Clone SP142,Ventana	TC:M	Percentage of positive TCs(>1%)	NE	Y	Y
Klein et al.	2021	Germany/58	22C3 clone,Dako	TC:M	Intensity of staining (ranging 0-3,[?]2)	lip cancer	Y	Y
Satgunaseelan et al.	2016	Australia/217	E1L3N-XP-Rb mAb	TC: M	Percentage of positive cells ([?]5%)	Tongue,floor of mouth, gingiva,buccal.	Y	Y
Takahashi et al.	2019	Japan/77	E1L3N, Cell signaling	TC:M	Semiquantitative evaluation([?]10%)	NE	Y	Y
de Sa et al.	2021	Brazil/48	NS	TC:M	Intensity of staining(>0%)	Only tongue	N	Y
Wilms et al.	2020	Sweden/101	Clone SP263, Ventana	TC,IC: M&C	H-score(ranging0-300,[?]18,[?]6)	Only tongue	Y	Y
Huang et al.	2020	China/152	Abcam,ab52587	TC,IC: M&C	H-score([?]2)	Only tongue	Y	Y
Hanna et al.	2017	USA/81	Clone 9A11	TC:M&C	Percentage of positive cells ([?]10%)	NE	N	Y
Maruse et al.	2018	Japan/97	Clone E1L3N, Cell Signaling	TC:M&C	Percentage of positive cells ([?]5%)	NE	N	Y
Ahn et al.	2017	Korea/68	Clone ab153991, Abcam	TC:M&C	Semiquantitative evaluation([?]10%)	NE	Y	Y
Balermpas et al.	2017	Germany/41	Clone E1L3N	TC:NS	Percentage of positive cells(>5%)	NE	N	Y
Foy et al.	2017	France/44	Clones SP142, 28.8	TC:NS	Percentage of positivecells([?]1,[?]5,and[?]10%)	NE	Y	N
Kogashiwa et al.	2017	Japan/84	Clones SP142	TC:M&C	Percentage of positive cells(>5%)	NE	Y	Y

Mattox et al.	2017	USA/53	Clone 5H1	TC: M	Percentage of positive TCs and/or TILs (> 1%)	Only tongue	Y	Y
Moratin et al.	2019	Germany/175	Cell signaling	TC:NS	H-score([?]4)	NE	Y	Y
Naruse et al.	2019	Japan/121	Abcam(clone ab156361)	TC:C&N	Percentage of positive TCs(>5%)	Only tongue	Y	Y
Oliveira-Costa et al.	2015	Brazil/96	Abcam(clone ab28753)	TC:M, C	Percentage of positive cells (>5%)	NE	Y	Y
Schneider et al.	2018	Austria/36	Clone 5H1	TC:M	Percentage of positive cells (>5%)	Only tongue	N	Y
Tsai et al.	2019	Taiwan, China/173	NS	NS	H-score	NE	N	Y
Udeabor et al.	2018	Nigeria/20	28-8 Abcam	TC:NS	H-score([?]0)	NE	Y	N
Manikhas et al.	2018	Russia/82	clone BCDdx1020 by ICH	TC:NS	Percentage of positive cells (TC:[?]1%;[?]5%)	NE	N	Y
Ahmadi et al.	2019	Australia/255	E1L3N-XP-Rb mAb	TC:M	Percentage of positive TCs ([?]1%)	NE	Y	Y
Subramaniam et al.	2021	India/64	NS	TC,TILs:M&C	Intensity of staining (ranging 0-3,[?]1)	Tongue and buccal mucosa.	Y	Y
Chen X-J et al.	2019	China/41	clone SP142, abcam	TC:C	Percentage of positive cells ([?]5%)	Only tongue	N	Y

Zhao et al.	2019	China/46	ab205921,abcam	TC:M&C	Percentage of positive TCs ([?]50%)	Only tongue	Y	N
Abbreviations: TC: tumor cell; IC: immune cell; IHC: immunohistochemistry; M: membranous; C: cytoplasmic; N:nuclei; NS:not stated; NE: not examined. H:defined as staining intensity × the percentage of staining cells; CPS: combined positive score; TPS: tumour proportion score.	Abbreviations: TC: tumor cell; IC: immune cell; IHC: immunohistochemistry; M: membranous; C: cytoplasmic; N:nuclei; NS:not stated; NE: not examined. H:defined as staining intensity × the percentage of staining cells; CPS: combined positive score; TPS: tumour proportion score.	Abbreviations: TC: tumor cell; IC: immune cell; IHC: immunohistochemistry; M: membranous; C: cytoplasmic; N:nuclei; NS:not stated; NE: not examined. H:defined as staining intensity × the percentage of staining cells; CPS: combined positive score; TPS: tumour proportion score.	Abbreviations: TC: tumor cell; IC: immune cell; IHC: immunohistochemistry; M: membranous; C: cytoplasmic; N:nuclei; NS:not stated; NE: not examined. H:defined as staining intensity × the percentage of staining cells; CPS: combined positive score; TPS: tumour proportion score.	Abbreviations: TC: tumor cell; IC: immune cell; IHC: immunohistochemistry; M: membranous; C: cytoplasmic; N:nuclei; NS:not stated; NE: not examined. H:defined as staining intensity × the percentage of staining cells; CPS: combined positive score; TPS: tumour proportion score.	Abbreviations: TC: tumor cell; IC: immune cell; IHC: immunohistochemistry; M: membranous; C: cytoplasmic; N:nuclei; NS:not stated; NE: not examined. H:defined as staining intensity × the percentage of staining cells; CPS: combined positive score; TPS: tumour proportion score.	Abbreviations: TC: tumor cell; IC: immune cell; IHC: immunohistochemistry; M: membranous; C: cytoplasmic; N:nuclei; NS:not stated; NE: not examined. H:defined as staining intensity × the percentage of staining cells; CPS: combined positive score; TPS: tumour proportion score.	Abbreviations: TC: tumor cell; IC: immune cell; IHC: immunohistochemistry; M: membranous; C: cytoplasmic; N:nuclei; NS:not stated; NE: not examined. H:defined as staining intensity × the percentage of staining cells; CPS: combined positive score; TPS: tumour proportion score.	Abbrev TC: tumor cell; IC: immune cell; IHC: immunohistochemistry; M: membranous C: cyto- plasmic N:nucle NS:not stated; NE: not examined. H:define as staining intensit × the percent age of staining cells; CPS: combined positive score; TPS: tumour proportion score.

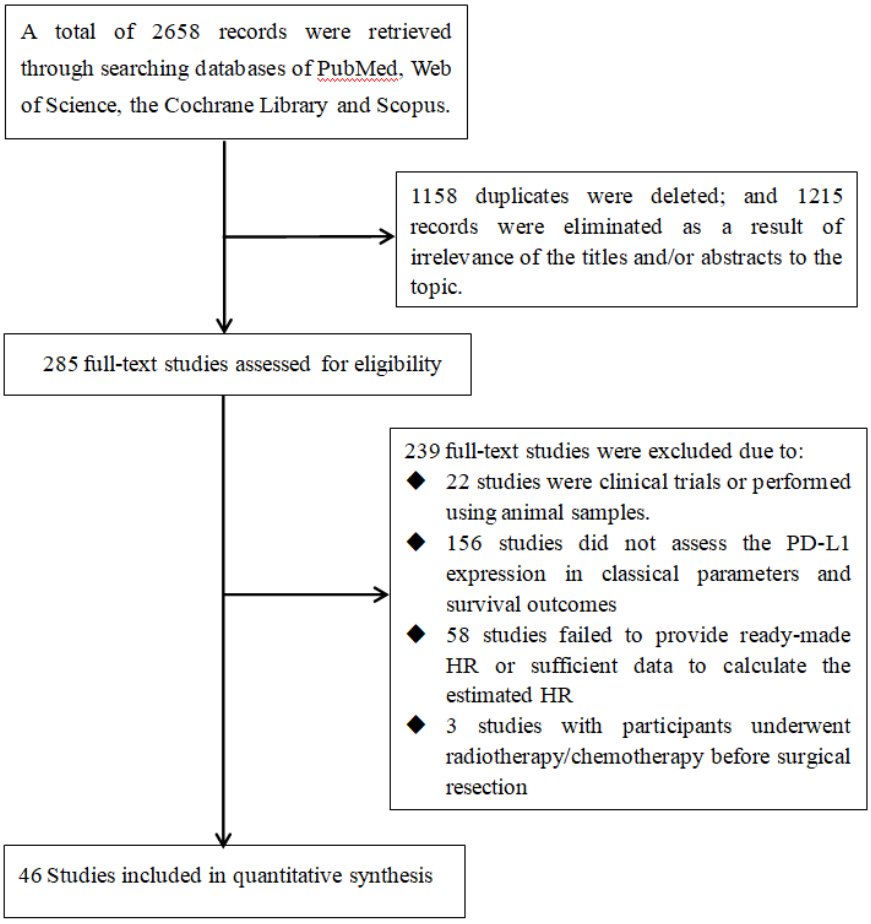
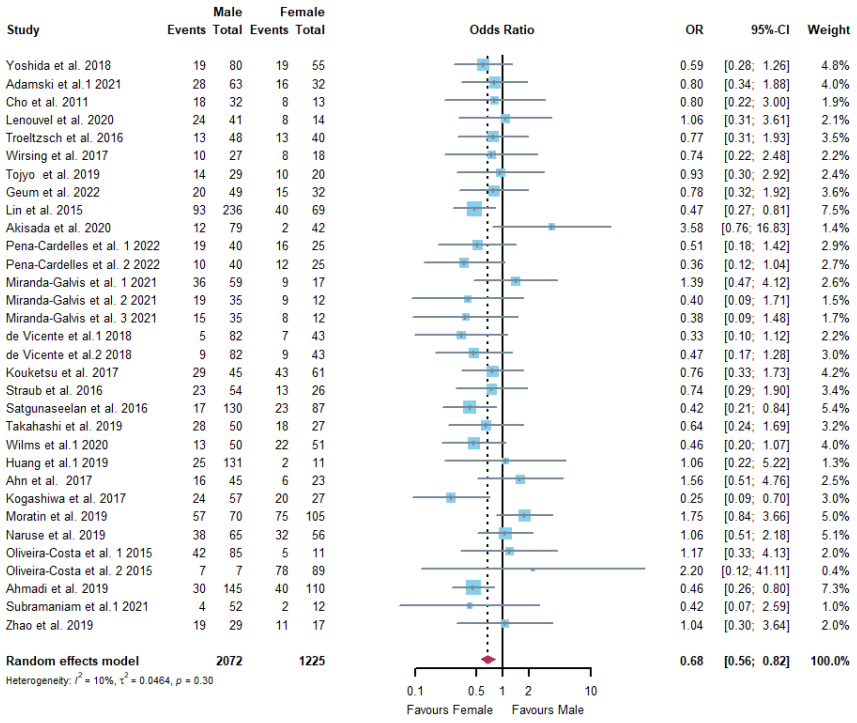


Fig 1. Flow chart presented the detailed process of study selection

(A)



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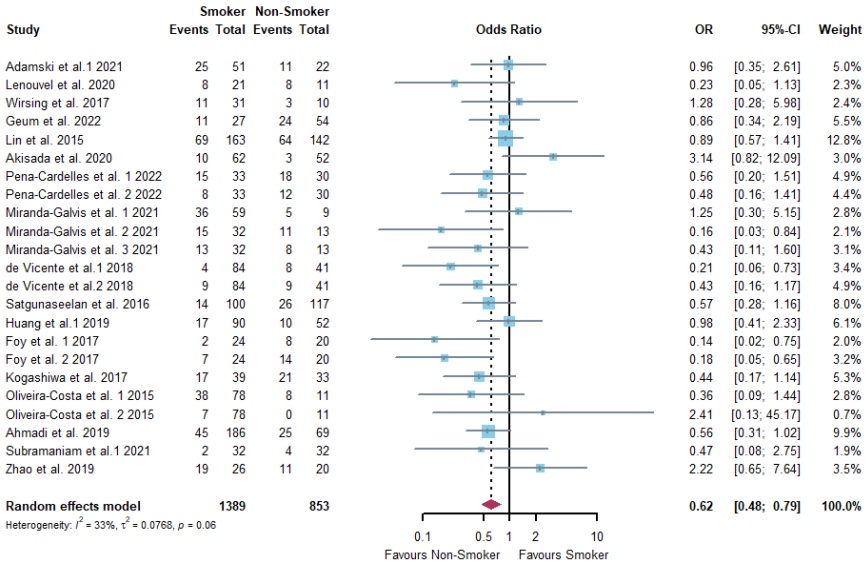

(C)

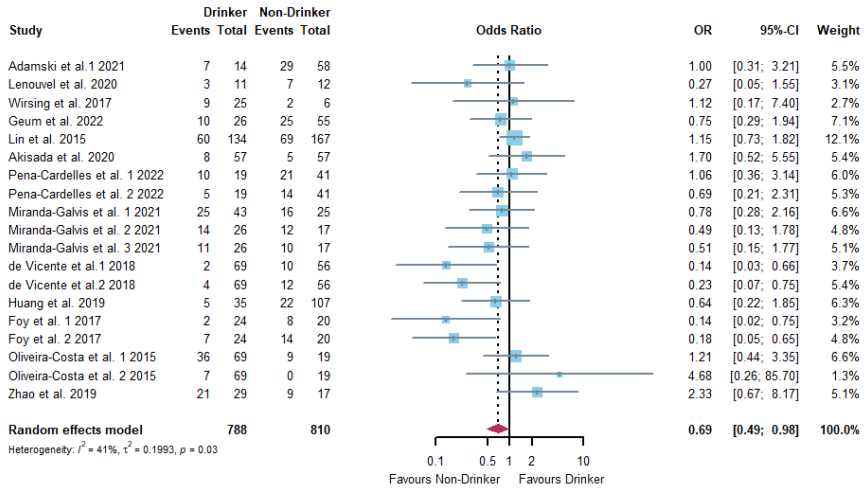

Fig 2. Forest plot showing association between PD-L1 expression in TCs and gender(A), smoking(B), drinking(C).

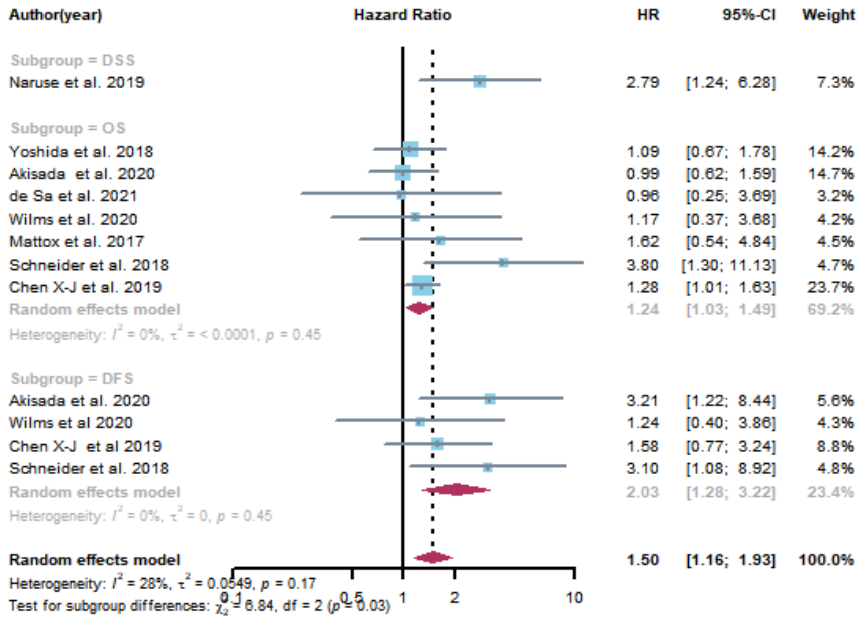

Fig 3. Forest plot showing association between PD-L1 expression in tumor cells and prognosis in TCs of tongue of OSCC