The Prognostic Value of Immune Checkpoints in Oral Squamous Cell Carcinoma

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8 Institute of Dentistry, University of Misurata, Misurata, Libya.
+ These authors jointly supervised the study.
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Running title: The Prognostic Value of Immune Checkpoints

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Keywords: oral squamous cell carcinoma (OSCC), immune checkpoint, systematic review, prognosis, programmed death ligand 1 (PD-L1), B7-H3

ABSTRACT

Background: Despite the importance of immune checkpoints in immunotherapy, the prognostic value of these molecules remains controversial in oral squamous cell carcinoma (OSCC). We performed a systematic review to investigate the prognostic significance of the immune checkpoints in OSCC.

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**Materials:** A systematic search was conducted in Ovid Medline, Scopus and Cochrane libraries and all studies that evaluated the prognostic significance of immune checkpoints in OSCC were systematically retrieved.

**Results:** Twelve immune checkpoints/modulators were studied for their prognostic values in OSCC patients between 1985 to 2017. Seven immune checkpoints (FKBP51, B7-H4, B7-H6, ALHD1, PD-L1, B7-H3, IDO1) were reported to be associated with poor patients’ survival in at least one study, and 5 (CTLA-4, TLT-2, VISTA, PD-L2, PD-1) did not have a significant prognostic value. PD-L1 results were controversial as it was reported to be associated with both better and worse patients’ survival.

**Conclusions:** Even though immune checkpoint markers had high expectation for OSCC prognostication, our systematic review revealed that the majority of them had been studied only once. The other molecules, which had been studied more than once had controversial findings, except B7-H3.

1. **INTRODUCTION**

Oral cancers arising from the oral cavity and lip are the ninth most common malignancy globally and have an annual incidence of >300 000 (Ferlay *et al*, 2012; Roser *et al*, 2015). Approximately 90% of the oral malignancies are squamous cell carcinomas (SCC). The association between oral (O) SCC and alcohol and tobacco abuse has been confirmed in several studies (Hashibe *et al*, 2007; Wyss *et al*, 2013). Despite advanced knowledge in cancer therapy, there are still approximately over 145 000 deaths annually due to oral cancer (Roser *et al*, 2015). The 5-year survival rate for OSCC in most countries is approximately 50% (Warnakularsuriya, 2009). Unfortunately, this rate has not improved in recent decades; new treatments and therapeutic approaches are thus needed (Warnakularsuriya, 2009).

Escape from immune-mediated destruction is an important step for cancer growth and metastasis (Mittal *et al*, 2014). Programmed death receptor 1 (PD-1), programmed death ligand 1 (PD-L1), indoleamine-2,3 dioxygenase (IDO1), and B7-H3 are immune checkpoints. They induce immune tolerance, prevent induction of autoimmune diseases, and protect tissues from immune collateral damage (Topalian *et al*, 2017). In cancer, immune checkpoints play a predominant role in immune surveillance and escape of the cancer cells (Mittal *et al*, 2014).
Oral cancer has two approved immunotherapies that target immune checkpoints, namely the PD-1/PD-L1 inhibitors nivolumab (Opdivo®) and pembrolizumab (Keytruda®) (Polverini et al, 2018). Several other immune checkpoint inhibitors are being developed and are in different phases of clinical trials. To improve the survival outcome of patients with OSCC, the prognostic value of immune checkpoints in OSCC has been studied. A recent review analysed the prognostic value of PD-L1 in head and neck cancers and concluded that there are technical and biological challenges in the evaluation of this molecule as a prognostic marker (De Meulenare et al, 2017). To the best of our knowledge, this is the first systematic review analyzing all immune checkpoints molecules that were examined for prognostication of OSCC.

2. MATERIALS AND METHODS

Search strategy

A search strategy combining the following terms was developed: (“immune checkpoint” OR “CTLA-4” OR “PD-L1” OR “PD-1” OR “IDO1” OR “B7-H4” OR “VISTA” OR “VTCN1” OR “A2AR” OR “B7-H3” OR “KIR” OR “LAG3”) AND (“oral cancer” OR “mouth neoplasms” OR “oral squamous cell carcinomas”). We used both the abbreviated and the full name of each immune checkpoint. The search terms were entered into Ovid Medline, Scopus, and Cochrane Library (1985-2017 December) with no language restrictions. For Ovid Medline, we also used the exploded mesh words for each immune checkpoint combined with the various sites of the oral cavity. In advanced search, the following search fields were included: abstract, title, subject heading, and keyword. The Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) were utilized in this study (Moher et al, 2009).

In case of multiple publications on the same patient cohort, only the most recent publication was included. Two independent researchers (MS and RA) screened the retrieved hits, discarded duplicates, and verified that the selected studies met the inclusion criteria. The review article on PD-L1 expression in OSCC was screened for papers missed in the search strategy (De Meulenare et al, 2017). To be included in the systematic review, the studies needed to pass the inclusion and exclusion criteria listed in Supplement Table 1.
Data extraction

For relevant articles, we extracted the following information: (1) basic article information including publication year, study period, follow-up duration, and the first author; (2) patient and tumour information, including total patient number, age, gender, number of patients included in the analysis, name and source of the antibody (and its dilution), method of sample preservation (paraffin embedded or frozen), tumour size, and disease stage; (3) outcome measures including survival data, Kaplan-Meier curves, metastasis, recurrence, statistical results (estimated hazard ratio [HR], 95% confidence interval [CI], and p-values), and number of events; (4) other variables including the methods of quantitative immune checkpoint measurement and the definition of positivity (cut-off value). The adapted guidelines from REMARK were used to evaluate the quality of the eligible studies as previously described (Altman et al., 2012; Almangush et al., 2017). The selected and applied guidelines taken from the REMARK criteria are summarized in Supplement Table 2, as previously reported (Almangush et al., 2017).

For studies that reported only Kaplan-Meier curves without a hazard ratio (HR) estimate, we first extracted numerical information by extrapolating the Kaplan-Meier curves with Engauge Digitizer Version 10.6 software and further estimated the HR and its standard error (SE) following the approach presented by Tierney et al, 2007. In our tables, these estimates are highlighted by Italic font style.

3. RESULTS

Our search retrieved a total of 284 studies from three electronic databases (159 studies from Ovid Medline, 119 studies from Scopus, and 6 studies from Cochrane libraries) (Figure 1). After applying the inclusion and exclusion criteria, 25 studies that evaluated twelve immune checkpoints/modulators in OSCC remained (Supplement Figure 1). Of these, only 4 checkpoints (PD-L1, PD-1, IDO1, B7-H3; 33.3%) were studied more than once. Eight immune checkpoints (66.6%) were studied in only one patient cohort (Table 1). All studies were assessed for their quality based on the adapted REMARK criteria (Supplement Table 2).

Out of the twelve immune checkpoints/modulators found in our search, seven molecules (FKBP51, B7-H4, B7-H6, ALHD1, PD-L1, B7-H3, IDO1) associated with poorer OSCC patients´ survival in at least one study. The other five molecules (CTLA-4, TLT-2, VISTA, PD-L2, PD-1) did not have significant prognostic value. PD-L1 had controversial results.

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IDO1 was studied in three articles (Table 2). While one article (Laimer et al, 2011) offered weak evidence for IDO1 as a prognostic marker for all-stage OSCC, another study (Seppälä et al, 2016) revealed that IDO1 was a prognostic marker only in early-stage oral tongue squamous cell carcinoma (OTSCC). The third article (Kuales et al, 2011) did not present a statistical analysis of the data.

Two articles on PD-1 (Straub et al, 2016; Ahn et al, 2017) reported an association with patient survival (Table 3) but both concluded that PD-1 is not valid for prognostication. The prognostic value of PD-L1 in oral cancer was studied in thirteen articles (Table 4). While 4 studies showed that high expression of PD-L1 associates with better patients’ survival, 2 reported opposite results. Five studies showed insignificant data for PD-L1 as prognostic marker and the other 2 studies did not provide survival data.

B7-H3 was studied in three articles, two of them provided survival data. Both of the two articles reported that B7-H3 is a negative prognostic marker for OS (Table 5).

4. DISCUSSION
Immune checkpoints are a group of molecules that regulate several functions of immune cells and participate in crosstalk between cancer and immune cells. Immune checkpoints are thus regarded as important targets in immunotherapy. Furthermore, several immune checkpoint inhibitors/blockers are already in clinical trials in OSCC and some of them (PD-1/PD-L1 inhibitors) are approved for clinical use (Sikora, 2016). Despite the importance of immune checkpoints, their expression and prognostic value in OSCC is currently unclear. The present systematic review, which is the first systematic review on immune checkpoints molecules in OSCC, revealed that 12 immune checkpoints had been studied for their prognostic value in OSCC. Among these, four molecules (PD-L1, PD-1, IDO1, B7-H3) were studied more than once.

Out of the 12 immune checkpoints/modulators found in our search, seven molecules are associated with poorer OS of OSCC patients in at least one study (FKBP51, B7-H4, B7-H6, ALHD1, PD-L1, B7-H3, IDO1). The other five molecules (CTLA-4, TLT-2, VISTA, PD-L2, PD-1) did not have prognostic value in OSCC patients. Interestingly, PD-L1 was reported as both negative and positive prognostic marker.
IDO1 is an immune checkpoint that has been used as a target for immunotherapy. At present, there are three ongoing clinical trials (NCT03343613, NCT03325465, NCT03358472 at ClinicalTrials.gov) for IDO1 inhibition in head and neck SCC. Unfortunately, according to the recent clinical trials in ovarian cancer, melanoma, non-small cell lung carcinoma, and urothelial cancer (NCT01685255, NCT01604889, NCT02298153 at ClinicalTrials.gov), IDO1 inhibition showed unpromising data and the phase 1 to 2 trials were terminated. The present systematic review revealed that IDO1 had been studied in three articles, where only one reported weak evidence as a prognostic marker in all stages of oral cancer (Laimer et al, 2011). In another study (Seppälä et al, 2016), IDO1 was a prognostic marker only in early-stage OTSCC.

PD-L1, which is the most studied immune checkpoint, was reported as both positive and negative marker for OSCC patients’ survival. The results of the PD-L1 was even dependent on the molecule location at the cell, as the cytoplasmic localization of the PD-L1 associated with better OS and the membranous one associated with the worse OS (Oliveira-Costa et al 2015). Additionally, PD-1, one of the most important targets for immunotherapy, was not found to be a statistically significant prognostic marker by either of the two articles which studied this molecule.

B7-H3 is an immune checkpoint with immune regulatory properties that affects activation of T cells (Loos et al, 2010). Its exact mechanisms are still unknown but there is evidence for co-stimulatory and co-inhibitory signalling for adaptive immune system activation under different tumour contexts (Wang et al, 2014). Although B7-H3 was studied only twice in OSCC for its prognostic value (Chen J.T. et al, 2015; Mao et al, 2017), it was more promising than the other molecules as a prognostic marker as both studies reported consistent evidence for its prognostic value.

Prognostic molecular biomarkers for OSCC have been studied for several years; during this time over 100 biomarkers have been introduced as prognosticators (Almangush et al, 2017; Søland and Brusevold, 2013). However, none of these biomarkers are in clinical use. One of the main problems in the field of prognostic biomarkers is missing validation. This was also observed in the present study as among 12 molecules, only four had been analysed more than once. Paucity of prospective studies was noted. Lack of multicentre studies with small number of cases was also another shortness in the published studies. Further research on immune checkpoint of OSCC should consider well-designed studies (both retrospective and prospective) with appropriate multivariate analysis of large cohorts.

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5. ACKNOWLEDGMENTS

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6. REFERENCES


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Satgunaseelan, L., Gupta, R., Madore, J., Chia, N., Lum, T., Pame, C. E., ... Clark, J. R. (2016) Programmed cell death-ligand 1 expression in oral squamous cell carcinoma is associated with an inflammatory phenotype. Pathology, 48(6), 574-580. doi: 10.1016/j.pathol.2016.07.003


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**FIGURE AND TABLE LEGENDS:**

**Figure 1.** PRISMA flowchart: studies included and excluded along the various steps.

**Table 1.** Summary of studies with only one patient cohort of an immune checkpoint/modulator.

**Table 2.** Summary of studies addressing the expression, prognostic value and clinicopathological features of IDO1 in OSCC.

**Table 3.** Summary of studies addressing the expression, prognostic value and clinicopathological features of PD-1 in OSCC.

**Table 4.** Summary of studies addressing the expression, prognostic value, and clinicopathological features of PD-L1 in OSCC.

**Table 5.** Summary of studies addressing the expression, prognostic value and clinicopathological features of B7-H3 in OSCC.

**Supplement Table 1.** Inclusion and exclusion criteria for systematic analysis.

**Supplement Table 2.** Evaluation criteria used to assess the quality of studies included in the systematic review of the studied immune checkpoints for their prognostic value in OSCC (adapted from REMARK guidelines; Almangush et al, 2017).

**Supplement Figure 1.** Immune checkpoints/modulators investigated in OSCC for expression, prognostic significance, and/or clinicopathological significance.

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Table 1. Summary of studies with only one patient cohort of an immune checkpoint/modulator.

<table>
<thead>
<tr>
<th>(Authors, year)</th>
<th>Country</th>
<th>Immune checkpoint</th>
<th>Stage/ tumour size</th>
<th>Primary antibody</th>
<th>No. cases</th>
<th>Expression of immune checkpoint</th>
<th>End-point</th>
<th>Survival analysis</th>
<th>Result interpretation</th>
<th>Compliance to REMARK guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Moreira et al, 2010)</td>
<td>Brazil</td>
<td>CTLA-4</td>
<td>T2-T4</td>
<td>anti-CTLA-4 1:1200, Santa Cruz Biotechnology</td>
<td>18</td>
<td>Positive cases 3.39% ± 0.46 in OSCC</td>
<td>OS</td>
<td>Univariate:</td>
<td>No difference in survival between the high and low CTLA-4 groups</td>
<td>Checklist number 6 not fulfilled</td>
</tr>
<tr>
<td>(Zhang et al, 2015)</td>
<td>China</td>
<td>TLT-2</td>
<td>T1-T4</td>
<td>anti-TLT-2 1:200, Santa Cruz Biotechnology</td>
<td>76</td>
<td>Higher expression in OSCC than in normal mucosa</td>
<td>-</td>
<td>-</td>
<td>Significantly higher expression levels of TLT-2 in OSCC than in normal mucosa</td>
<td>Checklist number 4-6 not fulfilled</td>
</tr>
<tr>
<td>(Russo et al, 2017)</td>
<td>Italy</td>
<td>FKBP51</td>
<td>T1-T4</td>
<td>anti-FKBP51 1:200, Santa Cruz Biotechnology</td>
<td>72</td>
<td>Percentage of positive tumour cells: mean value 48.2% (95% Confidence Interval CI for the mean 41.4%–54.9%), median value 51% (95% CI for the median 33.9%–70%)</td>
<td>OS</td>
<td>Univariate:</td>
<td>Area under the ROC curve 0.097 (95% CI 0.806–0.966), p&lt;0.0001</td>
<td>High FKBP51 expression associated with death in 5 years from diagnosis with a sensitivity of 88.46% and a specificity of 91.67%,</td>
</tr>
<tr>
<td>(Wu et al, 2016)</td>
<td>China</td>
<td>B7-H4</td>
<td>-</td>
<td>anti-B7-H4 1:800, Cell Signaling Technology</td>
<td>165</td>
<td>Significantly greater in OSCC than epithelial dysplasia and normal mucosa</td>
<td>OS</td>
<td>Unadjusted HR 1.784 (95% CI 1.018–3.017), p&lt;0.05</td>
<td>High B7-H4 expression associated with poor overall survival.</td>
<td>Checklist number 2, 6 not fulfilled</td>
</tr>
<tr>
<td>(Wu et al, 2017)</td>
<td>China</td>
<td>VISTA</td>
<td>T1-T4</td>
<td>anti-VISTA 1:400, Cell Signaling Technology</td>
<td>165</td>
<td>Significantly greater in OSCC than epithelial dysplasia and normal mucosa</td>
<td>OS</td>
<td>Univariate:</td>
<td>p=0.8799</td>
<td>High VISTA expression was not independently associated with poor prognosis.</td>
</tr>
</tbody>
</table>

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Table 1, continued.

<table>
<thead>
<tr>
<th>(Authors, year)</th>
<th>Country</th>
<th>Immune checkpoint</th>
<th>Stage/ tumour size</th>
<th>Primary antibody</th>
<th>No. cases</th>
<th>Expression of immune checkpoint</th>
<th>Endpoint</th>
<th>Survival analysis</th>
<th>Result interpretation</th>
<th>Compliance to REMARK guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Wang et al., 2017)</td>
<td>China</td>
<td>B7-H6</td>
<td>I-IV</td>
<td>anti-B7-H6 1:200, Abcam</td>
<td>50</td>
<td>Positive cases 48%</td>
<td>OS</td>
<td>Univariate: p=0.0057</td>
<td>Multivariate: HR 5.03 (95% CI 1.53-16.54), ( p=0.007 )</td>
<td>Fulfilled all items</td>
</tr>
<tr>
<td>(Koga-shiwa et al., 2017)</td>
<td>Japan</td>
<td>PD-L2</td>
<td>III-IV</td>
<td>Polyclonal rabbit anti-PD-L2 1:10 (Sigma-Aldrich, USA)</td>
<td>84</td>
<td>Positive cases 23.8%</td>
<td>OS</td>
<td>Univariate: HR 0.442 (95% CI 0.132-1.486), ( p=0.187 )</td>
<td>PD-L2 did not significantly associate with PFS or OS.</td>
<td>Fulfilled all items</td>
</tr>
<tr>
<td>(Tsai et al., 2017)</td>
<td>ALDH1</td>
<td>III-IV</td>
<td>141 (tumor stage III-IV)</td>
<td>anti-ALDH1</td>
<td>141</td>
<td>Positive cases 43%</td>
<td>OS</td>
<td>Univariate: p&lt;0.001</td>
<td>Multivariate: HR 2.27 (95% CI 1.21-4.28), ( p=0.011 )</td>
<td>Checklist number 3 not fulfilled</td>
</tr>
</tbody>
</table>

Abbreviations: IHC=immunohistochemistry, OS = overall survival, PFS=patient free survival, DSF=disease-free survival ROC= Receiver Operating Characteristic, HR = hazard ratio, CI = confidence interval, CTLA-4=cytotoxic T-lymphocyte-associated protein 4, TLT2=TREM-like transcript-2, FKBP51=FK506-binding protein 51, VISTA=V-domain Ig Suppressor of T cell Activation, PDL2=programmed death-ligand 2, ALDH1=Aldehyde dehydrogenase 1, CSC=cancer stem cells, MDSC=myeloid-derived suppressor cells
Table 2. Summary of studies addressing the expression, prognostic value and clinicopathological features of IDO1 in OSCC.

<table>
<thead>
<tr>
<th>(Authors, year)</th>
<th>Country</th>
<th>Stage/tumour size</th>
<th>Primary antibody</th>
<th>Cutoff value</th>
<th>No. cases/ in IHC</th>
<th>No. IDO1+ Cases</th>
<th>End point</th>
<th>Unadjusted analysis</th>
<th>Adjusted analysis</th>
<th>Result interpretation</th>
<th>Compliance to REMARK guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Laimer et al, 2011) Austria</td>
<td>T1-T4</td>
<td>IDO1, Chemicon, 1:500</td>
<td>10 %</td>
<td>88, 88</td>
<td>56%</td>
<td>OS</td>
<td>RR 1.7 (95% CI 1.058-2.817), ( p=0.029 )</td>
<td>RR 1.7 (95% CI 1.267-3.230), ( p=0.030 )</td>
<td>IDO1+ had a poorer median OS than IDO1-</td>
<td>Checklist number 1 not fulfilled</td>
<td></td>
</tr>
<tr>
<td>(Seppälä et al, 2016) Finland</td>
<td>T1-T4</td>
<td>IDO1, Chemicon International Inc. 1:200</td>
<td>1 %</td>
<td>108, 58</td>
<td>~35%</td>
<td>DSS, OS</td>
<td>Did not affect survival; ( p&gt;0.05 )</td>
<td>-</td>
<td>In all cancer stages, IDO1+ staining did not affect survival</td>
<td>Checklist numbers 5 and 6 not fulfilled</td>
<td></td>
</tr>
<tr>
<td>(Kuales et al, 2011) Germany</td>
<td>T1-T2</td>
<td>IDO1, Monoclonal, Millipore, 1:150</td>
<td>-</td>
<td>47, 47</td>
<td>26%</td>
<td>Clinical outcome</td>
<td>IDO1+ 12 alive and 0 dead; IDO1- 1 dead and 34 alive</td>
<td>-</td>
<td>-</td>
<td>Checklist numbers 3 to 6 not fulfilled</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: OS=overall survival, DSS=disease-specific survival, RR=relative risk, CI=confidence interval.
Table 3. Summary of studies addressing the expression, prognostic value and clinicopathological features of PD-1 in OSCC.

<table>
<thead>
<tr>
<th>Authors, year</th>
<th>Country</th>
<th>Stage/tumor size</th>
<th>Primary antibody</th>
<th>Cutoff point</th>
<th>No. cases in IHC</th>
<th>No. PD-1+ Cases</th>
<th>End point</th>
<th>Unadjusted analysis</th>
<th>Result interpretation</th>
<th>Compliance to REMARK guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ahn et al, 2017) Korea</td>
<td>I-IV T1-T4</td>
<td>PD-1 #6796-1 Epitomics 1:300</td>
<td>Counted no of TILs</td>
<td>68, 68</td>
<td>Mean No. 6.8 +/-6.9</td>
<td>DFS</td>
<td>0.94 (95% CI 0.85-1.04), (p=0.213)</td>
<td></td>
<td></td>
<td>Checklist numbers 3 and 5 not fulfilled</td>
</tr>
<tr>
<td>(Straub et al, 2016) Germany</td>
<td>T1-T4</td>
<td>PD-1 11RQ22 Cell Marque, 1:50</td>
<td>Counted no of TILs</td>
<td>80, 79</td>
<td>52%, mean 6% (3-20%) stained of the TILs</td>
<td>RFS</td>
<td>-</td>
<td></td>
<td></td>
<td>Checklist numbers 3 and 5 not fulfilled</td>
</tr>
<tr>
<td>(Mattox et al, 2017) USA</td>
<td>I-II T1-T2</td>
<td>PD-1, clone M3</td>
<td>-</td>
<td>53, 53</td>
<td>100%</td>
<td>OS</td>
<td>Not significant data not shown in article</td>
<td>PD-1+ not relevantly associated with OS and RFS</td>
<td></td>
<td>Checklist numbers 3, 5, and 6 not fulfilled</td>
</tr>
<tr>
<td>(Troeltzsch et al, 2017) Germany</td>
<td>T1-T4</td>
<td>PD-1, Monoclonal, MEDAC, 1:80</td>
<td>Counted no of TILs (&gt;10)</td>
<td>88, 88</td>
<td>83%</td>
<td>OS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Checklist numbers 4 to 6 not fulfilled</td>
</tr>
</tbody>
</table>

Abbreviations: TILs=tumour infiltrating lymphocytes, IHC=immunohistochemistry, DFS=disease-free survival, OS=overall survival, RFS=recurrence-free survival, HR=hazard ratio, CI=confidence interval.
<table>
<thead>
<tr>
<th>Authors, year</th>
<th>Country</th>
<th>Stage/tumor size</th>
<th>Primary antibody</th>
<th>Cutoff point</th>
<th>No. cases/ in IHC</th>
<th>No. PD-L1+ cases</th>
<th>End-point</th>
<th>Unadjusted analysis</th>
<th>Adjusted analysis</th>
<th>Result interpretation</th>
<th>Compliance to REMARK guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ahn et al, 2017)</td>
<td>Korea</td>
<td>I-IV T1-T4</td>
<td>PD-L1, Polyclonal rabbit, ab153991 Abcam, 1:1000</td>
<td>10%</td>
<td>68, 68</td>
<td>66%</td>
<td>DFS</td>
<td>HR 0.25 (95% CI 0.06-1.12) p=0.070</td>
<td>-</td>
<td>High PD-L1 expression was associated with good OS</td>
<td>Fulfilled all items</td>
</tr>
<tr>
<td>(Kogashiwa et al, 2017)</td>
<td>Japan</td>
<td>III-IV</td>
<td>PD-L1, Monoclonal rabbit, Spring Bioscience, 1:100</td>
<td>5%</td>
<td>84, 84</td>
<td>52%</td>
<td>PFS</td>
<td>HR 0.576 (95% CI 0.274-0.956) p=0.0372</td>
<td>HR 0.541 (95% CI 0.278-0.894) p=0.0315</td>
<td>PD-L1+ associated significantly with DFS and OS</td>
<td>Fulfilled all items</td>
</tr>
<tr>
<td>(Lin et al, 2015)</td>
<td>Taiwan</td>
<td>I-IV T1-T4</td>
<td>PD-L1, GeneTex, 1:100</td>
<td>-</td>
<td>305, 305</td>
<td>44%</td>
<td>OS</td>
<td>HR 1.209 (95% CI 0.890-1.643) p=0.2254</td>
<td>HR 1.345 (95% CI 0.987-1.834) p=0.0609</td>
<td>PD-L1+ associated with distant metastasis; could be an independent prognostic marker in males or smokers</td>
<td>Checklist number 3 not fulfilled</td>
</tr>
<tr>
<td>(Straub et al, 2016)</td>
<td>Germany</td>
<td>T1-T4</td>
<td>PD-L1, Monoclonal rabbit, Cell Signaling, 1:100</td>
<td>5%</td>
<td>80, 80</td>
<td>45%</td>
<td>RFS</td>
<td>p=0.05</td>
<td>-</td>
<td>PD-L1+ associated with nodal metastasis, OS, and RFS</td>
<td>Checklist number 5 not fulfilled</td>
</tr>
<tr>
<td>(Hanna et al, 2018a)</td>
<td>USA</td>
<td>T1-T4</td>
<td>PD-L1, Monoclonal mouse, 9A11, 1:200</td>
<td>5%, ♂ 10% ♂ 32 ♂ 87%</td>
<td>Only ♂ OS</td>
<td>-</td>
<td>OS</td>
<td>HR 2.12 (95% CI 0.670-6.69) p=0.20, p=0.01</td>
<td>-</td>
<td>Subjects (♀) with greater membranous PD-L1+ and the presence of tumour-infiltrating lymphocytes had a decreased risk of recurrence and improved survival</td>
<td>Checklist number 5 not fulfilled</td>
</tr>
</tbody>
</table>

Table 4. Summary of studies addressing the expression, prognostic value, and clinicopathological features of PD-L1 in OSCC.
Table 4, continued.

<table>
<thead>
<tr>
<th>Authors, year</th>
<th>Country</th>
<th>Stage/tumor size</th>
<th>Primary antibody</th>
<th>Cutoff point</th>
<th>No. cases/ in IHC</th>
<th>No. PD-L1+ Cases</th>
<th>End-point</th>
<th>Unadjusted analysis</th>
<th>Adjusted analysis</th>
<th>Result interpretation</th>
<th>Compliance to REMARK guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Chen TC et al., 2015) Taiwan</td>
<td>III-IV</td>
<td>PD-L1, Monoclonal rabbit, Proteintech Group Inc.</td>
<td>&gt;5%</td>
<td>218, 218</td>
<td>64%</td>
<td>DFS</td>
<td>27.4%, b</td>
<td>Not analysed</td>
<td>PD-L1+ in metastatic LN worsens DS and OS</td>
<td>Checklist numbers 3 and 5 not fulfilled</td>
<td></td>
</tr>
<tr>
<td>(Cho et al., 2011) Korea</td>
<td>I-IV T1-T4</td>
<td>PD-L1, Polyclonal rabbit, Abcam, 1:100</td>
<td>&gt;0 %</td>
<td>45, 45</td>
<td>87%</td>
<td>CS</td>
<td>HR 0.59, (95% CI 0.249-1.42)</td>
<td>p=0.25, p=0.501</td>
<td>PD-L1+ did not correlate with patient survival</td>
<td>Checklist numbers 1 and 5 not fulfilled</td>
<td></td>
</tr>
<tr>
<td>(Mattox et al., 2017) USA</td>
<td>I-II T1-T2</td>
<td>PD-L1, Monoclonal mouse, mAB 5H1, 2 µg/ml</td>
<td>&gt;1%</td>
<td>53, 53</td>
<td>73%</td>
<td>OS</td>
<td>PD-L1+ 64 months vs PD-L1+ 80.7 months, p=0.83</td>
<td>-</td>
<td>PD-L1+ not significant in survival</td>
<td>Checklist numbers 1 and 5 were not fulfilled</td>
<td></td>
</tr>
<tr>
<td>(Oliveira-Costa et al., 2015) Brazil</td>
<td>T1-T4</td>
<td>PD-L1, Polyclonal goat, Abcam, 1:25</td>
<td>5%</td>
<td>142, 96</td>
<td>56%</td>
<td>DSS(a)</td>
<td>-</td>
<td>HR 0.426 (95% CI 0.186-0.977), p=0.044</td>
<td>-</td>
<td>PD-L1+ was an independent prognostic factor in this cohort</td>
<td>Fulfilled all items</td>
</tr>
<tr>
<td>(Satguna-seelan et al., 2016) Australia</td>
<td>I-IV T1-T4</td>
<td>PD-L1, Monoclonal rabbit, E1L3N-XP-Rb mAb Cell Signaling Technology, 1:500</td>
<td>&gt;5%</td>
<td>243, 217</td>
<td>18%</td>
<td>DSS(b)</td>
<td>HR 1.15 (95% CI 0.713-1.85), p=0.57, p=0.62</td>
<td>-</td>
<td>PD-L1+ not significantly associated with DS and OS</td>
<td>Checklist number 5 not fulfilled</td>
<td></td>
</tr>
<tr>
<td>(Stasikowska-Kanicka et al., 2017) Poland</td>
<td>G1-G3</td>
<td>PD-L1, Polyclonal rabbit, Abcam, 1:400</td>
<td>-</td>
<td>78, 78</td>
<td>79%</td>
<td>DSS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>PD-L1+ associated significantly with poor prognosis OSCC (p&lt;0.011)</td>
<td>Checklist numbers 1 and 3 to 6 not fulfilled</td>
</tr>
<tr>
<td>(Takakura et al., 2017) Japan</td>
<td>I-IV T1-T4</td>
<td>PD-L1, Monoclonal mouse, 27A2 MBL</td>
<td>&gt;0%</td>
<td>18, 18</td>
<td>78%</td>
<td>DFS</td>
<td>p=0.82</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Checklist numbers 4 to 6 not fulfilled</td>
</tr>
<tr>
<td>(Troeltzsch et al., 2017) Germany</td>
<td>T1-T4</td>
<td>PD-L1, Monoclonal rabbit, E1L3N Cell Signaling Technology, 1:100</td>
<td>5%</td>
<td>88, 88</td>
<td>30%</td>
<td>DSS</td>
<td>p=0.937</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Checklist numbers 5 and 6 not fulfilled</td>
</tr>
</tbody>
</table>

Abbreviations: IHC=immunohistochemistry, DFS=disease-free survival, OS=overall survival, DSS=disease-specific survival, CS=cumulative survival, PFS=progression-free survival, RFS=recurrence-free survival, HR=hazard ratio, CI=confidence interval. Italic font style was used to separate the calculated HRs and CI.

\(a\) article was published ahead 09/2017
\(b\) article only had Kaplan-Meier rates in percent.
Table 5. Summary of studies addressing the expression, prognostic value and clinicopathological features of B7-H3 in OSCC.

<table>
<thead>
<tr>
<th>(Authors, year)</th>
<th>Country</th>
<th>Stage/ tumour size</th>
<th>Primary antibody</th>
<th>Cutoff point</th>
<th>No. cases</th>
<th>No. B7-H3+ Cases</th>
<th>End point</th>
<th>Unadjusted analysis</th>
<th>Result interpretation</th>
<th>Compliance to REMARK guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Chen JT et al, 2015) Taiwan</td>
<td>Taiwan</td>
<td>I-IV T1-T4</td>
<td>B7-H3, Polyclonal goat, R&amp;D Systems, 1:100</td>
<td>55%</td>
<td>72</td>
<td>67%</td>
<td>OS</td>
<td>HR 3.85, 95% CI (1.12-13.29), p=0.033, p=0.005</td>
<td>B7-H3+ associated with poor survival rate</td>
<td>Checklist numbers 5 and 6 not fulfilled</td>
</tr>
<tr>
<td>(Mao et al, 2017) China</td>
<td>China</td>
<td>-</td>
<td>B7-H3, Cell Signaling Technology</td>
<td>-</td>
<td>165</td>
<td>50%</td>
<td>OS</td>
<td>HR 1.49, CI95% (0.923-2.42), p=0.10, p=0.039</td>
<td>B7-H3+ associated with poor OS in OSCC.</td>
<td>Checklist numbers 1 to 3 and 5 and 6 not fulfilled</td>
</tr>
<tr>
<td>(Zhang et al, 2015) China</td>
<td>China</td>
<td>T1-T4</td>
<td>B7-H3, Polyclonal goat, R&amp;D Systems, 1:200</td>
<td>-</td>
<td>76</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Significantly higher expression levels of B7-H3 in OSCC than in normal mucosa</td>
<td>Checklist number 4-6 not fulfilled</td>
</tr>
</tbody>
</table>

Abbreviations: OS=overall survival, HR=hazard ratio, CI=confidence interval, Italic font style was used to separate the calculated HRs and CI.
Studies identified through database searching
n=284

Additional records identified through other sources
n=6

51 Duplicates removed; studies extracted with author, title and abstract information and screened
n=239

Studies excluded:
- 200 Not in English
- 96 Not OSCC
- 23 Immunotherapy
- 26 Samples not from human tissue
- 32 Wrong article type
- 18 Otherwise irrelevant

Full-text articles assessed for eligibility
n=39

Full-text articles excluded:
- 14 Insufficient data
- 1 not OSCC
- 1 Samples not from human tissue

Studies included in systematic review and quality assessment:
n=25