Elsevier Editorial System(tm) for Oral Oncology

Manuscript Draft

Manuscript Number: OO-D-18-692R2

Title: The Prognostic Role of PD-L1 Expression for Survival in Head and Neck Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis

Article Type: Original Research Article

Section/Category: Translational

Keywords: Head and neck cancer; squamous cell carcinoma; programmed death-ligand 1; PD-L1; HPV; tumor-infiltrating lymphocytes; TIL; survival; meta-analysis.

Corresponding Author: Dr. Yu-xiong Richard Su, MD, DDS, PhD

Corresponding Author's Institution: the University of Hong Kong

First Author: Wei-fa Yang

Order of Authors: Wei-fa Yang; May Wong, Professor; Peter J Thomson, Professor; Kar-Yan Li; Yu-xiong Su, Clinical Associate Professor

Abstract: Background
We aimed to review comprehensively the prognostic role of programmed death-ligand 1 (PD-L1) in tumor cells detected by immunohistochemistry (IHC) assays for survival outcomes in head and neck squamous cell carcinoma (HNSCC).
Methods: All studies before 2018 March 31 were systematically identified and screened. Included studies were assessed using the Quality in Prognosis Studies (QUIPS) tool. Survival outcomes were combined in HRs with 95% CI using the generic inverse-variance method.
Results: Twenty-three studies with 3105 patients were analysed. The overall positive rate of PD-L1 in HNSCC was 0.42 (95% CI: 0.36-0.48). There was no significant difference between PD-L1-positive and -negative HNSCC patients in overall survival (OS; hazard ratio [HR]: 0.98; 95% confidence interval [CI]: 0.71-1.37; p=0.93), disease-free survival (DFS; HR: 1.07; 95% CI: 0.68-1.70; p=0.76), and disease-specific survival (DSS; HR: 0.90; 95% CI: 0.63-1.29; p=0.56). An improved progression-free survival (PFS) was observed in patients with positive PD-L1 (HR: 0.71; 95% CI: 0.55-0.93; p=0.01). In patients with low CD8+ tumor-infiltrating T cells, a poorer OS was detected in patients with positive PD-L1 expression (HR: 1.90; 95% CI: 1.07-3.36; p=0.03). Patients with HPV-positive HNSCC were associated with increased PD-L1 expression (odds ratio [OR]: 1.99; 95% CI: 1.50-2.64; p<0.001). However, PD-L1 expression showed no significant benefit on OS in HPV-positive HNSCC (HR: 1.04; 95% CI: 0.65-1.65; p=0.88).
Conclusions: PD-L1 expression was not recommended to predict survival in HNSCC patients. However, positive PD-L1 may predict better PFS. The combined effects of PD-L1 expression and CD8+ tumor-infiltrating T cells should be further elucidated.

Highlights

PD-L1 detected by IHC was not a robust prognostic factor for survival in HNSCC;

PD-L1 expression might predict progression-free survival in advanced HNSCC;

The combined roles of PD-L1 and CD8+ tumor-infiltrating T cells in HNSCC should be further elucidated.

*Title Page (with author names and affiliations)

The Prognostic Role of PD-L1 Expression for Survival in Head and Neck Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis

Wei-fa Yang[1], May CM Wong[2], Peter J Thomson[1], Kar-Yan Li[2], Yu-xiong Su[1]*

[1]Oral and Maxillofacial Surgery, Faculty of Dentistry, The University of Hong Kong, Hong Kong Special Administrative Region, China

[2]Dental public health, Faculty of Dentistry, The University of Hong Kong, Hong Kong Special Administrative Region, China

***Correspondence to:**

Dr Yu-xiong Su, Clinical Associate Professor, Oral and Maxillofacial Surgery, Faculty of Dentistry, The University of Hong Kong, Prince Philip Dental Hospital, 34 Hospital Rd, Sai Ying Pun, Hong Kong Special Administrative Region, China. Tel: +852-28590267; E-mail: richsu@hku.hk

Word Count: 3986

Declaration of interests

We declare no competing interests.

Acknowledgement

This study was funded by Hong Kong Research Grant Council General Research Fund (No. 17120718) and seed fund for basic research, the University of Hong Kong (No.201511159130).

The Prognostic Role of PD-L1 Expression for Survival in Head and Neck Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis

Abstract

Background

Programmed death-ligand 1 (PD-L1) is an immune checkpoint that is primarily located on the surface of tumor cells. PD-L1 expression detected by immunohistochemistry (IHC) assays has been widely studied to predict survival outcomes in head and neck squamous cell carcinoma (HNSCC) recently. We aimed to review comprehensively the prognostic role of PD-L1 expression for survival in HNSCC.

Methods

We systematically searched PubMed, Embase, Web of Science, Cochrane Library and Scopus to identify studies investigating the prognostic role of PD-L1 expression in HNSCC. All studies published before 2018 March 31 were screened. Included studies were assessed using the Quality in Prognosis Studies (QUIPS) tool. Data were extracted and overall survival (OS), disease-free survival (DFS), progression-free survival (PFS), disease-specific survival (DSS) were combined and presented as hazard ratios (HR) with 95% confidence interval (CI) using the generic inverse-variance method.

Results

Twenty-three studies with 3105 patients were analysed. The overall positive rate of PD-L1 in HNSCC was 0.42 (95% CI: 0.36-0.48). There was no significant difference between PD-L1-positive and -negative HNSCC patients in OS (HR: 0.98; 95% CI: 0.71-1.37; p=0.93), DFS (HR: 1.07; 95% CI: 0.68-1.70; p=0.76), and DSS (HR: 0.90; 95% CI: 0.63-1.29; p=0.56). An improved PFS was observed in patients with positive PD-L1 expression (HR: 0.71; 95% CI: 0.55-0.93; p=0.01). In patients with low CD8+ tumor-infiltrating T cells, a poorer OS was detected in patients with positive PD-L1 expression (HR: 1.90; 95% CI: 1.07-3.36; p=0.03). Patients with HPV-positive HNSCC were associated with increased PD-L1 expression (OR: 1.99; 95% CI: 1.50-2.64; p<0.001). However, PD-L1 expression showed no significant benefit on OS in HPV-positive HNSCC (HR: 1.04; 95% CI: 0.65-1.65; p=0.88).

Conclusions

PD-L1 expression detected by IHC was not recommended to predict survival in HNSCC patients. However, the positive PD-L1 expression might predict better PFS in patients with advanced HNSCC. The combined effects of PD-L1 expression and CD8+ tumor-infiltrating T cells should be further elucidated.

Keywords

Head and neck cancer; squamous cell carcinoma; programmed death-ligand 1; PD-L1; HPV; tumor-infiltrating lymphocytes; TIL; survival; meta-analysis.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is one of the most common malignancies, with an annual incidence of over 600,000 worldwide.[1] Despite recent advances in multidisciplinary regimens, treatment outcomes remained poor and barely improved over the past decades. Even with an accumulated knowledge of cancer genomics, the identification of clinical prognostic factors has still been a major challenge to improve risk stratification and personalized treatment in HNSCC.[2]

Other than the conventional studies of biomarkers on tumor cells, new insights have emphasized the prospective predictive role of tumor immune microenvironment.[3, 4] HNSCC is typically characterized by diverse profiles of tumor-infiltrating lymphocytes (TILs), which render tumor cells in the distinct "inflamed" or "non-inflamed" condition.[5, 6] Since different cohorts of TILs display different functions, the prognostic values of TILs are not unchanging in different settings.[5, 7] For instance, as the most prominent cohort, the cytotoxic CD8+ T cells tend to exert an anti-cancer effect through recognizing and killing cancer cells, therefore underlining the positive prognostic role of CD8+ T cells in HNSCC.[8]

The anti-cancer functions of CD8+ T cells, however, can be counterbalanced by the compensatory expression of programmed death-ligand 1 (PD-L1) on tumor cells.[9, 10]

The PD-L1 is a transmembrane protein that can be triggered by interferon gamma (IFNγ), which is predominantly released from activated CD8+ T cells in the tumor microenvironment.[11] The overexpression of PD-L1, in turn, can induce T cell anergy and apoptosis by interacting with programmed death protein 1 (PD-1), which is an immune checkpoint expressed on the surface of immune cells.[11, 12] Generally, the PD-L1 serves as an immune gatekeeper in regulating the dynamic interrelationship between TILs and tumor cells, which indicates a potential prognostic role of PD-L1 for survival in HNSCC.

Considering the profound roles of PD-1/PD-L1, the cancer immunotherapy based on PD-1/PD-L1 checkpoint blockade has been developed to renovate CD8+ T cells in advanced or refractory cancers.[13, 14] In the most recent clinical trials, the overall response rates to PD-1/PD-L1 blockade were 13.3%-17.7% in recurrent and metastatic HNSCC, and survival outcomes were improved with a prolonged duration of response.[15-17] Moreover, in patients with positive PD-L1 expression, an improved response rate was observed when compared to patients with negative PD-L1, although further clinical verification shall be required.[16-18] Taken all together, before clarifying the predictive role of PD-L1 for PD-1/PD-L1 blockade immunotherapy, it is worthwhile to investigate the prognostic role of PD-L1 expression for survival in patients with HNSCC.

Expression of PD-L1 in cellular membrane or cytoplasm of tumor cells, detected by immunohistochemistry (IHC) assays, has been widely investigated in recent years. IHC was used to semi-quantitatively examine the localization and expression of PD-L1 in tumor tissues.[19] Numerous studies have been published, showing inconsistent findings regarding the prognostic value of PD-L1 expression for survival in HNSCC. Compelling evidence is needed to further guide clinical practice and research. Therefore, in the present study, we aimed to conduct a systematic review and meta-analysis to evaluate comprehensively the prognostic role of PD-L1 expression detected by IHC for survival in patients with HNSCC.

Methods

Search strategy

We performed a comprehensive search in PubMed, Embase, Web of Science, Cochrane Library, and Scopus for relevant studies using different combinations of keywords from the following four domains: head and neck, squamous cell carcinoma, PD-L1, and prognosis. The detailed search strategy in PubMed is shown in **Supplementary Table 1**. All studies published before 2018 March 31 were screened in the initial stage by title and abstract. Any potential studies were subsequently reviewed by full-text reading. The reference lists of eligible studies were further hand-searched. This process was conducted by two authors and repeated until no additional studies were detected.

Selection criteria

In order to identify original studies investigating the prognostic role of PD-L1 expression for survival in HNSCC, two authors independently reviewed and selected studies according to the inclusion and exclusion criteria. The inclusion criteria were: (1) patients were diagnosed with squamous cell carcinoma of the nasal cavity, paranasal sinuses, oral cavity, oropharynx, hypopharynx, and larynx; (2) the expression of PD-L1 in tumor cells was measured using IHC techniques; (3) accessible study outcomes included the overall survival (OS), disease-free survival (DFS), progression-free survival (PFS) and disease-specific survival (DSS).

The exclusion criteria included: (1) neoplasms were derived from the nasal vestibule, salivary glands, thyroid, nasopharynx, and skin, due to their heterogeneous biological properties from HNSCC; (2) the PD-L1 expression was only detected in immune cells; (3) the PD-L1 expression was measured by the quantification of mRNA level, which has not been validated with IHC results;[20] (4) the number of patients at risk was less than 10. Any discrepancies between the two authors were resolved through discussion and consensus.

Data extraction

Two authors independently extracted data from the included studies using a pre-established form. The extracted information included: author, year of publication,

patient source, tumor sites and stages, sample size, human papillomavirus (HPV) status, treatment regimens, tissue sections, IHC method, antibody and dilution, scoring strategy, cut-off value, follow-up period, and survival outcomes. Survival data were expressed as hazard ratios (HR) of observed events on PD-L1-positive versus PD-L1-negative cohorts, which was the preferential statistical parameter for time-to-event data.[21] We obtained the HR and confidence interval (CI, 95% level), and associated statistics directly, or indirectly, from results of log-rank and Cox proportional hazards regression models in each study using the methods illustrated by Tierney.[21] The completed forms were checked by a third author and statistical outcomes were checked by a statistician.

Assessment of risk of bias in included studies

The Quality In Prognosis Studies (QUIPS) tool was adopted to assess the risk of bias in included studies.[22] The QUIPS tool contains six domains, including study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding, and statistical analysis and reporting. In each domain, 3-7 prompting items are assessed to facilitate an overall judgment. Two authors independently assessed the risk of bias in included studies using the electronic QUIPS tool. The risk of bias for each domain was determined by counting the number of inadequate items rated "no". According to our predefined criteria (**Supplementary Table 2**), a domain would be rated as "low risk" only when there is no inadequate item; "moderate risk" with at least one item (1-3 items depends on domains). Any

discrepancies were resolved by consensus. All eligible studies were pooled in meta-analysis irrespective of the potential risk of bias so as to detect precise results, which would be interpreted carefully by taking the risk of bias into consideration.[23]

Statistical analysis

The primary outcome of the present meta-analysis was OS, followed by the DFS, PFS, and DSS as secondary outcomes. OS was defined as the time from diagnosis to any-cause death, DFS as the time from therapy completion to death or cancer recurrence, PFS as the time from the treatment commencement to cancer progression, and DSS as the time from diagnosis to cancer-related death. For all survival outcomes, the natural logarithms of HRs and standard errors were obtained and combined using the generic inverse-variance method.[23] If both unadjusted and adjusted HRs were available, the adjusted values were combined for analysis. The positive rates of PD-L1 expression in HNSCC were pooled using the score method.[24] The positive rates of PD-L1 were compared using the odds ratio (OR) with 95% CI.[23] Subgroup analyses were conducted to examine the roles of cancer sites, HPV conditions, and CD8+ TILs. The effects of patient sources, types of tissue sections, primary antibodies, localizations of positive PD-L1 expression, and cut-off values of PD-L1 expression were also investigated where information was available. Heterogeneity was computed using the Pearson Chi^2 and I^2 tests. If the p-value of the Pearson Chi^2 test was smaller than 0.1 or the I^2 was bigger than 50%, the random-effects analyses were performed. Otherwise, the fixed-effects analyses were performed. If the number of included studies was small,

the fixed-effects analyses were preferred to provide the best pooled outcome estimates.[25] All statistics were performed using Stata (version 13; College Station, Texas, United States), Review Manager (version 5.3; the Nordic Cochrane Centre, the Cochrane Collaboration; Oxford, United Kingdom) and SPSS Statistics (version 22.0; SPSS, IBM Corporation, Chicago, IL).

Results

Selection of studies

Of 3424 records initially identified from database searching, and 3 additional reports retrieved through reference lists, 3056 records were retained after duplicates removed. By title and abstract screening, 2997 studies were excluded in the first stage. The remaining 59 articles were reviewed by full text in the second stage, among which 36 were excluded due to various reasons as shown in **Figure 1**. According to the predefined inclusion and exclusion criteria, 23 studies with 25 independent cohorts, a total of 3105 patients, were included in the present meta-analysis.[26-48]

Characteristics of included studies

The characteristics of the included studies were shown in **Table 1**. A total of 3105 patients from 25 independent cohorts were diagnosed with HNSCC, with various primary sites including the oral cavity, oropharynx, hypopharynx, and larynx. Patient

cohorts were from Korea, Germany, France, Taiwan, Japan, Belgium, Brazil, Canada, Greece and the United States of America (USA). Since the HPV infection status had been established as an independent prognosis factor in oropharyngeal squamous cell carcinoma (OPSCC), the HPV status was investigated by measuring the p16 protein or HPV mRNA in all OPSCC patients except in one cohort from Muller 2017.[32] In the study by Solomon 2018, all recruited patients were HPV-positive due to the study design.[45]

As to the detection of PD-L1 expression, the IHC assays were performed in all studies. Tumor samples were mainly from formalin-fixed paraffin-embedded tissues (FFPE). Eleven study cohorts used surgical specimens from excisional surgery, three cohorts used biopsy specimens, and other eleven cohorts used both surgical and biopsy specimens. The primary antibodies used were heterogeneous across studies, among which the rabbit monoclonal antibody (E1L3N®, Cell Signaling Technology, United States) was mostly used, followed by the rabbit monoclonal antibody (SP142, Roche, Switzerland) and the mouse monoclonal antibody (5H1) from Dr. Lieping Chen's laboratory.[49] In the study by Meulenaere 2017, two different antibodies were used in the same cohort of patients, and thus the outcome values were combined before pooled with data from other studies to prevent bias.[41]

The definitions of positive staining of PD-L1 were also different among studies since there has been no consensus so far. In the studies by Kogashiwa 2017 and Ou 2017, the

positive IHC stains in both tumor cells and TILs were counted,[38, 39] whilst in the other studies, only the positive stains in tumor cells were deemed valid. The cellular localizations of positive PD-L1 stains mainly involved the membrane and cytoplasm. In the study published by Oliveira-Costa 2015, the PD-L1 expression in cellular membrane and cytoplasm were separately counted and investigated, and therefore, the prognostic roles of PD-L1 expression in membrane and cytoplasm were combined before pooled with outcomes from other studies.[27]

The cut-off values in different studies were also inconsistent, with the most commonly adopted value of 5%. Meanwhile, a preliminary estimation of the positive rate of PD-L1 expression in HNSCC was calculated by combining data from each study regardless of the cut-off values. The proportions of samples with positive PD-L1 expression in different studies were varied as shown in **Supplementary Figure 1**. The overall positive rate of PD-L1 expression in HNSCC was 0.42 (95% CI: 0.36-0.48), which did not change significantly with different primary cancer sites (heterogeneity between groups: $p=0.962$). However, the overall heterogeneity between studies was significant ($p<0.05$), indicating the heterogeneity of PD-L1 measurement and interpretation in most current studies.

Risk of bias in included studies

The 23 included studies were assessed using the QUIPS tool (**Figure 2**). As shown in **Figure 2**, only one "high risk" was rated to the study by Badoual 2013, due to the

inadequate description of study participation.[42] The other studies were rated with 1-4 “moderate risk” domains. Besides, studies with an intentional selection of patients, like if only late-staged patients included, were considered as inadequate participation by eligible patients. As to the study attrition, since nearly all studies were performed in the retrospective setting, the attrition domain was not relevant and studies were rated “low risk”. However, in the study by Ou 2017 and Steuer 2018, some samples were lost to PD-L1 evaluation for unspecified reasons.[39, 46] In assessing the bias of prognostic factor measurement, a clear definition of positive PD-L1 expression should be provided including the specific protocols, cellular localizations, positive and negative controls, whole tissue sections or tissue microarrays (TMA), and cut-off values. Tissue specimens were harvested from either definitive surgery or biopsy. Although small biopsy specimens might not fully reflect the overall status of PD-L1 expression, we did not count it as a risk of bias as no information of specimen size were available among all included studies. Any studies using TMA without adequate rectification were rated “moderate risk”.[50] Since no consensus of cut-off values of positive PD-L1 exists, a standard cut-point was not compulsory. For the outcome measurement, the follow-up duration should be indicated and blind measurement should be performed to prevent bias.[22] To minimize study confounding effects, the study cohorts should be matched, or the potential confounding factors should be accounted for in the analysis. The multivariable Cox regression model was adopted to adjust the prognostic performance of PD-L1 in most included studies. Especially in OPSCC, the role of HPV was

supposed to be well accounted for. For data reporting, survival endpoints should be reported in accordance with study design to avoid the risk of selective reporting.

Prognostic role of PD-L1 expression for survival in HNSCC

Overall Survival (OS)

A total of 19 studies with 21 patient cohorts investigated the prognostic role of PD-L1 expression for OS in HNSCC (**Figure 3**). Due to the significant heterogeneity (I^2=76.0%, p<0.001), the random-effect analysis was used. No significant difference was found for OS comparing the PD-L1-positive and -negative patients (HR: 0.98; 95% CI: 0.71-1.37; p=0.93). In subgroup analyses based on different primary cancer sites, the prognostic roles of PD-L1 expression for OS were not significant in oral cavity squamous cell carcinoma (OSCC; HR: 0.77; 95% CI: 0.34-1.77; p=0.54), OPSCC (HR: 1.07; 95% CI: 0.77-1.50; p=0.68), hypopharyngeal squamous cell carcinoma (HPSCC; HR: 0.54; 95% CI: 0.28-1.03; p=0.06). Only one study focused on laryngeal squamous cell carcinoma (LSCC), in which the prognostic role of PD-L1 expression was significant (HR: 0.57; 95% CI: 0.33-0.98; p=0.04).[30] The cumulative meta-analysis confirmed that the positive PD-L1 expression did not exert benefits to OS in HNSCC (**Supplementary Figure 2**). The Begg's funnel plot and Egger's test were employed to identify publication bias and no significant publication bias was detected (**Supplementary Figure 3**; Begg's test: p=0.566; Egger's test: p=0.217). Meanwhile, no significant finding was detected in the subgroup analyses, including patient sources from Europe and Asia, types of tissue sections, primary antibody, positive PD-L1

expression in tumor cells, different cellular localizations of positive PD-L1 expression, and the cut-off value of 5%.

Disease-free survival (DFS)

Seven studies examined the prognostic role of PD-L1 expression for DFS in HNSCC (**Supplementary Figure 4**). The random-effect analysis was used since the heterogeneity was significant (I^2=65.5%, p=0.01). There was no significant difference in DFS between patients with or without positive PD-L1 expression (HR: 1.07; 95% CI: 0.68-1.70; p=0.76), which was also confirmed in the cumulative meta-analysis (**Supplementary Figure 5**). No significant publication bias was detected (**Supplementary Figure 6**; Begg's test: p=0.548; Egger's test: p=0.685). Similarly, in OSCC, no significant benefits were found for positive PD-L1 expression (HR: 1.33; 95% CI: 0.72-2.43; p=0.36). However, when data from the study of Ahn 2017 was excluded, the heterogeneity decreased from 64% to 37%, and the positive PD-L1 expression was related with poorer DFS in OSCC (fixed-effect model; HR: 1.44; 95% CI: 1.07-1.95; p=0.02).[31] Only one study focused on LSCC and presented the significant prognostic role of PD-L1 expression (HR: 0.53; 95% CI: 0.30-0.94; p=0.03).[30]

Progression-free survival (PFS)

Six studies with seven cohorts, which mainly consisted of advanced cancers, were combined to analyze the prognostic role of PD-L1 expression for PFS in HNSCC (**Figure 4**). The fixed-effect model was used due to low heterogeneity (I^2=32.9%,

p=0.18). According to the pooled result, patients with positive PD-L1 expression showed an improved PFS when compared to those with negative PD-L1 expression (HR: 0.71; 95% CI: 0.55-0.93; p=0.01), which was confirmed with the accumulation of studies along the time (**Supplementary Figure 7**). Only one study focused on locally advanced OSCC, which indicated the significant prognostic role of PD-L1 expression (HR: 0.54; 95% CI: 0.33-0.90; p=0.02).[38]

Disease-specific survival (DSS)

Only four studies investigated the prognostic role of PD-L1 expression for DSS in HNSCC and the fixed-effect model was used to combine data (**Supplementary Figure 8; Supplementary Figure 9**). The difference of DSS between PD-L1-positive and -negative patients was not significant (HR: 0.90; 95% CI: 0.63-1.29; p=0.56).

Prognostic role of PD-L1 expression for survival in HNSCC with high/low CD8+ TILs

Previous studies had revealed the positive association between high CD8+ TILs with better survival outcomes in HNSCC.[8] While recently, the PD-L1 was supposed to more accurately stratify HNSCC in combination with the density of CD8+ TILs.[51] We combined available data from three studies using the fixed-effect models (**Figure 5; Supplementary Figure 10; Supplementary Figure 11**). In these three studies, the densities of CD8+ TILs were categorized into “high” or “low” based on the cut-off value of median density. Due to limited sample sizes, the study by Meulenaere 2017

was merely included in the meta-analysis of OS in HNSCC with low CD8+ TILs.[41] In HNSCC with high CD8+ TILs, there was no significant difference of OS (HR: 2.23; 95% CI: 0.83-5.98; p=0.11) and PFS (HR: 1.49; 95% CI: 0.60-3.70; p=0.39) between PD-L1-positive and -negative patients. No significant difference was observed neither for PFS (HR: 1.56; 95% CI: 0.74-3.27; p=0.24) in HNSCC with low CD8+ TILs. However, in HNSCC with low CD8+ TILs, a worse OS was observed in patients with positive PD-L1 expression (HR: 1.90; 95% CI: 1.07-3.36; p=0.03) (**Figure 5; Supplementary Figure 10**).

Prognostic role of PD-L1 expression for OS in HPV-positive HNSCC

The HPV infection had defined an independent cohort of HNSCC which tended to have a favorable survival outcome. As HPV was speculated to induce adaptive immune suppression through the PD-1/PD-L1 axis, it deserved to be explored whether the PD-L1 expression would serve as a new biomarker for risk stratification in HPV-positive HNSCC. In the present meta-analysis, the HPV-positive HNSCC were associated with the positive PD-L1 expression (OR: 1.99; 95% CI: 1.50-2.64; p<0.001) as shown in **Supplementary Figure 12, Supplementary Figure 13**. However, when the prognostic roles of PD-L1 expression for OS in HPV-positive HNSCC were pooled from six studies which mainly comprised OPSCC, the positive PD-L1 expression showed no additional benefits for OS in HPV-positive HNSCC (HR: 1.04; 95% CI: 0.65-1.65; p=0.88). (**Figure 6; Supplementary Figure 14**)

Discussion

The PD-1/PD-L1 blockade immunotherapy has recently renovated the therapeutic regimens in recurrent and metastatic HNSCC. To promote personalized treatment, robust biomarkers of treatment response are necessitated to guide patient selection. The PD-L1 expression assessed by IHC is the most common clinically used biomarker for treatment response. According to the current evidence in HNSCC,[15-17, 52] patients with positive PD-L1 expression were indicated to have better responses or survival outcomes of PD-1/PD-L1 blockade therapy. The FDA had even approved diagnostic PD-L1 IHC assays for PD-1/PD-L1 blockade therapy. However, there is still a lack of sufficient evidence to establish the definite predictive role of PD-L1 expression for PD-1/PD-L1 blockade therapy in HNSCC.

Before clarifying the predictive role of PD-L1 expression for PD-1/PD-L1 blockade therapy, numerous studies have investigated the prognostic role of PD-L1 expression for survival in HNSCC. A previous meta-analysis by Li et al. found no significant correlation between PD-L1 expression and survival endpoints of head and neck cancer.[53] With the accumulation of studies in the past two years, we focused on the role of PD-L1 expression in HNSCC using IHC technique, so as to minimize clinical heterogeneity and facilitate interpretation of findings.[23] This meta-analysis showed that the prognostic role of PD-L1 expression varied widely among studies. Overall, PD-L1 expression was not positively or negatively correlated with OS, DFS, and DSS

in HNSCC patients. Although patients with positive PD-L1 expression showed improved PFS, the favorable effect should be further confirmed due to the limited number of studies. Especially when considering the heterogeneity of tumor sites, stages, treatment regimens, and IHC platforms, these results should be interpreted carefully.

PD-L1 plays a central role in mediating the interrelationship between TILs and tumor cells. Upregulation of PD-L1 suppresses T cell function and promotes immune evasion in cancer.[9, 11-13] The PD-L1-induced suppression of immune surveillance is anticipated to be an indicator of tumor progression and poor survival. However, upregulation of PD-L1 expression could be an adaptive response to the vigorous immune microenvironment which renders anti-cancer effects.[10, 54, 55] In addition, there are multiple cell cohorts (including malignant, stromal and immune cells) and immune checkpoints that impose more complexity on the tumor microenvironment.[6, 8] Therefore, PD-L1 expression in tumor cells alone may not be a robust prognostic factor in HNSCC. We attempted to identify subgroups in which PD-L1 expression could predict prognosis by taking into account the primary cancer sites, HPV status and cellular localizations of positive PD-L1 expression. However, there were no significant differences in survival outcomes. Our results did not show that PD-L1 expression in tumor cells detected by IHC assays could be used to assess the OS, DFS, and DSS of HNSCC patients. Only one study investigated the role of PD-L1 expression in LSCC and indicated the favorable prognosis in PD-L1-positive patients.[30] More research is warranted to confirm the results.

In this meta-analysis, we detected an improvement in PFS in HNSCC with positive PD-L1 expression. PFS is mainly used in patients with advanced staged or recurrent/metastatic cancer, as among our included cohorts. Presumably, the improved PFS might be associated with a better OS. However, we did not observe an improvement of OS in the same cohorts. More research is needed to confirm and clarify the results. At the same time, caution should be taken when illustrating the PFS endpoint in PD-1/PD-L1 blockade immunotherapy clinical trials. The PD-1/PD-L1 blockade immunotherapy was mainly administered in recurrent/metastatic cancer, and the PFS was widely used in these studies.[15, 16, 52] However, the improved PFS in patients receiving PD-1/PD-L1 blockade therapy might partly be due to the intrinsic PD-L1 expression level instead of the treatment outcome of PD-1/PD-L1 blockade. In all, the role of PD-L1 expression in PD-1/PD-L1 blockade immunotherapy deserved to be appropriately accounted for in the study design or in the analysis.

Recent studies have suggested the PD-L1 and TILs can be combined to categorize the tumor microenvironment into four types, based on different PD-L1 expression levels and varied TILs abundances.[51] The first type is characterized by intense TILs with positive PD-L1 expression, which is entitled “adaptive immune resistance” and patients in this type are most likely to benefit from PD-1/PD-L1 blockade immunotherapy.[55] On the contrary, those patients with intense TILs but negative PD-L1 is classified as the fourth type, which is designated as “immune tolerance” and the suppressed immunity is

supposed to be dominated by other immunosuppressive pathways rather than the PD-1/PD-L1 axis.[51] Previous studies have indicated that patients with intense CD8+ TILs tended to display better survival outcomes.[8] In the present meta-analysis, we found that the PD-L1 expression did not add value to risk stratification among patients with intense CD8+ TILs. No significant difference in survival outcomes was detected between patients with the first and fourth type of tumor microenvironment. We speculated that, in the fourth type, some other underlying molecular mechanisms might play an equivalent role as the PD-1/PD-L1 axis, which should be further elucidated in future studies.

The second and third types of tumor microenvironment are exempt from TILs. Due to the lack of immune response, both the second and third type cancers are predicted to have a very poor prognosis.[51] The second type is “immunological ignorance” which denotes the lack of TILs and negative PD-L1 expression,[51, 56, 57] while the third type exhibits positive PD-L1 expression induced by intrinsic oncogenic pathways.[11, 58-60] In this meta-analysis, the poorer OS was detected in the third type patients with positive PD-L1 expression and sparse CD8+ TILs. It is suggested that the intrinsic oncogenic pathways that stimulate PD-L1 expression, including aberrant STAT3 and EGFR activation, or ALK translocation, may contribute to poorer outcomes in HNSCC.[11, 61-63] Although the intrinsic oncogenic pathways of PD-L1 expression are still under research and yet to be elucidated, the intrinsic oncogenic pathways do

highlight the complexity of PD-L1 expression,[64] which alone may not be a predictor of survival in HNSCC.

HPV infection has been recognized as an independent risk factor for HNSCC. Chronic persistent HPV infection is believed to evade local immunosurveillance through various mechanisms.[65, 66] As recent studies confirm the activation of PD-1/PD-L1 during chronic infection of virus,[67] it is worth exploring the role of PD-L1 in HPV-related HNSCC. In this meta-analysis, we found that HPV infection was associated with positive PD-L1 expression. This HPV-enhanced PD-L1 expression could be stimulated by chronic immune cell infiltration in the highly specialized reticular epithelium in tonsil and tongue base lymphoid tissues.[61, 67, 68] We further examined whether PD-L1 could improve risk stratification in the HPV-positive HNSCC. However, there was no difference in OS between PD-L1-positive and -negative patients. The potential explanation was that other immunosuppressive pathways exist and that PD-L1 might not be sufficient as an independent prognostic factor.[51]

Some limitations of included studies cannot be ignored in interpreting main findings of this meta-analysis. The significant heterogeneity of studies limits the validity of outcomes, which could be attributed to the diversity of patient sources, different primary cancer sites and stages, and various treatment regimens. We have performed subgroup analyses while the reliability decreased due to limited studies. An individual

participant data meta-analysis is warranted to improve the quality of data and confirm the findings. Meanwhile, although IHC is a simple and sophisticated assay that is accessible on different platforms, the heterogeneity of IHC protocols cannot be underestimated.[50] Currently, there are multiple PD-L1 antibodies, which inevitably result in inhomogeneous results including cellular localization and staining intensity.[49, 50, 69] We suggest the specific IHC protocols in each platform should be calibrated and operation techniques should be standardized, in order to arrive at more comparable results while reducing heterogeneity in future studies. Another point to emphasize is the dynamic PD-L1 expression in tumor cells.[60] Some studies suggest that DNA or mRNA aberration may better predict the expression of PD-L1, which may be limited in clinical application and should be further investigated.[20, 49, 70]

Overall, based on the results of this meta-analysis, PD-L1 expression detected by IHC was not recommended for use in clinical practice to predict survival outcomes in HNSCC patients. It was not recommended to aid risk stratification in HPV-related HNSCC neither. Whilst, the positive PD-L1 expression might predict better PFS in patients with advanced HNSCC. The combined effects of PD-L1 and CD8+ TILs should be further elucidated. Due to the heterogeneity of included studies and the limited sample size, these findings should be interpreted with caution and more high-quality researches are still warranted.

Contributors

Yu-xiong Su designed the study, assisted in data inclusion and extraction, supervised the work of all contributors, and has primary responsibility for the study. Wei-fa Yang performed the study and contributed to the first draft. May CM Wong supervised the data analysis and contributed to data interpretation. Peter J Thomson helped data interpretation and discussion of results. Kai Yan Li did data analysis. All authors contributed to and approved final version of the manuscript.

Declaration of interests

We declare no competing interests.

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Acknowledgment

This study was funded by Hong Kong Research Grant Council General Research Fund (No. 17120718) and seed fund for basic research, the University of Hong Kong (No.201511159130).

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer

statistics, 2012. CA: a cancer journal for clinicians. 2015;65:87-108.

[2] Kang H, Kiess A, Chung CH. Emerging biomarkers in head and neck cancer in the era of genomics. NAT REV CLIN ONCOL. 2015;12:11-26.

[3] Mandal R, Chan TA. Personalized Oncology Meets Immunology: The Path toward Precision Immunotherapy. CANCER DISCOV. 2016;6:703-13.

[4] Ferris RL. Immunology and Immunotherapy of Head and Neck Cancer. J CLIN ONCOL. 2015;33:3293-304.

[5] Lei Y, Xie Y, Tan YS, Prince ME, Moyer JS, Nor J, et al. Telltale tumor infiltrating lymphocytes (TIL) in oral, head & neck cancer. ORAL ONCOL. 2016;61:159-65.

[6] Puram SV, Tirosh I, Parikh AS, Patel AP, Yizhak K, Gillespie S, et al. Single-Cell Transcriptomic Analysis of Primary and Metastatic Tumor Ecosystems in Head and Neck Cancer. CELL. 2017;171:1611-24 e24.

[7] Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. IMMUNITY. 2013;39:782-95.

[8] de Ruiter EJ, Ooft ML, Devriese LA, Willems SM. The prognostic role of tumor infiltrating T-lymphocytes in squamous cell carcinoma of the head and neck: A systematic review and meta-analysis. ONCOIMMUNOLOGY. 2017;6:e1356148.

[9] Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J EXP MED. 2000;192:1027-34.

[10] Llosa NJ, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. CANCER DISCOV. 2015;5:43-51.

[11] Concha-Benavente F, Srivastava RM, Trivedi S, Lei Y, Chandran U, Seethala RR, 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 RES. 2016;76:1031-43.

[12] Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. NAT MED. 2002;8:793-800.

[13] Chen L, Han X. Anti-PD-1/PD-L1 therapy of human cancer: past, present, and future. J CLIN INVEST. 2015;125:3384-91.

[14] Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. NATURE. 2014;515:568-71.

[15] Ferris RL, Blumenschein G, Jr., Fayette J, Guigay J, Colevas AD, Licitra L, et al. Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. The New England journal of medicine. 2016;375:1856-67.

[16] Bauml J, Seiwert TY, Pfister DG, Worden F, Liu SV, Gilbert J, et al. Pembrolizumab for Platinum- and Cetuximab-Refractory Head and Neck Cancer: Results From a Single-Arm, Phase II Study. J CLIN ONCOL. 2017;35:1542-9.

[17] Chow LQM, Haddad R, Gupta S, Mahipal A, Mehra R, Tahara M, et al. Antitumor Activity of Pembrolizumab in Biomarker-Unselected Patients With Recurrent and/or Metastatic Head and Neck Squamous Cell Carcinoma: Results From the Phase Ib KEYNOTE-012 Expansion Cohort. J CLIN ONCOL. 2016;34:3838-45.

[18] Gandini S, Massi D, Mandala M. PD-L1 expression in cancer patients receiving anti PD-1/PD-L1 antibodies: A systematic review and meta-analysis. Critical reviews in oncology/hematology. 2016;100:88-98.

[19] Buttner R, Gosney JR, Skov BG, Adam J, Motoi N, Bloom KJ, et al. Programmed Death-Ligand 1 Immunohistochemistry Testing: A Review of Analytical Assays and Clinical Implementation in Non-Small-Cell Lung Cancer. J CLIN ONCOL. 2017;35:3867-76.

[20] Budczies J, Bockmayr M, Denkert C, Klauschen F, Groschel S, Darb-Esfahani S, et al. Pan-cancer analysis of copy number changes in programmed death-ligand 1 (PD-L1, CD274) - associations with gene expression, mutational load, and survival. Genes, chromosomes & cancer. 2016;55:626-39.

[21] Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. TRIALS. 2007;8:16.

[22] Hayden JA, van der Windt DA, Cartwright JL, Cote P, Bombardier C. Assessing bias in studies of prognostic factors. ANN INTERN MED. 2013;158:280-6.

[23] Higgins JP. Green S. Cochrane handbook for systematic reviews of interventions version 5.1.0. The cochrane collaboration. 2011.

[24] Nyaga VN, Arbyn M, Aerts M. Metaprop: a Stata command to perform

meta-analysis of binomial data. Arch Public Health. 2014;72:39.

[25] Yang WF, Liao GQ, Hakim SG, Ouyang DQ, Ringash J, Su YX. Is Pilocarpine Effective in Preventing Radiation-Induced Xerostomia? A Systematic Review and Meta-analysis. International journal of radiation oncology, biology, physics. 2016;94:503-11.

[26] Lin YM, Sung WW, Hsieh MJ, Tsai SC, Lai HW, Yang SM, et al. High PD-L1 Expression Correlates with Metastasis and Poor Prognosis in Oral Squamous Cell Carcinoma. PLOS ONE. 2015;10:e0142656.

[27] Oliveira-Costa JP, de Carvalho AF, Da SDG, Amaya P, Wu Y, Park KJ, et al. Gene expression patterns through oral squamous cell carcinoma development: PD-L1 expression in primary tumor and circulating tumor cells. ONCOTARGET. 2015;6:20902-20.

[28] Kim HS, Lee JY, Lim SH, Park K, Sun JM, Ko YH, et al. Association Between PD-L1 and HPV Status and the Prognostic Value of PD-L1 in Oropharyngeal Squamous Cell Carcinoma. CANCER RES TREAT. 2016;48:527-36.

[29] Ock CY, Kim S, Keam B, Kim M, Kim TM, Kim JH, et al. PD-L1 expression is associated with epithelial-mesenchymal transition in head and neck squamous cell carcinoma. ONCOTARGET. 2016;7:15901-14.

[30] Vassilakopoulou M, Avgeris M, Velcheti V, Kotoula V, Rampias T, Chatzopoulos K, et al. Evaluation of PD-L1 Expression and Associated Tumor-Infiltrating Lymphocytes in Laryngeal Squamous Cell Carcinoma. CLIN CANCER RES. 2016;22:704-13.

[31] Ahn H, Yang JM, Kim H, Chung JH, Ahn SH, Jeong WJ, et al. Clinicopathologic implications of the miR-197/PD-L1 axis in oral squamous cell carcinoma. ONCOTARGET. 2017;8:66178-94.

[32] Muller T, Braun M, Dietrich D, Aktekin S, Hoft S, Kristiansen G, et al. PD-L1: a novel prognostic biomarker in head and neck squamous cell carcinoma. ONCOTARGET. 2017;8:52889-900.

[33] Ono T, Azuma K, Kawahara A, Sasada T, Hattori S, Sato F, et al. Association between PD-L1 expression combined with tumor-infiltrating lymphocytes and the prognosis of patients with advanced hypopharyngeal squamous cell carcinoma. ONCOTARGET. 2017;8:92699-714.

[34] Balermpas P, Rodel F, Krause M, Linge A, Lohaus F, Baumann 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. 2017;141:594-603.

[35] Chen TC, Wu CT, Wang CP, Hsu WL, Yang TL, Lou PJ, et al. Associations among pretreatment tumor necrosis and the expression of HIF-1alpha and PD-L1 in advanced oral squamous cell carcinoma and the prognostic impact thereof. ORAL ONCOL. 2015;51:1004-10.

[36] 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 ONCOL. 2011;47:1148-53.

[37] Fiedler M, Weber F, Hautmann MG, Haubner F, Reichert TE, Klingelhoffer C, et al. Biological predictors of radiosensitivity in head and neck squamous cell carcinoma. Clin Oral Investig. 2018;22:189-200.

[38] Kogashiwa Y, Yasuda M, Sakurai H, Nakahira M, Sano Y, Gonda K, et al. PD-L1 Expression Confers Better Prognosis in Locally Advanced Oral Squamous Cell Carcinoma. ANTICANCER RES. 2017;37:1417-24.

[39] Ou D, Adam J, Garberis I, Blanchard P, Nguyen F, Levy A, et al. Clinical relevance of tumor infiltrating lymphocytes, PD-L1 expression and correlation with HPV/p16 in head and neck cancer treated with bio- or chemo-radiotherapy. ONCOIMMUNOLOGY. 2017;6:e1341030.

[40] Satgunaseelan L, Gupta R, Madore J, Chia N, Lum T, Palme CE, et al. Programmed cell death-ligand 1 expression in oral squamous cell carcinoma is associated with an inflammatory phenotype. PATHOLOGY. 2016;48:574-80.

[41] De Meulenaere A, Vermassen T, Aspeslagh S, Deron P, Duprez F, Laukens D, et al. Tumor PD-L1 status and CD8(+) tumor-infiltrating T cells: markers of improved prognosis in oropharyngeal cancer. ONCOTARGET. 2017;8:80443-52.

[42] Badoual C, Hans S, Merillon N, Van Ryswick C, Ravel P, Benhamouda N, et al. PD-1-expressing tumor-infiltrating T cells are a favorable prognostic biomarker in HPV-associated head and neck cancer. CANCER RES. 2013;73:128-38.

[43] 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. 2013;7:113-21.

[44] Straub M, Drecoll E, Pfarr N, Weichert W, Langer R, Hapfelmeier A, et al. CD274/PD-L1 gene amplification and PD-L1 protein expression are common events in squamous cell carcinoma of the oral cavity. ONCOTARGET. 2016;7:12024-34.

[45] Solomon B, Young RJ, Bressel M, Urban D, Hendry S, Thai A, et al. Prognostic Significance of PD-L1(+) and CD8(+) Immune Cells in HPV(+) Oropharyngeal Squamous Cell Carcinoma. CANCER IMMUNOL RES. 2018.

[46] Steuer CE, Griffith CC, Nannapaneni S, Patel MR, Liu Y, Magliocca KR, et al. A correlative analysis of PD-L1, PD-1, PD-L2, EGFR, HER2, and HER3 expression in oropharyngeal squamous cell carcinoma. MOL CANCER THER. 2018.

[47] Chen SC, Chang PM, Wang HJ, Tai SK, Chu PY, Yang MH. PD-L1 expression is associated with p16(INK4A) expression in non-oropharyngeal head and neck squamous cell carcinoma. ONCOL LETT. 2018;15:2259-65.

[48] Maruse Y, Kawano S, Jinno T, Matsubara R, Goto Y, Kaneko N, et al. Significant association of increased PD-L1 and PD-1 expression with nodal metastasis and a poor prognosis in oral squamous cell carcinoma. Int J Oral Maxillofac Surg. 2018.

[49] Velcheti V, Schalper KA, Carvajal DE, Anagnostou VK, Syrigos KN, Sznol M, et al. Programmed death ligand-1 expression in non-small cell lung cancer. LAB INVEST. 2014;94:107-16.

[50] McLaughlin J, Han G, Schalper KA, Carvajal-Hausdorf D, Pelekanou V, Rehman J, et al. Quantitative Assessment of the Heterogeneity of PD-L1 Expression in Non-Small-Cell Lung Cancer. JAMA ONCOL. 2016;2:46-54.

[51] Teng MW, Ngiow SF, Ribas A, Smyth MJ. Classifying Cancers Based on T-cell

Infiltration and PD-L1. CANCER RES. 2015;75:2139-45.

[52] Seiwert TY, Burtness B, Mehra R, Weiss J, Berger R, Eder JP, 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;17:956-65.

[53] Li J, Wang P, Xu Y. Prognostic value of programmed cell death ligand 1 expression in patients with head and neck cancer: A systematic review and meta-analysis. PLOS ONE. 2017;12:e0179536.

[54] Howitt BE, Shukla SA, Sholl LM, Ritterhouse LL, Watkins JC, Rodig S, et al. Association of Polymerase e-Mutated and Microsatellite-Instable Endometrial Cancers With Neoantigen Load, Number of Tumor-Infiltrating Lymphocytes, and Expression of PD-1 and PD-L1. JAMA ONCOL. 2015;1:1319-23.

[55] Taube JM, Anders RA, Young GD, Xu H, Sharma R, McMiller TL, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. SCI TRANSL MED. 2012;4:127ra37.

[56] Bald T, Landsberg J, Lopez-Ramos D, Renn M, Glodde N, Jansen P, et al. Immune cell-poor melanomas benefit from PD-1 blockade after targeted type I IFN activation. CANCER DISCOV. 2014;4:674-87.

[57] Huang RR, Jalil J, Economou JS, Chmielowski B, Koya RC, Mok S, et al. CTLA4 blockade induces frequent tumor infiltration by activated lymphocytes regardless of clinical responses in humans. CLIN CANCER RES. 2011;17:4101-9.

[58] Atefi M, Avramis E, Lassen A, Wong DJ, Robert L, Foulad D, et al. Effects of MAPK and PI3K pathways on PD-L1 expression in melanoma. CLIN CANCER RES. 2014;20:3446-57.

[59] Parsa AT, Waldron JS, Panner A, Crane CA, Parney IF, Barry JJ, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. NAT MED. 2007;13:84-8.

[60] Rech AJ, Vonderheide RH. Dynamic interplay of oncogenes and T cells induces PD-L1 in the tumor microenvironment. CANCER DISCOV. 2013;3:1330-2.

[61] Lu C, Talukder A, Savage NM, Singh N, Liu K. JAK-STAT-mediated chronic inflammation impairs cytotoxic T lymphocyte activation to decrease anti-PD-1 immunotherapy efficacy in pancreatic cancer. ONCOIMMUNOLOGY. 2017;6:e1291106.

[62] Barsoum IB, Smallwood CA, Siemens DR, Graham CH. A mechanism of hypoxia-mediated escape from adaptive immunity in cancer cells. CANCER RES. 2014;74:665-74.

[63] Lui VW, Peyser ND, Ng PK, Hritz J, Zeng Y, Lu Y, et al. Frequent mutation of receptor protein tyrosine phosphatases provides a mechanism for STAT3 hyperactivation in head and neck cancer. Proc Natl Acad Sci U S A. 2014;111:1114-9.

[64] Wellenstein MD, de Visser KE. Cancer-Cell-Intrinsic Mechanisms Shaping the Tumor Immune Landscape. IMMUNITY. 2018;48:399-416.

[65] Alcami A, Koszinowski UH. Viral mechanisms of immune evasion. Immunol Today. 2000;21:447-55.

[66] Tindle RW. Immune evasion in human papillomavirus-associated cervical cancer. NAT REV CANCER. 2002;2:59-65.

[67] Lyford-Pike S, Peng S, Young GD, Taube JM, Westra WH, Akpeng B, et al. Evidence for a role of the PD-1:PD-L1 pathway in immune resistance of HPV-associated head and neck squamous cell carcinoma. CANCER RES. 2013;73:1733-41.

[68] Lim SO, Li CW, Xia W, Cha JH, Chan LC, Wu Y, et al. Deubiquitination and Stabilization of PD-L1 by CSN5. CANCER CELL. 2016;30:925-39.

[69] Ratcliffe MJ, Sharpe A, Midha A, Barker C, Scott M, Scorer P, et al. Agreement between Programmed Cell Death Ligand-1 Diagnostic Assays across Multiple Protein Expression Cutoffs in Non-Small Cell Lung Cancer. CLIN CANCER RES. 2017;23:3585-91.

[70] Erber R, Stohr R, Herlein S, Giedl C, Rieker RJ, Fuchs F, et al. Comparison of PD-L1 mRNA Expression Measured with the CheckPoint Typer(R) Assay with PD-L1 Protein Expression Assessed with Immunohistochemistry in Non-small Cell Lung Cancer. ANTICANCER RES. 2017;37:6771-8.

Figure 1. Flowchart of study selection in the meta-analysis.

Figure 2. Risk of bias assessment using the Quality In Prognosis Studies (QUIPS) tool.

Figure 3. Meta-analysis of the prognostic role of PD-L1 expression for OS in HNSCC using the random-effect analysis.

Figure 4. Meta-analysis of the prognostic role of PD-L1 expression for PFS in HNSCC using the fixed-effect analysis.

Figure 5. Meta-analysis of the prognostic role of PD-L1 expression for OS in HNSCC with (A) high CD8+ TILs and (B) low CD8+ TILs using the fixed-effect analysis.

Figure 6. Meta-analysis of the prognostic role of PD-L1 expression for OS in HPV-positive HNSCC using the fixed-effect analysis.

Table

Table 1. Main characteristics of studies included in meta-analysis.

Study Year (ref)	Patient source	Cancer subtype	N (PD-L1+/-)	HPV status	Immunohistochemistry				PD-L1+		
					Tissue section	Test	Antibody	Dilution	Cell type	Location	Cut-off value
Ahn 2017	Korea	OSCC	68 (22/46)	NA	SS-FFPE-TMA	IHC	Rabbit PAb (ab153991, Abcam)	1:1000	TC	M&C	10%
Badoual 2013	France	HNSCC	64 (33/31)	50%	S/BS-FFT	IFA	Goat PAb (R&D Systems)	15µg/ml	TC	NA	20%
Balermpas 2017	Germany	HNSCC	161 (63/98)	37.9%	SS-FFPE	IHC	Rabbit MAb (E1L3N®, CST)	1:50	TC	M&C	5%
Chen 2015	Taiwan	OSCC	218 (139/79)	NA	SS-FFPE	IHC	Rabbit PAb (Proteintech Group)	NA	TC	M	5%
Chen 2018	Taiwan	HNSCC	106 (34/72)	20.8%	SS-FFPE-TMA	IHC	Rabbit MAb (E1L3N®, CST)	1:200	TC	M&C	5%
Cho 2011	Korea	OSCC	45 (22/23)	NA	SS-FFPE	IHC	Rabbit PAb (ab82059, Abcam)	1:100	TC	M&C	Median
Fiedler 2017	Germany	HNSCC	81 (31/50)	9.8%	BS-FFPE-TMA	IHC	Rabbit MAb (E1L3N®, CST)	1:200	TC	M	5%
Kim 2015	Korea	OPSCC	133 (90/43)	66.9%	S/BS-FFPE	IHC	Mouse MAb (5H1)	1:1000	TC	M	20%
Kogashiwa 2017	Japan	OSCC	84 (44/40)	NA	SS-FFPE	IHC	Rabbit MAb (SP142, Roche)	1:100	TC&TIL	M&C	5%
Lin 2015	Taiwan	OSCC	305 (133/172)	NA	SS-FFPE-TMA	IHC	Rabbit PAb (104763, GeneTex)	1:100	TC	M&C	Stg/mod vs. weak/nil
Maruse 20108	Japan	OSCC	97 (63/34)	NA	BS-FFPE	IHC	Rabbit MAb (E1L3N®, CST)	1:200	TC	M	5%
Meulenaere 2017	Belgium	OPSCC	99 (22/72) 99 (33/64)	19.2%	S/BS-FFPE	IHC	Rabbit MAb (SP142, Roche) Mouse MAb (22C3, Agilent)	1:1 1:100	TC	M&C	5%
Muller(1) 2017	Germany	HNSCC	98 (15/83)	NA	S/BS-FFPE-TMA	IHC	Rabbit MAb (ab174838, Abcam)	1:75	TC	M&C	Stg vs. low/nil
Muller(2) 2017	Germany	HNSCC	195 (54/141)	8.2%	S/BS-FFPE-TMA	IHC	Rabbit MAb (ab174838, Abcam)	1:75	TC	M&C	Stg vs. low/nil
Ock(1) 2016	Korea	HNSCC	50 (32/18)	30%	S/BS-FFPE	IHC	Rabbit MAb (E1L3N®, CST)	NA	TC	M	5%
Ock(2) 2016	Korea	HNSCC	91 (59/32)	36.3%	S/BS-FFPE	IHC	Rabbit MAb (E1L3N®, CST)	NA	TC	M	5%
Oliveira-Costa 2015	Brazil	OSCC	96 (7/89) 96 (47/49)	NA	SS-FFPE-TMA	IHC	Goat PAb (ab28753, Abcam)	1:25	TC	M C	5%
Ono 2017	Japan	HPSCC	83 (26/57)	NA	S/BS-FFPE	IHC	Rabbit MAb (E1L3N®, CST)	1:50	TC	M&C	1%
Ou 2017	France	HNSCC	38 (19/19)	39.5%	BS-FFPE	IHC	Rabbit MAb (E1L3N®, CST)	1:1500	TC&TIL	M&C	5%
Satgunaseelan 2016	Australia	OSCC	217 (40/177)	0.9%	SS-FFPE-TMA	IHC	Rabbit MAb (E1L3N®, CST)	1:500	TC	M	5%
Solomon 2018	Australia	OPSCC	182 (86/96)	100%	S/BS-FFPE	IHC	Rabbit MAb (SP142, Roche)	1:500	TC	M	1%
Steuer 2018	USA	OPSCC	95 (24/71)	71.1%	S/BS-FFPE	IHC	Rabbit MAb (E1L3N®, CST)	1:20	TC	M	Stg/mod/weak vs. nil
Straub 2016	Germany	OSCC	80 (36/44)	6.3%	SS-FFPE-TMA	IHC	Rabbit MAb (E1L3N®, CST)	1:100	TC	M	5%
Ukpo 2012	Canada	OPSCC	181 (84/97)	79.3%	S/BS-FFPE-TMA	IHC	Mouse MAb (5H1-A3)	1:300	TC	M&C	5%
Vassilakopoulou 2016	Greece	LSCC	238 (98/140)	NA	SS-FFPE-TMA	IFA	Mouse MAb (5H1)	1:500	TC	NA	58%

Abbreviations: reference (ref); No. of patients (N); not applicable (NA); surgical specimen (SS); biopsy specimen (BS); surgical or biopsy specimen (S/BS); formalin-fixed paraffin-embedded tissue (FFPE); tissue microarrays (TMA); fresh frozen tissue (FFT); immunohistochemistry staining (IHC); immunofluorescence assay (IFA); Cell Signaling Technology (CST); monoclonal antibody (MAb); polyclonal antibody (PAb); strong (stg); moderate (mod); tumor cells (TC); tumor-infiltrating lymphocytes (TIL); membrane (M); cytoplasm (C); head and neck squamous cell carcinoma (HNSCC); oral squamous cell carcinoma (OSCC); oropharyngeal squamous cell carcinoma (OPSCC); hypopharyngeal squamous cell carcinoma (HPSCC); laryngeal squamous cell carcinoma (LSCC).

Figure1

Click here to download high resolution image

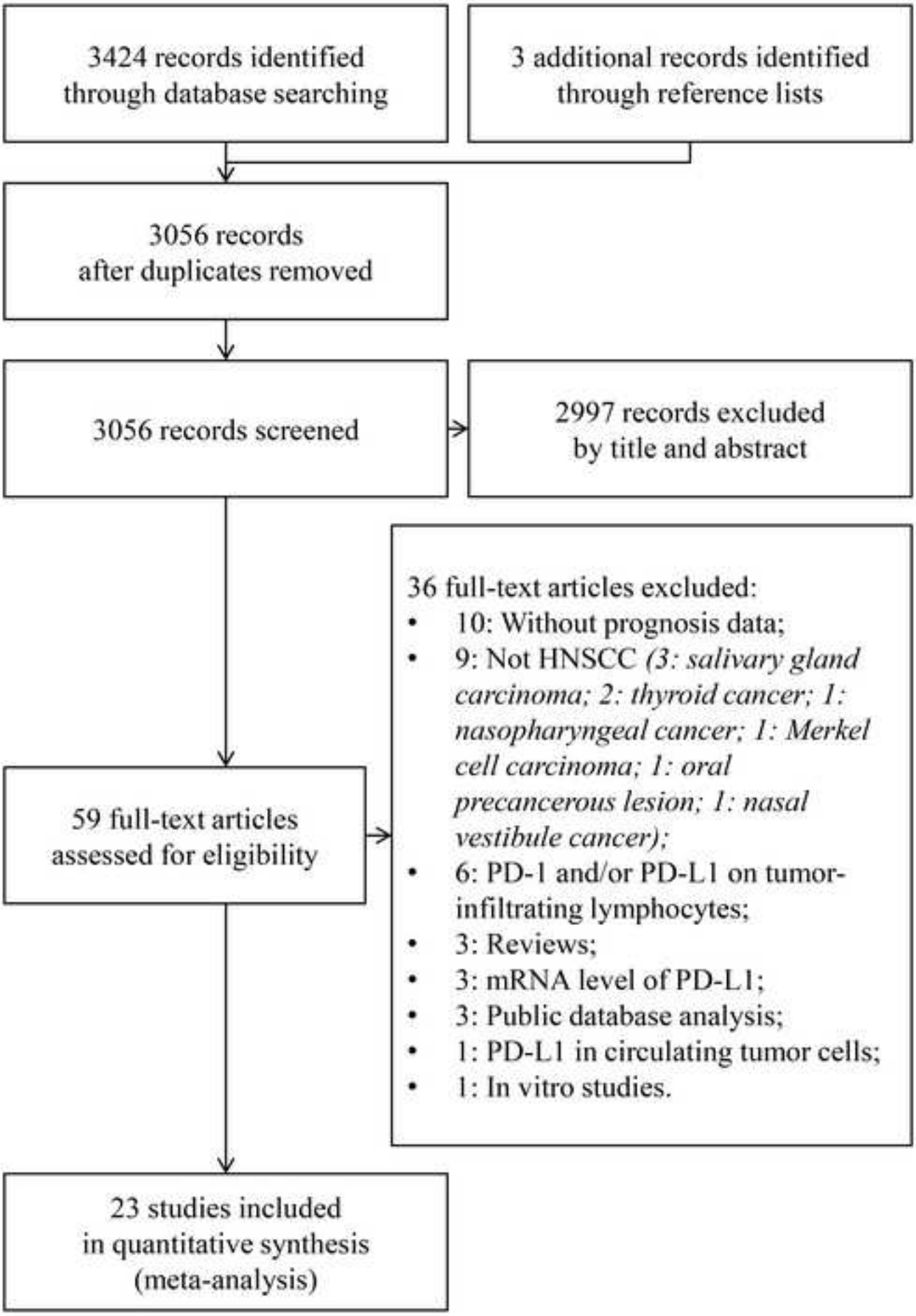


Figure2

Click here to download high resolution image

Figure3

Click here to download high resolution image

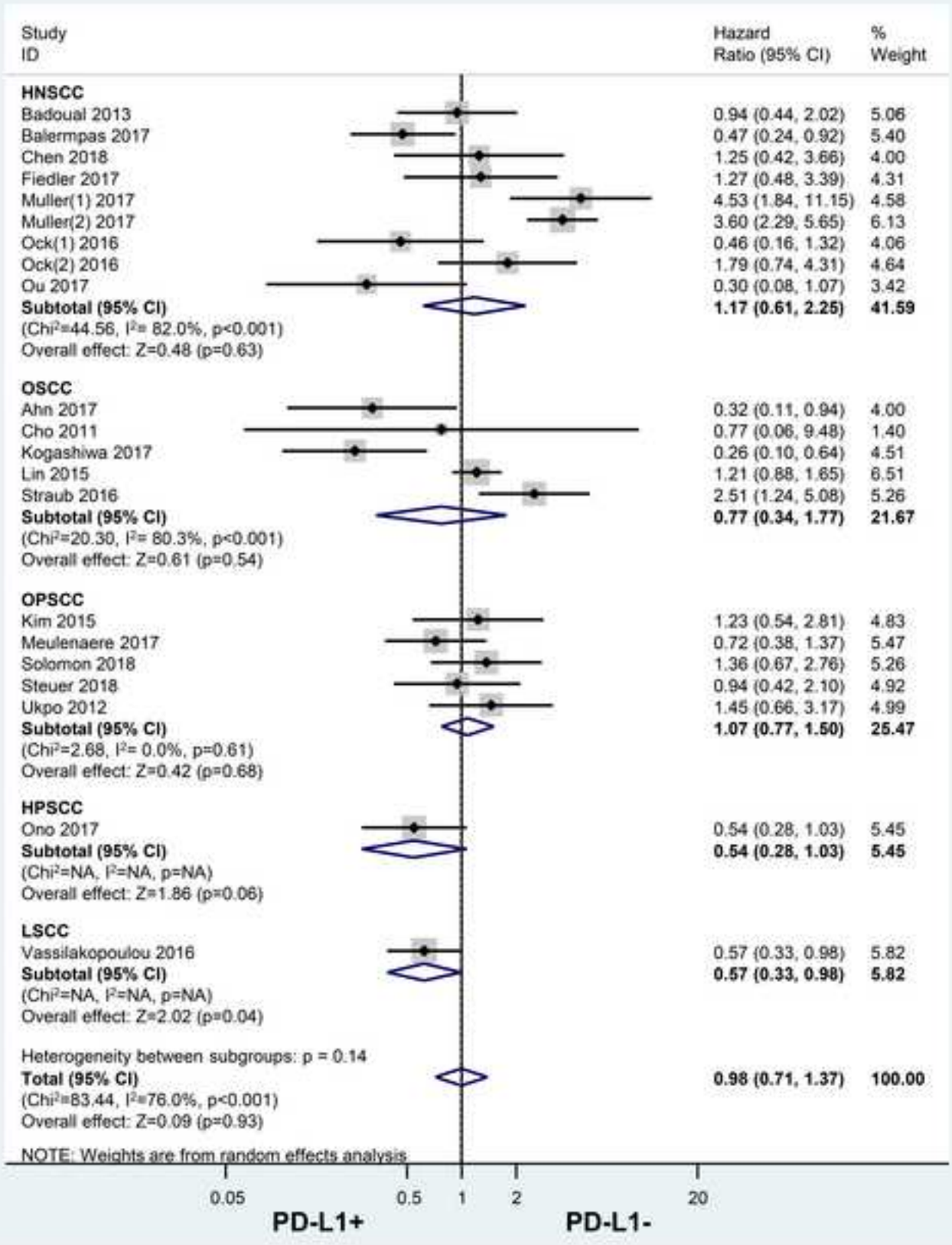


Figure4

Click here to download high resolution image

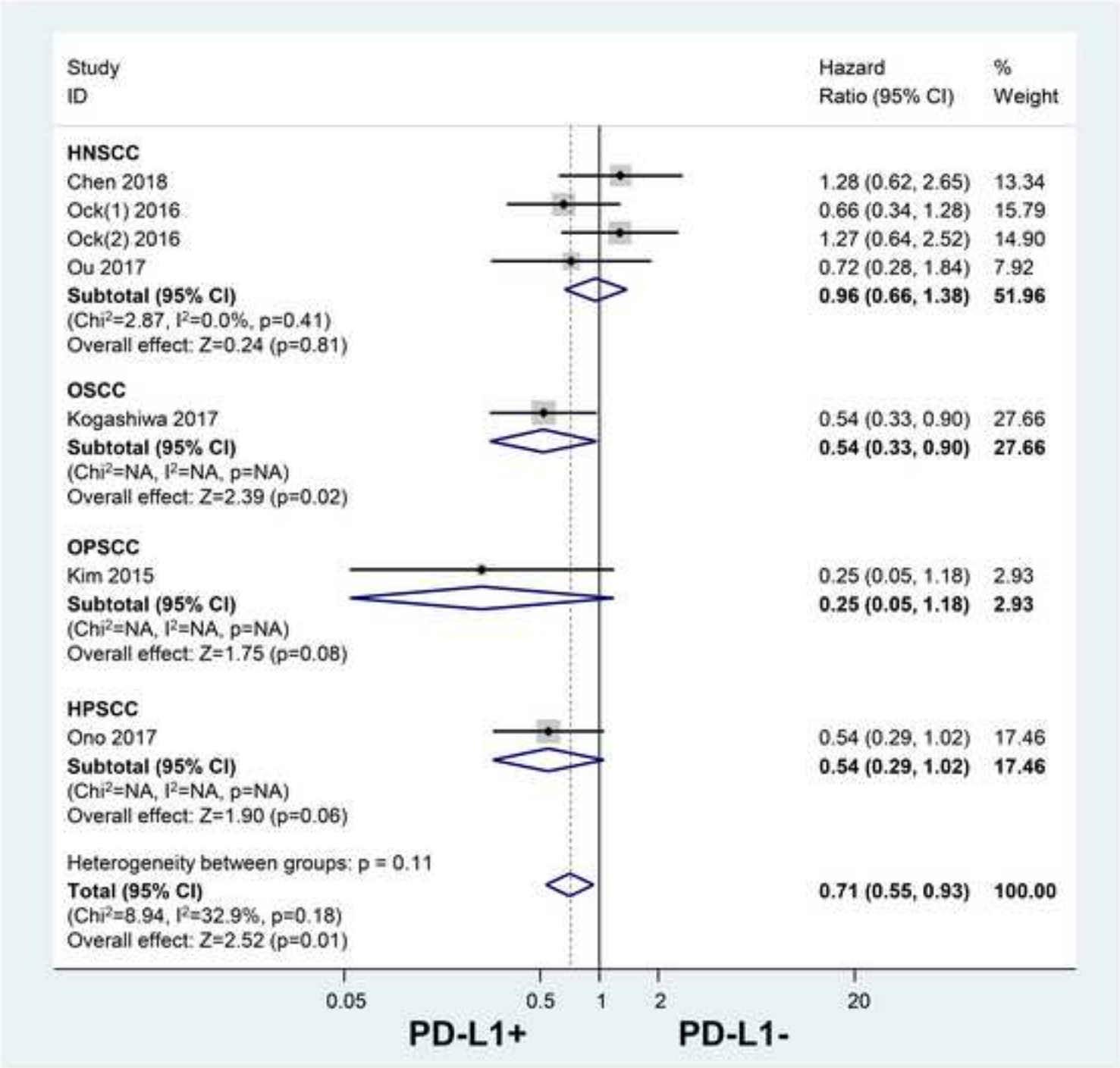


Figure5

Click here to download high resolution image

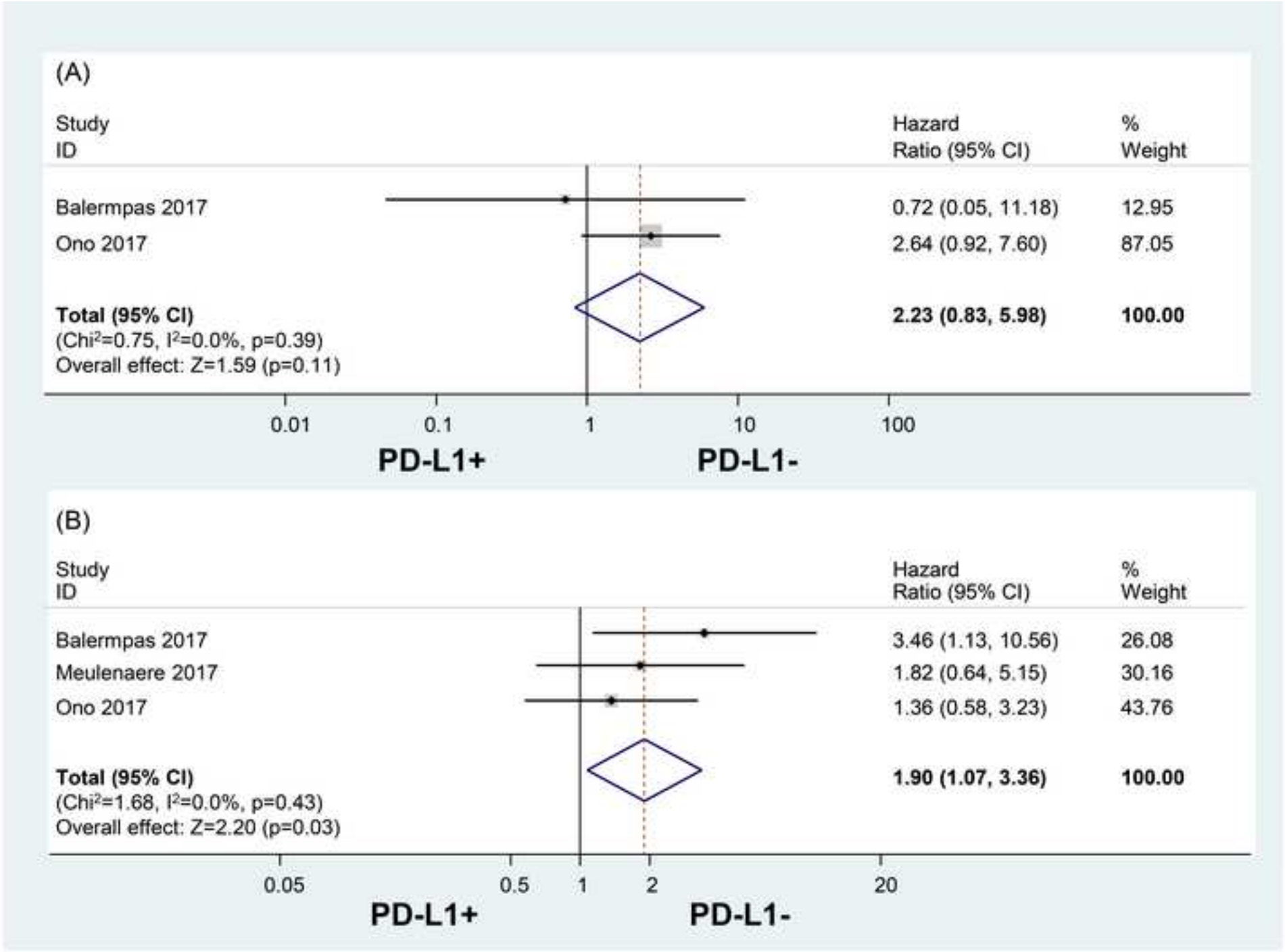
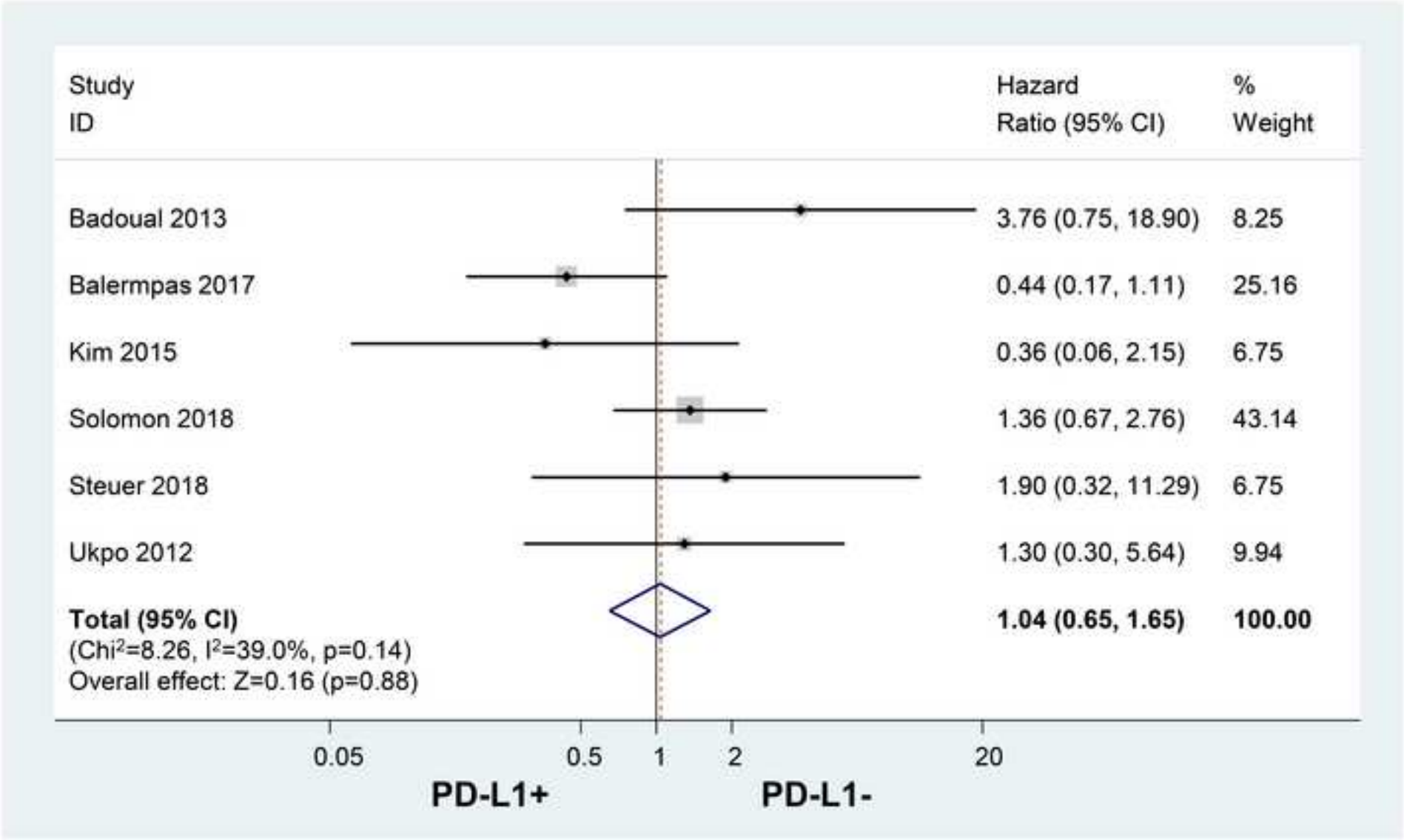


Figure6

Click here to download high resolution image



Supplementary Data (online only)

Click here to download Supplementary Data (online only): Appendix.docx