OPEN ACCESS

PD-L1 expression in recurrent or metastatic head and neck squamous cell carcinoma in China (EXCEED study): a multicentre retrospective study

Haizhen Lu ,[1] Dong Kuang,[2] Ping Zhou,[3] Jing Zeng,[4] Qingxin Xia,[5] Jian Wang ,[6] Pei Duan,[2] Lili Jiang ,[3] Shengbing Zang,[4] Yiping Jin,[5] Xiangnan Jiang,[6] Jielin Li,[7] Wenmin Tang,[7] Jiansong Zhou ,[7] Jihua Chen,[7] Jianming Ying[1]

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jcp-2023-209059).

For numbered affiliations see end of article.

Correspondence to
Dr Jianming Ying, Department of Pathology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China; jmying@cicams.ac.cn

HL, DK, PZ and JZ contributed equally.

Received 21 July 2023
Accepted 12 October 2023

ABSTRACT

Aims Programmed death-ligand 1 (PD-L1) is known to be highly expressed in various malignancies, including head and neck squamous cell carcinoma (HNSCC). We aimed to determine the prevalence of PD-L1 expression in recurrent or metastatic HNSCC (R/M HNSCC) among Chinese patients.

Methods This multicentre, retrospective analysis of data from six centres in China included patients with R/M HNSCC treated from 9 August 2021 to 28 February 2022. PD-L1 expression in tumour tissue was assessed and represented using a combined positive score (CPS). The χ^2 and Cochran-Mantel-Haenszel χ^2 tests were used to compare the prevalence of different PD-L1 expression statuses according to related co-variables.

Results For all 402 examined patients with R/M HNSCC, 168 cases (41.8%) had PD-L1 expression with a CPS ≥20, and 337 cases (83.8%) had PD-L1 expression with a CPS ≥1. Between the PD-L1 CPS ≥20 group and PD-L1 CPS <20 group, statistically significant differences were observed for variables of sex (p<0.001), smoking habit (p=0.0138 for non-smokers vs current smokers) and primary tumour site (p<0.001 for hypopharynx vs oral cavity and p=0.0304 for larynx vs oral cavity, respectively).

Conclusion PD-L1 with CPS ≥20 was expressed in about 41.8% of cases with R/M HNSCC among Chinese patients, and PD-L1 expression was significantly associated with sex, smoking history and primary tumour site. Our findings regarding the variables related to PD-L1 expression level provide insight for clinical practice and a solid basis for future research on immunotherapy in HNSCC.

Trial registration number ISRCTN10570964.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Head and neck squamous cell carcinomas (HNSCCs) are reported to be the sixth most common cancer worldwide. Programmed death-ligand 1 (PD-L1) expression is the guiding factor for the use of immune checkpoint inhibitors, but the PD-L1 expression status in Chinese patients with HNSCC is largely unknown.

WHAT THIS STUDY ADDS

⇒ This study provided data regarding the expression of PD-L1 among Chinese patients with HNSCC as well as how PD-L1 expression is associated with sex, smoking history and primary tumour site.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The insights derived from this study's data may help inform the use of immune checkpoint inhibitors and serve as a basis for future research on immunotherapy in HNSCC.

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is reported to be the sixth most common cancer worldwide.[1] Furthermore, according to the National Central Cancer Registry of China, 142 000 new cases of HNSCC occurred in China, along with nearly 75 000 deaths, in 2020.[2 3] Around half of cases with HNSCC have regional lymph node involvement at diagnosis (ie, recurrent or metastatic HNSCC (R/M HNSCC)) with poor prognosis: the median survival is only about 6–15 months depending on patient-related and disease-related factors.[4]

Programmed death ligand 1 (PD-L1) is the principal ligand of programmed death 1 (PD-1).[5] Additionally, PD-L1 can prevent the hyperactivation of uncontrolled T cells and protect normal cells.[6] PD-L1 can be found in malignant cells, including HNSCC, but was not found to be associated with poor prognosis in previous studies.[7–9] However, it was reported that PD-1/PD-L1 checkpoint inhibitors (nivolumab and pembrolizumab) have remarkable antitumour activity against PD-L1-expressing tumours.[10 11] Therefore, PD-L1 expression is the guiding factor for the use of immune checkpoint inhibitors.[12] Both the prevalence and prognostic value of PD-L1 expression in HNSCC are still subjects of contention, though several studies have reported that patients with PD-L1-positive tumours are more likely to respond to PD-1/PD-L1 inhibitors.[13–17] Moreover, the most recent results from the KEYNOTE-048 study showed that pembrolizumab was effective as a first-line treatment for patients with R/M HNSCC with PD-L1-positive cancer.[18]

The expression of PD-L1 on non-tumour cells in the tumour microenvironment has been described in some studies.[19 20] However, the distribution of PD-L1 on non-malignant cells in HNSCC and the

To cite: Lu H, Kuang D, Zhou P, *et al*. *J Clin Pathol* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/jcp-2023-209059

clinical phenotype most likely to benefit from immunotherapy remain unclear.[21] Additionally, the main cause of HNSCC in Europe and the USA is oral human papillomavirus (HPV) infection, but in China, smoking or drinking are the leading causes.[1] It is necessary to collect solid evidence regarding PD-L1 expression in HNSCC for distinguishing the predictive subgroups more likely to benefit from different treatments. A combined positive score (CPS), which was defined in the KEYNOTE-055 study as 'percentage of tumour and mononuclear inflammatory cells within the tumour nests and adjacent supporting stroma expressing PD-L1 at any intensity',[22] was proposed to be the most appropriate score for determining PD-L1 expression in HNSCC.[23] Because the PD-L1 expression status in Chinese patients with HNSCC is largely unknown, our study aimed to determine the prevalence of PD-L1 high expression (determined by CPS and assessed by immunohistochemistry (IHC) with PD-L1 IHC 22C3 pharmDx kit on ASL48 staining platform) in Chinese patients with advanced R/M HNSCC.

METHODS

Study design

This study was a multicentre retrospective analysis of data from six centres in China. The list of participating centres can be found in online supplemental appendix 1. The protocol and protocol amendments of this study were reviewed and approved by each study site's Independent Ethics Committee. All patients signed the informed consent form (ICF) before enrollment into the study. The present trial was registered at the ISRCTN registry.

Participants

We screened patients with R/M HNSCC in six participating centres in China from 9 August 2021 to 28 February 2022. The inclusion criteria included: (1) having a histologically or cytologically confirmed diagnosis of R/M HNSCC that was considered incurable by local therapies (patient could not have a primary tumour site of the nasopharynx with any histology); (2) age ≥18 years at diagnosis of R/M HNSCC; (3) having an available formalin-fixed, paraffin-embedded (FFPE) tumour specimen obtained via core or excisional biopsy; (4) missing data for related medical information less than 5%[24–26] and (5) previously providing written informed consent form, giving consent for his/her sample to be used in a future study, unless the patient was under conditions accepted by the institutional review board/ethics review committee(IRB/ERC) to waive the need for an ICF. Otherwise, the patient must have provided a specific written informed consent for this study. The exclusion criterion of the patients was who have only a specimen obtained via fine needle aspirate (FNA) or a cytologic specimen. Notably, AJCC Cancer Staging Manual, 8th edition was used for disease stage.[27]

PD-L1 measurement

The samples for PD-L1 measurement were FFPE tumour specimens obtained via core or excisional biopsy, which represented a newly obtained biopsy specimen (within 90 days prior to start of PD-L1 (IHC) evaluation), or at least the archival tissue block had to be no older than 2 years. Regarding the sample site, we preferred tumour specimens collected from the primary site rather than the metastatic site. Furthermore, decalcified bony specimens were not accepted.

A minimum of three freshly cut (within 1 month of assay, prefer within 1 week) 4 µm sections from selected FFPE specimens were required for each patient. Two unstained slides were used for PD-L1 detection and negative control, and one would be stained with H&E to assess tissue histology and preservation quality. However, four unstained slides and one matched H&E-stained slide were preferred to allow for rapid retesting. The detailed procedures for use of the PD-L1 IHC 22C3 pharmDx kit (Agilent Technologies, Santa Clara, California, USA) for tissue collection, processing and shipment were consistent with those applied in the KEYNOTE-048 study.[18] The IHC analysis and CPS scoring were performed locally at each site by a pathologist who had been trained and certified through a training programme. Details regarding PD-L1 expression analysis, including the date of analysis and CPS, were electronically captured in a central database and merged with other key variables.

The PD-L1 expression level was determined in all samples using the PD-L1 IHC 22C3 pharmDx kit. Clone 22C3 is intended for detection of PD-L1 protein in FFPE tissues using the EnVision FLEX visualisation system on Autostainer Link 48. PD-L1 protein expression was represented by the CPS, which is the number of PD-L1-stained cells (tumour cells, lymphocytes, macrophages) divided by the total number of viable tumour cells, multiplied by 100, according to the following equation:

$$\text{CPS} = \frac{\#\ \text{PD-L1 staining cells (tumour cells, lymphocytes, macrophages)}}{\text{Total}\ \#\ \text{of viable tumour cells}} \times 100$$

Outcomes

The primary outcome was the prevalence of PD-L1 CPS ≥20 expression in R/M HNSCC. The secondary outcomes were the prevalence of PD-L1 CPS ≥1 expression in R/M HNSCC and the factors associated with expression of PD-L1 in R/M HNSCC.

Variables

We collected relevant demographic characteristics (eg, age at diagnosis, sex, family history of the studied disease and history of tobacco use), clinicopathological parameters (eg, primary tumour site, tumour stage, histology and grade, metastatic location and number, site and type of tumour tissue sample), details of treatment status (eg, previous lines of therapy and prior curative treatments) and other available biomarkers (eg, HPV status) from each centre's electronic medical records (EMR) system or by chart review if no EMR existed.

Tobacco and alcohol consumption

The definitions used to define the alcohol consumption status of patients are as follows:

- Alcohol dependent, sometimes known as 'alcoholism', is the most serious form of drinking problem and describes a strong, often uncontrollable, desire to drink.
- Heavy drinker: a person who has frequent episodes of heavy drinking (>5 drinks at a time), which can be defined by sex and periodicity.
- Occasional drinkers: they drank less than 1 day per week (for instance once or twice per month) and drinking a maximum of 3 drinks for men and 2 drinks max for women. Binge drinkers were incorporated into the heavy drinker category.
- Abstinent: abstinence from alcohol lasting 6 months or longer.

The definitions used to define the tobacco consumption status of patients are as follows:

- Current smoker: an adult who has smoked 100 cigarettes in his or her lifetime and who currently smokes cigarettes.
- Former smoker: an adult who has smoked at least 100 cigarettes in his or her lifetime but who had quit smoking at the time of interview.
- Non-smoker: an adult who has never smoked, or who has smoked less than 100 cigarettes in his or her lifetime.

Bias

To decrease selection bias, we preferred the tumour specimen collected from the primary site rather than the metastatic site. Newly obtained specimens were preferred to archived samples (90 days prior to start of PD-L1 IHC examination). In addition, a training programme on IHC analysis and CPS scoring was performed locally at each site by a trained and certified pathologist to reduce inter-group heterogeneity. Finally, imputation was performed to deal with the missing data bias related to the medical characteristic information.

Sample size

According to a prevalence of 44% of HNSCC samples with PD-L1 (CPS ≥20) expression based on Chinese subgroup data from the KEYNOTE-048 study, a total sample size of 396 cases with HNSCC was required to estimate the PD-L1 expression prevalence with ±5% precision with a 95% CI.

Statistical analyses

The categorical variables are presented as count with proportion or percentage, and continuous variables are described as mean and SD. The prevalence of PD-L1 expression is presented in the form of percentage of patients with PD-L1 CPS ≥20 and with CPS ≥1, separately. In addition, 95% Clopper-Pearson intervals were calculated for the prevalence, to describe PD-L1 expression according to baseline demographic characteristics, clinicopathological characteristics, treatment status and other available biomarkers. The χ^2 test and Cochran-Mantel-Haenszel χ^2 test were used to compare the prevalence of related variables (eg, demographic characteristics, clinicopathological parameters and treatment status) among patients with different PD-L1 expression statuses to explore the potential factors associated with PD-L1 expression (CPS ≥20) in R/M HNSCC.

Data availability

Study data are available from the corresponding author as appropriately required.

RESULTS

Patients

In the six participating sites, we screened a total of 406 patients with R/M HNSCC. No patient was excluded according to the inclusion/exclusion criteria. Of these patients, 123 had previously signed an ICF for use of her/his sample for future research and 283 were under conditions accepted by the IRB/ERC for waiving the ICF. During PD-L1 IHC analysis, four patients were excluded from the final analysis for insufficient viable tumour cells on the slides (figure 1).

Among all 402 patients, the median age at initial diagnosis was 56.8 years (range 26–91 years). The primary tumour site was the oral cavity for nearly half of the patients (189/402, 47.0%), and more than one-third (138/402, 34.3%) had an Eastern Cooperative Oncology Group Performance Status of 1. For most cases (380/402, 94.5%), samples were available from the primary tumour sites, while 14 samples were from metastatic lesion and 8 from lymph node. Among the patients, 27 had new biopsies while 375 had archival tissues within the last 2 years. The detailed baseline characteristics of the included patients are described in table 1.

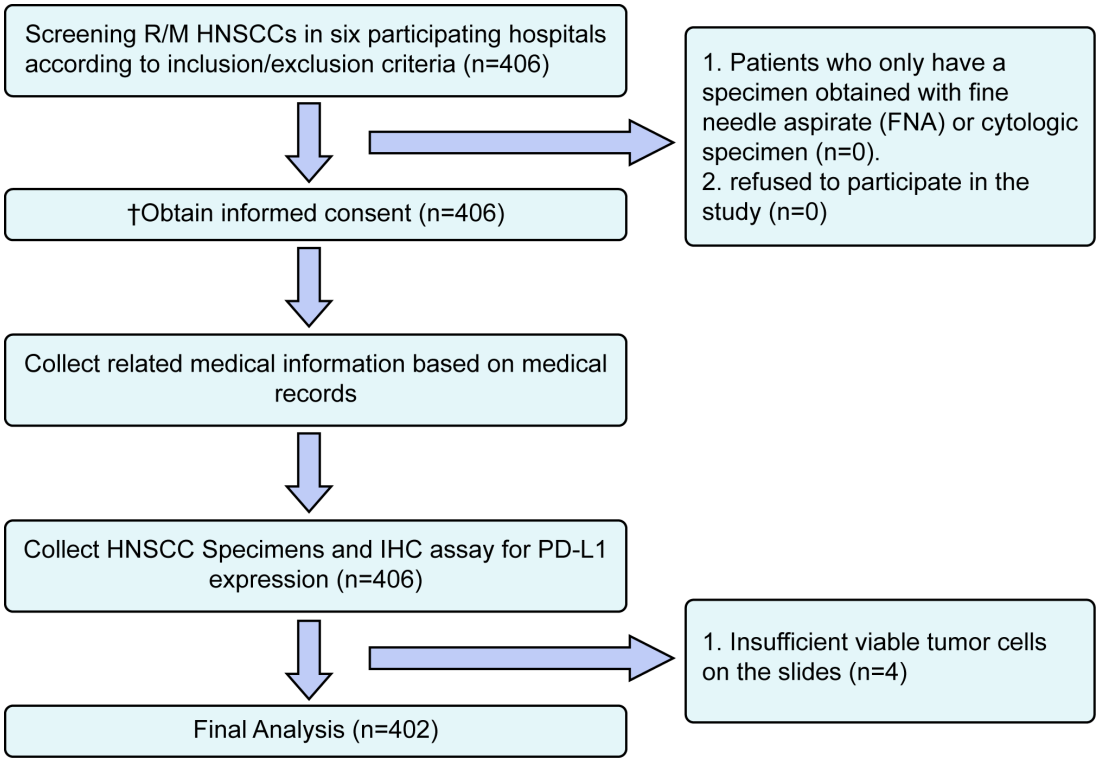

Figure 1 Study workflow chart. †For patients who gave consent for his/her sample to be used in future study in an ICF signed previously or under conditions (eg, lost to follow-up or died prior to the study initiation) accepted by IRB/ERC, the requirement of specific informed consent for this study was waived. ICF, informed consent form; IHC, immunohistochemistry; IRB/ERC, institutional review board/ethics review committee; PD-L1, programmed death-ligand 1; R/M HNSCCs, recurrent or metastatic head and neck squamous cell carcinomas.

Table 1 Baseline characteristics of the included patients with R/M HNSCC (n=402)

Variables	Number of patients, n (%) or specified (n=402)
Age at initial diagnosis (years), mean±SD	56.8±10.8
≤60 years	256 (63.8)
>60 years	145 (36.2)
Missing	1 (0.2)
Female	56 (13.9)
Male	346 (86.1)
Primary tumour site	
Oral cavity	189 (47.0)
Oropharynx	22 (5.5)
Hypopharynx	70 (17.4)
Larynx	99 (24.6)
Others	22 (5.5)
Biopsy timing	
New biopsy	27 (6.7)
Archival tissue within 2 years	375 (93.3)
Bio-specimen site	
Primary tumour	380 (94.5)
Lymph node	8 (2.0)
Metastatic lesion	14 (3.5)
HPV status	
Positive	15 (3.7)
Negative	41 (10.2)
Unknown	346 (86.1)
Clinical stage at initial diagnosis	
I	4 (1.1)
II	10 (2.6)
III	132 (34.7)
IVA	58 (15.3)
IVB	10 (2.6)
IVC	1 (0.3)
Unknown	165 (43.4)
Missing	22 (5.5)
Disease status	
Recurrent disease with no distant metastases	126 (33.2)
Recurrent disease with distant metastases	12 (3.2)
Newly diagnosed metastatic disease	241 (63.6)
Missing	23 (5.7)
Metastatic sites	
Lung	11 (2.7)
Liver	1 (0.2)
Others	282 (70.1)
Unknown	24 (6.0)
ECOG PS	
0	2 (0.5)
1	138 (34.3)
2	4 (1.0)
Unknown	258 (64.2)
Treatment history	
Surgery	319 (79.4)
Radiation	51 (12.7)
Chemotherapy	59 (14.7)
Immunotherapy	11 (2.7)
Targeted therapy	13 (3.2)
Others	1 (0.2)
Smoking habit	
Current smoker	94 (23.4)
Former smoker	125 (31.1)
Non-smoker	177 (44.0)
Unknown	6 (1.5)
Alcohol consumption	
Alcohol dependent	26 (6.5)
Heavy drinker	47 (11.7)
Occasional drinker	49 (12.2)
Abstinent	273 (67.9)
Unknown	7 (1.7)

ECOG PS, Eastern Cooperative Oncology Group Performance Status; HPV, human papillomavirus; R/M HNSCC, recurrent or metastatic head and neck squamous cell carcinoma.

Prevalence of PD-L1 expression

For all 402 examined patients with HNSCC, the prevalence of PD-L1 expression with a CPS ≥20 was 41.8% (95% CI: 36.9% to 46.8%), and the prevalence of PD-L1 expression with a CPS ≥1 was 83.8% (95% CI: 79.9% to 87.3%).

Potential associated factors

Between the PD-L1 CPS ≥20 and CPS <20 patient groups, statistically significant differences were observed in variables of sex (p<0.001), smoking habit (p=0.0138, non-smokers vs current smokers) and primary tumour site (p<0.001 for hypopharynx vs oral cavity and p=0.0304 for larynx vs oral cavity). On univariate logistic regression analyses, the prevalence of PD-L1 CPS ≥20 expression was significantly higher in women versus men (69.6% vs 37.3%; OR, 3.86; 95% CI: 2.097 to 7.101; p<0.001) and in non-smokers versus current smokers (50.8% vs 35.1%; OR, 1.91; 95% CI: 1.141 to 3.230; p=0.0138). Conversely, there was a lower prevalence of PD-L1 CPS ≥20 expression in patients whose primary tutor sites were the hypopharynx (18.6% vs 51.9%; OR, 0.21; 95% CI: 0.109 to 0.412; p<0.001) or larynx (38.4% vs 51.9%; OR, 0.58; 95% CI: 0.352 to 0.949; p=0.0304) compared with those whose primary tutor site was the oral cavity. And between the PD-L1 CPS ≥1 and CPS <1 patient groups, the percentage of patients with CPS ≥1 was higher among patients who had a history of surgery than among patients who did not have a history of surgery (86.2% vs 74.7%; OR, 2.12; 95% CI: 1.175 to 3.813; p=0.0125). The details of the comparisons between the PD-L1 CPS ≥20 and CPS <20 patient groups are listed in table 2 and online supplemental appendix 2.

DISCUSSION

The present EXCEED study revealed the prevalence of PD-L1 expression status in Chinese patients with R/M HNSCC, and we found that the prevalence of PD-L1 CPS ≥20 expression was 41.8% and that of PD-L1 CPS ≥1 expression was 83.8%, which was consistent with the findings of the KEYNOTE-055 study (82% for CPS ≥1%) and the KEYNOTE-048 study (43% for CPS ≥20 and 85% for CPS ≥1).[18 22] The Food and Drug Administration(FDA) approved pembrolizumab (KEYTRUDA, Merck) for the first-line treatment of R/M HNSCC on 10 June 2019, for use in combination with platinum and fluorouracil

Table 2 PD-L1 expression in cases with R/M HNSCC according to patients' characteristics (with statistically significant differences)

Variables	Overall (n=402)	PD-L1 with CPS ≥20 (n=168)	PD-L1 with CPS <20 (n=234)	OR (95% CI)*	P value*
Sex					
Male	346	129 (37.3%)	217 (62.7%)	3.86 (2.097 to 7.101)	<0.0001
Female	56	39 (69.6%)	17 (30.4%)		
Smoking habit					
Current smoker	94	33 (35.1%)	61 (64.9%)	1	
Former smoker	125	43 (34.4%)	82 (65.6%)	0.97 (0.553 to 1.700)	0.9135
Non-smoker	177	90 (50.8%)	87 (49.2%)	1.91 (1.141 to 3.203)	0.0138
Primary tumour site					
Oral cavity	189	98 (51.9%)	91 (48.1%)	1	
Oropharynx	22	7 (31.8%)	15 (68.2%)	0.43 (0.169 to 1.111)	0.0817
Hypopharynx	70	13 (18.6%)	57 (81.4%)	0.21 (0.109 to 0.412)	<0.0001
Larynx	99	38 (38.4%)	61 (61.6%)	0.58 (0.352 to 0.949)	0.0304
Others	22	12 (54.5%)	10 (45.5%)	1.11 (0.459 to 2.704)	0.8109

Footnote: The row percentage is calculated.
*OR, 95% CI or p value are calculated by univariate logistic regression. P values are rounded to four decimal places. OR values are rounded to two decimal places. The lower and upper bounds of CI are rounded to three decimal places.
CPS, combined positive score; PD-L1, programmed death-ligand 1; R/M HNSCC, recurrent or metastatic head and neck squamous cell carcinoma.

(FU) for all patients and as a single agent for patients whose tumours express PD-L1 (CPS ≥1) as determined by an FDA-approved test, whereas the National Medicine Products Administration approved pembrolizumab monotherapy for treating advanced R/M HNSCC with PD-L1 CPS ≥20. Our findings filled in time of the data gap for PD-L1 expression in Chinese patients with R/M HNSCC and closely aligned with current indications in China, providing a solid foundation for healthcare professionals to develop suitable detection and treatment strategies and for future research. The present study further identified several potential factors associated with PD-L1 CPS ≥20 expression in R/M HNSCC, including sex, smoking habit and primary tumour site. The sex difference in PD-L1 expression was previously reported in patients with non-small cell lung cancer.[28] However, no sex difference in PD-L1 expression in nasopharyngeal carcinoma was reported by Feng *et al*.[29] Despite the inconsistent relationship between sex and PD-L1 expression, the sex-based differences in response to anti-PD-1 or PD-L1 treatment have been confirmed by systematic reviews.[30 31] The rationale may be related to sex differences in innate and adaptive immune responses.[32] Our findings revealed that the sex difference may be related to PD-L1 expression in Chinese patients with R/M HNSCC, which will be a good indicator for healthcare providers in making diagnosis and treatment decisions. Another factor associated with PD-L1 expression, smoking, has been reported to reduce PD-L1 expression in lung epithelial cells in vitro and in vivo.[33] The rationale for the influence of smoking on PD-L1 expression may be related to the aryl hydrocarbon receptor (AhR), which can be affected by smoking and contributes to immunosuppression via regulation of important immune checkpoints (eg, AhR can mediate tobacco-induced PD-L1 expression and transcriptional control CD39 expression) in oral squamous cell carcinoma.[34] We found that non-smokers had a higher PD-L1 (CPS ≥20) ratio than patients who currently smoke, which was consistent with the previous findings that PD-L1 expression appears to be higher in female patients, non-smokers, non-drinkers reported by Lenouvel *et al*.[35] Finally, our findings indicate that the primary tumour sites of the hypopharynx and larynx may have less PD-L1 expression than the oral cavity, without existing evidence to support this statement, further research is needed to draw stronger conclusions.

The existing literature indicates that pathologists are currently facing several challenges when evaluating PD-L1 expression in patients with HNSCC. Among them, the most important one is concordance among assays.[36] Despite a clear concomitant diagnosis (CDx) being approved, many laboratories work on other platforms and/or with other clones, which creates variability in detected PD-L1 expression in patients with HNSCC (ranges from 18% to 100%). The main published findings to date indicate that the degree of concordance varies from fair to substantial among the reference assay 22C3 pharmDx and two alternate Ventana assays (SP262 and SP142), while limited evidence is available concerning the 28-8 assay.[36] Hence, further work is needed to draw stronger conclusions on the interchangeability of PD-L1 assays in HNSCC.[36 37] In our study, we adopted 22C3 pharmDx on the Dako IHC platform. CPS was scored by trained and certified pathologists and provided reliable data concerning the prevalence of PD-L1 expression status in Chinese patients with R/M HNSCC.

The second challenge lies in concordance among pathologists. CPS's apparent higher sensitivity at lower cutoffs of positivity supported its widespread adoption in testing patients with HNSCC. Indeed, CPS is more complex and less intuitive than Tumor Proportion Score (TPS), as it requires specific counting of tumour and immune cells to calculate the score.[36] Not surprisingly, training could narrow both interobserver and intraobserver differences. Also, better concordance was found among trained pathologists, with the reproducibility being higher in assessing CPS.[38] In addition, digital tools such as artificial intelligence and algorithms may serve certain important aid for pathologists evaluating PD-L1 expression, since automated algorithms can be trained to recognise different types of cells (tumour vs immune; with macrophages to be excluded from other cell types) and provide a more objective quantification of CPS.[36 39]

The third challenge is the intrinsic heterogeneity of PD-L1 expression in HNSCC, including temporal and spatial heterogeneity. First, PD-L1 expression is heterogeneous within primary tumours, thus choosing samples that are representative of the whole tumour is important. Concordance between PD-L1 expression in primary HNSCC and neck Lymph node metastasis (LNM) is fair to moderate, and PD-L1 may be over-expressed in LNM even in cases that have negative primary tumours.

Consequently, clinicians should undertake a careful evaluation of PD-L1 both in primary HNSCC and on LNM if the latter is available.[40] Furthermore, by comparing PD-L1 expression between freshly biopsied tissue and the same tissue later in time, Karpathiou *et al* provided evidence of PD-L1 expression diminishing, in both tumour and immune cells, over time in storage.[41] Therefore, an immediate rather than a retrospective assay of PD-L1 expression should be preferable in routine practice. To minimise heterogeneity, we preferred that the tumour specimen be collected from the primary site rather than the metastatic site when possible, with 380 samples (94.5%) from the primary tumour, 8 samples (2.0%) from the lymph node and 14 samples (3.5%) from metastatic lesions, as relatively uniform sample sourcing could make the result more homogeneous. In addition, we aimed to consider the time factor by selecting newly obtained specimens preferred to archived samples (90 days prior to start of PD-L1 IHC examination). However, due to various limitations such as difficult cases and/or ethical considerations, only 27 specimens (6.7%) were newly obtained and the other 375 (93.3%) were archived. In an effort to compensate for the heterogeneity this may have caused, the most recent sample available was always selected whenever using archived samples, and none of them were older than 2 years.

The potential impact of already administered therapy on PD-L1 expression is also a concern.[40] To date, findings regarding the effects of anti-tumour Chemotherapy (CT) and Radiation Therapy (RT) on PD-L1 expression in HNSCC have been conflicting.[42–44] And history of systemic therapy and RT treatment hardly had an impact on PD-L1 expression in our study. PD-L1 expression in tumour cells of patients undergoing combined therapy with a platinum agent tends to be higher, though further studies are needed to clarify this issue.[45–47] Moreover, in contrast to many previous studies using TPS which showed conflicting results, only a few recent papers and two USCAP abstracts used the CPS scoring method and judged the correlations between biopsy and resection specimens and between primary and metastasis sites as generally modest.[40 48–50] However, Liu *et al* found that the concordance between FNA of metastatic lymph nodes and histological material was 76% when analysed with both CPS and TPS. In their study, the positive predictive value was 100% for both scoring assessments, but the negative predictive value was only 54% and 50%, with most of discordant cases being negative in histological material.[51] The evaluation of PD-L1 expression using CPS has not been well established, mainly because of challenges in identifying tumour-associated immune cells in the absence of tissue architecture.[37] Recent evidence suggests that many other markers and mechanisms are involved in the dysregulation of the cancer-related immune system and affect the expression of related markers such as PD-L1.[52] It is then foreseeable that in the near future, growing evidence will provide more quantitative, reliable and practical evidence of PD-L1 expression in HNSCC.[53] For the treatment history before bio-specimen collection, 319 (79.4%) of the included patients received surgical treatment, 51 (12.7%) received radiation treatment, 59 (14.7%) received chemotherapy, 11 (2.7%) received immunotherapy, 13 (3.2%) underwent targeted therapy and 1 patient (0.2%) was given other treatment. The results showed that patients who had a surgical treatment history ($p=0.0125$) had a higher PD-L1 (CPS ≥ 1) ratio than patients who had no surgical treatment history. Furthermore, we quite agree on the consistency of PD-L1 using CPS scoring between core biopsy and resection specimens, but not FNA cell block or cytological material, for PD-L1 positive immune cells, PD-L1 tends to gather at the tumour–stroma interface and be easily missed by FNA or cytological procedures.[49] Therefore, the exclusion criteria in our study included that 'availability of only a specimen obtained with fine needle aspirate (FNA) or cytologic specimen', and more robust evidence is needed to draw clear conclusions.

It is now well demonstrated that alcohol consumption, smoking and viral infections, such as HPVs are major risk factors for HNSCC development. Particularly, HPV16 is responsible for more than 90% of virus-driven HNSCC, and the HPV-driven HNSCC incidence is increasing in Europe and North America.[54–56] Findings in the literature about the association of HPV with PD-L1 expression and prognosis have yet to reach a consensus, and the present study explored the relationship between HPV infection and PD-L1 expression in HNSCC. Xu *et al* reported that of 112 HPV (+) oropharyngeal squamous cell carcinoma (OPSCC) tumours, high (CPS ≥ 20), intermediate ($1 \leq CPS < 20$) and low ($CPS < 1$) PD-L1 expression was seen in 29.5%, 43.8% and 26.8% Chinese cases, respectively.[57] However, with limited data, PD-L1 expression was observed in the majority of patients with OPSCC regardless of HPV status. Moreover, a trend of higher PD-L1 (CPS ≥ 20) ratio in HPV-positive patients whose primary tumour site were oral cavity than HPV-negative ones was noted among the Chinese patients, and whether this is related to race or geography remains to be confirmed.

To our best knowledge, this study is the first clinical study aimed at exploring the PD-L1 expression condition in R/M HNSCC among Chinese patients. Our study provides solid insight for healthcare providers to use in making diagnosis and treatment decisions in Chinese R/M HNSCC cases. In addition, we applied the CPS to score PD-L1 expression, which can reflect the integrated condition under the specific R/M HSNSCC immune environment. However, this study still has some limitations. First, as this is a multicentre retrospective analysis, there may be variability in data quality and completeness across centres including inter-rater variability for PD-L1 scoring. Additionally, selection bias may be a potential concern. Also, even though proper training was provided to all participating pathologists, some amount of bias was inevitably present in the subjective process of imaging interpretation.

CONCLUSION

According to our findings, more than 40% of Chinese patients with R/M HNSCC had PD-L1 expression with a CPS ≥ 20, which was significantly associated with sex, smoking history, and primary tumour site. Our findings regarding the variables related to the PD-L1 expression level provide insight for clinical practice and a solid basis for future research on the use of immunotherapy in HNSCC.

Author affiliations
[1]Department of Pathology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
[2]Department of Pathology, Tongji Hospital, Tongji Medical College, Huazhong University of Science & Technology, Wuhan, Hubei, China
[3]Department of Pathology, West China Hospital, Sichuan University, Chengdu, Sichuan, China
[4]Department of Pathology, Sun Yat-Sen University Cancer Center, Guangzhou, Guangdong, China
[5]Department of Pathology, Henan Cancer Hospital, Zhengzhou, Henan, China
[6]Department of Pathology, Fudan University Shanghai Cancer Center, Shanghai, China
[7]MRL Global Medical Affairs, MSD China, Shanghai, China

Handling editor Munita Bal.

Acknowledgements Editorial assistance was provided by Medjaden, Inc. This assistance was funded by MSD China.

Contributors JY is responsible for the overall content as the guarantor. HL, JW, JL, WT, JC and JY contributed to conception, design or planning of the study. HL, DK, PZ, JZ, QX, PD, LJ, SZ, YJ, XJ and JY contributed to acquisition of the data. HL, YJ, XJ, JZ and JY contributed to analysis of the data. HL, LJ, JZ, JC and JY contributed to interpretation of the results. HL and JY contributed to drafting of the manuscript. All authors contributed to reviewing or revising the manuscript for important intellectual content. HL, LJ, SZ, YJ and XJ contributed to provision of study materials. JW and WT contributed to administrative, logistical or technical support. All authors read and approved the final version of the manuscript.

Funding The study was funded by MSD China.

Competing interests JL, WT, JZ and JC are employees of MSD China; all other authors have none to declare.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants and was approved. The protocol and protocol amendment of this study were reviewed and approved by each study site's Independent Ethics Committee (Ethics Committee of National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (approval no. 20/377-2573), Ethics Committee of Tongji Hospital,Tongji Medical College, Huazhong University of Science & Technology (approval no. 2020-S332), Ethics Committee of Biomedical Research, West China Hospital of Sichuan University (approval no. 2021-343), Ethics Committee of Sun Yat-Sen University Cancer Center (approval no. B2021-087-01), Ethics Committee of Henan Cancer Hospital (approval no. 2020103012-001), Ethics Committee of Fudan University Shanghai Cancer Center (approval no. 2102231-16)). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. The data generated in this study are available upon request from the corresponding author.

ORCID iDs
Haizhen Lu http://orcid.org/0000-0001-7564-794X
Jian Wang http://orcid.org/0000-0003-4082-7750
Lili Jiang http://orcid.org/0000-0002-0359-0873
Jiansong Zhou http://orcid.org/0009-0009-8820-7711

REFERENCES

1 Johnson DE, Burtness B, Leemans CR, *et al*. Head and neck squamous cell carcinoma. *Nat Rev Dis Primers* 2020;6:92.
2 Xia C, Dong X, Li H, *et al*. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. *Chin Med J (Engl)* 2022;135:584–90.
3 Cao W, Chen H-D, Yu Y-W, *et al*. Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020. *Chin Med J (Engl)* 2021;134:783–91.
4 Larkins E, Blumenthal GM, Yuan W, *et al*. FDA approval summary: pembrolizumab for the treatment of recurrent or metastatic head and neck squamous cell carcinoma with disease progression on or after platinum-containing chemotherapy. *Oncologist* 2017;22:873–8.
5 Kythreotou A, Siddique A, Mauri FA, *et al*. PD-L1. *J Clin Pathol* 2018;71:189–94.
6 Cha J-H, Chan L-C, Li C-W, *et al*. Mechanisms controlling PD-L1 expression in cancer. *Mol Cell* 2019;76:359–70.
7 Crosta S, Boldorini R, Bono F, *et al*. PD-L1 testing and squamous cell carcinoma of the head and neck: a multicenter study on the diagnostic reproducibility of different protocols. *Cancers (Basel)* 2021;13:292.
8 Higashino M, Kawata R, Nishikawa S, *et al*. Programmed death Ligand-1 expression is associated with stage and histological grade of parotid carcinoma. *Acta Otolaryngol* 2020;140:175–80.
9 Mukaigawa T, Hayashi R, Hashimoto K, *et al*. Programmed death Ligand-1 expression is associated with poor disease free survival in salivary gland carcinomas. *J Surg Oncol* 2016;114:36–43.
10 Park J-E, Kim S-E, Keam B, *et al*. Anti-tumor effects of NK cells and anti-PD-L1 antibody with antibody-dependent cellular cytotoxicity in PD-L1-positive cancer cell lines. *J Immunother Cancer* 2020;8:e000873.
11 Ganbaatar O, Konnai S, Okagawa T, *et al*. PD-L1 expression in equine malignant melanoma and functional effects of PD-L1 blockade. *PLoS One* 2020;15:e0234218.
12 Doroshow DB, Bhalla S, Beasley MB, *et al*. PD-L1 as a biomarker of response to immune-checkpoint inhibitors. *Nat Rev Clin Oncol* 2021;18:345–62.
13 Kujan O, van Schaijik B, Farah CS. Immune checkpoint inhibitors in oral cavity squamous cell carcinoma and oral potentially malignant disorders: a systematic review. *Cancers (Basel)* 2020;12:1937.
14 Jiang S, Li X, Huang L, *et al*. Prognostic value of PD-1, PD-L1 and PD-L2 deserves attention in head and neck cancer. *Front Immunol* 2022;13:988416.
15 He J, Chen X-F, Xu M-G, *et al*. Relationship of programmed death ligand-1 expression with clinicopathological features and prognosis in patients with oral squamous cell carcinoma: a meta-analysis. *Arch Oral Biol* 2020;114:104717.
16 Tang H, Zhou X, Ye Y, *et al*. The different role of PD-L1 in head and neck squamous cell carcinomas: a meta-analysis. *Pathol Res Pract* 2020;216:152768.
17 Patel JJ, Levy DA, Nguyen SA, *et al*. Impact of PD-L1 expression and human papillomavirus status in anti-PD1/PDL1 Immunotherapy for head and neck squamous cell carcinoma-systematic review and meta-analysis. *Head Neck* 2020;42:774–86.
18 Burtness B, Harrington KJ, Greil R, *et al*. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. *Lancet* 2019;394:1915–28.
19 Taube JM, Anders RA, Young GD, *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.
20 Carey CD, Gusenleitner D, Lipschitz M, *et al*. Topological analysis reveals a PD-L1-associated microenvironmental niche for reed-sternberg cells in hodgkin lymphoma. *Blood* 2017;130:2420–30.
21 Cavalieri S, Rivoltini L, Bergamini C, *et al*. Immuno-oncology in head and neck squamous cell cancers: news from clinical trials, emerging predictive factors and unmet needs. *Cancer Treat Rev* 2018;65:78–86.
22 Bauml J, Seiwert TY, Pfister DG, *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.
23 Evrard D, Hourseau M, Couvelard A, *et al*. PD-L1 expression in the microenvironment and the response to checkpoint inhibitors in head and neck squamous cell carcinoma. *Oncoimmunology* 2020;9:1844403.
24 Rubin DB, Schenker N. Multiple imputation in health-care databases: an overview and some applications. *Stat Med* 1991;10:585–98.
25 Duffy ME. Handling missing data: a commonly encountered problem in quantitative research. *Clin Nurse Spec* 2006;20:273–6.
26 Beesley LJ, Bondarenko I, Elliot MR, *et al*. Multiple imputation with missing data indicators. *Stat Methods Med Res* 2021;30:2685–700.
27 Amin MB, Edge SB, Greene FL, *et al*. *AJCC cancer staging manual*. Springer Cham, 2017.
28 Gu Y, Tang YY, Wan JX, *et al*. Sex difference in the expression of PD-1 of non-small cell lung cancer. *Front Immunol* 2022;13:1026214.
29 Feng C, Zhang Y, Huang J, *et al*. The prognostic significance of APOBEC3B and PD-L1/PD-1 in Nasopharyngeal carcinoma. *Appl Immunohistochem Mol Morphol* 2021;29:239–44.
30 Conforti F, Pala L, Pagan E, *et al*. Sex-based differences in response to anti-PD-1 or PD-L1 treatment in patients with non-small-cell lung cancer expressing high PD-L1 levels. A systematic review and meta-analysis of randomized clinical trials. *ESMO Open* 2021;6:100251.
31 Conforti F, Pala L, Bagnardi V, *et al*. Cancer Immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. *Lancet Oncol* 2018;19:737–46.
32 Klein SL, Morgan R. The impact of sex and gender on immunotherapy outcomes. *Biol Sex Differ* 2020;11:24.
33 Wang G-Z, Zhang L, Zhao X-C, *et al*. The aryl hydrocarbon receptor mediates tobacco-induced PD-L1 expression and is associated with response to Immunotherapy. *Nat Commun* 2019;10:1125.
34 Kenison JE, Wang Z, Yang K, *et al*. The aryl hydrocarbon receptor suppresses immunity to oral squamous cell carcinoma through immune checkpoint regulation. *Proc Natl Acad Sci U S A* 2021;118:e2012692118.
35 Lenouvel D, González-Moles MÁ, Ruiz-Ávila I, *et al*. Clinicopathological and prognostic significance of PD-L1 in oral cancer: a preliminary retrospective immunohistochemistry study. *Oral Dis* 2021;27:173–82.
36 Girolami I, Pantanowitz L, Barberis M, *et al*. Challenges facing pathologists evaluating PD-L1 in head & neck squamous cell carcinoma. *J Oral Pathol Med* 2021;50:864–73.
37 Marletta S, Fusco N, Munari E, *et al*. Atlas of PD-L1 for pathologists: indications, scores, diagnostic platforms and reporting systems. *J Pers Med* 2022;12:1073.
38 Eccher A, Fontanini G, Fusco N, *et al*. Digital slides as an effective tool for programmed death ligand 1 combined positive score assessment and training: lessons

learned from the "programmed death ligand 1 key learning program in head-and-neck squamous cell carcinoma" *J Pathol Inform* 2021;12:1.
39 Inge LJ, Dennis E. Development and applications of computer image analysis algorithms for scoring of PD-L1 immunohistochemistry. *Immunooncol Technol* 2020;6:2–8.
40 Paolino G, Pantanowitz L, Barresi V, *et al*. PD-L1 evaluation in head and neck squamous cell carcinoma: insights regarding specimens, heterogeneity and therapy. *Pathol Res Pract* 2021;226:153605.
41 Karpathiou G, Vincent M, Dumollard JM, *et al*. PD-L1 expression in head and neck cancer tissue specimens decreases with time. *Pathol Res Pract* 2022;237:154042.
42 Leduc C, Adam J, Louvet E, *et al*. TPF induction chemotherapy increases PD-L1 expression in tumour cells and immune cells in head and neck squamous cell carcinoma. *ESMO Open* 2018;3:e000257.
43 Ock C-Y, Kim S, Keam B, *et al*. Changes in programmed death-ligand 1 expression during cisplatin treatment in patients with head and neck squamous cell carcinoma. *Oncotarget* 2017;8:97920–7.
44 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. *Pathol Oncol Res* 2020;26:735–42.
45 Li S, Ji J, Zhang Z, *et al*. Cisplatin promotes the expression level of PD-L1 in the microenvironment of hepatocellular carcinoma through YAP1. *Mol Cell Biochem* 2020;475:79–91.
46 Liang Y, Yu M, Zhou C, *et al*. Variation of PD-L1 expression in locally advanced cervical cancer following neoadjuvant chemotherapy. *Diagn Pathol* 2020;15:67.
47 Fournel L, Wu Z, Stadler N, *et al*. Cisplatin increases PD-L1 expression and optimizes immune check-point blockade in non-small cell lung cancer. *Cancer Lett* 2019;464:5–14.
48 Hirshoren N, Al-Kharouf I, Weinberger JM, *et al*. Spatial Intratumoral heterogeneity expression of PD-L1 antigen in head and neck squamous cell carcinoma. *Oncology* 2021;99:464–70.
49 Paintal AS, Brockstein BE. PD-L1 CPS scoring accuracy in small biopsies and aspirate cell blocks from patients with head and neck squamous cell carcinoma. *Head Neck Pathol* 2020;14:657–65.
50 Abstracts from USCAP 2019: head and neck pathology. *Modern Pathology* 2019;32:1–59.
51 Liu Z, Williams M, Stewart J, *et al*. Evaluation of programmed death ligand 1 expression in cytology to determine eligibility for immune checkpoint inhibitor therapy in patients with head and neck squamous cell carcinoma. *Cancer Cytopathol* 2022;130:110–9.
52 Cramer JD, Burtness B, Ferris RL. Immunotherapy for head and neck cancer: recent advances and future directions. *Oral Oncol* 2019;99:104460.
53 Girolami I, Marletta S, Fiorentino V, *et al*. Effect of radio-chemotherapy on PD-L1 immunohistochemical expression in head and neck squamous cell carcinoma. *J Pers Med* 2023;13:363.
54 Galati L, Chiocca S, Duca D, *et al*. HPV and head and neck cancers: towards early diagnosis and prevention. *Tumour Virus Res* 2022;14:200245.
55 Castellsagué X, Alemany L, Quer M, *et al*. HPV involvement in head and neck cancers: comprehensive assessment of biomarkers in 3680 patients. *J Natl Cancer Inst* 2016;108:djv403.
56 Hashibe M, Sturgis EM. Epidemiology of oral-cavity and oropharyngeal carcinomas: controlling a tobacco epidemic while a human papillomavirus epidemic emerges. *Otolaryngol Clin North Am* 2013;46:507–20.
57 Xu S-M, Shi C-J, Xia R-H, *et al*. Analysis of immunological characteristics and genomic alterations in HPV-positive oropharyngeal squamous cell carcinoma based on PD-L1 expression. *Front Immunol* 2021;12:798424.