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The Prognostic Value of Immune Checkpoints in Oral Squamous Cell Carcinoma

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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.

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 e*t 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.

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.

5. ACKNOWLEDGMENTS

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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.

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

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

Table 1, continued.

(Authors, year) Country	Immune check-point	Stage/ tumour size	Primary antibody	No. cases	Expression of immune checkpoint	End-point	Survival analysis	Result interpretation	Compliance to REMARK guidelines
(Wang *et al*, 2017) China	B7-H6	I-IV	anti-B7-H6 1:200, Abcam	50	Positive cases 48%	OS	Univariate: p=0,0057 Multi-variate: HR 5.03 (95% CI 1.53-16.54), *p*=0.007	multivariate survival analysis by COX proportional hazard regression model revealed that the recurrence, differentiation, and expression of B7-H6 were related to the prognosis. High B7-H6 expression associated with poor OS and DSF.	Fulfilled all items Fulfilled all items
(Koga-shiwa *et al*, 2017) Japan	PD-L2	III-IV	Polyclonal rabbit anti-PD-L2 1:10 (Sigma-Aldrich, USA)	84	Positive cases 23.8%	OS	Univariate: HR 0.442 (95% CI 0.132-1.486), *p*=0.187	$PD\text{-}L2^{+}$ did not significantly associate with PFS or OS.	Fulfilled all items
(Tsai *et al*, 2017)	ALDH1	III-IV	anti-ALDH1	141 (tumour stage III-IV)	Positive cases 43%	OS	Univariate: p<0,001 Multi-variate: HR 2.27 (95% CI 1.21-4.28), *p*=0.011	High ALDH1 expression associated with poor prognosis and linked to treatment resistance.	Checklist number 3 not fulfilled

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, ALHD1=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.

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

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.

(Authors, year) Country	Stage/ tumour size	Primary antibody	Cutoff point	No. cases/ in IHC	No. PD-1$^+$ Cases	End point	Unadjusted analysis	Result interpretation	Compliance to REMARK guidelines
(Ahn *et al*, 2017) Korea	I-IV T1-T4	PD-1 #6796-1 Epitomics 1:300	Counted no of TILs	68, 68	Mean No. 6.8 +/-6.9	DFS	HR 0.94 (95% CI 0.85-1.04), *p*=0.213	-	Checklist numbers 3 and 5 not fulfilled
						OS	HR 1.01 (95% CI 0.95-1.06), *p*=0.826		
(Straub *et al*, 2016) Germany	T1-T4	PD-1 11RQ22 Cell Marque, 1:50	Counted no of TILs	80, 79	52%, mean 6% (3-20%) stained of the TILs	RFS	Not significant data not shown in article	PD-1$^+$ not relevantly associated with OS and RFS	Checklist numbers 3 and 5 not fulfilled
						OS			
(Mattox *et al*, 2017) USA	I-II T1-T2	PD-1, clone M3	-	53, 53	100%	-	-	-	Checklist numbers 3,5, and 6 not fulfilled
(Troeltzsch *et al*, 2017) Germany	T1-T4	PD-1, Mono-clonal, MEDAC, 1:80	Counted no of TILs (>10)	88, 88	83%	-	-	-	Checklist numbers 4 to 6 not fulfilled

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

(Authors, year) Country	Stage/ tumour size	Primary antibody	Cutoff point	No. cases/ in IHC	No. PD-L1+ cases	End-point	Unadjusted analysis	Adjusted analysis	Result interpretation	Compliance to REMARK guidelines
(Ahn *et al*, 2017) Korea	I-IV T1-T4	PD-L1, Polyclonal rabbit, ab153991 Abcam, 1:1000	10%	68, 68	66%	DFS	HR 0.25 (95% CI 0.06-1.12) *p*=0.070	-	High PD-L1 expression was associated with good OS	Fulfilled all items
						OS	HR 0.32 (95% CI 0.11-0.94) *p*=0.039	NS		
(Kogashi-wa *et al*, 2017) Japan	III-IV	PD-L1, Monoclonal rabbit, Spring Bioscience, 1:100	5%	84, 84	52%	PFS	HR 0.576 (95% CI 0.274-0.956), *p*=0.0372	HR 0.541 (95% CI 0.278-0.894) *p*=0.0315	PD-L1$^+$ associated significantly with DFS and OS	Fulfilled all items
						OS	HR 0.257 (95% CI 0.102-0.649), *p*=0.006	HR 0.256 (95% CI 0.101-0.646), *p*=0.008		
(Lin *et al*, 2015) Taiwan	I-IV T1-T4	PD-L1, GeneTex, 1:100	-	305, 305	44%	OS	HR 1.209 (95% CI 0.890-1.643), *p*=0.2254	HR 1.345 (95% CI 0.987-1.834), *p*=0.0609	PD-L1$^+$ associated with distant metastasis; could be an independent prognostic marker in males or smokers	Checklist number 3 not fulfilled
(Straub *et al*, 2016) Germany	T1-T4	PD-L1, Monoclonal rabbit, Cell Signaling, 1:100	5%	80, 80	45%	RFS	*p*=0.05	-	PD-L1$^+$ associated with nodal metastasis, OS, and RFS	Checklist number 5 not fulfilled
						OS	*HR 2.12 (95% CI 0.670-6.69), p=0.20*, *p*=0.01	-		
(Hanna *et al*, 2018[a]) USA	T1-T4	PD-L1, Monoclonal mouse, 9A11, 1:200	5%, ♀10%	81, ♀32	♀ 87%	Only ♀ OS	-	HR 0.58 (95% CI 0.45-0.74), *p*<0.001	Subjects (♀) with greater membranous PD-L1$^+$ and the presence of tumour-infiltrating lymphocytes had a decreased risk of recurrence and improved survival	Checklist number 5 not fulfilled

Table 4, continued.

(Authors, year) Country	Stage/ tumor size	Primary antibody	Cutoff point	No. cases/ in IHC	No. PD-L1+ Cases	End-point	Unadjusted analysis	Adjusted analysis	Result interpreta-tion	Compliance to REMARK guidelines
(Chen TC *et al*, 2015) Taiwan	III-IV	PD-L1, Monoclonal rabbit, Proteintech Group Inc.	>5%	218, 218	64%	DFS	27,4%, [b] *p*=0.02	Not analysed	PD-L1$^+$ in metastatic LN worsens DS and OS	Checklist numbers 3 and 5 not fulfilled
						OS	27,6%, *p*=0.11	Not analysed		
(Cho *et al*, 2011) Korea	I-IV T1-T4	PD-L1, Polyclonal rabbit, Abcam, 1:100	>0 %	45, 45	87%	CS	*HR 0.59, (95% CI 0.249-1.42) p=0.25*, *p*=0.501	-	PD-L1$^+$ did not correlate with patient survival	Checklist numbers 1 and 5 not fulfilled
(Mattox *et al*, 2017) USA	I-II T1-T2	PD-L1, Monoclonal mouse, mAB 5H1, 2 µg/ml	>1%	53, 53	73%	OS	PD-L1$^+$ 64 months vs PD.L1$^-$ 80.7 months, *p*=0.83	-	PD-L1$^+$ not significant in survival	Checklist numbers 1 and 5 were not fulfilled
(Oliveira-Costa *et al*, 2015) Brazil	T1-T4	PD-L1, Polyclonal goat, Abcam, 1:25	5%	142, 96	56%	DSS[c]	-	HR 0.426 (95% CI 0.186-0.977), *p*=0.044	PD-L1$^+$ was an independent prognostic factor in this cohort	Fulfilled all items
						DSS[m]	-	HR 2.628 (95% CI 0.473-14.1613), *p*=0.270		
(Satguna-seelan *et al*, 2016) Australia	I-IV T1-T4	PD-L1, Monoclonal rabbit, E1L3N-XP-Rb mAb Cell Signaling Techology, 1:500	>5%	243, 217	18%	DSS	*HR 1.15 (95%CI 0.713-1.85), p=0.57*, *p*=0.62	-	PD-L1$^+$ not significantly associated with DS and OS	Checklist number 5 not fulfilled
						DFS	*p*=0.82	-		
						OS	*p*=0.93	-		
(Stasikow-ska-Kanicka *et al*, 2017) Poland	G1-G3	PD-L1, Polyclonal rabbit, Abcam, 1:400	-	78, 78	79%	-	-	-	PD-L1$^+$ associated significantly with poor prognosis OSCC (p<0.011)	Checklist numbers 1 and 3 to 6 not fulfilled
(Takakura *et al*, 2017) Japan	I-IV T1-T4	PD-L1, Monoclonal mouse, 27A2 MBL	>0%	18, 18	78%	-	-	-	-	Checklist numbers 4 to 6 not fulfilled
(Troeltzsch *et al*, 2017) Germany	T1-T4	PD-L1, Monoclonal rabbit, E1L3N Cell Signaling Technology, 1:100	5%	88, 88	30%	DSS	*p*=0.937	-	PD-L1$^+$ in OSCC might differ depending on its anatomic origin	Checklist numbers 5 and 6 not fulfilled

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.

[c] cytoplasmic, [m] membranous

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

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

Abbreviations: OS=overall survival, HR=hazard ratio, CI=confidence interval, Italic font style was used to separate the calculated HRs and CI.