Prognostic biomarkers in oral squamous cell carcinoma: a systematic review

Running title: Biomarkers for oral cancer

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CONFLICT OF INTEREST STATEMENT: The authors had no conflict of interest concerning the topic under consideration in this article.
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ABSTRACT

Over the years, several tumor biomarkers have been suggested to foresee the prognosis of oral squamous cell carcinoma (OSCC) patients. Here, we present a systematic review to identify, evaluate and summarize the evidence for OSCC reported markers. Eligible studies were identified through a literature search of MEDLINE/PubMed until January 2016. We included primary articles reporting overall survival, disease-free survival and cause-specific survival as outcomes. Our findings were analysed using REporting recommendations for tumor MARKer prognostic studies (REMARK), QuickGo tool and SciCurve trends. We found 41 biomarkers, mostly proteins evaluated by immunohistochemistry. The selected studies are of good quality, although, any study referred to a sample size determination. Considering the lack of follow-up studies, the molecules are still potential biomarkers. Further research is required to validate these biomarkers in well-designed clinical cohort-based studies.

Keywords. mouth neoplasms; oral cancer; oral squamous cell carcinoma; biomarkers, tumor; review, systematic
INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common malignancy of the head and neck (excluding non-melanoma skin cancer), with more than 300,000 new cases reported annually worldwide[1]. The disease has a high morbidity rate (37.8%) five years after diagnosis (http://www.cancer.gov/statistics/find - 2003-2009 data); despite the progress in research and therapy, survival has not improved significantly in the last few decades [2]. The search for prognostic markers represents a continuing challenge for biomedical science.

A cancer biomarker may be a molecule secreted by a tumor cell or a specific response of the body to the presence of cancer [3]. Biomarkers can be used for patient assessment in multiple clinical settings, including estimating the risk of disease and distinguishing benign from malignant tissues [4]. Cancer biomarkers can be classified based on the disease state, including predictive, diagnosis and prognosis biomarkers [5]. A prognostic biomarker informs about a likely cancer outcome (e.g., overall survival, disease-free survival, and cause-specific survival) independent of treatment received [6].

According to the NCI Dictionary of Cancer Terms (https://www.cancer.gov/publications/dictionaries/cancer-terms) the overall survival (OS) corresponds to the length of time from either the date of diagnosis or the start of treatment for cancer, which patients diagnosed with the disease are still alive. Disease-free survival (DFS, also called relapse-free survival) offers the length of time after primary treatment ends that the patient survives without any signs or symptoms of that cancer. Cause-specific survival (CSS) is the length of time from either the date of diagnosis or the start of treatment for cancer to the date of death from the disease.
From the identification of a promising biomarker to its clinical use, there is a long pathway involving many complicated hurdles, such as estimating the number of patients needed for the validation phase and statistical validation, among others [7, 8]. This validation and qualification are responsible for linking the promising biomarker with a biological process to clinical endpoints [9].

Considering several tumor biomarkers have been suggested to predict the prognosis of OSCC patients, we performed a systematic review, which is widely accepted as a ”gold standard” in medicine based on evidence [10], to identify, evaluate and summarize the evidence for OSCC reported markers.

MATERIALS AND METHODS

We performed a systematic review to conduct this investigation. The independent variables were prognostic biomarkers; the dependent variables were OSCC outcomes.

Search strategy

A systematic review allows critical analysis of multiple research studies. Aiming to answer the question “what are the biomarkers of OSCC?”, a systematic literature search based on keywords was performed. As PubMed comprises more than 26 million citations from the biomedical literature from MEDLINE, it is the search engine of choice to initiate queries in the health sciences. To identify all the primary research studies that evaluated candidate biomarkers in OSCC, we searched the MEDLINE/PubMed (http://www.ncbi.nlm.nih.gov/pubmed) medical literature database up to January 18, 2016. The search strategy was based on combinations of the
following keywords: “mouth neoplasms” [MeSH] and "biomarkers” [MeSH] and (risk ratio [Title/Abstract] or relative risk [Title/Abstract] or odds ratio [Title/Abstract] or risk [Title/Abstract]) and ("humans"[MeSH Terms] and English [lang]).

**Inclusion criteria**

Articles were included based on a previously published protocol [11]. Briefly, studies were selected if they examined the impact of a potential biological marker on at least one of the features in OSCC patients: OS, DFS or CSS. These definitions were assessed among the selected papers. In addition, if a study was focused on isolated or combined (multiple) tumor biomarkers, it must have been subjected to multivariable analysis with one or more additional variables.

**Exclusion criteria**

Articles were excluded from the present review for the following reasons: i) lack of the terms “oral cancer” and “risk” in their titles, abstracts or keywords; ii) absence of risk ratios and iii) unclear defining criteria for groups and variables.

**Potential prognostic biomarker**

To determine whether a biomarker is potentially prognostic, the selected articles showed: i) a formal test (binary logistic regression or Cox proportional hazards model) and ii) a statistically significantly association between the biomarker and outcome [6]. The computed risk (odds ratio, OR or hazard ratio, HR) was reported as the risk of a specific outcome from the biomarker group versus the reference group, with OR/HR>1 indicating increased risk and OR/HR<1 indicating decreased risk.
Data extraction

One investigator reviewed all the eligible studies and carefully extracted the study characteristics, including the article citation information, biomarker name and classification, condition or outcome, laboratory technique, sample size, number of clinical outcomes, status of biomarker expression, statistical test method, computed risk and its p-value and 95% confidence interval (CI). The main biological processes in which the biomarkers are involved were obtained using QuickGo (http://www.ebi.ac.uk/QuickGO).

Quality assessment

Quality assessment was performed in duplicate for each eligible study by three independent reviewers using operationalized prognostic biomarker reporting the REMARK guidelines [12] and extracted details on 20 items. The inter-observer agreement was evaluated using Kappa statistics.

Publication trends

To observe the publication trends in the selected potential OSCC biomarkers, we searched the scholarly literature in SciCurve Open (http://www.scicurve.com). SciCurve Open is a search engine that transforms a systematic literature review into an interactive and comprehensible environment [13].
RESULTS

Studies searching for OSCC biomarkers: proteins are the most analysed molecules

The keyword search strategy identified 403 suitable abstracts, from which 320 were excluded by reviewing the title and abstract during the screen because they did not meet the eligibility criteria. Full text articles were obtained for 83 studies (34 with single markers and 49 with multiple or combined markers).

Forty-five of these articles were excluded for different reasons, including: out of goal (3 articles), unavailability online (2 articles), lack of multivariable analysis (18 articles) and model inconsistencies (22 articles). Figure 1 shows a PRISMA diagram for this review (for details, see Supplemental file S1).

The selected studies were screened, and specific study characteristics and remarks were recorded. These parameters are summarized in Table 1 (the article context is grouped according to the hallmarks of cancer [14]). Thirty-eight papers examined 41 biomarkers [15-52]. Most of them were proteins determined using immunohistochemistry (IHC) in paraffin-embedded tissues (36 of 38 studies).

The included studies were conducted in Poland, India, Germany, Taiwan, Korea, Japan, Australia, Spain, China, Portugal, Brazil, UK, USA and Finland. Variable cohort sizes were used, ranging from 34 to 208 patients. $n$, outcome event number, statistical test, CIs and p-values, risk values and Google scholar citations were extracted (see Supplemental file S1). The main results of the included articles are summarized in Table 2. The biomarker high vs. low levels was defined differently in each study.
Fourteen clinicopathologic group factors were incorporated in 48 multivariate analyses (38 studies generated 48 significant models and 210 covariables). The most commonly included prognostic factors for model adjustment were the histopathological features (excluding the WHO histological differentiation degree) in 30 models (62.5%), protein (27 models, 53.3%) AJCC clinical stage (22 models, 45.8%) and WHO histological differentiation degree (21 models, 43.8%) (Figure 2). For complete details, see Supplemental file S1.

Quality of study reports: studies do not clear determine the sample size

The result of this agreement was 0.87, which is classified as almost perfect. Differences were resolved by consensus. Most study analyses reported details of the objective/hypothesis, patient source, population characteristics, assay method, cut-off point, and relationship of the potential marker to standard prognostic variables, as well as discussed the implications for future research and clinical value (for details, see Supplemental file S2). Notably, no study referred to a statistical sample size, which is key for biomarker validation.

Proposed OSCC biomarkers

None of the studied molecules presented an analysis of validation, so we called them “potential biomarkers”. A narrative review of the proposed biomarkers is presented in Table 3.

Trends: potential biomarkers with more publications and citations

To explore the publication trends in our OSCC potential protein biomarkers, we searched the scholarly literature in SciCurve Open. SciCurve uses PubMed’s library of 23 million references to generate visually pleasing graphs and curves that help grasp trends in the literature [53]. It is
associated with the following main functionalities: publications, citations, most prolific authors and countries.

According to Figure 3, MMP-2 is the most researched field, followed by MMP-1, cadherin-1 and mucin-1. The countries with the largest contributions are the USA, Japan and China.

**DISCUSSION**

We have summarized the results on the association between biomarkers and oral cancer outcomes using a systematic review. Overall, our results suggest 41 prognostic molecules involved with OSCC endpoints. These markers may be candidates for long-term studies.

OSCC is the most relevant epithelial malignancy for dental surgeons. It has late clinical detection and poor prognosis, and the available therapeutic alternatives are highly expensive and disfiguring [54].

OSCC is a very complex subtype of cancer with high heterogeneity [55]. Several risk factors are implicated in its aetiology, among which tobacco, alcohol, viruses and diet are highlighted [2]. These factors related to genetic inheritance may have a carcinogenic effect on the normal cells of the respiratory and digestive systems. This type of carcinoma can occur anywhere in the mouth, although the most affected sites are the tongue, lower lip and mouth floor [2, 56]. These regions are great facilitators of carcinoma spreading to regional lymph nodes and/or distant organs [57]. At present, the diagnosis of OSCC is based on comprehensive clinical examination and histological analysis of suspicious areas [58]. Recently, The Cancer Genome Atlas (TCGA) showed that a large dataset of proteomics/genomics did not improve the prognosis potential of classic clinical variables in patients with different types of cancer [59]. Some studies seeking
biomarkers in oral cancer are still in the discovery phase, requiring validation to be accepted in clinical practice.

Currently, biomarkers are a subject of particular interest because they may represent the most important part in the diagnosis step. In the future, specific and personalised diagnostics can guide treatment against the disease and consequently improve the chance of curing the disease.

In response to the need for tumor biomarkers for OSCC that can be readily evaluated in routine clinical practice, we performed a systematic review (PubMed keyword-base query) of the published literature to identify single or multiple biomarkers for OSCC outcomes: overall survival, disease-free survival, relapse-free survival and cause-specific survival. The main finding was the identification of 38 studies describing multivariate survival analysis for 41 biomarkers. From these articles, MMP-2, MMP-1, cadherin-1, mucin-1, GLUT-1 (SLC2A1), mucin-4, interleukin-8, HPV-16, EGFR and p53 have received great interest from the scientific community. Of these, up to now, it is accepted that the HPV status have a clinical utility [60], suggesting that HPV positive head and neck squamous cell carcinomas form a distinct clinical entity with better treatment outcome [61].

The malignant progression to OSCC is characterized by the acquisition of progressive and uncontrolled growth of tumor cells. Predicting whether premalignant lesions will progress to cancer is crucial to make appropriate treatment decisions. The first detectable clinical changes that can indicate that an epithelium is on the way to establish OSCC is the occurrence of malignant disorders, including leukoplakia (most common) [2]. In this context, we emphasize the results associated with Rho GTPase-activating protein 7, retinal dehydrogenase 1/prominin-1 (combined biomarkers), podoplanin, cortactin/focal adhesion kinase 1 (combined biomarkers) and catenin
These proteins show a potential role as a marker of oral cancer risk and malignant transformation [17, 26-28, 39, 40, 42].

There are thousands of papers reporting cancer biomarker discovery, but only few clinically useful biomarkers have been successfully validated for routine clinical practice [62]. Quality assessment tools have been developed for prognostic studies to help identify study biases and causes of heterogeneity when performing meta-analysis. We chose to use the REMARK reporting guidelines, which provide a useful start for assessing tumor prognostic biomarkers (all included studies were prognostic). We found that the investigations reported an average of 19 of 20 REMARK items. However, all studies failed to report the sample size calculation. In the absence of this calculation, the findings of each research should be interpreted with caution [63]. The sample size requirements that allow the identification of a benefit beyond existing biomarkers are even more demanding [64].

In our review, none of the articles that created prediction models had internal or external validation. In general, studies recruited cases of OSCC from a clinical setting as well as controls without a clearly defined diagnosis. Under this circumstance, any differences in the biomarker levels between OSCC patients and controls could simply reflect individual differences rather than cancer-related differences. The lack of biomarker validation strategies and standard operating procedures for sample selection in the included studies represent an important pitfalls and limitations, leading us to use the term "potential biomarkers" instead of biomarker in our article title.

It is important to highlight that our research searched only one database, which means that only studies available in MEDLINE were included. Additionally, due to the heterogeneity among the studies, a meta-analysis that combined the results of different studies could not be performed.
In addition, our research included results from observational studies, and their evaluation may have been problematic if the confounder variables were not adjusted because they were not measured [65].

CONCLUSION

Recent research in OSCC has identified a multitude of potential markers that have a significant role in prognosis. In this systematic review, despite the inherent limitations, we identified several potential biomarkers of particular interest that appear to carry prognostic significance. Considering the validation step as a process of assessing the biomarker and its measurement performance characteristics, and determine the range of conditions under which this biomarker can provide reproducible data [9], our results show biomarkers in the discovery phase, thereby leading us to call them OSCC “potential biomarkers”. Nevertheless, it is urgent to apply validation methods to provide clinically useful oral cancer biomarkers.

ACKNOWLEDGMENT

CONICYT Becas-Chile Scholarship 8540/2014, PNPD/CAPES 33003033009P4, and FAPESP Grants 2016/07846-0, 2014/06485-9 and 2015/12431-1, supported this work.
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**FIGURE CAPTIONS**

![Flow diagram representing systematic literature search on biomarkers and oral cancer outcomes. Studies were included if they examined the impact of a potential biomarker on at least one of overall survival, disease free survival or cause-specific survival in oral squamous cell carcinoma patients.](image)

**Fig. 1.** Flow diagram representing systematic literature search on biomarkers and oral cancer outcomes. Studies were included if they examined the impact of a potential biomarker on at least one of overall survival, disease free survival or cause-specific survival in oral squamous cell carcinoma patients.
Fig. 2. A. Adjustment variables. Frequencies with which adjustments were performed for OSCC outcomes. The heat map combines the most frequent factors for adjustments and survival models. The most commonly included factor was “histopathological features” (excluding the WHO histological differentiation degree). Higher numbers represent intense and saturated colors.

B. Trends in oral cancer biomarkers (top ten). Compared with other biomarkers, MMP-2 is the most researched field with 15,057 publications and 46,368 citations (1997-2017). MMP-2 is followed by MMP-1 (14,650 publications/43,762 citations) and cadherin-1 (14,531/43,422).
Table 1. Characteristics of the included studies.

| Reference        | Biomarker*                                      | Change | Design and method | Study remarks                                                                 |
|------------------|-------------------------------------------------|--------|-------------------|-------------------------------------------------------------------------------|
| **Sustaining proliferative signaling** |                                                |        |                   |                                                                                |
| Gontarz M et al., 2014 | Proliferation marker protein Ki-67 or ki-67 (MKI67) | (+)    | Poland. Retrospective. IHC. | May be useful in the selection of patients at a higher risk of recurrence who would benefit from postoperative radiotherapy |
| Ramshankar V et al. 2014 | Cyclin-dependent kinase inhibitor 2A or p16 (CDKN2A) | (+)    | India. Retrospective. IHC and RT-qPCR. | CDKN2A overexpression is a single important prognostic variable in defining a high risk group. CDKN2A expression should possibly not be used as a surrogate marker for HPV infection in tongue cancers. |
| Tripathi SC et al., 2012 | Rho GTPase-activating protein 7 or DLC1 (DLC1) | (-)    | India. Retrospective. IHC. | Loss of expression emerged as an important biomarker for predicting patients diagnosed with oral dysplasia at high risk of transformation. Is a poor prognostic marker for oral squamous cell carcinoma patients. |
| Freudlsperger C et al., 2011 | MKI67                                           | (+)    | Germany. Retrospective. IHC. | Expression level could be used to identify a subgroup of surgically treated patients with stage I OSCC who might benefit from treatment intensification. |
| Kok SH et al., 2010 | Protein CYR61 (CYR61)                            | (+)    | Taiwan. Retrospective. IHC. | Is a positive growth modulator of OSCC and overexpression is an independent prognostic indicator. |
| Shah NG et al. 2009 | Cellular tumor antigen p53 or p53 (TP53)         | (+)    | India. Retrospective. IHC. | TP53 was independently associated with DFS and OS, and CDKN2A with DFS only. |
|                    | CDKN2A                                          | (-)    |                   |                                                                                |
| Kim SJ et al. 2007 | Carbonic anhydrase 9 (CA9) and MKI67 combined    | (+)    | Korea. Retrospective. IHC. | The expression of CA9 and MKI67may be useful for predicting prognosis in squamous cell carcinoma of the tongue. |
| Shiraki M et al. 2005 | TP53, G1/S-specific cyclin-D1 (CCND1), epidermal growth factor receptor (EGFR) combined | (+)    | Japan. Retrospective. IHC. | Simultaneous expression of these markers in oral cancers might prove to be a useful indicator for identification of low- or high-risk patients. |
| Study                          | Gene          | Abbreviation | Country | Study Type | Method | Findings                                                                                     |
|-------------------------------|---------------|--------------|---------|------------|--------|---------------------------------------------------------------------------------------------|
| Myo K et al., 2005            | CCND1 (+)     | Japan        | Retrospective. FISH. | Aberrations in gene numbers appear to be valuable in identifying patients at high risk of late lymph node metastasis in stage I and II OSCCs. |
| Pande P et al. 2002           | TP53 (+)      | India        | Prospective. IHC. | RB1 loss and TP53 overexpression may serve as adverse prognosticators for disease free survival of the patients. |
| Bova RJ et al. 1999           | CCND1 (+)     | Australia    | Retrospective. IHC. | CCND1 overexpression and loss of CDKN2A expression predict early relapse and reduced survival in squamous cell carcinoma of the anterior tongue |
| Pérez-Sayáns M et al., 2014   | Myc oncogene  | Spain        | Retrospective. IHC. | Its determination can be valuable when used together with other markers to assess the prognosis of OSCC patients. |
| Liu W et al. 2013             | Retinal dehydrogenase 1 or ALDH1 (+) | China        | Prospective. IHC. | Expression of cancer stem cell markers ALDH1A1 and PROM1 correlate with a high risk of malignant transformation in a large series of patients with premalignant oral leukoplakia. |
| Feng JQ et al. 2013           | ALDH1A1 (+)   | China        | Retrospective. IHC. | Expression pattern was associated with malignant transformation, suggesting that it may be valuable predictors for evaluating the risk of oral cancer. |
| Suzuki F et al., 2005         | Protein S100-A2 (-) | Japan       | Retrospective. IHC. | Patients with stage I or II invasive OSCC without expression should be considered a high-risk group for late cervical metastasis when a wait-and-see policy for the neck is being considered. |
| Tsai ST et al., 2005          | S100-A2 (-)   | Taiwan       | Retrospective. IHC. | Loss of nuclear expression may serve as an independent prognostic marker for early-stage oral cancer patients at high risk of recurrence. A more aggressive treatment modality and intensive follow-up may be recommended for the patients with reduced expression of in tumor cell nuclei. |
| Moura IM et al., 2014         | Cell division cycle protein 20 homolog (CDC20) (+) | Portugal    | Retrospective. IHC. | High expression is associated with poor prognosis in OSCC, may be used to identify high-risk OSCC patients, and may serve as a therapeutic target. |
| Study Authors        | Protein                                                                 | Location | Study Design | Findings                                                                 |
|----------------------|-------------------------------------------------------------------------|----------|--------------|-------------------------------------------------------------------------|
| Tang JY et al., 2013 | Microtubule-associated proteins 1A/1B light chain 3A or LC3 (MAP1LC3A) | Taiwan.  | Retrospective. IHC. | Elevated expression, which corresponds to increased level of autophagy activity, is a frequent event and an indicator of poor prognosis in human OSCC. |
| de Carvalho-Neto PB et al. 2013 | Tumor necrosis factor receptor superfamily member 6 or FAS (FAS) | Brazil.  | Retrospective. IHC. | DFS and CSS were significantly correlated with FAS/FASL expression profiles. The high risk category was an independent marker for earlier disease relapse and disease-specific death. |
|                       | Tumor necrosis factor ligand superfamily member 6 or FASL (FASLG)      |          |              |                                                                         |
| **Inducing angiogenesis** | **Yanagawa T et al., 2004**                                    | Japan.   | Retrospective. IHC. | Could be used clinically as a marker for tumors possessing the potential for lymph node metastasis. This method could prove useful as an adjuvant method to detect lymph node metastasis and may help reduce the number of surgeries by indicating when surgery is unnecessary. |
|                       | **Heme oxygenase 1 (HMOX1)**                                        |          |              |                                                                         |
| **Activating invasion and metastasis** | **de Vicente JC et al., 2013**                               | Spain.   | Retrospective. IHC. | Could be a valuable biomarker for risk assessment of malignant transformation in patients with oral leukoplakia along with histological assessment |
|                       | Podoplanin (PDPN)                                                     |          |              |                                                                         |
| de Vicente JC et al. 2012 | Src substrate cortactin (CTTN) and focal adhesion kinase 1 (PTK2) combined | Spain.   | Retrospective. IHC. | Strong immunoexpression of CTTN and PTK2, and not only one of them, is a predicting factor for increased cancer risk in oral premalignant lesions. |
|                       | Mucin-4 (MUC4)                                                       |          |              |                                                                         |
| Hamada T et al., 2012  | Catenin delta-1 (CTNND1)                                             | China.   | Retrospective. IHC. | May serve as a useful marker for the identification of a high risk of potentially malignant oral lesions progressing to OSCC. |
| Ma LW et al., 2012     | Actin, aortic smooth muscle or SMA (ACTA2)                           | UK.      | Retrospective. IHC. | An positive, myofibroblastic stroma is the strongest predictor of OSCC mortality. |
| Author                | Protein                        | Location | Technique | Findings                                                                                                                                                                                                 |
|----------------------|--------------------------------|----------|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Zhang Z et al. 2011  | Interstitial collagenase or MMP-1 (MMP1) | China    | Retrospective. IHC. | Up-regulation of MMP1, MMP2 might be important features of OSCC progression.                                                                                                                               |
|                      | 72 kDa type IV collagenase or MMP-2 (MMP2) |          | (+)       |                                                                                                                                                                                                             |
| Liu LK et al. 2010   | Vimentin (VIM)                  | China    | Retrospective. IHC. | The high expression of VIM and low expression of CDH1 were associated with survival and were independent prognostic factors in multivariate analyses.                                                      |
|                      | Cadherin-1 (CDH1)               |          | (-)       |                                                                                                                                                                                                             |
| Kawaguchi H et al., 2008 | PDPN                            | USA      | Retrospective. IHC. | Together with histology, may serve as a powerful biomarker to predict the risk for oral cancer development in patients with oral leukoplakia.                                                           |
| Pukkila M et al., 2007 | VCAN protein (VCAN)             | Finland  | Retrospective. IHC. | Correlated with both increased risk for disease recurrence and shortened survival. High stromal expression may thus be considered an independent and adverse prognostic marker in OSCC. |
| Endo K et al. 2006   | E3 ubiquitin-protein ligase AMFR (AMFR) | Japan    | Retrospective. IHC. | Is valuable in identifying patients at high risk for tongue SCC recurrences                                                                                                                                   |
|                      |                                |          | (+)       |                                                                                                                                                                                                             |
| **Reprogramming of energy metabolism** |                                |          | (+)       |                                                                                                                                                                                                             |
| Hamada T et al., 2012 | Mucin-1 (MUC1)                  | Japan    | Retrospective. IHC. | Is a risk factor for subsequent lymph node metastasis in patients with OSCC and therefore may represent an indication for elective neck dissection                                                                  |
|                      | Hypoxia-inducible factor 1-alpha (HIF1A) or HIF-1α and Solute carrier family 2, facilitated glucose transporter member 1 or GLUT-1 (SLC2A1) combined | Germany  | Retrospective. IHC. | Coexpression of high levels of HIF1A and SLC2A1 is significantly correlated with prognosis in OSCC patients.                                                                                               |
| Eckert AW et al., 2011 | HIF1A                           | Germany  | Retrospective. IHC. | Immunohistochemical detection appears to improve diagnosis and to provide prognostic information in addition to the TNM – system and histological grade of OSCC.                                                |
| Fillies T et al., 2005 | HIF1A                           | Germany  | Retrospective. IHC. | Overexpression is an indicator of favorable prognosis in T1 and T2 SCC of the oral floor. Node negative patients lacking expression may therefore be considered for adjuvant radiotherapy. |
| Tumor-promoting inflammation |                                |          | (+)       |                                                                                                                                                                                                             |

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| Authors          | Protein/Target                                      | Location   | Study Type | Method          | Findings                                                                 |
|------------------|-----------------------------------------------------|------------|------------|-----------------|---------------------------------------------------------------------------|
| Kwon M et al., 2015 | Interleukin-4 receptor subunit alpha (IL4R)         | Korea      | Retrospective | IHC.            | High expression of IL4R correlated with increased recurrence, while high IL13RA1 expression had an inverse relationship to recurrence and disease-specific survival in OSCC patients. |
|                  | Interleukin-13 receptor subunit alpha-1 (IL13RA1)   |            |            |                 |                                                                           |
| Fujita Y et al. 2014 | Interleukin-8 (CXCL8)                               | Japan      | Retrospective | IHC.            | These factors in addition to N status may have prognostic value in patients with resectable OSCC. |
|                  | Scavenger receptor cysteine-rich type 1 protein M130 (CD163) |            |            |                 |                                                                           |
| Lai WM et al. 2013 | Myeloperoxidase (MPO)                               | Taiwan     | Retrospective | IHC.            | Higher MPO expression in buccal mucosal SCC is a risk factor for second primary tumors. |
| Huang SF et al. 2012 | Serpin B3 (SERPINB3) and C-reactive protein (CRP) combined | Taiwan     | Retrospective | Immunoassay.     | High levels of both preoperative SERPINB3 and CRP levels act as a predictor for DFS and OS. |

The articles are grouped according to the hallmarks of cancer. *UniProt Knowledgebase or common name. HGNC name between parentheses. (+) Up-regulated/overexpressed, (-) Down-regulated/down-expressed, CSS, cause-specific survival; OS, overall survival; DFS, disease free survival; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization.
Table 2. Data extracted from selected studies.

| Article                          | Biomarker* | N** | Cases vs. reference group | Outcome | HR   | CI     | p-value |
|---------------------------------|------------|-----|--------------------------|---------|------|--------|---------|
| **Sustaining proliferative signaling** |            |     |                          |         |      |        |         |
| Gontarz M et al., 2014          | MKI67      | 34  | IHC. High vs. low.       | DFS     | 5.42 | 1.18–24.83 | 0.029   |
|                                 |            |     |                          | CSS     | 9.02 | 1.99–40.93 | 0.004   |
| Ramshankar V et al. 2014        | CDKN2A     | 156 | IHC. Overexpression vs. low | OS      | 2.34 | 1.30–4.40  | 0.005   |
|                                 |            |     |                          | DFS     | 2.58 | 1.44–4.64  | 0.002   |
|                                 | HPV16      |     | RT-qPCR. Negative vs. positive | OS      | 0.61 | 0.38–0.99  | 0.049   |
| Tripathi SC et al., 2012        | DCL1       | 181 | IHC. Loss vs. expression | DFS     | 2.10 | 1.2–3.9  | 0.023   |
| Freudlsperger C et al., 2011    | MKI67      | 69  | IHC. High vs. low        | DFS     | 4.24 | NS      | 0.029   |
| Kok SH et al., 2010             | CYR61      | 93  | IHC. High vs. low        | OS      | 2.44 | 1.2–4.95  | 0.010   |
| Shah NG et al. 2009             | TP53       | 135 | IHC. Positive vs. negative | OS      | 2.71 | 1.29–5.72 | 0.009   |
|                                 | CDKN2A     |     |                          | DFS     | 2.45 | 1.28–4.69 | 0.007   |
| Kim SJ et al. 2007              | CA9 and MKI67 combined | 60  | IHC. High vs. low        | OS      | 4.04 | NS      | 0.005   |
|                                 |            |     |                          | DFS     | 2.39 | NS      | 0.007   |
| Shiraki M et al. 2005           | TP53, CCND1 and EGFR combined | 140 | IHC. Co-expression of all three markers vs. 0-2 markers | OS | 3.56 | 1.59–7.93 | 0.002   |
| Myo K et al., 2005              | CCND1      | 45  | Numerical aberration positive vs. negative | DFS | 8.69 | 2.23–33.80 | 0.002   |
| Pande P et al. 2002             | TP53       | 50  | IHC. Positive vs. negative | DFS | 2.98 | 1.04–8.47 | 0.041   |
|                                 | RB1        |     | IHC. Negative vs. positive | DFS | 2.94 | 0.12–0.92 | 0.034   |
| Bova RJ et al. 1999             | CCND1      | 147 | IHC. Positive vs. negative | DFS | 2.48 | 1.0–6.15  | 0.005   |
|                                 | CDKN2A     | 143 | IHC. Negative vs. positive | OS | 3.15 | 1.65–7.50 | 0.001   |
| **Evading growth suppressors**   |            |     |                          |         |      |        |         |
| Pérez-Sayáns M et al., 2014     | MYC        | NA  | NA                       | OS      | 1.15 | 1.06–1.25 | <0.001  |
| Liu W et al. 2013               | ALDH1A1    | 141 | IHC. Positive vs. negative | DFS | 4.17 | 1.96–8.90 | <0.001  |
|                                 | PROM1      |     | IHC. Positive vs. negative | DFS | 2.86 | 1.48–5.55 | 0.002   |
| Feng JQ et al. 2013             | ALDH1A1    | 34  | IHC. Positive vs. negative | DFS | 8.89 | 1.67–47.41 | 0.011   |
|                                 |            |     |                          |         |      |        |         |
| Suzuki F et al., 2005           | S100-A2    | 52  | IHC. Negative vs. positive | DFS | 0.20 | 0.08–0.53 | 0.001   |
| Tsai ST et al., 2005            | S100-A2    | 70  | IHC. Low vs. high        | DFS     | 4.36 | 1.52–12.49 | 0.006   |
| **Resisting cell death**        |            |     |                          |         |      |        |         |
| Moura IM et al., 2014           | CDC20      | 65  | IHC. Positive vs. negative | CSS | 2.36 | 1.08–5.17 | 0.032   |
| Authors                              | Gene      | IHC/Expression | Tissue   | OS       | DFS1     | DFS2     | p       |
|--------------------------------------|-----------|----------------|----------|----------|----------|----------|---------|
| Tang JY et al., 2013                 | MAP1LC3A  | 90             | High vs. low | OS       | DFS      | CSS      | p       |
| de Carvalho-Neto PB et al., 2013     | FAS       | 60             | IHC. Negative vs. positive | DFS      | CSS      | **        | **       |
| de Carvalho-Neto PB et al., 2013     | FASLG     |                | IHC. Negative vs. positive | CSS      | **        | **        | **       |
| Yanagawa T et al., 2004              | HMOX1     | 54             | IHC. Low vs. high | DFS      | **        | **        | **       |
| de Vicente JC et al., 2013           | PDPN      | 58             | IHC. Score 2–3 vs. 0–1 | DFS      | **        | **        | **       |
| de Vicente JC et al., 2013           | CTTN and PTK combined | 50 | IHC. High co-expression of vs. negative to moderate | DFS | 6.30 | 1.55–25.58 | 0.01 |
| Hamada T et al., 2012                | MUC4      | 150            | IHC. Positive vs. negative | OS       | 1.62 | 1.12–2.41 | 0.002 |
| Ma LW et al., 2012                   | CTNND1    | 68             | Phosphorylated. IHC. High vs. low | DFS | 3.43 | 1.40–8.41 | 0.007 |
| Marsh D et al. 2011                  | ACTA2     | 208            | IHC. High vs. low | OS       | 3.06 | 1.65–5.66 | 0.002 |
| Zhang Z et al. 2011                  | MMP1      | NS.            | IHC intensity in cancer tissue (continuous variable) | DFS | 1.09 | 1.03–1.16 | 0.003 |
| Liu LK et al. 2010                   | VIM       | 83             | IHC. High vs. negative/low | OS | 1.61 | 1.02–2.55 | 0.042 |
| Kawaguchi H et al., 2008             | PDPN      | 150            | IHC. Oral leukoplakia positive vs. negative | DFS | 3.09 | 1.53–6.23 | 0.002 |
| Pukkila M et al., 2007               | VCAN      | 139            | IHC. High vs. low | CSS | 1.80 | 1.01–3.30 | 0.048 |
| Endo K et al. 2006                   | AMFR      | 99             | IHC. Positive vs. negative | DFS | 2.07 | 1.04–4.11 | 0.038 |

**Activating invasion and metastasis**

| Authors                              | Gene      | IHC/Expression | Tissue   | OS       | DFS1     | DFS2     | p       |
|--------------------------------------|-----------|----------------|----------|----------|----------|----------|---------|
| Hamada T et al., 2012                | MUC1      | 206            | IHC. Positive vs. negative | OS | 2.09 | 1.04-4.29 | 0.040 |
| Eckert AW et al. 2011                | HIF1A and SLC2A1 combined | 55 | IHC. High co-expression vs. low | CSS | 5.13 | 1.33–19.79 | 0.017 |
| Eckert AW et al., 2010               | HIF1A     | 80             | IHC. Moderate or strong vs. negative or weak | CSS | 3.49 | NS | 0.016 |
| Fillies T et al., 2005               | HIF1A     | 85             | IHC. Low vs. very high | OS | 0.20 | 0.10–0.50 | 0.000 |

**Reprogramming of energy metabolism**

| Authors                              | Gene      | IHC/Expression | Tissue   | OS       | DFS1     | DFS2     | p       |
|--------------------------------------|-----------|----------------|----------|----------|----------|----------|---------|
| Hamada T et al., 2012                | MUC1      | 206            | IHC. Positive vs. negative | OS | 2.09 | 1.04-4.29 | 0.040 |
| Eckert AW et al. 2011                | HIF1A and SLC2A1 combined | 55 | IHC. High co-expression vs. low | CSS | 5.13 | 1.33–19.79 | 0.017 |
| Eckert AW et al., 2010               | HIF1A     | 80             | IHC. Moderate or strong vs. negative or weak | CSS | 3.49 | NS | 0.016 |
| Fillies T et al., 2005               | HIF1A     | 85             | IHC. Low vs. very high | OS | 0.20 | 0.10–0.50 | 0.000 |
| Study                                      | Marker   | N   | Assay                      | DFS Results (HR) | OS Results (HR) | p-Value |
|-------------------------------------------|----------|-----|----------------------------|------------------|----------------|---------|
| Kwon M et al., 2015                       | IL4R     | 186 | IHC. High vs. low          | DFS 2.34 1.38-3.97 | OS 0.26 0.14-0.48 | <0.001  |
|                                           | IL13RA1  |     | IHC. High vs. low          | DFS 0.33 0.17-0.67 | OS 0.33 0.17-0.67 | <0.001  |
| Fujita Y et al. 2014                      | CXCL8    | 50  | IHC. Positive vs. negative | DFS 0.27 0.08-0.89 | OS 0.33 0.17-0.67 | 0.031   |
|                                           | CD163    |     | IHC. Invasive front, high vs. low | DFS 2.63 1.31-5.25 | OS 2.63 1.31-5.25 | 0.006   |
| Lai WM et al. 2013                        | MPO      | 173 | IHC. High vs. low          | DFS 3.89 1.33-11.39 | OS 3.89 1.33-11.39 | 0.013   |
| Huang SF et al. 2012                      | SERPINA3 and CRP combined | 99  | Immunoassay. Positive vs. negative | DFS 8.43 3.94-18.01  | OS 6.25 2.60-15.01  | <0.001  |

The articles are grouped according to the hallmarks of cancer. *HGNC database recommended names were used. **N, number of subjects in the contrast. ***Odds ratio (multiple logistic regression). HR and OR values are reported as they originally appear in the selected articles. NS, not specified. NA, not apply. DFS, disease free survival; CSS, cause-specific survival; OS, overall survival; 1Recurrence and 2lymph node metastasis.
Table 3. Overview of proposed biomarkers

| Name* | Biological processes | Cancer context |
|-------|----------------------|----------------|
| MKI67 | Cell cycle, cell proliferation. | Marker of the growth fraction for a certain cell population [1]. The labelling index is considered one of the best prognostic factors of the survival rate and recurrence [2]. |
| CDKN2A| Cell cycle, cell cycle arrest. | This gene is frequently mutated or deleted in a wide variety of tumors, and is known to be an important tumor suppressor gene [3]. |
| HPV16 | High-risk HPV type. | Is emerging as an important factor in the rise of oropharyngeal tumors affecting non-smokers in developed countries. Patients with HPV(+) tumors demonstrated favorable outcomes compared to TP53 mutants and 11q13/CCND1-amplified tumors [4]. |
| DLC1 | Negative regulation of cell proliferation and migration. | Acts as a tumor suppressor in a number of common cancers, including liver cancer [5]. |
| CYR61 | Regulation of cell growth and adhesion. | Can function as an oncogene or a tumour suppressor, depending on the origin of the cancer [6]. |
| TP53 | Cell cycle, cell cycle arrest. | Tumor-suppressor protein. Mutations in this gene are associated with a variety of human cancers [3]. |
| CA9 | Response to hypoxia. | Is the most widely expressed gene in response to hypoxia. Its role in intracellular pH maintenance represents the means by which cancer cells adapt to the toxic conditions of the extracellular environment [7]. |
| CCND1 | Cell cycle, cell division. | Is frequently deregulated in cancer and is a biomarker of cancer phenotype and disease progression [8]. |
| EGFR | Positive regulation of cell proliferation. | EGFR overexpression is a significant finding in cancer, particularly in head and neck cancer, where it is also associated with a poor prognosis [9]. |
| RB1 | Cell cycle, cell cycle arrest. | Tumor-suppressor protein. Defects in this gene are a cause of childhood cancer retinoblastoma (RB), bladder cancer, and osteogenic sarcoma [3]. |
| MYC | Positive regulation of cell proliferation. | Its oncogenic reputation stems from its frequent deregulation in a host of human cancers and from a suite of activities that place this protein at the nexus of cell growth, proliferation, metabolism, and genome stability [10]. |
| ALDH1A1 | Ethanol oxidation. | Play a key role in the regulation of growth and differentiation of both normal tissue stem cells and cancer stem cells [11]. |
| PROM1 | Retina layer formation. | Maintaining stem cell properties by suppressing differentiation [3]. |
| S100-A2 | Endothelial cell migration. | In epithelial tissue, S100-A2 expression is decreased remarkably in tumours compared with normal specimens [12]. S100-A2 promotes p53 transcriptional activity, and its loss of expression has been associated with a poorer prognosis and shorter survival [13]. |
| CDC20 | Cell cycle, positive regulation of cell proliferation. | The role of CDC20 expression in tumours is not known, but many studies have reported that CDC20 regulates apoptosis, leading to genetically instability [14]. |
| Gene    | Function                                      | Expression/Implications                                                                                                                                 |
|---------|-----------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| MAP1LC3A| Autophagy.                                    | Strong positive expression in the peripheral area of pancreatic cancer tissue had a shorter overall and disease-free survival; correlations with tumour size, poor differentiation, blood vessel infiltration and tumour necrosis were noted [15]. |
| FAS     | Apoptotic process.                            | Cancer cells can never lose FAS or FASLG. FAS and/or FASLG expression promotes tumor growth and favors the establishment of tumor metastases [16].          |
| FASLG   |                                              |                                                                                                                                                         |
| HMOX1   | Angiogenesis.                                 | Many human tumours produce HMOX1, and its expression is usually higher in cancer cells than in surrounding healthy tissues [17].                        |
| PDPN    | Lymhangiogenesis.                             | Is commonly used in the identification of lymphatic endothelial differentiation in vascular endothelial neoplasms and lymphatic invasion by tumours [18]. Recent evidence have identified podoplanin as a marker of cancer-associated fibroblasts [19]. |
| CTTN    | Cell motility and focal adhesion assembly.    | Is overexpressed in breast cancer and squamous cell carcinomas of the head and neck [3].                                                                 |
| PTK2    | Angiogenesis assembly.                       | Promotes tumor progression and metastasis through effects on cancer cells, as well as stromal cells of the tumor microenvironment [20].                 |
| MUC4    | Cell adhesion.                                | An aberrant expression of MUC4 has been reported in various carcinomas [21].                                                                             |
| CTNN1D  | Cell adhesion.                                | Evidence is emerging that complete loss, downregulation or mislocalization of CTNND1 correlates with the progression of different types of human tumours [22]. |
| ACTA2   | Mesenchyme migration.                         | Patients with lung adenocarcinomas and high ACTA2 expression showed significantly enhanced distant metastasis and unfavorable prognosis [23].           |
| MMP1    | Proteolysis.                                  | Imbalance between matrix metalloproteinases and their inhibitors play the important role in progression of head and neck cancer [24].                  |
| MMP2    | Angiogenesis, response to hypoxia and proteolysis. |                                                                                                                                                       |
| VIM     | Movement of cell or subcellular component.    | Has been recognized as a marker for epithelial-mesenchymal transition. Overexpression in cancer correlates well with accelerated tumor growth, invasion, and poor prognosis [25]. |
| CDH1    | Cell adhesion.                                | Loss of function of this gene is thought to contribute to cancer progression by increasing proliferation, invasion, and/or metastasis [3].             |
| VCAN    | Cell adhesion.                                | Is strongly associated with a poor outcome for many different cancers. Depending on the cancer nature, is expressed either by cancer cells themselves or by stromal cells surrounding the tumour [26]. |
| AMFR    | Movement of cell or subcellular component.    | Is a tumor motility-stimulating protein secreted by tumor cells [3].                                                                                   |
| MUC1    | DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest. | Is aberrantly glycosylated and overexpressed in various epithelial cancers and plays a crucial role in the progression of the disease [27]. MUC1 is often used as a diagnostic marker for metastatic progression [28]. |
| Gene  | Process                                                                 | Function/Implication                                                                 |
|-------|--------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| HIF1A | Angiogenesis, response to hypoxia.                                       | Up-regulates the expression of proteins that promote angiogenesis, anaerobic metabolism, and many other survival pathways [29]. |
| SLC2A1| Glucose transport.                                                        | Was significantly correlated with depth of invasion and clinical stage in patients with gastric cancer [30]. |
| IL4R  | Immune system process and regulation of cell proliferation.              | The IL4/IL4R signaling axis is a strong promoter of pro-metastatic phenotypes in epithelial cancer cells including enhanced migration, invasion, survival, and proliferation [31]. |
| IL13RA1| Cell surface receptor signaling pathway.                                  | Glioblastoma samples presented higher IL13RA1 and IL13RA2 expression levels compared to lower grades astrocytomas and non-neoplastic cases [32]. |
| CXCL8 | Angiogenesis, movement of cell or subcellular component and chemotaxis.  | Neovascularisation is now recognised as a critical function of CXCL8 in the tumour microenvironment [33]. |
| CD163 | Inflammatory response.                                                   | Could be used as a general anti-inflammatory myeloid marker with prognostic impact for breast cancer patients [34]. |
| MPO   | Defense response.                                                        | Myeloperoxidase-positive cell infiltration in colorectal carcinogenesis is an indicator of colorectal cancer risk [35]. |
| SERPINB3 | Positive regulation of cell proliferation.                               | Promotes oncogenesis and epithelial-mesenchymal transition [36].                     |
| CRP   | Inflammatory response.                                                   | Patients with a high baseline CRP had a greater risk of early death compared with those with low CRP levels [37]. |

*HGNC database recommended names were used. **Representative processes from QuickGo (http://www.ebi.ac.uk/QuickGO).

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