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REVIEW ARTICLE

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World Workshop on Oral Medicine VII: Prognostic biomarkers in oral leukoplakia and proliferative verrucous leukoplakia—A systematic review of retrospective studies

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Abstract

Objective: To systematically review retrospective studies examining prognostic potentials of candidate biomarkers to stratify malignant progression of oral leukoplakia (OL) and proliferative verrucous leukoplakia (PVL).

Materials and Methods: A systematic literature search of PubMed, EMBASE, Evidence-Based Medicine and Web of Science databases targeted literature published through 29 March 2018. Inter-rater agreement was ascertained during title, abstract and full-text reviews. Eligibility evaluation and data abstraction from eligible studies were guided by predefined PICO questions and bias assessment by the Quality in Prognosis Studies tool. Reporting followed Preferred Reporting Items for Systematic Review and Meta-Analysis criteria. Biomarkers were stratified based on cancer hallmarks.

Results: Eligible studies (n = 54/3,415) evaluated 109 unique biomarkers in tissue specimens from 2,762 cases (2,713 OL, 49 PVL). No biomarker achieved benchmarks for clinical application to detect malignant transformation. Inter-rater reliability was high, but 65% of included studies had high "Study Confounding" bias risk.

Conclusion: There was no evidence to support translation of candidate biomarkers predictive of malignant transformation of OL and PVL. Systematically designed, large, optimally controlled, collaborative, prospective and longitudinal studies with a priori-specified methods to identify, recruit, prospectively follow and test for malignant transformation are needed to enhance feasibility of prognostic biomarkers predicting malignant OL or PVL transformation.

KEYWORDS

early detection of cancer, mouth neoplasms, prognosis

1 | INTRODUCTION

Oral leukoplakia (OL) is the most prevalent oral potentially malignant disorder (OPMD) with an estimated global prevalence of 4.11% (Mello et al., 2018) and is currently defined as *"a white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer"* (Warnakulasuriya, Johnson, & van der Waal, 2007). In addition to homogeneous and non-homogeneous OL phenotypes, proliferative verrucous leukoplakia (PVL) is a particularly aggressive clinical variant (Hansen, Olson, & Silverman, 1985). PVL is associated with a high probability of recurrence and a malignant transformation rate exceeding 70% (Cerero-Lapiedra, Balade-Martinez, Moreno-Lopez, Esparza-Gomez, & Bagan, 2010).

Currently, the gold standard for assessing risk and the strongest predictive factors for malignant transformation (MT) of OLs (MT-OL) and PVL (MT-PVL) of clinically evident mucosal change are non-homogeneous clinical appearance (Dost, Le Cao, Ford, & Farah, 2013) and histopathological determination of oral epithelial dysplasia (OED) on surgical biopsy (Amagasa, Yamashiro, & Uzawa, 2011; Dost, Le Cao, Ford, Ades, & Farah, 2014; Napier & Speight, 2008; Scully, 2014; Speight, Khurram, & Kujan, 2018; Warnakulasuriya et al., 2011). However, current clinical practice in management of OL and PVL lacks precision due to limitations in clinical and histopathological assessment (Dost et al., 2014). The presence or the degree of OED are not sufficiently predictive of malignant transformation (Dost et al., 2014; Holmstrup, Vedtofte, Reibel, & Stoltze, 2007) with up to 3.5% of non-dysplastic lesions developing oral squamous cell carcinoma (OSCC) (Hsue et al., 2007). A subset of OL presenting with "genotypic dysplasia" lacking histopathological evidence of "phenotypic dysplasia" was first identified by Farah et al. (2019) in a study exploring transcriptomic differences between dysplastic and non-dysplastic OL (Farah & Fox, 2019). Additionally, leukoplakia without dysplasia (termed keratosis of unknown significance [KUS] by some authors) has been shown to share genomic features with dysplastic OLs (Villa, Hanna, et al., 2019). Collectively, these studies support the notion that some leukoplakias may be precancerous regardless of whether dysplasia is present on biopsy.

With the advent of precision medicine, a mounting evidence base has evaluated candidate predictive and prognostic biomarkers for capability to assess risk for MT of OL and PVL (Srivastava & Grizzle, 2010). Candidate biomarkers include those in relevant biochemical pathways associated with malignant transformation and potentially leveraged for the development of targeted therapies. However, the strength of the current scientific evidence with respect to clinical utility of potential biomarkers explored to date remains equivocal. Therefore, we conducted a systematic review of retrospective studies that specifically aimed to:

1. Assess whether prognostic biomarkers could accurately stratify the risk of progression of OL and PVL to cancer,
2. Assess whether prognostic biomarkers could independently predict malignant transformation of OL and PVL without relying on the presence of oral epithelial dysplasia.

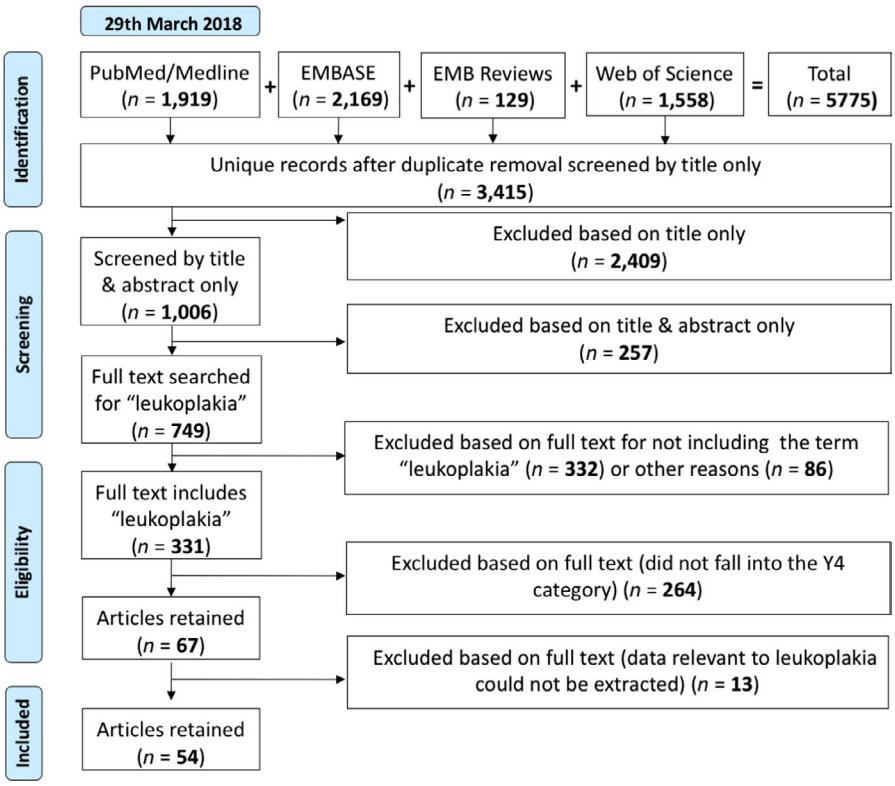

FIGURE 1 Selection of studies for systematic review of prognostic biomarkers for malignant transformation of oral leukoplakia (Moher et al., 2009) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Findings from the 54 eligible studies using biomarkers with oral leukoplakia specimens

Author Year, Country	Biomarker	Specimen Type	Sample Size *N* (#M/#F)	Risk Factors (#Y/#N/#NR) (%Y/%N/%NR)	Mean Age (± *SD*) (Range) Years
PROLIFERATIVE VERRUCOUS LEUKOPLAKIA (PVL)					
Fettig et al. (2000), USA (Fettig et al., 2000)	p53, Ki−67, HPV DNA	Tissue (paraffin)	10 (6/4)	Smoking: (3/5/2) (30%/50%/20%)	65.2 (±10.3) years Range NR
Gouvea et al. (2010), Brazil (Gouvea et al., 2010)	p53, Ki−67, Mcm−2, Mcm−5	Tissue (paraffin)	12 (0/12)	Smoking: (1/11/0) (8%/92%/0%) Past: 2 (17%) Alcohol use: (3*/9/0) (25%/75%/0%)	69.7 (NR) years (51–85 years) (50% >70 years)
Thennavan, Byatnal, Solomon, and Radhakrishnan (2015) , India (Thennavan et al., 2015)	Ki−67, p16, CD34, Bcl−2, COX−2	Tissue (paraffin)	7 (1/6)	Smoking: (3/4/0) (42.9%/57.1%/0%) (all BQ or beedi)	63.7 (± NR) yeras (54–76 years)
Upadhyaya et al. (2018), USA (Upadhyaya et al., 2018)	p16INK4A, p53 genes	Tissue (paraffin)	20 (6/14)	Smoking: (12/5/3) (60.0%*/25.0%/15%) *25% quit at diagnosis	62.8 (±11.6) years Range NR
ORAL LEUKOPLAKIA (OL)					
Abdel-Salam, Mayall, Chew, Silverman, and Greenspan (1988), USA (Abdel-Salam et al., 1988)	Nuclear chromatin pattern features	Tissue (paraffin)	13 (9/4)	NR	61.4 (± 10.5) years Range NR
Bakri, Cannon, Holmes, and Rich (2014) New Zealand (Bakri et al., 2014)	Candida ADH1 & ADH2 RNAs	Tissue (paraffin)	28 (NR/NR)	NR	NR
Bremmer et al. (2011), Netherlands (Bremmer et al., 2011)	DNA ploidy	Tissue (paraffin)	62 (22/40)	Smoking: (43/NR) (69%/ NR)	56 (± NR) yeras (24–88 years)
Brouns et al. (2012), Netherlands (Brouns et al., 2012)	DNA ploidy	Tissue (paraffin)	41 (20/21)	Smoking: (26/11/4) (63%/27%/10%) Alcohol use: (16/8/17) (39%/20%/41%)	59 (± NR) yeras (36–78 years)
Cao et al. (2011), China (Cao et al., 2011)	EZH2	Tissue (paraffin)	76 (42/34)	Smoking: 14/51/11 (18%/67%/15%) Alcohol use: (17/ 48/11 (22%/63%/15%)	55.1 (± 13.6) yeras Median 53.5 years (25–82 years)

Anatomical Site: Location # Cases	Histopathology: Type# Cases	Outcomes/Results/Comments
Gingiva: 10 (9 only gingiva, w/1 extending to FoM	Dys: 7	Lesion proliferation indices showed modest increases vs normal epithelium; Pos p53 staining evident in 4/10 cases indicating keratinocyte cell cycle disruption, but mechanism underlying p53 expr not determined
Alveolus: 8 BM: 5,5, Tongue: 6 Lip: 2 Hard palate: 1 Soft palate: 1 Gingival sulcus: 2 FoM: 3 Gingiva: 4	Of 47 biopsies: HK & AC: 6 Mild dys: 27 Mod dys: 3 Sev dys: 4 SCC: 7 Of 18 biopsies: Mild dys: 10 Mod dys: 3	Immunohistochemical findings showed higher pos for p53, Ki−67, Mcm−2 & Mcm−5 in SCC. But some pts w/mild or mod dys, especially pts who develop SCC, had high Mcm−2 & Mcm−5 expr. High immunoexpr of Mcm−2 & Mcm−5 in mild & mod dys could be helpful to predict MT of PVL [*All non-habitual alcohol use]
BM: 7 Gingiva: 6 Vestibule: 3 Palate: 1 Retromolar pad: 1 Tongue: 1 Lip: 1	Hyperplasia, VH &VH w/ dys: 6. (unspecified degree of dys)	Latest labelling index of Ki−67 in cases: 8.18–12.6; p16 pos in 3/7 cases, Bcl−2 expr mod pos in 2/7 cases; all cases intensely pos for COX−2 staining; microvascular density assessed by CD34 staining: 11–20/high-power fields; 1 case w/MT into SCC showed increased Ki−67, Bcl−2, COX−2 & CD34 expr, but tested neg for p16 & Bcl−2 expr; these markers suggest imbalance btw proliferation apoptosis dynamics of lesion, accompanied by increase in inflam & angiogenesis as aspects of molecular pathogenesis along PVL spectrum
Most pts had multiple involv sites: Gingiva: 85% Palate: 45% Tongue: 35% BM or alveolus: 25% each FoM: 10%	Initial biopsy: Grade 2:12 (equivalent to HK w/ little or no dys); Grade 4:3 (representing VH w/little or no dys), Grade 5:1	p16INK4A gene expr was considered neg w/≥ 50%−65% immuno-reactivity observed in only 3 cases that progr to malign; no expr of H-R HPV was detected, whereas p53 staining was pos in < 25% of the cells demonstrating gene expr; no definite assoc btw PVL & H-R HPV infection was established All lesions gradually progr ranging in severity from grades 3–10
BM: 3; FoM: 2 Tongue: 4; Palate: 4; Alveolus: 2 Labial commis:1	Dys: 13 (Mild dys in 6 UT cases)	Mean clearing index/margination/form factor different in transform & non-transform lesions; DNA & chromatin distribution predict oral lesion malign potential w/high accuracy (87.5%)
NR	Dys: 10 (level unknown)	RT-PCR confirmed sign correlation btw CaADH1 mRNA (p = .0001), but not CaADH2 mRNA (p = .056) expr; C albicans presence in CHC lesions assoc w/expr of C albicans genes involv in acetaldehyde metabolism, esp CaADH1; no assoc btw Candida presence & MT
Tongue: 26 Non-tongue: 36 BM: 13; FoM: 13 Alveolus: 8; Palate: 1 soft/ 1 hard	No dys: 35, Mild dys: 16, Mod dys: 7, Sev dys: 4	Abnormal DNA in lesions (7/13) progr to OSCC; aneuploidy assoc w/ cancer develop (HR = 3.7; CI: 1.1–13.0); DNA-ICM some value in predicting progr for individual pt (sens 54%, spec 60%, PPV 26%; NPV 83%); pt-related factors (sex/age/tobacco use/lesion site) not assoc w/cancer progr risk; DNA ploidy status alone limited value to predict OL progr to cancer
Tongue: 10, FoM: 7; BM: 5 Hard palate: 3 Upper/lower alveolus: 4, Multiple sites: 12	No dys: 21 Mild/Mod dys: 14 Sev dys: 6	FCM-DNA assessed DNA aneuploidy occurred sign more often at high-risk locations (p = .03) & stat sign assoc w/dys; no stat sign assoc btw pt factors & DNA ploidy assessed w/both FCM-DNA & ICM-DNA; Image cytometry more sensitive & clin relevant than flow cytometry.
Low-risk areas: BM, lip mucosa, gingiva & palate: 31 High-risk areas: FoM, lat & ventral tongue: 45	No dys: 19 Dys: 57	EZH2 expr in OLs: 45% strong, 34% mod, 21% weak/ absent; greater EZH2 levels strongly assoc w/dys (p < .001) & OSCC develop (p < .0001); EZH2 expr = only independent factor for OSCC develop in multivar anal (p < .0001); 5 years post diagnosis, 80% pts w/strong EZH2 developed OSCC vs 24% w/mod or weak/no EZH2 (p < .0001); EZH2 plays important role in OL malign transform & may predict OSCC.

(Continues)

TABLE 1 Continued

Author Year, Country	Biomarker	Specimen Type	Sample Size *N* (#M/#F)	Risk Factors (#Y/#*N*/#NR) (%Y/%N/%NR)	Mean Age (± *SD*) (Range) Years
Cervigne et al. (2009), Canada (Cervigne et al., 2009)	miR–1, miR–106b, miR–133a, miR–133b, miR–146a, miR–17–5p, miR–181b, miR–184, miR–196a, miR–206, miR–21 miR–345 miR–518b, miR–520g, miR–649	Tissue (paraffin)	29 M: Block 1/2: NR Block 3: 2 F: Block 1/2: NR Block 3: 3	NR	Block 1/2: NR Block 3: 55.8 (± NR) yeras (40–78 years)
Cervigne et al. (2014), (Country NR) (Cervigne et al., 2014)	DNA copy number alterations, Genes: KHDRBS1, PARP1, RAB1A, HBEGF, PAIP2, BTBD7,	Tissue (paraffin)	25 (13/12) 20 MT OLs from 5 pts, 5 UT OLs from 5 pts	Smoking: MT OLs: 16/4/0 (80%/20%/0%), UT OLs: 0/5/0 (0%/100%/0%)	62.9 (± 15.9) yeras (32–83 years)
Chang, Lin, Kwan, and Wong (2000), Taiwan (Chang et al., 2000)	p53, p21WAF1	Tissue (paraffin)	53 (52/1)	Tobacco & BQ & alcohol use: 35 (66%) Tobacco & BQ use: 39 (75%) Non-smokers BQ users: 9 (17%) Smoking: 2 (4%)	51.7 (± NR) yeras (31–79 years)
Cruz et al. (1998), Netherlands (Cruz et al., 1998)	p53	Tissue (paraffin)	32 (10/22)	Smoking: (12*/12/8) (37%/37%/26%) *In smokers: <10 cig/day: 3, 10–20 cig/day: 8, >20 cig/day: 1	63.8 (±15.7) yeras Range NR
Daley, Lovas, Peters, Wysocki, and McGaw (1996), Canada (Daley et al., 1996)	Depth of ductal dys	Tissue (paraffin)	11 (9/2)	NR	60.42 (±9.1) Range NR

Anatomical Site: Location # Cases	Histopathology: Type# Cases	Outcomes/Results/Comments
Block 1: Tonsil: 1 Alveolus & FoM: 1 Anterior FoM: 2 Tongue: 9 Lat tongue: 3 left, 2 right FoM: 1; BM: 1 Left BM: 7 Left tongue: 3 Right tongue: 13 Block 2: Right ventral tongue:1 Mand mucosa: 1 Tongue: 1; FoM: 2 Normal: Tongue: 3; BM: 2 Lip mucosa: 1 Lower lip mucosa: 1	MT: No dys: 6 Dys: 23	4 over-expressed miRs (miR–21, miR–181b, miR–345, miR–146a) found & clustered together in progr OL & OSCCs, but not in normal oral mucosa or non-progr OL; has-miR–21, has-miR–181b & has-miR–345 expr levels increased w/greater lesion severity; qRT-PCR confirmed over-expr of 8/15 miRs (miR–146a, miR–181b, miR–184, miR–21, miR–345, miR–518b, miR–520g & miR–649) in progr dys & OSCCs; 5miRs (miR–1, miR–133a, miR–133b, miR–196a & miR–206) had differential expr levels btw progr dys & OSCC (p = .005); miR–196a & miR–206 sign over-expr in OSCCs (p = .0016 & 0.00014, respectively), but under-expr in progr dys; miR–1, miR–133a (MT p = 1, OSCC p = .99) & miR–133b (MT p = 1, OSCC p = 1) under-expressed in both groups at sign different levels; global miR expr profiles distinguished progr OL/OSCC from non-progr OL/normal tissues;; 109 miRs were highly expr only in progr OL & invasive OSCC. Findings suggest role for miRs in malign transform
Tonsil: 1 Alveolus & FoM: 1 Anterior FoM: 2 Tongue: 5 Right lat tongue: 2 FoM: 3 BM: 2 Left BM: 7 Right tongue: 5 Mand gingiva: 1 Mand lingual mucosa: 1	No dys: 4 Dys (Mild, Mod or Sev): 16 MT OLs: Mild: 4 Mod: 3 Sev: 6	Recurrent DNA copy number gains identified on 1p (20/25) w/minimal, high-level amplification regions on 1p35 & 1p36; other regions of gains frequently observed: 11q13.4 (68%), 9q34.13 (64%), 21q22.3 (60%), 6p21 & 6q25 (56%) & 10q24, 19q13.2, 22q12, 5q31.2, 7p13, 10q24 & 14q22 (48%); DNA losses seen in > 20% samples, mainly detected on 5q31.2 (35%), 16p13.2 (30%), 9q33.1 & 9q33.29 (25%) & 17q11.2, 3p26.2, 18q21.1, 4q34.1 & 8p23.2 (20%); amplification of BTBD7, KHDRBS1, PARP1 & RAB1A only detected in progr OL & corr OSCC; validation of CNAs identified by aCGH revealed 14 amplified genes (BTBD7, CAMSAP1L1, CHRDL2, GMPK2, FBXO7, HBEGF, IRF9, KHDRBS1, NPM3, PAIP2, PARP1, RAB1A, REC8 & TBRG4); 2 genes (CSMD1 & MYO5B) deleted in progr OL lesions & paired OSCCs, but not non-progr samples. Modifications mapped in all dys grades of that progr & their corr OSCCs, in 70% pts, indicating potential assoc w/disease progr; potential genomic markers identified on chromosomes 1p, 2p, 5q, 8p, 11q, 14q, 18q & 22q may be drivers involv in oral cancer progr.
BM: 35 Non-BM: 11 Tongue: 7	No dys: 46 Dys: 7	Immunohistochemical anal revealed aberrant p53 & p21WAF1 immuno-reactivity in 51% (n = 27) & 75% (n = 40) cases, respectively. Sign differences in frequency of OSCC progr/recurrence noted in lesions exhibiting aberrant expr of either p53 (93% vs 42%; p = .00008) or p21WAF1 (80% vs 32%; p = .002) compared w/lesions w/no immune-reactivity. Aberrant p53 & p21WAF1 may align w/OVL alterations & impact outcome of this lesion.
Tongue: 16 Tongue/FoM: 3 FoM: 5 Other location: 11	No/Mild dys: 17 Mod/Sev dys: 18	p53 staining confined to basal cell layer in benign lesions & normal mucosa; PVL (7/35 = 20%) exhibited p53 expr above the basal cell layer & 6 (86%) developed carcinomas; suprabasal p53 expr found in 3 lesions w/no or mild dys that developed carcinomas; All carcinomas derived from premalign lesions w/p53 suprabasal expr showed p53 expr in neoplastic cells; combined histo parameters (dys presence) w/p53 expr patterns showed highest sens for detection of progr lesions (91%); p53 expr alone showed higher spec (96% vs 54%) & PPV (86% vs 44%) for detection of MT than histo assessment alone.
FoM: 12 FoM/Warton duct: 6 Retromolar pad: 2 BM: 1 Lat border tongue: 2 Soft palate: 2 Tonsillar pillar: 1	Mild/Mod. dys: 4, Sev dys: 7	Dyspl cases exhibited unequivocal ductal involv occurring w/higher likelihood in FoM lesions & those exhibiting sev dys or CiS; Clin FU found recurrence rate of pre-invasive lesions w/ductal involv same as SCC; ductal dys depth did not correlate w/recurrence; salivary gland duct involv oral epithelial dys & CiS uncommon yet sign; surgical stripping or ablation should extend ≥ 3mm below surface to eradicate reservoirs of dys cells.

(Continues)

TABLE 1 Continued

Author Year, Country	Biomarker	Specimen Type	Sample Size *N* (#M/#F)	Risk Factors (#Y/#N/#NR) (%Y/%N/%NR)	Mean Age (± *SD*) (Range) Years
de Rosa et al. (1999), Italy, UK (de Rosa et al., 1999)	Silver-stained nucleolar organizer regions (AgNORs), PCNA, p53, c-myc	Tissue (paraffin)	3 (1/2)	Smoking: (2/1) (67%/33.3%)	69.7 (± 2.1) Range NR
de Vicente et al. (2013), Spain (de Vicente et al., 2013)	Podoplanin	Tissue (paraffin)	58 (37/21) M/UT:23 F/UT:22	Smoking: Yes: 35 (60%) (mean 20 cig/day) Alcohol use: Yes: 28 (48%)	64 (± 12.9) yeras (39–87 years); UT only: 63.8 (± 12.7) yeras
Gissi, Gabusi, Servidio, Cervellati, and Montebugnoli (2015) Italy (Gissi et al., 2015)	p53, Ki−67	Tissue (paraffin)	77 (34/43)	Smoking: (35/42/0) (45%/55%/0%)	61.6 (± 13.8) yeras (26–95 years)
Habiba et al. (2017), Japan (Habiba et al., 2017)	ALDH1, Podoplanin	Tissue (paraffin)	79 (25/54)	NR	70 (± 12) yeras (median: 72 years) Range NR
Hamidi et al. (2000), Canada, Finland (Hamidi et al., 2000)	αvβ6 integrin, Integrins: β1, β3, β4, β5; Fibronectin, Tenascin	Tissue (paraffin)	29 (NR/NR)	Smoking: (11*/9/9) (38%*/31%/31%) *current or past	NR
Jiang, Fujii, Shirai, Mega, and Takagi (2001), Japan (Jiang et al., 2001)	LOH	Tissue (paraffin)	13 (7/6)	NR	61.5 (±8.8) yeras (48–78 years
Kaur, Srivastava, and Ralhan (1997), India (Kaur et al., 1997)	HSP70, p53, p53-HSP70 complexes	Tissue (paraffin) Whole blood, Serum, Cell lines	25 (17/8)	NR	Mean NR (25–65 years)

Anatomical Site: Location # Cases	Histopathology: Type# Cases	Outcomes/Results/Comments
Lip: 3	Sev dys: 3	Size & numbers of AgNORs & percentage PCNA-pos cells showed sens for discriminating btw potentially malign lesions & SCC, & for prognostic sub-typing of lower lip SCC; p53 pos found more often in high-grade carcinomas, & p53-pos cellular clones identified in potentially malign lesions which may be at increased risk of malign progr; c-myc pos found only in some high-grade carcinomas/metastases, appeared correlated w/ later-phase lip carcinogenesis; combined evaluation of proliferation status, p53 & c-myc onco-proteins expr represent candidates for prognostic evaluation of potentially malign lesions of the lip.
NR	Mild dys: 43 Mod dys: 7 Sev dys/CiS: 8	Podoplanin expr correlated w/dys grade ($p < .0005$) & risk of progr to oral cancer ($p < .0005$); in multivariate survival anal, only premalign oral lesions w/pos podoplanin expr showed sign increased risk of developing OSCC (HR = 8.738, $p = .007$); histo assessment & podoplanin expr anal may be candidate biomarkers risk of MT
BM: 8 Tongue: 5 Gingiva: 19 Hard palate 3 Lip: 1	OLs w/signs of dys not included in study population	At BL p53 over-expr was seen in cases ($n = 3$) that progr to OSCC next 30–60mos; additional cases ($n = 4$) w/high Ki67/p53 ratio develop OSCC ≤ 6 mos; No OL w/normal p53 expr or Ki67/p53 ratio evolv to OSCC; samples w/p53 over-expr combined w/high Ki67/p53 ratio achieved stat sign ($Chi^2 = 5.3$; $p < .02$); Immunohistochemical expr of p53 & Ki67 proteins may represent molecular markers for early detection of non-dys OL at risk of develop oral cancer
Tongue: 28 Gingiva: 18 BM: 21; FoM: 5 Other: 7	Low-grade dys: 27 High-grade dys: 52,	ALDH1 (61% pts) & podoplanin (67% pts) expr assoc w/3.02- & 2.62-fold increased risk of MT, respectively; 66% pts w/expr of both ALDH1 & podoplanin develop oral cancer, suggesting they may be useful biomarkers to identify OL w/high oral cancer risk3.02- & 2.62-fold increased risk of MT, respectively; 66% pts w/expr of both ALDH1 & podoplanin develop oral cancer, suggesting they may be useful biomarkers to identify OL w/ high oral cancer risk
Gingiva: 11 BM or alveolus: 9 Tongue: 9	Mild dys: 15 Mod dys: 6 Sev dys: 1 Other types: 7	Integrin avb6 highly expr in 90% SCC lesions, not in normal specimens; αvβ6 integrin expr in 41% of OL specimens, not in tissues w/inflam hyperplasia or chronic inflam; OL pts w/initial avb6 integrin-pos (but not avb6 integrin-neg) often had disease progr in 1–4 years; avb6 integrin expr could herald MT of OL
Tongue: 7 Mand gingiva: 2, FoM: 2 BM: 1 Hard palate: 1	Mild dys: 7 Mod dys: 6	LoH seen in foci (11/13 cases) & allelic divergence (2/13 cases) during early MT of OL; LoH seen at 9p21 (66.7%), 3p14–25 (61.5%), 4q31–32 (45.5%) & 17p12–14 (44.4%); LoH at 5q21–23 sign diff in OL lesion & in foci w/early signs of malign ($p = .0137$, Fisher exact test); Microsatellite instability seen at low levels in 3 cases. Mean fractional allelic loss in OL diff sign from that in the foci of early MT within OL plaques (0.02, $p = .05$, Student t test). High incidence of LoH in OL indicated premalign potential of this lesion. Cumulative increase of LoH assoc w/transition from OL to malignant foci suggests a role in MT & suggested that both lesions were potentially derived from a common clone.
OSCC: BM: 10 Tongue: 6 FoM: 5 Alveolus: 5 Lip: 4 OLs: BM: 12 Tongue: 6 FoM: 4 Alveolus: 3	Mild dys: 5 Mod dys:11 Sev dys: 9	Circulating anti-p53 antibodies seen in 7/30 cancer pts & 3/25 pts w/dys lesions. Over-expr of p53 protein in matched oral lesions seen in 22/30 cancer pts & 14/25 pts w/dys lesions; no detectable levels of p53 protein or anti-p53 antibodies seen in normal subjects ($n = 15$); elevated HSP70 levels seen in 23/30 oral tumours & 17/25 dys lesions. All anti-p53–antibody-seropos cases had elevated levels of p53 & HSP70 proteins & formation of p53-HSP70 complexes, in matched dys or malign lesions, suggesting that these molecular alterations may be early events in oral tumorigenesis, eliciting p53-specific humoral immune response; anti-p53–antibody-seropos cases showed poor prognosis & sign decreased overall disease-free survival vs seroneg cases

(Continues)

TABLE 1 Continued

Author Year, Country	Biomarker	Specimen Type	Sample Size *N* (#M/#F)	Risk Factors (#Y/#*N*/#NR) (%Y/%N/%NR)	Mean Age (± *SD*) (Range) Years
Kaur, Srivastava, and Ralhan (1998), India (Kaur et al., 1998)	P53	Tissue Formalin fixed tissue, Snap-frozen tissue	75 (52/23)	None: 11 (15%) Betel & areca nut: 8 (11%) Tobacco only: 23 (31%) Betel & areca nut &tobacco: 33 (44%)	Mean NR (25–85 years)
Khanal et al. (2017), USA (Khanal et al., 2017)	Cytologic Score (CS), High-risk (H-R) HPV, p16	Tissue (paraffin)	3 (2/1)	Smoking: (2/1/0) (67%/33%*0%) *Past: 1 Alcohol use: (2/0/1) (67%/0%/33%)	56.3 (± 10.0 years) Range NR
Kil, Kim, Kim, Nam, and Cha (2016), Korea (Kil et al., 2016)	Copy number variations (CNV)	Tissue (paraffin)	27 (18/9)	NR	54.3 (± 12.6) yeras Range NR
Kreppel et al. (2012), Germany (Kreppel et al., 2012)	Podoplanin	Tissue (paraffin)	60 (32/28)	Smoking: Current/former/never (28/11/21) (46%/18%/35%) Alcohol use: (31/29/0) (52%*/48%/0%)	58.6 (± 16.7) yeras (median 60.8 y) Range NR
Lima, Correa, Klingbeil, and de Sousa (2016), Brazil (Lima et al., 2016)	c-Jun, pc-Jun, p27	Tissue (paraffin)	73 (36/36/ 1 NR)	Smoking: Current/ former/never (39*/0/34) (53%*/0%/47%) *1) Smokers: Mean (range): Dys lesions: 20 (2–60) cig/day; 30 (12–53) yeras Non-dys lesions: 25 (10–40) cig/day 48 (20–59) yeras	1)Smokers: Dys lesions: 55 (+NR) yeras (43–82 years) Non-dys lesions: 49.5 (+NR) yeras (28–73 years) 2)Never-smokers: Dys lesions: 67 (+NR) yeras (38–89 years) Non-dys lesions: 54 (+NR)y (40–85 years)
Liu et al. (2012), China (Liu et al., 2012)	ATP-binding cassette G2 Subfamily (ABCG2), BMI–1	Tissue (paraffin)	135 (64/71) M: [UT: 53; MT: 11] F: [UT: 50; MT: 21]	Smoking: Never: 97 (72%) UT:71 (73%) MT: 26 (87%) Current/former: 30 (22%) UT: 26 (26%) MT: 4 (13%)] NR: 8 (6%) Alcohol use: Never: 115 (85%) UT: 88 (92%) MT: 27 (90%) Current/former: 11(8%); UT:8 (8%) MT: 3 (10%) NR: 9 (7%)	UT: 52.9 (± 10.7) yeras (21–77 years) MT: 54.2 (± 12.5) (26–79 years)

Anatomical Site: Location # Cases	Histopathology: Type# Cases	Outcomes/Results/Comments
BM: 36 Tongue: 19 FoM: 12 Lip: 8	Mild dys: 18 Mod dys: 34 Sev dys: 23	Pts w/OSCC (70%) & oral dys (52%) vs 3% w/normal oral tissues had over-expr of p53 protein; over-expr of p53 protein in premalign oral lesions showed sign correlation w/dys severity ($p < .001$), suggesting loss of p53function is relevant early in neoplastic transform of OSCs in H & *N* carcinogenesis prior to signs of overt neoplasia; FU studies presented showed shorter median transition time in p53 pos cases com-pared with p53 neg cases ($p = .0131$); immunohisto detection of p53 protein in premalign lesions may represent a biomarker for identifying pts at high risk for cancer
FoM: 2 Ventral tongue: 1	Sev dys: 2 CiS: 1	Sign increased HR-HPV prevalence ($p = .047$) for lesions w/CS > 5.3; HPV16 predominated in HR-HPV-pos cases (90.5%); Increasing CS assoc w/slightly younger age ($p = .04$) & increased p16 expr ($p = .005$); CS & p16 expr were highly specific (but not sensitive) predictors for HR-HPV presence; Based on limited FU information, HPV-OED does not differ in clinical aggressiveness compared w/conventional OED
Tongue: 12 BM: 8; Mand gingiva: 4; max gingiva: 2 Palate: 1	All mild or mod dys. Lesions w/sev dys were excluded	CNV frequent at 3p, 9p & 13q loci in progressing dys; CNV at multiple (not single) loci is characteristic of progressing dys. Genetic abnormalities of true precancer demonstrate progr risk that cannot be delineated by current histopathological diagnosis.
FoM: 9 Tongue: 6 Upper & lower gingiva: 12 Hard palate: 7 Soft palate: 10 BM: 16	SIN (classification: Epithelial hyperplasia): 31 SIN I: 8 SIN II: 12 SIN III: 9	High podoplanin in pretreatment biopsies assoc w/MT (Chi^2-test; $p = .003$) & increasing SIN classification ($p = .009$); podoplanin expr in OL sign impact on OCFS ($p = .009$) (univariate anal); 5-y OCFS rate decreased from 100% for pts w/no podoplanin expr to 41.7% for pts w/highest level of podoplanin expr; podoplanin expr & SIN classification served as factors to predict MT in OL pts in univariate anal, but no sign impact was found for both factors in multivariate anal
1)Smokers: Dys lesions: Tongue: 8 BM: 4; FoM: 3 Palate: 4 Gingiva: 4 No data: 2 Non-dys lesions: Tongue: 1; BM: 6 Palate: 1; Gingiva: 6; No data: 1 2)Never-smokers: Dys lesions: Tongue: 6; BM: 3FoM: 8, Gingiva: 3, Non-dys lesions: Tongue: 7; BM: 5Gingiva: 1 No data: 1	High-risk dys: Smokers: 15 Never-smokers: 10 Low-risk dys: Smokers: 9 Never-smokers: 10	Sign correlation btw smoking status & frequency of c-Jun ($p = .0356$) & pc-Jun ($p = .0216$); Expr more intense in cases w/MT (6/47); 100% of lesions w/confirmed MT had > 20% c-Jun pos cells (41.5% median, 26.2–58% range); 66.6% had > 20% pc-Jun pos cells (25.8% median, 0–60% range), but 83.3% of these lesions had < 20% p27-pos cells (5.5% median, 0–32.8% range). Smoking habits may be linked to expr of proteins directly assoc w/cell cycle progr.
Tongue 73 Cheek:38 Gingiva:11 Palate:8 FoM: 5	Low-grade dys: 103 [UT:84; MT: 19] High-grade dys: 32 [UT:19; MT 13]	ABCG2 & BMI−1 expr seen in 43% & 33% of pts (*N* = 135), respectively; sign correlation btw ABCG2 & BMI−1 expr ($p = .024$); 37.9% of pts w/ ABCG2 pos develop cancer vs 13.0% pts w/ABCG2 neg ($p = .014$, log-rank test); about 41% pts w/BMI−1 pos develop cancer vs 15% pts w/BMI−1 neg ($p = .029$, log-rank test); ABCG2 & BMI−1 expr assoc w/3.24-fold (CI: 1.31–7.98; $p = .011$) & 4.03-fold (CI: 1.59–10.26; $p = .003$) increased risk of MT, respectively (multivariate anal); ABCG2 & BMI−1 expr assoc w/ develop of oral cancer in a large cohort of OL pts w/long-term FU; data suggest ABCG2 & BMI−1 may be candidate predictors of OL transform

(Continues)

TABLE 1 Continued

Author Year, Country	Biomarker	Specimen Type	Sample Size *N* (#M/#F)	Risk Factors (#Y/#*N*/#NR) (%Y/%*N*/%NR)	Mean Age (± *SD*) (Range) Years
Liu et al. (2013), China (Liu et al., 2013)	ALDH1, CD133	Tissue (paraffin)	141 (68/73)	Diet: Bland: 109 (77%) Spicy: 22 (16%) *N*/A: 10 (7%) Smoking: Never: 33 (72%) Never: 99 (70%), Current/former: Current/former: 34 (24%) *N*/A: 8 (6%) Alcohol use: Never: 118 (84%), Current/former: 14 (10%) *N*/A: 9 (6%)	UT: 53.0 (± 10.7) yeras (21–77 years) MT: 53.7 (± 11.9) yeras (26–79 years)
Liu et al. (2017), China (Liu et al., 2017)	OCRI2 (oral cancer risk index)	Tissue (paraffin) Cytological smear	110 (56/54) M: TS: 19 VS: 37 F: TS: 9 VS: 45	Smoking: 1)TS: (16/12/0) (57%/43%/0%) 2)VS: (29/53/0) (35%/65%/0% Alcohol use: 1)TS: (9/19/0) (32%/68%/0%) 2)VS: (19/63/0) (23%/77%/0%)	1)TS: 57.7 (± 13.5) yeras (26–77 years) 2)VS: 58.2 (± 11.5) yeras (25–85 years)
Lopez et al. (2004), Spain (Lopez et al., 2004)	p53	Oral rinse, Cytological smear (brush), Hair root	34 (20/14)	Smoker only: 4 (12%) Smoker/drinker: 17 (50%) Drinker only: 3 (9%) No habits: 10 (29%)	OL: 54.8 (± 14.3) yeras OL pts w/ OSCC history: 59.4 (± 13.2) yeras Ranges NR
Mogi et al. (2003), Japan (Mogi et al., 2003)	p53	Tissue (paraffin)	60 (M/MT:6 F/MT:7, 47 NR)	NR	58.1 (±14.7) yeras Range NR
Montebugnoli et al. (2010), Italy (Montebugnoli et al., 2010)	p16INK4A	Tissue (paraffin)	20 (11/9)	NR	67.6 (± 9.3) yeras Range NR
Nasser et al. (2011), Germany (Nasser et al., 2011)	pRb, p53, p16INK4a, Cyclin D1, Ki−67	Tissue (paraffin)	41 (NR/NR)	NR	NR

Anatomical Site: Location # Cases	Histopathology: Type# Cases	Outcomes/Results/Comments
Tongue: 76 BM: 39 Gingiva: 13 Palate: 8 FoM: 5	Low-grade dys: 109, High-grade dys: 32	ALDH1 & CD133 expr seen in 38.3% & 22.7% of OL pts (N = 141), respectively; 48.1% pts w/ALDH1-pos develop oral cancer vs 12.6% w/ LDH1-nega (p < .001); 59.4% pts w/CD133-pos develop oral cancer vs 16.5% pts w/CD133-neg (p < .001); ALDH1 & CD133 expr assoc/w 4.17-fold (CI: 1.96–8.90; p < .001) & 2.86-fold (CI: 1.48–5.55; p = .002) increased risk of OL transform, respectively (multivariate anal);ALDH1 & CD133 expr correlated w/MT in large series of OL pts w/long-term FU, suggesting their utility as predictors that identify OL at high risk for oral cancer develop
BM: 30 Gingiva: 44 Lip: 1 Palate: 7 Tongue: 28	No dys: 38 Mild dys: 38 Mod dys: 34	36.4% of 11 OL pts w/OCRI2 > 0.5 develop cancer during FU (23 ± 20mos) vs 5.3% of 57 OL pts w/OCRI2 < 0.5 developed cancer (32 ± 31 mos); OCRI2 is better than other methods in predicting OSCC during FU; OCRI2 can predict future OSCC better than traditional methods & OCRI
Gingiva: 7 BM: 7 Palate: 4 FoM: 12 Tongue: 6 Retromolar pad: 1	NR	11 mutations in p53 gene in oral cytological specimens were detected only in brush cytology samples in pts without previous carcinoma, but in both rinse & brush samples in pts w/prior carcinoma (among whom 3 pts had recurrence); these non-invasive techniques may be useful in FU of at-risk pts as molecular markers before malignant lesions are clinically apparent
MT: Tongue: 6 BM: 2 Max gingiva: 3 Mand gingiva: 2	Mild dys: 33 Mod-sev dys: 27	50% OL lesions, tested pos for p53 protein; 13/60 lesions develop SCC & 78% of them exhibited p53-pos staining prior to MT; Over-expr of p53 protein may be a useful diagnostic tool for monitoring OL w/high probability of MT
Tongue: 8 BM: 8 Gingiva: 3 FoM: 1	HK: 8 Mild dys: 4 Mod dys: 5 Sev dys: 3	All control cases p16INK4A-neg; 45% of oral lesions p16INK4A-pos; No sign relationship btw p16INK4A-pos & dys; 45% of OSCC p16INK4A-pos; p16INK4A staining in both OSCC & lesions preceding OSCC sign correlated; p16INK4A immunohistochemistry has potential role in detecting a subset of p16INK4A-pos lesions w/malignant potential; Neg immunostaining is not informative for the risk of develop OSCC; observations require validation
NR	OLs: No dys: 37 Mild dys: 4 OSCC: Mild dys: 4 Mod dys: 3 Sev dys: 2	Increased expr of p53, Ki−67 & Cyclin D1 & loss of p16INK4a seen in 45.9%, 38.9%, 29.4% & 32.4% of OL without dys, respectively; all alterations increased w/progr, but had poor PPV; combined p53/p16INK4a/Ki−67 aberration occurred in only 3 (9%) cases & 2/3 pts experienced progr to dys & CiS; combined p53/p16INK4a/Ki−67 alteration had NPV, 100% sensitivity, 97% specificity & PPV of 67%; by contrast, combined p53 /p16INK4a/Cyclin D1 alteration had 97% NPV, 50% sensitivity, 90% specificity & only 25% PPV; loss of pRb and concomitant over-expr of p16INK4a were not observed, suggesting lack of involv of HPV in OL; authors proposed the combined p53/p16INK4a/Ki−67 alteration as a basic marker to identify high-risk OL pts; lesions not showing this alteration appear to be benign. Future studies should validate these findings and search for proteins that can further improve the PPV of the proposed basic marker.

(Continues)

TABLE 1 Continued

Author Year, Country	Biomarker	Specimen Type	Sample Size *N* (#M/#F)	Risk Factors (#Y/#*N*/#NR) (%Y/%*N*/%NR)	Mean Age (± *SD*) (Range) Years
Nguyen et al. (2017), Japan (Nguyen et al., 2017)	LAMC2	Tissue (paraffin) Fresh frozen tissue Tissue microarray	93 (NR/NR)	NR	NR
Nielsen et al. (1996), Denmark (Nielsen et al., 1996)	HPV genus-specific antigen, HPV DNA type 16	Tissue (paraffin)	39 (23/16)	Smoking: MT: Yes: 3 (8%) *N*/A: 36 (92%)	NR
Nogami, Kuyama, and Yamamoto (2003), Japan (Nogami et al., 2003)	AI, IK, MI, Ki−67, p53, Bcl−2; BAX	Tissue (paraffin)	13 (5/8)	NR	61.4 (± 10.6) yeras (46−84 years)
Ögmundsdóttir, Björnsson, et al. (2009), Iceland (Ögmundsdóttir, Hilmarsdottir, et al., 2009)	TP53	Tissue (paraffin)	45 (NR/NR)	NR	57.3 (NR) yeras (11−89 years)
Ögmundsdóttir, Björnsson, et al., 2009, Iceland (Ögmundsdóttir, Hilmarsdottir, et al., 2009)	TP53	Tissue (paraffin)	4 (1/3)	Smoking: (2/2/0) (50%/50%/0%)	73.5 (± 6.6) yeras Range NR
Öhman et al. (2015), Sweden (Öhman et al., 2015)	CD3 + T cells, CD1a + LCs, Ki−67, p53	Tissue (paraffin)	16 (10/6)	NR	UT: NR (median 68 years) (50−73 years) MT: NR (median 71 years) (58−86 years)

Anatomical Site: Location # Cases	Histopathology: Type# Cases	Outcomes/Results/Comments
OLs: Tongue: 6 Control: Tongue: 2 Gingiva: 2 BM: 2	Mild dys: 11, Mod dys: 78, Sev dys: 4	LAMC2 upregulated in OSCC at the cancer–stroma interface; grade of LAMC2 expr was sign assoc w/pattern & invasion depth of OSCC ($p < .0001$); number & size of LAMC2-pos foci sign assoc w/dys grade ($p = .0003$ & 0.0002, respectively); LAMC2-pos foci sign predictive factor for the malign progr of OL (Cox, $p = .002$); LAMC2-pos OL assoc w/~11-fold increased risk of malign vs LAMC2-neg OL; Value of LAMC2 as a marker of invasive cancer proposed w/LAMC2-pos foci in OL suggestive of imminent risk of cancer; LAMC2 immunostaining is expected to contribute to a more precise assessment of malign OL
OPMDs: BM/lip: 18 Sulcus: 1; Sub-lingual region: 21 Tongue: 4; Palate: 5 Controls: BM/lip: 14 Sublingual region: 3, Tongue: 3	OPMDs: No dys: 24 Slight dys: 11 Mod dys: 9 Sev dys: 4 CiS: 1 Controls: No dys: 20	HPV seen in 62.5% of OVL, 50.0% of erythroplakias, 45.5% of homogeneous OL, 33.3% of erythroleukoplakias & 12.5% of the nodular leukoplakias; HPV detected in 40.8% of examined premalign lesions; all control samples were HPV-neg; HPV may be a cofactor in oral cancer develop, since 100% pts who develop oral cancers within 4–12 years were all pos for HPV; one pt tested pos for HPV–16
Normal: Gingiva: 4 Tongue 1; OLs: Gingiva: 5 Tongue: 5; BM: 3	Unknown dys: 5 Mild dys: 4 Mod dys: 3 Sev dys: 1	Peak of mitotic & Ki–67 indices & p53 expr shifted basally, possibly due to MT, but peak of apoptosis & expr of apoptotic-related proteins in OL showed no transform; frequent Bcl–2 expr in OL w/MT combined w/ reduction in # apoptotic cells indicated that malignancy occurred due to absence of apoptosis; high levels of Bax expr in OL without MT indicated that the Bcl family may play a role in disease progr
OSCC: Lip: 13 Tongue: 15 FoM: 4, Gingiva/edentulous ridge: 14 BM: 5; Palate: 4	No dys: 22 Mild dys: 20 Mod dys: 3	29% OL pts tested pos for TP53-mutation; 1 TP53-mutated OL pt develop OSCC at a different site; 13.6% pts w/HK (clinical leukoplakia) exhibited mutations; 1 HK pt w/no mutation develop OSCC in same site; TP53 mutations can exist in benign oral mucosal lesions for many years without malign progr; no assoc btw TP53 protein expr or TP53 mutation & recurrence of OSCC or disease-related survival; survival was reduced in pts w/pos TP53 protein expr
Case #: #1: BM, mand ridge #2: BM, tongue #3: gingiva #4: BM, hard & soft palate, lip, tongue #5: FoM, hard palate, (OLP-like lesions BM, tongue, vermillion border) #6: gingiva #7: BM, tongue, tuberosity, hard palate, FoM; #8: tongue, edentulous ridge	No dys: 5 Dys: 2 OSCC: 1	7 pts had TP53 mutations, 3 of them on repeated occasions; all 4 pts who develop SCC had mutations; 2 of them had mutated premalign lesions, 1 of them previously had a non-mutated cancer; 3 pts had 2 different primary cancers, only 1 of them mutated; 1 pt develop mutated cancer 5 years after last mutation-free biopsy; of the cancer-free pts, a suspicious lesion in 1 case was mutated; in another pt, 2 OL lesions were mutated, the 3rd had 5 biopsies taken during 8 years, all non-mutated; TP53 mutations may occur early or late in the develop of OSCC
UT: BM: 2, Gingiva: 1 Lat border tongue: 5 MT: BM: 2 FoM: 3 Lat border tongue: 3	UT: Mild dys: 2 Mod dys: 4 Sev dys: 1 CIS: 1 MT: Mild dys: 3 Mod dys: 4 Sev dys: 1	Quantitative analyses showed sign lower numbers of CD3 + T cells in UT OLs than in MT OLs. No sign differences btw MT OLs & UT OLs regarding CD1a+, p53+ & Ki–67 + cells; number of CD3-expr T cells may be important for preventing MT of OL

(Continues)

TABLE 1 Continued

Author Year, Country	Biomarker	Specimen Type	Sample Size *N* (#M/#F)	Risk Factors (#Y/#*N*/#NR) (%Y/%N/%NR)	Mean Age (± *SD*) (Range) Years
Philipone et al. (2016), USA (Philipone et al., 2016)	MicroRNAs: 208b–3p, 204–5p, 129–2–3p, 3065–5p	Tissue (paraffin)	97 (36/62) M: TS: UN: 4 MT: 5 VS: UN: 13 MT: 13 F: TS: UN: 6 MT: 5 VS: UN: 26 MT: 25	NR	a)TS: UT: 58.9 (± 11) yeras MT: 63.2 (± 23.2) yeras b)VS: UT: 59.6 (± 12.6) yeras MT: 66.5 (± 17.5) yeras
Rich, Kerdpon, and Reade (1999), Australia (Rich et al., 1999)	p53	Tissue (paraffin)	41 (NR/NR)	NR	NR
Ries et al. (2001), Germany, USA (Ries et al., 2001)	Telomerase activity	Tissue (paraffin) Snap-frozen tissues, Cell lines	8 (NR/NR)	NR	NR
Ries, Agaimy, Vairaktaris, Kwon, et al. (2012), Germany (Ries, Agaimy, Vairaktaris, Gorecki, et al., 2012)	MAGE-A 1–4, 6, 10, 12	Tissue (paraffin)	98 (57/41) M: [MT: 31 UT: 26] F: [MT: 1 UT: 24]	NR	53.7 (± NR) yeras Range NR
Ries, Agaimy, Vairaktaris, Kwon, et al. (2012), Germany (Ries, Agaimy, Vairaktaris, Kwon, et al., 2012)	MAGE-A 1–4, 6, 10, 12	Tissue (paraffin)	74 (41/33) M: [MT: 15; UT: 26] F: [MT: 9; UT: 24]	NR	53.7 (± NR) yeras Range NR

Anatomical Site: Location # Cases	Histopathology: Type# Cases	Outcomes/Results/Comments
Mild dys High-risk site: (tongue, FoM) TS: UT: 7, MT: 7 VS: UT: 21, MT: 26 Low-risk site: (BM, vestibule, gingiva, palate, lip mucosa) TS: UT: 3, MT: 3 VS: UT: 19, MT: 14	Mild dys: TS: UT: 2 MT: 3 VS: UT: 4 MT:14	4 candidate miRNAs−208b−3p, 204−5p, 129−2−3p & 3065−5p were identified. Combining these 4 miRNAs as a panel w/age & histo dx ($p < .004$), authors' final model had a predictive value for the area under ROC curve (AUC) of 0.792, sensitivity of 76.9% & specificity of 73.7% to accurately identify non- & low-grade dys lesions at risk of cancer progr. This predictive capacity is an improvement over histopathological examination alone (AUC of 0.645); further investigation is needed
NR	p53-pos: Mild dys: 12 Mod dys: 4 Sev dys: 10 p53-neg: Mild dys: 4	All normal oral mucosa cases were p53-neg; 94% OSCC cases expr p53; among dys or hyperplasia cases, 85% & 36% expr p53 hyperplasia, respectively; intensity of p53 staining progr decreased: cancer > dys>hyperplasia; differential p53 expr was noted in hyperplastic (basal & suprabasal region) & dys lesions; proportion of cases w/pos p53 expr decreased: hyperplasia < dys<OSCC; presence/absence of p53 staining has utility in predicting the outcome of potentially malign oral mucosal lesions
Tongue: 5 FoM: 1 Alveolus: 1 BM: 1	No dys: 3 Mild dys: 3 Mod dys: 1 Sev dys:1	50% of OL & 46% of OSCC showed telomerase activity; 1 pt w/pos, high dys OL develop OSCC 11mos later; 1/3 specimens of adjacent tissue presented activity & recurrence occurred > 6 mos; 2/10 tissues exhibited activity in both distal normal mucosa & the corr tumour; detection of telomerase reactivation may support early detection of immortalized cell clones & malign cells in histopathologically normal oral squamous epithelium
NR	No dys: 41 Mild dys: 32 Mod dys: 18 Sev dys: 7	Correlation btw MT & MAGE-A occurrence in OL was stat sign ($p < .0001$); detection of MAGE-A may support identification of OL at high risk for MT
NR	UT OLs: No dys: 38 Mild dys: 26 Mod dys: 7 Sev dys:3 MT OLs: No dys: 12 Mild dys: 5 Mod dys: 4 Sev dys: 3	46% progr lesions expr ≥ 1 MAGE-A antigens, but no expr seen in non-progr OL lesions & normal specimens; correlation btw MT & MAGE-A expr stat sign ($p = .00001$); Also, 42% of progr OLs without dys expr ≥ 1 MAGE-A antigen; Correlation btw dys grade & MAGE-A staining in MT group was not sign ($p = .08$); detection of ≥ 1 MAGE-A antigen may allow identification of H-R lesions that may progr into carcinoma over time

(Continues)

TABLE 1 Continued

Author Year, Country	Biomarker	Specimen Type	Sample Size *N* (#M/#F)	Risk Factors (#Y/#*N*/#NR) (%Y/%N/%NR)	Mean Age (± *SD*) (Range) Years
Ries et al. (2013), Germany (Ries et al., 2013)	EGFR	Tissue (paraffin)	98 (59/39) Group 1: (34/19) Group 2: (25/20)	NR	Groups 1 + 2: 55.8 (± NR) yeras Group 1 only: 61.8 (± NR) yeras Group 2 only: 49.8 (± NR) yeras
Schaaij-Visser et al. (2010), Netherlands (Schaaij-Visser et al., 2010)	Cornulin, Keratin 4, Keratin 13, dys grading	Tissue (paraffin)	48 (NR/NR)	NR	NR
Seoane, Bascones, Asenjo, Garcia-Pola, and Varela-Centelles (1998), Spain (Seoane et al., 1998)	DNA ploidy	Tissue (paraffin)	41 (NR/NR)	NR	NR
Siebers et al. (2013), Netherlands (Siebers et al., 2013)	Chromosome instability	Tissue (paraffin)	102 (54/48) UT: (45//41) M: MT: 9] F: MT: 7]	Smoking: Yes: 63 (62%) [UT: 54, MT: 9], No: 37 (38%) [UT:32, MT: 5], Past: 10 (10%) [UT:9, MT: 1] *N*/A: 3 (3%) [UT: 2, MT: 1]; Alcohol use: Yes: 46 (45%) [UT:39, MT: 7], No: 48 (47%) [UT:40, MT: 8] *N*/A: 5 (5%) [UT:4, MT: 1]	UT: 51.9 (± NR) yeras Range NR MT: 57.8 (± NR) yeras Range NR
Tanimoto et al. (2000), Japan (Tanimoto et al., 2000)	FHIT gene	Tissue (paraffin) fresh tissue	6 (3/3)	Non-smoker/ drinker: 1 (17%) Smoker/non-drinker: 1 (17%) Smoker/drinker: 1 (17%) Non-smoker/non-drinkers: 3 (50%)	59.5 (± 10.1) yeras Range NR

Anatomical Site: Location # Cases	Histopathology: Type# Cases	Outcomes/Results/Comments
Group 1: OLs: Oral cavity: 40 Oropharynx: 13 Group 2: UT OLs: 100% Originated from the oral cavity: 45	All OLs: No dys: 37 Mild: 33 Mod dys: 17 Sev dys:11 Group 1 (MT OLs): No dys:15 Mild dys: 14 Mod dys: 13 Sev dys: 11 Group 2 (UT OLs): No dysp: 22 Mild dys: 19 Mod dys: 4	A sign different expr rate of EGFR was determined btw transformed & non-transformed OL (p = .017); Stat signt EGFR expr increase in low dys lesions in group 1 vs group 2 (D0, p = .013; D1, p = .049); optimal threshold value [cut-off point (COP)=44.96] for distinguishing transformed from non-transformed lesions was estimated (critical expr rate of EGFR) by calculation of ROC curve & determination of highest Youden index; using determined COP, the correlation btw high-risk lesions & detection of increased expr rates was sign (p = .001). In the future, assessment of EGFR over-expr in OL may allow identifying OL lesions w/increased risk of MT that may have been regarded harmless when only the dys grade were taken into account
NR	No dys: 28 Mild dys: 7 Mod dys: 7 Sev dys: 6	Neither loss of cornulin (p = .075), keratin 4 (p = .789), nor keratin 13 (p = .732) was sign assoc w/MT of OL lesions; However, decreased expr of cornulin (p = .001) & keratin 13 (p = .002) was sign assoc w/ presence of HK; only dys grading correlated sign w/malign progr of OL (p = .024); although detection of these markers in oral mucosa may be assoc w/premalign state, they do not predict MT of OL lesions; aberrant differentiation state of HK OL lesions may be responsible for decreased expr, obscuring putative assoc w/MT. These results support the sign of dys grading for the prediction of MT
NR	Out of 10: No dys: 5 Minimal dys: 4 Sev dys: 1	Aneuploid DNA pattern detected in 9.7% of tested specimens; DNA indices showed no stat sign difference w/respect to DNA ploidy related to dys presence or/ absence; only 1/10 OLs that transformed exhibited multiple pattern; this study presented no evidence to support value of applying DNA index to differentiate btw dys & non-dys OL
MT: FoM: 2 Tongue: 10 BM: 3 Inferior alveolus: 1	Hyperplastic: 66 D+: 16 D++: 17 D++++: 3	Chromosome instability strong individual marker of progr w/HRs of 7.2 & 6.8 for ICM & FISH, respectively. ICM has utility for monitoring lesions over time. Combining histopathology & chromosome instability enables subdivision of pts into 3 risk groups w/different probabilities of malign progr; chromosome instability detection seems a reliable method for risk assessment of oral premalign. Its application may contribute to better risk-counselling & inform appropriate treatment regimen or a watchful-waiting approach to clinical disease management
Upper gingiva: 1 BM: 1 Lower gingiva: 2 Tongue: 2	Hyperplasia: 2 Mild dys: 3 Mod dys: 1	Abnormal transcripts of FHIT gene found in 53% of oral SCCs; although these abnormal transcripts varied widely, deletion patterns incorporating a deletion of exon 5 were most common; LoH anal demonstrated that abnormal FHIT transcripts found in cancer cells were due to abnormalities of the FHIT gene; abnormal FHIT transcripts were also observed in 2/7 premalign lesions; In 1 case w/ premalign lesion showing abnormal FHIT transcript, oral SCC develop during 3 years FU; in 2 pts w/both OL & SCC samples taken simultaneously, abnormal FHIT transcripts were found only in the SCCs; Findings suggest that FHIT alteration may actually be involv in carcinogenesis of the oral epithelium

(Continues)

TABLE 1 Continued

Author Year, Country	Biomarker	Specimen Type	Sample Size *N* (#M/#F)	Risk Factors (#Y/#*N*/#NR) (%Y/%N/%NR)	Mean Age (± *SD*) (Range) Years
von Zeidler, de Souza Botelho, Mendonça, and Batista (2014), Brazil (von Zeidler et al., 2014)	E-cadherin	Tissue (paraffin)	31 (14/17)	Smoking: (25/6/0) (81%/19%/0%) Alcohol use: (13/18/0) (42%/58%0%)	50.9 (± NR) yeras (31–79 years)
Wagner et al. (2017), Brazil (Wagner et al., 2017)	TGF-β1, Ki67	Tissue (paraffin)	24 (16/8)	Smoking: (14/9/1) (58%*/38%/4%) *current/former Alcohol use: (11/12/1) (46%*/50%)/4%) *current/former Ethnicity: White: 23 (96%) Black: 1 (4%) Residence: Urban: 15 (62%) Rural: 9 (38%)	56.6 (± 14.9) yeras Range NR
Xia, Song, Wang, Li, and Mao (2013), China (Xia et al., 2013)	SMAD4, dys grading	Tissue (paraffin)	88 (37/51)	Smoking: (16/64/8) (18%*/73%/9%) *current/former Alcohol use: (16/64/7) (18%*/73%/8%)	55.9 (± 13) yeras (27–85 years)
Zhang, Kim, Zheng, Kim, et al. (2017), Korea (Zhang, Kim, Zheng, Bazarsad, et al., 2017)	P53, Ki–67, P16, b-catenin, c-jun, c-met, IMP–3, COX–2, Podoplanin, CA9	Tissue (paraffin)	160 (100/60)	NR	51.9 (±NR) yeras median:54 years (13–89 years at initial diagnosis)

Anatomical Site: Location # Cases	Histopathology: Type# Cases	Outcomes/Results/Comments
OLs: BM: 18 Tongue 13 OSCC: Tongue 31 Normal: BM: 28 Tongue: 3	OLs: No or Mild dys: 23 Mod/Sev dys: 8	Differences in E-cadherin expr seen among risk groups examined (p = .0001); in the low-risk OL group, reduction in the E-cadherin expr was seen mainly in the parabasal layer compared to normal oral mucosa (p = .006); in the high-risk OL group, E-cadherin expr was reduced in all epithelial layers; semi-quantitative anal revealed a sign reduction in E-cadherin expr in the high-risk group compared to the low-risk OL group (p = .019); there was a reduction in E-cadherin expr in the OCSCC N + group in the cell membrane of the neoplastic cells in invasive front of the tumour; cytoplasmic & nuclear staining was noted; Reduced E-cadherin expr was early phenomenon, observed in mod-sev dys, suggesting that loss of epithelial cohesion may be indicator of possible evolution assoc w/ dys changes & increased risk for MT & reduction in or loss of E-cadherin expr by keratinocytes occurs; therefore, E-cadherin could be a novel biomarker to identify OL lesions at increased risk for MT
UT OLs: Tongue/FoM: 10 Other sites: 10 MT OLs: FoM: 2 Tongue: 1 Palate: 1 OSCC: Tongue/FoM: 72 Other sites: 15	MT OLs: No dys: 1 Mild dys 1 Mod dys: 1 Sev dys: 1	TGF-β1 & Ki67 expr sign increased from normal mucosa, through OL to OSCC (p < .05 & 0.05, respectively); high TGF-β1 expr correlated w/ increase in proliferative labelling index; no assoc btw TGF-b1 expr & clinico-pathologic factors examined; TGF-β1 expr did not correlate w/ clinical outcome in either group; outcomes suggest that changes in TGF-β1 are assoc w/progr of oral carcinogenesis
Non-tongue: 31 Tongue: 57	Low-Mod dys: 66 High-grade dys: 22	SMAD4 expr & dys grade were sign predictors (log-rank test); strong SMAD4 expr & high dys grade predicted MT of OL better than either independently (p = .007); Both SMAD4 expr (weak vs strong) & lesion histol (low & mod-grade dys vs high-grade dys) were sign assoc w/ OL MT (univariate anal); SMAD4 expr was the most striking factor (p = .018 & 0.032, respectively); both SMAD4 expr & dys grade were independent factors for predicting OL MT(p = .013 & 0.021, respectively) (multivariate anal); results suggested that SMAD4 might be activated in early oral tumorigenesis but is insufficient to halt carcinogenic process. The combination of SMAD4 expr & histo dys grade showed good predictive capacity for MT of O
Gingiva: 72 BM: 44 Tongue: 44	No dys: 82 Low-grade dys: 54 High-grade dys: 24	All biomarkers examined were predictive of MT in OL (univariate Cox regression anal); simulation identified that P53 & CA9 expr combined w/ age & dys degree achieved highest predictive accuracy; a nomogram was develop for the candidate prognostic factors projecting prediction of 5, 10 & 15 years progr free survival of OL; Combination of P53 & CA9 w/other factors (e.g. age & degree of dys) achieved the highest prediction accuracy; The proposed nomogram may be useful for accurate, individual prediction of the transformation to SCC in OL pts & may inform appropriate treatment & FU in the clinical setting

(Continues)

TABLE 1 Continued

Author Year, Country	Biomarker	Specimen Type	Sample Size *N* (#M/#F)	Risk Factors (#Y/#*N*/#NR) (%Y/%*N*/%NR)	Mean Age (± *SD*) (Range) Years
Zhang, Kim, Zheng, Kim, et al. (2017), Korea (Zhang, Kim, Zheng, Kim, et al., 2017)	SNAI1, Axin2	Tissue (paraffin)	154 (96/58)	NR	NR median 55 years (13–89 years)

#, number; &, and; AC, acanthosis; anal, analysis, analyses, analysed; assoc, association, associated; AUC, area under the [ROC, receiver operating characteristic] curve; BL, baseline; BM, buccal mucosa; BQ, betel quid; btw, between; CI, 95% confidence interval; cig, cigarette, cigarettes; CiS, carcinoma in situ; clin, clinical, clinically; CNV, copy number variations; commis, commissure; corr, corresponding; CS, cytologic score; develop, developed, developing, development; diff, differ, differed, difference, different; DNA-ICM, DNA image cytometry; dys, dysplasia, dysplastic; dx, diagnosis; esp, especially; expr, express, expressed, expression; FoM, floor of mouth; FU, follow-up; H & N, head and neck; H-R, high-risk; histo, histologic, histological; HK, hyperkeratosis, hyperkeratotic; HR, hazard ratio; HR-HPV, high-risk human papilloma virus; ICM, image cytometry; inflam, inflammation, inflammatory; involv, involve, involvement, involving; lat, lateral; LoH, loss of heterozygosity; malign, malignancy, malignant; mand, mandibular; mod, moderate, moderately; mos, months; MT, malignant-transformed, malignant transformation; max, maxillary; multivar, multivariate; N/A, not applicable; neg, negative, negativity; NR, not reported; NPV, negative predictive value; occas, occasionally; OCFS, oral cancer-free survival; OCRI, oral cancer risk index; OED, oral epithelial dysplasia; OL, oral leukoplakia; OPMDs, oral potentially malignant disorders; OSCC, oral squamous cell carcinoma; OVL, oral verrucous leukoplakia; paraffin, paraffin-embedded biopsy specimen; pos, positive, positivity; PPV, positive predictive value; progr, progress, progresses, progression, progressive, progressively; pt, patient; pts, patients; PVL, proliferative verrucous leukoplakia; ROC, receiver operating characteristic; SCC, squamous cell carcinoma; *SD*, standard deviation; sens, sensitivity; sign, significant, significance, significantly; sev, severe; sign, significance, significant, significantly; spec, specificity; SIN, squamous intraepithelial neoplasia; stat, statistically; transform, transformation, transformed; TS, training set; UT, untransformed; VS, validation set; w/, with; wk, week, weeks.

This review expands on a previous systematic review undertaken by the World Workshop on Oral Medicine VII (WWOM VII) Precision Medicine Work Group that focused specifically on examination of prospective longitudinal studies of OL (n = 25) that examined prognostic capability of biomarkers to predict OSCC progression (Villa, Celentano, et al., 2019). In contrast, the current systematic review focuses on retrospective studies, also known as historic cohort studies.

2 | MATERIALS AND METHODS

This systematic review was conducted by a subgroup of the Precision Medicine Work Group participating in WWOM VII. Results are reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher, Liberati, Tetzlaff, & Altman, 2009).

2.1 | Study selection

2.1.1 | Inclusion criteria

The following PICOS/PECOS questions were used to formulate the following study inclusion criteria:

P = Patients/population: specimens from patients with OL or PVL.

I = Intervention, Interest/E = Exposure: biomarkers in human specimens.

C = Control/comparison group: specimens from healthy controls or patients with OSCC.

O = Outcome: development of OSCC.

S = Study design: case–control or retrospective cohort studies.

Eligible reports included findings from original case–control or retrospective cohort studies of human patients with OL or PVL and either healthy controls or patients with OSCC that evaluated biomarker expression in oral tissues, oral smear, saliva, blood, hair root and cell lines at two or more different time points.

2.1.2 | Exclusion criteria

Studies were excluded if:

1. Only non-human specimens were evaluated
2. They were not original case–control or retrospective cohort studies. Examples of excluded studies include prospective cohort studies, cross-sectional studies, reviews, case reports, commentaries, opinion articles, letters to the editor, meeting abstracts and withdrawn/retracted reports.

The 5-step screening process to identify studies eligible for inclusion in this systematic review is summarized in Figure 1.

Step 1: Electronic literature searches applying workgroup-defined search strategies aligned with PICO definitions were conducted by AC on 29 March 2018, in the databases PubMed (Ovid), Embase (Ovid), Evidence-Based Medicine (EBM) Reviews (Ovid) and Web of Science (ISI) with no restrictions placed on date of publication or language. Search strategies according to the syntaxes of each database are displayed in Table S1. Identified citations were imported into EndNote X8 reference management software (Clarivate Analytics, Philadelphia, PA,

Anatomical Site: Location # Cases	Histopathology: Type# Cases	Outcomes/Results/Comments
OLs: BM: 44 Tongue: 42 Gingiva 68 Normal: Gingiva: 68	NR	Increased Axin2 & Snail found in ~ 70% & 38% of OL pts, respectively; both Axin2 & Snail were independent risk factors for MT w/HRs of 7.47 (CI: 2.23–25.02; p = .001) & 4.41 (CI: 1.78–10.93; p = .001), respectively (multivariate anal); the increased abundance of Snail & Axin2 is highly correlated to MT of OL, making Snail & Axin2 novel biomarkers for predicting oral cancer develop in OL

USA). De-duplication was achieved by the Endnote automated procedure (AC) and manually by two reviewers (AC, AVil).

Step 2: Ineligible records were excluded based on sequential review of title only.

Step 3: Titles and abstract were reviewed.

(Steps 2-3 were conducted independently by two blinded reviewers (AC, AVil)).

Step 4: Full-text review was undertaken of studies retained following step 3 by AC and AVil with quality review by CSF. Exclusion categories were identified (Table S2), and studies meeting criteria for inclusion in alternative categories defined in Step 5 were assigned a category allocation code. At each step, the reviewers' decisions on a subset of articles were initially compared to identify discordant decisions and resolved by discussion among the two reviewers (AC, AVil) and the content expert (CSF) to establish standardized definitions. Cohen's kappa statistic was used to measure inter-rater reliability at each step.

Step 5: Articles retained for further review were categorized by the two reviewers (AC, AVil) by potential functional utility along the oncogenic trajectory including: risk prediction/surveillance, phenotypic marker heralding disease progression, diagnostic support, monitoring of pathophysiologic events or response to therapy as described in detail in our previous systematic review of prospective longitudinal studies (Villa, Celentano, et al., 2019).

Step 6: The principal reviewer (AC) extracted all relevant data from studies allocated to the "Y4" category "*phenotyping biomarker expression in progression of OL or PVL from premalignant status to OSCC in a retrospective study (case–control or retrospective cohort).*" Risk of bias was independently assessed by the principal reviewer (AC) and the content expert (CSF) applying the "Quality in Prognosis Studies" (QUIPS) tool that evaluates the following six domains: "study participation," "study attrition," "prognostic factor measurement," "outcome measurement," "study confounding" and "statistical analysis and reporting" (Hayden, van der Windt, Cartwright, Côté, & Bombardier, 2013). Any discord was resolved by achieving consensus upon discussion. The evidence level of each article was assessed using a score classification adapted from the Oxford Centre for Evidence-Based Medicine [available from: https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/].

2.2 | Statistical analysis

Data were tabulated into a Microsoft Excel spreadsheet, and simple descriptive analyses were performed (Microsoft Excel 2010). Absolute percentage inter-rater agreement and Cohen's kappa coefficient were calculated using IBM Statistics 23 (SPSS). Heterogeneity of the studies, high number of unique biomarkers identified and variability across studies in definition of diagnostic criteria applied precluded performance of any further quantitative analyses, such as meta-analyses.

3 | RESULTS

3.1 | Study selection

The process of selection of eligible studies is illustrated in Figure 1. Cohen's kappa statistic for inter-rater agreement and absolute agreement, respectively, was 0.95 (95% Confidence Interval [CI]:

0.94–0.96) and 96.7% for Step 2; 0.92 (95% CI: 0.91–0.94) and 96.7% for Step 3; and 0.59 (95% CI: 0.50–0.68) and 85.5% increasing to 100% upon a second revision for Step 5.

The reasons for exclusion of 418 of 749 studies in Step 4 are shown in Table S2. Step 5 resulted in allocation to one or more of the categories Y1-Y5 of 331 retained studies, of which the 67 studies in group Y4 are the focus of this report. The data extraction process further excluded 13 of 67 reports due to data deficits prohibiting quantitative analysis of OL cases (Table S3), thus identifying 54 studies included in this systematic review (Figure 1).

3.2 | Study characteristics

Table 1 provides an overview of key characteristics for the selected 54 studies of which 50 were conducted at a single centre each. The reports were published between 1988 and March 2018. Six studies were conducted in each of the countries USA, Japan and Canada, and 5 each in the Netherlands, Germany and China (Figure S1). Thirty-four studies met criteria for evidence level 3, while the remaining 20 were assigned evidence level 4.

A total of 50 studies assessed the role of biomarkers in samples from OL and four from PVL patients (Fettig et al., 2000; Gouvea, Vargas, Coletta, Jorge, & Lopes, 2010; Upadhyaya, Fitzpatrick, Islam, Bhattacharyya, & Cohen, 2018).

A total of 2,762 samples across the 54 studies were analysed including 2,713 from OL and 49 from PVL patients with 1,228 (44%) collected from males and 1,089 (39%) from females and sex unreported in 17% of samples. The sex distribution among OL specimens was 1,217 (45%) from males, 1,051 (39%) from females and 445 (16%) from patients with unreported sex vs 13 (27%) from males and 36 (73%) from females in VPL. Overall, the mean age at the time of diagnosis was about 60 years (range: 13–95 years) for OL, whereas it varied from 62.8 to 69.7 years in the four PVL studies (range: 51–85 years in the two PVL studies that reported range).

3.3 | Quality assessment of the included studies

Low risk of bias was predominantly observed in "Statistical analysis and reporting," "Outcome measurement," "Prognostic factor measurement" and "Study attrition" (62.96%, 81.48%, 62.96% and 68.52% of included studies, respectively); moderate bias risk was seen in "Study participation," "Prognostic factor measurement," "Statistical analysis and reporting" and "Study attrition" (53.70%, 37.03%, 31.48% and 31.48% of included studies, respectively), whereas high risk of bias was detected for "Study confounding" (64.81% of included studies) (Figure 2; Table S4).

3.4 | Specimen types

The types of specimen used to identify biomarkers included paraffin-embedded tissue (53/54), full blood sample (1/54), blood serum sample (1/54), cell lines (2/54), cryo-preserved tissue (2/54), oral rinse (1/54), cytological smear (2/54), hair root (1/54), fresh frozen tissue (1/54) and tissue microarray (1/54). The specific studies utilizing these specimen types are identified in Table 1 in the first and third columns from the left.

3.5 | Biomarkers identified

Some studies analysed multiple candidate biomarkers, and collectively, the 54 studies identified 109 unique biomarkers (Table 2) representing a range of biological categories, including transmembrane receptors, transporters, growth factors and enzymes among others. Biomarkers were stratified by hallmarks of cancer (Farah et al., 2019) and were generally categorized as follows: *Stem cell markers* (e.g. ABCG2, BMI-1, ALDH1, CD133, SNAI1, Axin2), *cellular adhesion and migration markers* (e.g. β-catenin, E-cadherin, Integrin αvβ6, LAMC2, Podoplanin), *apoptotic markers* (e.g. Bcl-2, Bax, telomerase), *biomarkers of genomic stability* (e.g. LOH, DNA copy number alterations, copy number variants (CNV), markers of chromosomal instability), *cell signalling markers* (e.g. c-Jun, pc-Jun, c-met), *cell cycle markers* (e.g. p21WAF1, p16INK4A, SMAD4, EZH2, MCM-2, MCM-5, p53), *cellular growth and proliferation markers* (e.g. EGFR, Ki-67), *immune and inflammatory markers* (e.g. CD3+ T cells, COX-2), *microRNAs* (e.g. miR-1, miR-106b, miR-133a, miR-133b, miR-146a, miR-17-5p, miR-181b, miR-184, miR-196a, miR-206, miR-21, miR-345, miR-518b, miR-520g, miR-649, 208b-3p, 204-5p, 129–2-3p, 3065-5p), *cellular markers* (e.g. dysplasia,

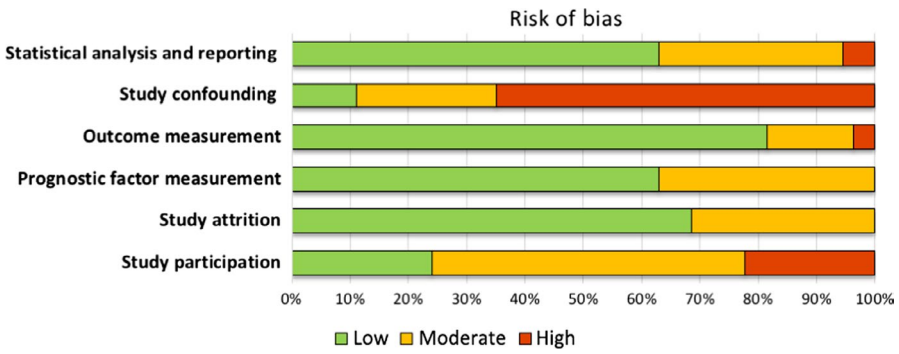

FIGURE 2 Summarized risk of bias in the 54 included studies according to the Quality in Prognosis Studies (QUIPS) criteria (Hayden et al., 2013). Individual ratings are displayed in table 4 [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 The 109 biomarkers assessed in oral leukoplakia specimens in the 54 included studies displayed in Table 1

Biomarker Acronym	#	Biomarker Name	Function
ABCG2	1	ATP-binding cassette superfamily G member 2	Membrane-associated transporter protein
AgNORs	1	Silver staining method for argyrophilic nucleolar organizer region-associated proteins (AgNORs)	AgNORs are loops of chromosomal DNA containing clusters of ribosomal RNA genes
ALDH1	2	Aldehyde dehydrogenase isoform 1	Enzyme of the major oxidative pathway of alcohol metabolism
AI	1	Apoptotic index	Measurement of extent of apoptosis
Axin2	1	Axis inhibition protein 2 (or "conductin")	Tumour suppressor protein that regulates stability of beta-catenin in the Wnt signalling pathway
β-catenin	1	Catenin beta−1	Protein involved in regulation and coordination of cell−cell adhesion and gene transcription
BAX	1	BCL2 Associated X	Protein coding gene, apoptosis regulator
Bcl−2	2	B-cell lymphoma 2	Protein that regulates cell death, by either inducing or inhibiting apoptosis
BMI−1	1	B lymphoma Mo-MLV insertion region 1 homolog, also known as polycomb group RING finger protein 4 or RING finger protein 51	A polycomb ring finger oncogene that regulates p16 and p19
BTBD7 gene	1	BTB Domain Containing 7	Protein coding gene that acts as a mediator of epithelial dynamics and organ branching by promoting cleft progression
c-Jun	2	c-Jun	In combination with c-Fos, forms the AP−1 early response transcription factor and plays a role in cellular proliferation and apoptosis
c-met	1	Tyrosine-protein kinase Met (or hepatocyte growth factor receptor (HGFR)	Activates a wide range of different cellular signalling pathways including those involved in proliferation, motility, migration and invasion on binding with its ligand, hepatocyte growth factor
c-myc	1	c-myc	Regulator genes and proto-oncogenes that code for transcription factors. The transcription factors activate expression of many pro-proliferative genes
CA9	1	Carbonic Anhydrase 9	Transmembrane protein, and is a tumour-associated carbonic anhydrase isoenzyme
Candida ADH1 mRNA	1	Alcohol dehydrogenase 1	Isozyme that catalyses conversion of primary unbranched alcohols to their corresponding aldehydes
Candida ADH2 mRNA	1	Alcohol dehydrogenase 2	Isozyme that catalyses conversion of primary unbranched alcohols to their corresponding aldehydes
CD133	1	CD133 (or) prominin−1	Transmembrane glycoprotein that organizes cell membrane topology
CD1a + LCs	1	Cluster of differentiation 1a	Transmembrane glycoprotein, structurally related to the major histocompatibility complex (MHC) proteins
CD3 + T cells	1	Cluster of differentiation 3	T cell co-receptor consisting of a protein complex that helps activate both the cytotoxic T cell (CD8 + naive T cells) and T helper cells (CD4 + naive T cells)
CD34	1	Cluster of differentiation 34	Transmembrane phosphoglycoprotein protein important as an adhesion molecule and required for T cells to enter lymph nodes
Chromosome instability	1	Chromosome instability	Genomic chromosomal instability leading to whole or partial chromosomal duplication or deletion.

(Continues)

TABLE 2 (Continued)

Biomarker Acronym	#	Biomarker Name	Function
Copy number variations	1	Copy number variations	Phenomenon in which sections of the genome are repeated. It is a type of structural variation: specifically, it is a type of duplication or deletion event that affects a considerable number of base pairs; see also "DNA copy number alterations"
Cornulin	1	Cornulin	Calcium-binding protein present in the upper layer of squamous epithelia. A survival factor, it has an important role in epidermal differentiation
COX–2	2	Cyclooxygenase–2, also known as prostaglandin-endoperoxide synthase (PTGS)	An enzyme responsible for the formation of prostanoids
CS	1	Cytologic Score	The average number of mitotic, karyorrhectic, and apoptotic cells per high-power field
Cyclin D1	1	Cyclin D1	Cyclin D1 is expressed in all adult human tissues except bone marrow-derived cells. Cyclins function as regulators of Cyclin-dependent kinase (CDK)
Depth of ductal dysplasia	1	Depth of ductal dysplasia	Spread of epithelial dysplasia along salivary gland ducts in oral epithelial dysplasia and squamous cell carcinoma
DNA copy number alterations	1	DNA copy number alterations	See "Copy number variations"
DNA ploidy	3		Measure of DNA content within tumour cells. DNA ploidy is the number of complete sets of chromosomes in a cell, and number of possible alleles for autosomal and pseudo-autosomal genes
Dysplasia grading	2	Dysplasia grading	Histopathological assessment of many combinations of dysplastic cellular features. To assign various degrees of epithelial dysplasia many grading systems have been proposed
E-cadherin	1	E-cadherin	Ca2+-dependent transmembrane glycoprotein which connects epithelial cells together at adherens junctions
EGFR	1	Epidermal growth factor receptor	A transmembrane protein receptor, activated by binding of its specific ligands, including epidermal growth factor and transforming growth factor α
EZH2	1	Enhancer of zeste homolog 2	A histone-lysine *N*-methyltransferase enzyme encoded by EZH2 gene that participates in histone methylation and ultimately, transcriptional repression.
FHIT gene	1	Fragile Histidine Triad gene protein product	Is the P1-P3-bis (5'-adenosyl) triphosphate hydrolase and functions in purine metabolism
Fibronectin	1	Fibronectin	High-molecular weight (~440kDa) glycoprotein of the extracellular matrix that binds integrins and other extracellular matrix proteins including collagen, fibrin and heparan sulphate proteoglycans
HBEGF gene	1	Heparin Binding EGF Like Growth Factor	A protein coding gene
HPV genus specific antigen	1	Human papilloma virus (HPV) antigen	It was detected by primary antibody with specificity for common papillomavirus antigen (rabbit anti-bovine BPV–1 antiserum)
HPV DNA (6, 11, 16, 18, 31 types)	1	Human papilloma virus DNA probe	Consisting of biotin-labelled HPV types including 6, 11, 16, 18, 31 and 33
HPV DNA (11, 16, 18, 51 types)	1	Human papilloma virus DNA	A biotin-labelled HPV-L1 consensus probe mixture consisting of full-length HPV types 11, 16, 18 and 51 L1 DNA
HPV (high risk) DNA	1	Human papilloma virus DNA of high-risk genotypes	Type-specific E7 HPV primers for HPV types 6, 16, 33 and 45 used with PCR-based sequencing
HSP70	1	70 kilodalton heat shock proteins or DnaK	Proteins that act as molecular chaperones and catalysts during protein folding

(Continues)

TABLE 2 (Continued)

Biomarker Acronym	#	Biomarker Name	Function
IK	1	Individual cell keratinization index	
IMP−3	1	Insulin-like growth factor II mRNA-binding protein	An oncofoetal protein and member of the IMP family encoded by a gene on chromosome 7p11.5 (4)
Integrin αvβ6	1	Epithelial-specific integrin	A receptor for the extracellular matrix (ECM) proteins fibronectin, vitronectin, tenascin and the latency-associated peptide (LAP) of TGF-β
Integrin β1	1	Integrin beta−1 also known as CD29	Cell surface receptor that associates with integrin alpha 1 and integrin alpha 2 to form integrin complexes that function as collagen receptors
Integrin β3	1	Integrin beta−3 or CD61	Integral cell-surface protein known to participate in cell adhesion and cell surface-mediated signalling
Integrin β4	1	Integrin, beta 4 (or CD104)	Non-covalently associated transmembrane glycoprotein receptors that mediate cell–matrix or cell–cell adhesion and transduced signals that regulate gene expression and cell growth
Keratin 13	1	Keratin 13 or cytokeratin 13	Type I cytokeratin, that pairs with keratin 4 found in the suprabasal layers of non-cornified stratified epithelia
Keratin 4	1	Keratin, type I cytoskeletal 4 (also known as cytokeratin−4 (CK−4) or keratin−4 (K4)	Type II cytokeratin specifically found in differentiated layers of the mucosal and oesophageal epithelia together with keratin 13
KHDRBS1 gene	1	KH RNA Binding Domain Containing, Signal Transduction Associated 1	Gene that encodes a member of the K homology domain-containing, RNA-binding, signal transduction-associated protein family
Ki−67	9	Antigen KI−67 (alternative names: Ki−67 or MKI67)	A nuclear protein associated with cellular proliferation and ribosomal RNA transcription
LAMC2	1	Laminin subunit gamma−2	Extracellular matrix glycoprotein: major non-collagenous constituent of basement membranes. Implicated in a wide variety of biological processes including cell adhesion, differentiation, migration, signalling and metastasis
LOH	1	Loss of heterozygosity	Cross chromosomal event that results in loss of the entire gene and the surrounding chromosomal region
MAGE-A: 1, 3, 4, 6,10,12	2	Melanoma-associated antigen 1,3,4,6,10,12	Members of the MAGE-A protein family sharing 50%–80% of sequence identity. MAGE-A is implicated in some hereditary disorders, (e.g. dyskeratosis congenita). MAGE-A enhances ubiquitin ligase activity of RING-type zinc finger-containing E3 ubiquitin-protein ligases and may play a role in embryonic development and tumour transformation or aspects of tumour progression
Mcm−2	1	DNA replication licensing factor MCM2	One of the highly conserved mini-chromosome maintenance proteins (MCM) that are involved in the initiation of eukaryotic genome replication
Mcm−5	1	DNA replication licensing factor MCM5	Protein structurally very similar to the CDC46 protein from S. cerevisiae, a protein involved in the initiation of DNA replication
MI	1	Mitotic index	Ratio between the number of cells in a population undergoing mitosis and total number of cells in a population

(Continues)

TABLE 2 (Continued)

Biomarker Acronym	#	Biomarker Name	Function
MicroRNAs: miR–1 miR–17–5p miR–21 miR–106b miR129–2–3p miR–133a miR–133b miR–146a miR–181b miR–184 miR–196a miR–204–5p miR–206 miR–208b–3p miR–3065–5p miR–345 miR–518b miR–520g miR–649	1	MicroRNAs: miR–1 miR–17–5p miR–21 miR–106b miR129–2–3p miR–133a miR–133b miR–146a miR–181b miR–184 miR–196a miR–204–5p miR–206 miR–208b–3p miR–3065–5p miR–345 miR–518b miR–520g miR–649	MicroRNAs (miRNAs) are short (20–24 nt) non-coding RNAs involved in post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of mRNAs
Nuclear chromatin pattern	1	Nuclear chromatin pattern	Features descriptive of the statistical and spatial distribution of nuclear chromatin
Oral cancer risk index 12 (OCRI2)	1	Oral cancer risk index 12 (OCRI2)	Statistical model and oral cancer risk index. Assesses the probability of OSCC for an unknown sample. The range of OCRI2 run from 0 to 1, with 0 indicating zero risk of OSCC and 1 indicating a 100% risk
p16	5	Cyclin-dependent kinase inhibitor 2A, (or "CDKN2A," "p16INK4A")	Tumour suppressor protein that inhibits cyclin D-dependent protein kinases, playing a vital role G1-S transition regulation
p16INK4A gene	1	Cyclin-dependent kinase Inhibitor 2A, (or "P16INK4A")	Gene that generates several transcript variants which differ in their first exons. At least three alternatively spliced variants encoding distinct proteins have been reported, two of which encode structurally related isoforms known to function as inhibitors of CDK4 kinase
p21WAF1	1	p21WAF1 protein	A broad-acting cyclin-dependent kinase inhibitor able to prevent the CDK2/cyclin E induced retinoblastoma protein (pRB) phosphorylation, thus inhibiting cell cycle progression at G1 phase
p27	1	(or "p27kip1" (cyclin-dependent kinase inhibitor 1B)	An inhibitor of cyclin-dependent kinase involved in cell cycle regulation
p53	17	Tumour protein p53 (also known as cellular tumour antigen p53," "phosphoprotein p53," "tumour suppressor p53," "antigen NY-CO–13" or "transformation-related protein 53")	Plays a role in regulation or progression through the cell cycle, apoptosis and genomic stability. Can activate DNA repair proteins. Can arrest growth by holding the cell cycle at the G1/S regulation point. Can initiate apoptosis. It is essential for the senescence response to short telomeres
p53 gene	1	Tumour Protein P53 gene	Gene that encodes a tumour suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains
P53-HSP70 complexes	1	P53-HSP70 complexes	p53-Hsp70 complex formation potentially stabilizes p53 protein, resulting in its increased levels in potentially malignant and malignant tumours
PAIP2 gene	1	Poly(A) Binding Protein Interacting Protein 2	Protein coding gene active in the TGF-Beta pathway and translational control
PARP1 gene	1	Poly(ADP-Ribose) Polymerase 1	This gene encodes a chromatin-associated enzyme, poly(ADP-ribosyl)transferase, which modifies various nuclear proteins by poly(ADP-ribosyl)ation

(Continues)

TABLE 2 (Continued)

Biomarker Acronym	#	Biomarker Name	Function
pc-Jun	1	phosphorylated c-Jun	c-Jun activity in stress-induced apoptosis and cellular proliferation is regulated by its *N*-terminal phosphorylation
PCNA	1	Proliferating cell nuclear antigen	DNA clamp that acts as a processivity factor for DNA polymerase δ in eukaryotic cells and is essential for replication
Podoplanin	4	Podoplanin	Mucin-type transmembrane protein expressed in multiple tissues during ontogeny and in adult animals and plays crucial roles in the biology of immune cells, including T cells and dendritic cells
pRb	1	Retinoblastoma protein	Tumour suppressor protein that represses gene transcription, required for transition from G1 to S phase, by directly binding to the transactivation domain of E2F and by binding to the promoter of these genes as a complex with E2F
RAB1A gene	1	Ras-Related Protein Rab–1A	Gene that encodes a member of the Ras superfamily of GTPases. Members of the gene family cycle between inactive GDP-bound and active GTP-bound forms. This small GTPase controls vesicle traffic from the endoplasmic reticulum to the Golgi apparatus
SMAD4	1	SMAD Family Member 4 also known as "mothers against decapentaplegic homolog 4"	In muscle physiology, plays a central role in the balance between atrophy and hypertrophy
SNAI1	1	Zinc finger protein SNAI1	Zinc finger transcriptional repressor downregulates the expression of ectodermal genes within the mesoderm
Telomerase activity	1	Telomerase activity (or terminal transferase)	Ribonucleoprotein that adds a species-dependent telomere repeat sequence to the 3' end of telomeres that protect the end of the chromosome from DNA damage or fusion with neighbouring chromosomes
Tenascin	1	Tenascin	Extracellular matrix glycoproteins abundant in the extracellular matrix of developing vertebrate embryos that reappear around healing wounds and in the stroma of some tumours
TGF-β1	1	Transforming growth factor beta (TGF β 1)	Secreted polypeptide member of the TGF β superfamily of cytokines that performs many cellular functions, including the control of cellular growth, proliferation, differentiation and apoptosis

#, number of studies assessing the biomarker.

nuclear chromatin pattern features, oral cancer risk index) and *miscellaneous* (e.g. MAGE-A 1–4, 6, 10, 12, HSP70, p53-HSP70 complexes, HPV genus-specific antigen, HPV DNA type 16).

4 | DISCUSSION

Our WWOM VII Precision Medicine Work Group previously systematically reviewed prospective longitudinal studies of prognostic biomarker candidates for stratification and long-term surveillance of MT-OL and MT-PVL (Villa, Celentano, et al., 2019). We identified 25 such eligible studies that reported on 31 unique biomarker candidates, but found insufficient evidence to support validated prognostic biomarkers for OL or PVL.

Despite extensive research efforts, the strongest predictive factors for OL transformation remain a non-homogeneous clinical appearance and histopathological detection of oral epithelial dysplasia. No intervention to prevent MT-OL and MT-PVL has currently been confirmed. Achievement of risk stratification and precision approaches for OL and PVL management potentially lies in biomarker discovery. While stratifying biomarkers according to the hallmarks of cancer (Farah et al., 2019), this current systematic review focused specifically on retrospective studies incorporating a longitudinal study design to assess the evidence to support a potential role for application of prognostic biomarkers to stratify risk prediction for MT-OL and MT-PVL. Each hallmark included multiple biomarker candidates, further complicating distillation of appropriate biomarkers for MT-OL and MT-PVL. The complexity and heterogeneity of study data precluded further quantitative synthesis of the findings, such as meta-analysis. For example, within the stem cell marker category, the six biomarkers ABCG2, BMI-1, ALDH1, CD133, SNAI1 and Axin2 were identified for oral cancer risk assessment, while β-catenin, E-cadherin, αvβ6-integrin, LAMC2 and podoplanin were promoted as candidate prognostic biomarkers within the cell adhesion and migration marker domain.

While p53 was most frequently studied biomarker for prediction of both MT-OL and MT-PVL, findings were variable and require further validation (Ögmundsdóttir, Hilmarsdottir, Björnsson, & Holbrook, 2009; Zhang, Kim, Zheng, Bazarsad, & Kim, 2017). Advancement of p53 into clinical practice as a biomarker for MT-OL prediction is precluded pending conclusive evidence and validation.

Zhang and colleagues showed that p53 could predict MT-OL, with the highest predictive accuracy (0.799) achieved for modelling p53 and CA9 with clinical factors including age and degree of dysplasia (Zhang, Kim, Zheng, Bazarsad, et al., 2017). Logistic regression analyses for this model achieved accuracy, sensitivity and specificity of 0.96, 0.82 and 0.98, respectively, and the positive (PPV) and negative predictive (NPV) were 0.90 and 0.97, respectively. Applying univariate Cox regression analysis showed that age (HR: 3.69; 95% CI: 1.36–10.02, $p = .01$) and p53 expression (HR: 29.00; 95% CI: 9.77–86.10, $p < .001$) were independent risk factors for malignant transformation of OL.

Cruz and co-authors demonstrated that the combined use of histological parameters (presence of dysplasia) with p53 distribution patterns showed the highest sensitivity for the detection of progressive OL lesions (91%) (Cruz et al., 1998). When analysed independently, p53 expression showed higher specificity than assessment of dysplasia alone (96% vs 54%) and higher PPV (86% vs 44%) for correct prediction of the malignant transformation of OLs.

Nasser, Flechtenmacher, Holzinger, Hofele, and Bosch (2011) showed that modelling of p53, p16INK4a and Ki-67 expression could define high-risk OL patients with NPV and sensitivity of 100%, specificity of 97% and PPV of 67% (Nasser et al., 2011). Conversely, Ögmundsdóttir, Hilmarsdottir, et al. (2009) reported no association of p53 expression with disease-specific prognosis, recurrence or cancer survival in a cohort of 144 patients including 45 OLs and 54 OSCCs (Ögmundsdóttir, Björnsson, & Holbrook, 2009).

Podoplanin assessed in a substantive cohort of patients in four independent studies remains a strong candidate (Habiba et al., 2017; Zhang, Kim, Zheng, Bazarsad, et al., 2017). Zhang, Kim, Zheng, Bazarsad, et al. (2017) assessed podoplanin expression in 160 OLs (22 malignant-transformed and 138 untransformed), and 18 control specimens derived from normal oral mucosa. Applying univariate Cox regression analysis, podoplanin achieved statistical significance for predicting MT-OL (HR: 6.019, 95% CI: 2.571–14.132, $p < .001$). Combined expression of podoplanin and ALDH1 was also analysed (Habiba et al., 2017). ALDH1 is crucial to maintaining the self-renewal properties and tumorigenicity of HNSCC-derived cancer stem cells. Podoplanin is involved in cell cytoskeleton remodelling mediated by actin and may promote cell invasion by increasing cell motility (Habiba et al., 2017). Multivariate analysis found that expression of podoplanin and ALDH1 was associated with 2.62- and 3.02-fold increased risk of malignant transformation, respectively, and HR increased to 3.64 when histology was modelled with co-expression of both proteins. Point-prevalence analysis revealed that 66% of patients with co-expression of podoplanin and ALDH1 developed oral cancer, suggesting that podoplanin and ALDH1 may be useful biomarkers to identify OL patients with a substantially high oral cancer risk (Habiba et al., 2017).

Notably, both p53 and podoplanin demonstrated increased sensitivity in combination with other candidate biomarkers. Advantages of using a combination of biomarkers was also reported for other biomarkers identified in this review, and is also reflected in the literature relative to other malignancies (Eftimie & Hassanein, 2018; Sun et al., 2017). These observations support the proposition that no single biomarker is likely to predict MT-OL independently. A finely tuned and appropriately tested predictive biomarker panel may hold higher predictive capacity.

Other biomarkers demonstrating potential clinical applicability are those related to genomic stability (Cervigne et al., 2014; Siebers et al., 2013). The evidence base continues to reflect that genetic susceptibility, represented by loss of heterozygosity, chromosomal instability, CNV and copy number alteration, may underlie predisposition for MT-OL (Cervigne et al., 2014; Siebers et al., 2013). Thus, monitoring for genetic susceptibility either alone or in combination with other candidate biomarkers may be used to: (a) predict status of OL, (b) stratify patient cohorts at low- or high-risk MT-OL and (c) ultimately inform precision medicine approaches to condition management (Farah et al., 2019; Zhang et al., 2012).

Although not captured in this review because of date of inclusion cut-off, growing evidence is building around emerging putative biomarkers defining conventional OL and MT-OL (Farah & Fox, 2019; Farah et al., 2019); however, few studies have assessed suitable biomarkers for PVL or MT-PVL (Fettig et al., 2000; Gouvea et al., 2010; Upadhyaya et al., 2018), notwithstanding the high rate of MT-PVL and disease recurrence.

The quest to carefully define potential candidate biomarkers in the context of rigorous phenotypic characterization is key to amplifying the capacity to identify malignant transformation and relative aggressiveness of OL and PVL lesions. This research is critically important to advance effective precision medicine approaches that support clinical management of oral cancer and its early heterogeneous presentation. Pivotal advances in molecular characterization of oral oncogenesis further support the importance of defining candidate biomarkers. For example, systems biology approaches with detailed pathway analysis for all biomarker categories are advancing understanding of molecular carcinogenesis and have applicability to OL and PVL. Gradually, bioinformatic tools are being developed that can leverage current understanding of genetic changes and consequences at a transcriptomic level to help decipher mechanistic pathways contributing to oncogenesis and facilitate capacity to interpret critical biological events. One such tool, Metabolizer, a recently published Web-based app with an interactive online interface, defines changes in metabolism associated with MT-OL (Cubuk et al., 2019). Such tools support capacity to model risk of disease progression and survival, define modes of action associated with genetic mutation, predict potential therapeutic targets and conduct in silico simulation of class prediction and optimal knockout interventions for reversal of an oncogenic phenotype. While these approaches are briefly presented here as examples supporting importance of biomarker research, further review falls outside the scope of this review, awaiting appraisal explored in future publications.

This systematic review identified the lack of support to promote any biomarker(s) for clinical translation because all explored candidate biomarkers require further clinical validation. Notably, most biomarkers included herein were each investigated in only a single study. Even among more extensively investigated biomarkers, including p53 or Ki-67 that were assessed in 17 and 9 independent reports, respectively, outcomes varied across studies. Particularly, the majority of reports were assigned a low evidence grade and hence did not provide strong and reliable support for their findings. All studies suffered from limited sample sizes, with the largest study reporting results derived from 160 patients (100 men, 60 women) (Zhang, Kim, Zheng, Bazarsad, et al., 2017). Further, geographical distribution of studies (as reported in Figure S1) could contribute occult bias since determination of OL/PVL prognosis may vary across populations due to genetic susceptibility or lifestyle variability.

4.1 | Limitations

Limitations and weaknesses in methodology were consistently present across studies. For example, wide variability was identified in control groups constituency, clinical OL/PVL feature definition and heterogeneous reporting of demographics, follow-up periods, histopathology documentation and modifiable risk factors. Some studies reported partially missing data. This outcome was extensively documented by studies scored with moderate and high risk of bias observed across multiple domains following the QUIPS assessment (Figure 2; Table S4). Studies further varied in detection methodology and tissue sample type. Tissue-based biomarkers predominated, with immunohistochemical analysis representing the most commonly applied analytical approach. Whereas protein analysis in fixed tissue using immunohistochemistry has been utilized since the 1930s, more sensitive approaches for MT detection in conjunction with systems level analysis are becoming feasible. More studies with larger sample sizes assessing a potential role for biomarkers in PVL patients should also be prioritized. The low collective sample size of only 49 patients across all PVL studies does not support substantial comparisons or definitive conclusions. Furthermore, differences in aetiopathogenetic mechanisms and risk factors between intraoral and perioral anatomical locations preclude definitive conclusions, as in the sole study reporting OLs of the lips (de Rosa et al., 1999). Given the significant amount of work required to undertake this systematic review as part of a global initiative by the WWOM VII, the cut-off date for inclusion of studies in this review can be viewed as a final limitation of the current study.

5 | CONCLUSION

Insufficient evidence precludes definitive conclusions surrounding the PICO questions addressed by this systematic review. Despite a burgeoning evidence base and the large number of retrospective studies included, we conclude that evidence is currently lacking to promote the advancement of any individual biomarker as an efficient tool for risk stratification of MT-OL or MT-PVL in the clinical setting. However, we acknowledge that biomarkers of genomic instability underlying predisposition for MT-OL broadly continue to be promising avenues to pursue. Future well-designed, prospective, multi-centre studies examining clinically translatable biomarkers in well-documented and appropriately defined cohorts of patients with adequate follow-up time are required to advance precision medicine approaches to these clinically important oral conditions.

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CONFLICTS OF INTEREST

None to declare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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