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Investigating the role of octamer binding transcription Factor-4 (Oct-4) in oral cavity squamous cell carcinoma: A systematic review and meta-analysis

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1. Introduction

Each year there is an estimate of 657,000 new cases of oral cavity and pharynx cancers and > 330,000 deaths [1]. Oral cancer is a prevalent cancer of the head and neck area, with over 90% of oral cancers being squamous cell carcinomas [2]. Major risk factors of oral squamous cell cancer include tobacco use, including pipes, cigars, cigarettes, and chewing tobacco, as well as excessive alcohol consumption. Although advances in detection and therapy have been made throughout the years, prognosis for oral cancer patients is dim with high occurrence of invasion to surrounding tissues, local and distant metastasis and high risk of second malignancy during a patient's lifetime [3].

Cancer stem cells (CSC), rare cells found in cancer that can act as both stem cells and cancer cells, have been shown to worsen prognosis of head and neck cancer. They are associated with tumor initiation, invasion, metastasis, progression, and recurrence [4–6]. Cell surface markers can be used to identify CSCs. Common CSC biomarkers within head and neck squamous cell carcinoma (HNSCC) include CD44, SOX2, Nanog, ALDH1, among others [7]. A notable CSC that belongs to the POU (Pit-Oct-Unc) transcription factor family, octamer-binding transcription factor 4 (OCT4) plays an important role in the regulation of pluoripotency in mammalian development. [8] Recent literature has shown OCT4 ubiquitination to be involved in the establishment of induced pluoripotent stem cells and determine their cell fate [9,10]. In the field of oncology, advances have shown high expressions of OCT4 in human tumors to be positively associated with poor prognosis [11,12]. However, up until now, no known meta-analysis to our knowledge has been conducted exclusively for prognostic value of OCT4 oral squamous cell carcinoma. To further investigate the role of OCT4 in prognosis of oral squamous cell carcinoma, we conducted a meta-analysis.

2. Methods

2.1. Study design

Our search was performed in accordance with the Cochrane Handbook for DTA Reviews chapter on searching [13]. Additionally, we followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines to identify, screen, and describe the protocols used in this systematic review [14]. We also followed the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) Checklist [15]. We designed the search strategy in collaboration with a librarian at the Zucker School of Medicine (WH). Our protocol was designed a priori and prospectively registered in an online systematic review database (PROSPERO Database CRD42018108572). Since this study was a systematic review and meta-analysis, registration with our Institutional Review Board (IRB) was not required.

2.2. Search strategy

PubMed (via the web), Scopus, Embase, Web of Science, and the Cochrane Library were searched on August 29, 2018. We searched all databases from their inception to the present, limited to articles written in English, and excluded grey literature. Variations of the following concepts were used: cancer and octamer-binding transcription factor 4 (OCT-4). The full search strategies can be found in the supplementary materials.

2.3. Article selection

In two phases, two of the authors (VG, MW) would select articles independently based on the search strategy. The first phase consisted of screening a list of titles and abstracts for full-text retrieval. During the first phase (title and abstract screening), our inclusion criteria included any study that reported a description of OCT4 in HNC, either in the title or abstract. The study was selected for full-text review if the abstract content was not clear. If an article passed the first phase of screening, it was selected for full-text retrieval and was assessed in the second phase of screening.

During the second phase, we screened full-text articles using predetermined inclusion and exclusion criteria. Inclusion criteria: [1] article reports on prognostic impact of.

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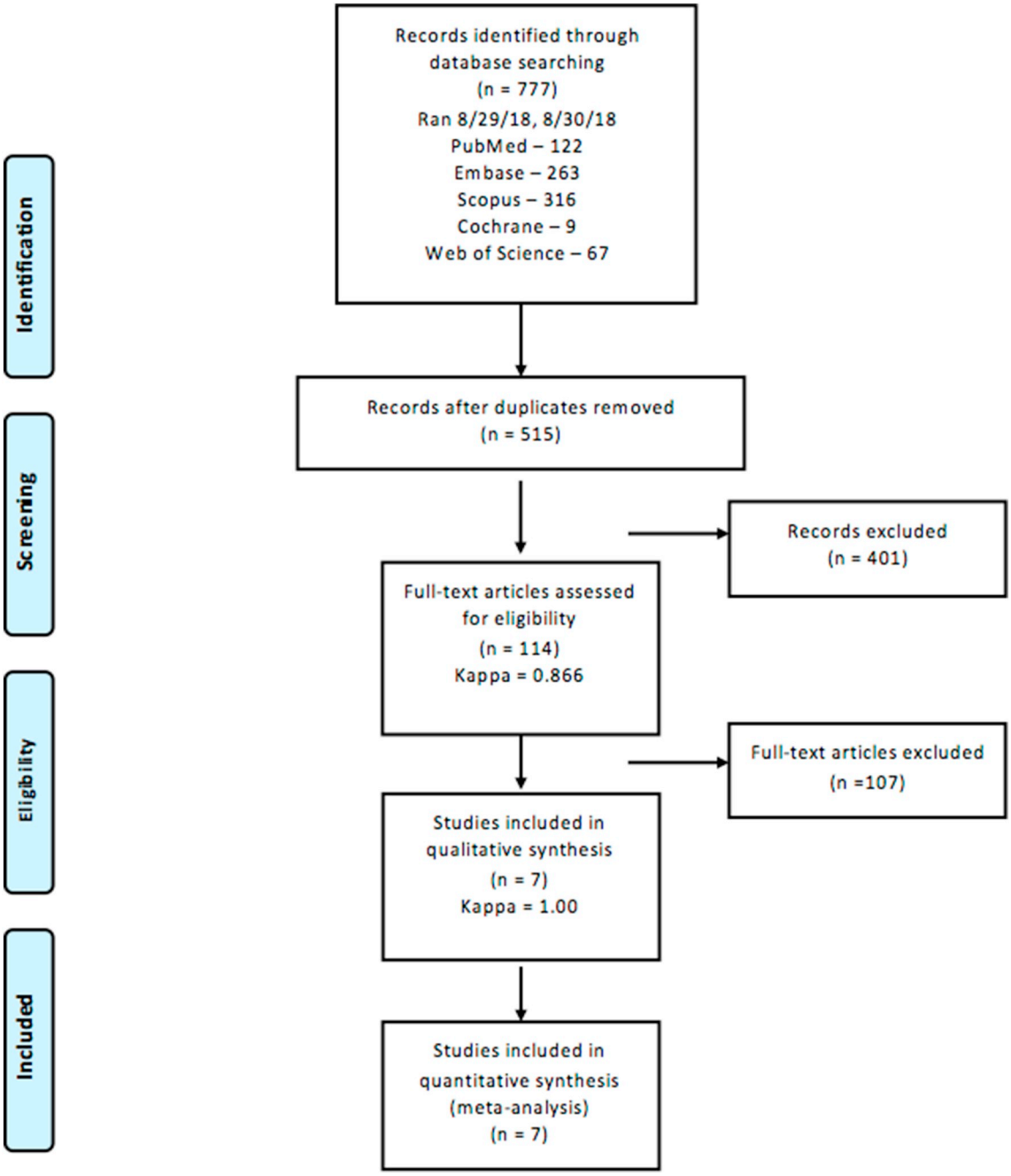

Fig. 1. PRISMA flowchart and study characteristics.

OCT4 in oral cavity (not including tongue base); [2] OCT4 treated as categorical variable; [3] OCT4 Hazard Ratio (HR) for overall survival (OS), with or without disease-specific survival (DSS), with or without disease-free survival (DFS) [4] 95% Confidence interval (CI) for survival statistic, with or without the *p* value; [5] available as full-text publication; [6] English language; [7] clinical trial, cohort, case–control.

Exclusion criteria: [1] case report, conference proceeding, letters, reviews/meta-analyses; [2] other types of head and neck tumors and thyroid and endocrine tumors; [3] animal studies; [4] laboratory studies; [5] duplicate literature and duplicate data; when multiple reports describing the same population were published, only the most recent or complete report was included; [6] incomplete data (No OCT4 HR for OS/DSS), although only excluded if contact with original author does not provide missing information or if we are unable to reconstruct the data. Disagreements were resolved via consensus.

The PRISMA flow chart for this systematic review can be found in Fig. 1. The initial search performed using our search strategies (Supplementary materials) yielded a total of 777 results. De-duplication was then performed, which reduced the initial number of results to 515. The first phase of screening was performed next on titles and abstracts, which reduced the number of results to 7. The level of agreement was strong for the first phase with a Kappa of 0.8666. The second phase of screening resulted in the exclusion of 107 results. The agreement was perfect for the second phase of screening with a Kappa of 1.00. Thus, a total of seven studies remained for further quantitative analysis.

2.4. Quality assessment

Risk of bias in the included papers was assessed by two authors (VG,

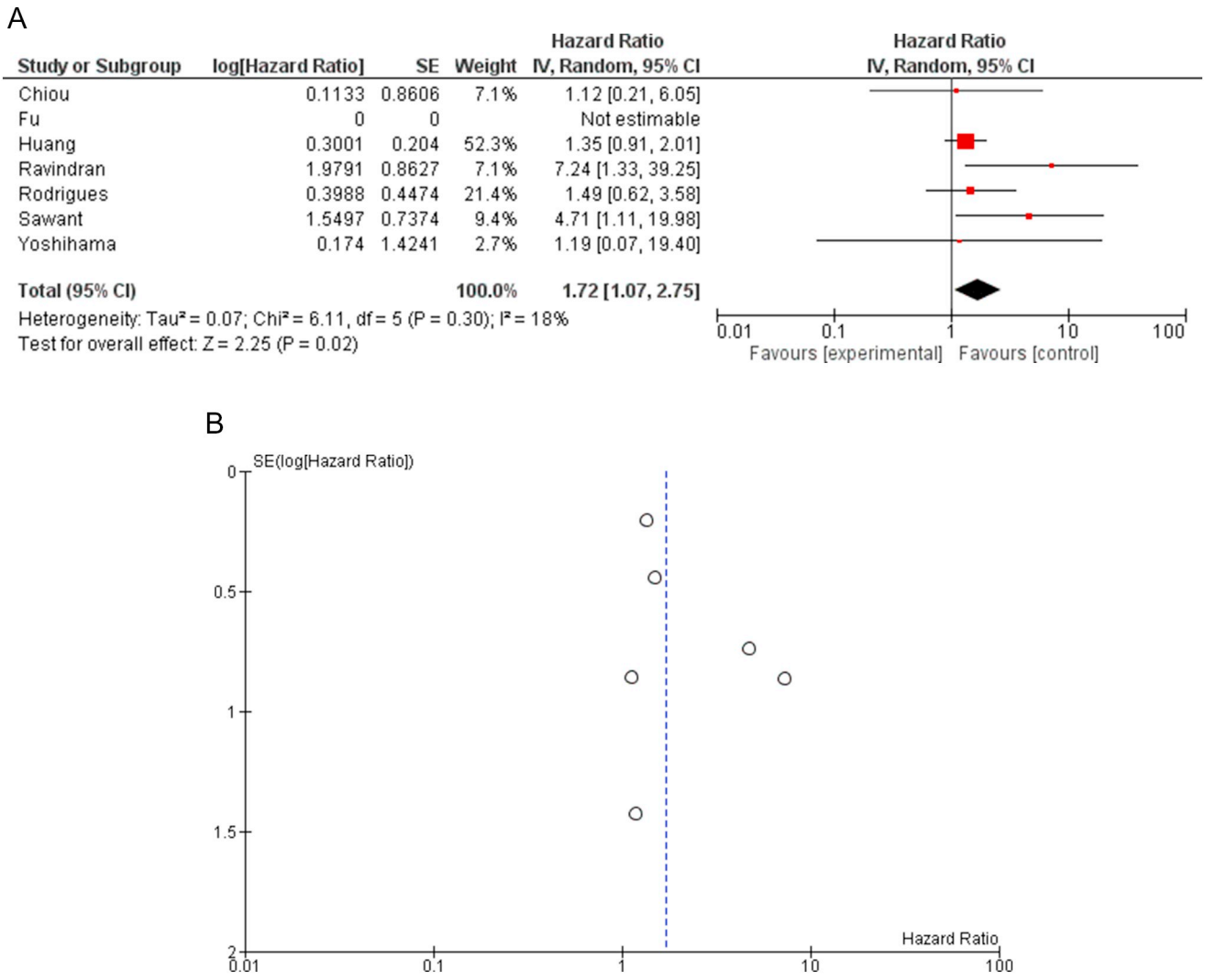

Fig. 2. A. Forest plot for OS

Forest plot is presented showing the pooled hazard ratio of the effect of high OCT4 expression on overall survival, with lines representing the 95% confidence interval and red boxes representing the hazard ratio.

B. Funnel plot for OS

The funnel plot is presented with the y-axis representing the size of the study and the x-axis representing the direction of effect of the study. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

MW). The assessment was made using the Quality In Prognosis Studies Tool (QUIPS). QUIPS consists of six domains: study participation, attrition, prognostic factor measurement, outcome measurement, study confounding, and statistical analysis and reporting. Each domain contained a checklist of 3–9 subdomains, which were used to render a score of low, moderate, or high risk of bias for the entire main domain. A detailed breakdown of the scoring criteria and subdomains is included with the supplementary materials. Disagreements in scoring of the domains were reconciled with a third author (TT).

2.5. Data extraction

Data forms were developed a priori as recorded in the PROSPERO registry [ID: CRD42018108572]. All of the full-text articles were reviewed individually by two authors (VG and MW) for the data extraction process. If there were disagreements about data points, a third author (TT) was consulted to resolve the disagreement. The following data points were collected: first author's name; year of publication; country (region) of the population studied; sample size; age; follow-up period; tumor stage; survival data HR OS, DSS, and DFS, with the associated 95% CI, *p* value; survival data reported with univariate or multivariate analysis; cut-off value used to define "elevated OCT4"; method of obtaining the cut-off value. For the analysis of the relationship between OCT4 and clinicopathological parameters, HR and 95% CI were combined as the effective value. If several estimates of OCT4 HR for OS/DSS were reported in the same article, we chose the most powerful one (multivariate analysis was superior to univariate analysis, and the latter one weighted over unadjusted Kaplan–Meier analysis). If the method of OCT4 cutoff was by done by dividing the continuous OCT4 data into percentile cutoffs, the lowest OCT4 percentile cutoff was chosen for data extraction. If the HR for OS was reported as HR of a patient with OCT4 below a specific cutoff experiencing the endpoint of death (versus HR of a patient with OCT4 above a specific cutoff experiencing endpoint of death), we took the reciprocal of the reported HR to make it comparable to the other studies. Lastly, if the study reported the Kaplan Meier (KM) curve without information on the HR, the survival statistics were reconstructed using the methods described by Parmar [16], and subsequently developed by Tierney [17].

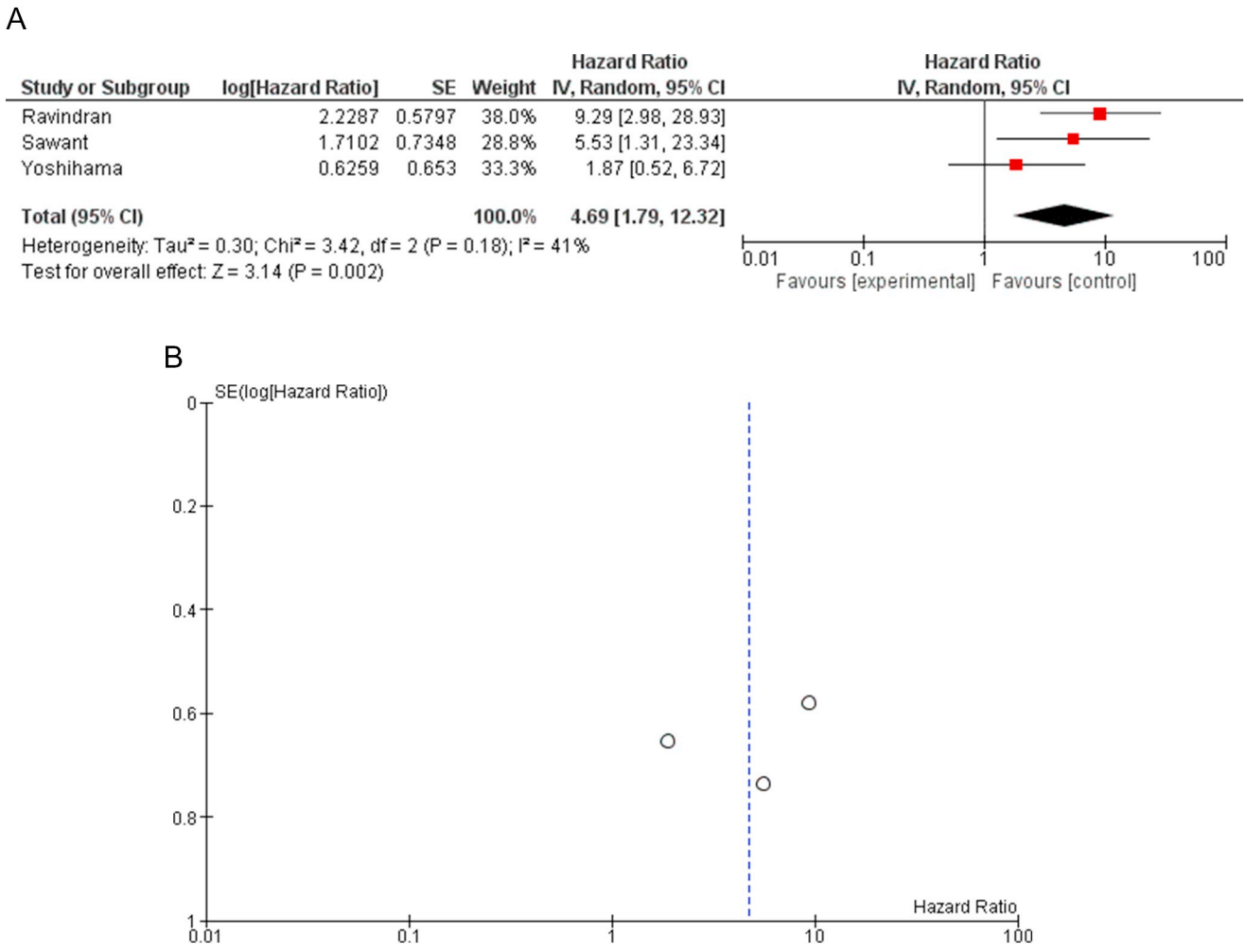

Fig. 3. A. DFS forest plot.
Forest plot is presented showing the pooled hazard ratio of the effect of high OCT4 expression on disease free survival, with lines representing the 95% confidence interval and red boxes representing the hazard ratio.
B. DFS Funnel plot
The funnel plot is presented with the y-axis representing the size of the study and the x-axis representing the direction of effect of the study. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.6. Statistical analysis

The logarithm of the HR with Standard Error (SE) was used as the primary summary statistic. To obtain the log[HR] and SE, the HR with 95% CI was extracted directly from the studies. Additional calculation to obtain the HR was required if the study reported the reciprocal of the HR. Estimates of log[HR] were weighted and pooled using the generic inverse-variance [13]. Because of anticipated heterogeneity, a more conservative approach applying the random effects model (DerSimonian and Laird method) was chosen for all analyses. Forest plots were constructed for all outcomes displaying the random-effects model of the summary effect measure and 95% CI. Heterogeneity was assessed using Cochran's Q and Higgins's I^2. Cochrane's Q p-value of < 0.2 and $I^2 > 50\%$ were considered as markers of significant heterogeneity. All analyses was done using the RevMan 5.3 analysis software (Cochrane Collaboration, Copenhagen, Denmark) [18] and Meta Essentials (ERASMUS Research Institute, Rotterdam, Netherlands) [19]. All statistical tests were two-sided, and a p-value of < 0.05 was considered statistically significant. No correction was made for multiple testing.

2.7. Publication bias

To assess publication bias, Begg's Funnel Plot and Egger's bias indicator test were used, if appropriate. If publication bias was detected, the influence of bias on the overall effect was assessed by Duval's "Trim and fill" method [20]. Tests for publication bias were performed by Meta-Essentials (ERASMUS Research Institute, Rotterdam, Netherlands) [21].

3. Results

3.1. High OCT4 expression is associated with poorer overall survival (OS) in oral squamous cell carcinoma

Pooled hazard ratios showed that high OCT4 Expression is associated with poorer OS in oral squamous cell carcinoma (HR: 1.72, 95% CI:1.07–2.75, $p = 0.02$). (Forest Plot Fig. 2A) This pooled result is associated with low heterogeneity, $I^2 = 18\%$, $p = 0.30$. On inspection, the Funnel plot indicated no publication bias (Funnel Plot Fig. 2B).

Of note, we also performed Duvall's trim and fill test, and the analysis indicated that there was 1 potential missing study in the literature. By taking into the account this missing study, OCT4 was not significantly associated with OS (log HR 95% CI: −0.33–1.24). (Supplementary material) Additionally, we also performed Egger's regression, and no publication bias was detected ($p = 0.35$). (Supplementary material):

Table 1
Study characteristics.

Author	Year	Country	Design	Site	Follow-up (months)	Age (years)	Total (n)	OCT4 analysis	Criteria for "High OCT4 Expression"	Stages I + II (n)	Stages III + IV (n)	Outcomes	OCT4 IHC information
Chiou S.H. et al. [23]	2008	China	RCS	OC	nr	nr	52	IHC	Two pathologists scored IHC blindly. Interpretation occurred in 5-high power views per slide	nr	nr	OS	Polyclonal rabbit anti OCT4 antibodies (Chemicon); recognizes OCT4 in human and mouse samples
Fu, T.Y. et al. [27]	2016	Taiwan	RCS	OC	48.5 (median)	nr	436	IHC	Extent of positivity scored as 0 when positive cells was < 5%; 1: 5–24%; 2: 25–49%; 3: 50–74%; 4: > 75%	282	154	DSS	Anti OCT4 mouse monoclonal antibody (dilution 1:50; Abcam, Cambridge, MA, USA); reacts with 45 kDa OCT4 and in a less extent 33 kDa OCT3b in human, mouse and rat samples
Huang, C.F. et al. [24]	2014	China	RCS	Tongue	nr	57	66	IHC	Assessed by percentage of positive cells (PPs) and staining intensity (SI) and rated as immunoreactive score = PP + SI	40	26	OS	Polyclonal rabbit anti-human antibody against OCT4 (dilution 1:50; Proteintech Group Inc., Chicago, IL, USA); reacts with OCT4 in human, mouse and rat samples
Ravindran, G. et al. [28]	2015	India	RCS	OC	31.9 (mean)	59.3 (mean)	60	IHC	Staining was assessed as 0 (negative), 1 (mild), 2 (moderate), 3 (intense)	nr	nr	OS, DFS	Mouse monoclonal IgG2b anti-Oct-4 (Santa Cruz Biotech, USA; sc-5279); reacts with OCT3/4 in mouse, rat and human samples
Rodrigues, M. et al. [25]	2018	Brazil	RCS	Tongue	61.2	nr	84	IHC + RT-qPCR	Sum of percentage of positive cells and staining intensity score (0: no staining; 1: weak; 2: moderate; 3: intense). Sum ranged from 0 to 7	nr	nr	OS	Monoclonal mouse anti-OCT4 (dilution 1:75, #2750; Cell Signaling Technology, Danvers, MA)
Sawant, S. et al. [29]	2015	India	PCS	OC	25 (median)	nr	87	IHC	Image J Software Analysis and manual microscopic counting of positively labeled cells	15	72	OS, DFS	Mouse monoclonal IgG1 antibody Oct3/4 (Clone 40, dilution 1:100 (Stem Cell technology, Canada V5Z 4 J7); reacts against human and mouse OCT4
Yoshihama, R. et al. [26]	2015	Japan	RCS	OC	60 (median)	62 (median)	108	IHC	Cutoff of 54.74 to delineate between high and low expression	nr	nr	OS, DFS, DSS	Polyclonal rabbit anti-human Oct4 (clone POU5F1; #2750; dilution 1:100; Cell Signaling Technology, Inc.); reacts against OCT4 in human samples

RCS retrospective cohort study, PCS prospective cohort study, OC lip and oral cavity, IHC immunohistochemistry, RT-qPCR reverse transcriptase-quantitative polymerase chain reaction, OS overall survival, DSS disease-specific survival, DFS disease-free survival.

Table 2
Summary of OCT4 endpoint data (OS, DFS, DSS).

Author	OS		DFS		DSS	
	HR (95% CI)	p-Value	HR (95% CI)	p-Value	HR (95% CI)	p-Value
Chiou S.H. et al. [23]	1.12 (0.21, 6.05)[a]	0.8952	nr	nr	nr	nr
Fu, T.Y. et al. [27]	nr	nr	nr	nr	0.79 (0.56, 1.12)	0.189
Huang, C.F. et al. [24]	1.35 (0.905, 2.016)[b]	0.141	nr	nr	nr	nr
Ravindran et al. [28]	7.236 (1.334, 39.243)	0.022	9.228 (2.982, 28.93)	0.001	nr	nr
Rodrigues, M. et al. [25]	1.49 (0.62, 3.57)[a]	0.37	nr	nr	nr	nr
Sawant, S. et al. [29]	4.71 (1.11, 19.91)	0.036	5.53 (1.31, 23.26)	0.02	nr	nr
Yoshihama, R. et al. [26]	1.19 (0.073, 20)[a]	0.9038	1.87 (0.52, 6.76)	0.3398	9.091 (0.011, > 100)	0.5195

HR hazard ratio, OS overall survival, DFS disease-free survival, DSS disease-specific survival.
[a] This study did not report HR and Kaplan Meier curves had to be reconstructed to extract these values [16,17].
[b] Relative risk was reported and reciprocal was taken to extract HR.

1. High OCT4 Expression is associated with poorer disease free survival (DFS) in oral squamous cell carcinoma.

Pooled hazard ratios showed that high OCT4 Expression is associated with poorer DFS in oral squamous cell carcinoma (HR: 4.69, 95% CI: 1.79–12.32, $p = 0.002$). (Forest Plot Fig. 3A).

This pooled result is associated with low heterogeneity, I2 = 41%, $p = 0.18$. On inspection, the Funnel plot indicated no publication bias (Funnel Plot Fig. 3B).

Of note, we also performed Duvall's trim and fill test, and the analysis indicated that there no potential missing studies. Additionally, we also performed Egger's regression, and no publication bias was detected ($p = 0.78$). (Supplementary material)

2. Disease specific survival (DSS)

Only two studies reported DSS, therefore, we were unable to pool the results for DSS.

3.2. Quality assessment

The six domains of QUIPs, a quality measurement tool for critical appraisal of prognostic studies, were used to assess the seven studies in this review. Analysis revealed a low risk of bias with regard to study attrition and a moderate risk of bias with regard to study participation. Prognostic factor measurement, outcome measurement, study confounding and statistical analysis and reporting were all at low-moderate risk of bias. The supplementary files show the overall summary of quality assessment grading and grading at the level of the individual study.

Five of the studies were retrospective cohort studies, which were determined to have an inherent risk of bias in study participation if the study did not adequately report the number of patients excluded during the initial patient selection or if they did not explain the reasons for exclusion. Study attrition rate was at low risk of bias since patients did not drop out after the initial exclusion during patient selection. Study confounding and statistical analysis and reporting were at low-moderate risk of bias, since almost half of the studies used Kaplan-Meier estimates with long-rank testing for the survival analysis. The other studies include such analysis with the addition of univariate and multivariate cox regression. Outcome measurement was at a low-to-moderate risk of bias as a little more than half of the studies had clear definitions and descriptions of their endpoints. Oct-4 expression value cut-off measurement was also at low-moderate risk since two studies did not have clear definitions of cutoff values.

4. Discussion

The aim of this metaanalysis was to determine the prognostic role of OCT4 in oral squamous cell carcinoma. Survival was analyzed in terms of overall survival and disease free survival. The pooled data determined that high OCT4 expression was associated with poorer OS in oral squamous cell carcinoma 1.72 (95% CI: 1.07–2.75, $p = 0.02$). This pooled result was associated with low heterogeneity with no publication bias. Of note, analysis indicated that there was 1 potential missing study in the literature. By taking into the account this missing study, OCT4 was not significantly associated with OS (logHR 95% CI: −0.33–1.24) with no publication bias. Pooled hazard ratios additionally showed that high OCT4 expression was associated with poorer DFS in oral squamous cell carcinoma 4.69 (95% CI: 1.79–12.32, $p = 0.002$). This pooled result was associated with low heterogeneity with no publication bias. Analysis indicated that there were no potential missing studies and no publication bias. Only two studies reported DSS. Therefore, we were unable to pool the results for DSS.

OCT4 has been implicated as a prognostic factor in numerous cancers including cervical carcinoma, renal cell carcinoma and breast cancer [11,12,22]. Immunohistochemistry was used in all papers to assess expression level of OCT4 in tumor samples. In each paper, OCT4 antibodies were taken from different sources and either polyclonal/monoclonal were used (Table 1). The antibodies used in four of the studies [23–26] are specific to Oct-4 only, and others were specific for both Oct-3 and Oct-4 [27–29].

Associations have been made with CSC factors CD44, SOX2, OCT4, Nanog, ALDH1, among others as common biomarkers used in head and neck cancer [7]. While studies have reported an association between high expression of OCT4 in tumors and poor clinical prognosis in oral squamous cell carcinoma, a clear link has not yet been established [23–29]. The results of our study showed that increased OCT4 expression was correlated with poor OS and DFS in OSCC patients.

CD44 and ALDH1 have been found to be prognostic for oral squamous cell invasion and metastasis [6]. These CSCs were going to be included in this study. However, literature has shown CD44 to be controversial as a marker of CSCs given that it is expressed by more differentiated cells and has been deemed to have no association with poor prognosis in the oral cavity by some [30]. Additionally, while the literature has shown ALDH1 to be associated with poor OS and DFS in head and neck cancer patients, fewer studies are available examining these survival metrics in OSCC patients [4].

For future research, studying the co-expression of OCT4 and SOX2 in the context of oral squamous cell carcinoma could reveal a new finding. OCT4 and SOX2 have been shown to be co-expressed [31]. An alternate paper studying high OCT4 and SOX2 expression has shown an association with an early stage of OSCC and better prognosis [27]. This was attributed to other reports being limited by “relatively small sample sizes and selection of certain pathological stages.” Meanwhile, other studies that studied OCT4 independently alongside other CSC markers, indicate worse prognosis [23–26,28,29].

Future studies should assess expression of OCT4 in conjunction with other CSC markers that have been implicated in oral cavity cancer. Such CSC markers include ALDH1, SOX2, and Nanog which have been shown

to be associated with progression of oral cancer [25,28].

Conducting an analysis of OCT4 alongside other notable CSC markers may prove to have greater benefit in therapeutics involving targeting these markers. In addition to analyzing a sole marker, other limitations of this study include the small number of patients involved in the studies this paper includes. This may be a result of conducting an analysis on a specific cancer within the larger field of head and neck and the sparseness of literature on prognosis of cancer stem cell markers in OSCC. Another limitation is that the majority of studies analyzed in this review involved a retrospective cohort (Table 2).

In summary, this meta-analysis shows there to be an association between high expression of OCT4 and poor prognosis of OS and DFS in OSCC patients. Given the few studies on CSC and OSCC, there is an indication for further research and exploring the role of OCT4 in tandem with other CSC markers in OSCC, particularly SOX2.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amjoto.2018.12.011.

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