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CLINICAL REVIEW

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Tumor budding to investigate local invasion, metastasis, and prognosis of head and neck carcinoma: A systematic review

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Abstract

The aim of this systematic review is to shed light on the role of tumor budding (TB) in the biology, behavior, and prognosis of head and neck squamous cell carcinoma (HNSCC). A search was run in PubMed, Scopus, and Embase databases following PRISMA guidelines. After full-text screening and application of inclusion/exclusion criteria, 36 articles were included. Several investigations support the prognostic role of TB, which might play a role in selecting rational treatment strategies. To achieve this goal, further research is needed for greater standardization in TB quantification. Although TB is not included as a negative prognostic factor in the current management guidelines, it might be reasonable to consider a closer follow-up for HNSCC cases with high histopathological evidence of TB.

KEYWORDS

epithelial to mesenchymal transition, head and neck squamous cell carcinoma, immunohistochemistry, oral carcinoma, tumor budding

1 | INTRODUCTION

Tumor budding (TB) is a histological phenomenon encountered in various cancers, for which individual malignant cells and/or small clusters of malignant cells are seen in the tumor stroma. TB is the presence of single cancer cells or small clusters of less than five cancer cells outside the main part of the tumor. Budding was first described in 1954 by Imai[1] but entered the mainstream of pathology only in the last decade.[2] Budding is associated with loss of cellular adhesion and has been postulated to

Elisabetta Zanoletti and Antonio Daloiso contributed equally to this study.

be closely associated with epithelial to mesenchymal transition (EMT).[2,3]

Budding is related to the presence of tumor invasion.[4–7] TB has been associated with poor prognosis in lung,[8,9] gastrointestinal,[10,11] breast,[12,13] colorectal,[14,15] and esophageal cancer.[16,17] At present, TB has been accepted as being an independent prognostic factor in colorectal cancer.[18,19] In head and neck squamous cell carcinoma (HNSCC), the role of TB has only been analyzed in recent years, highlighting the TB as being an independent prognostic factor for oral, laryngeal and hypopharyngeal HPV-negative squamous cell carcinomas.[20–25] Despite the promising prognostic significance of TB, its association with tumor local invasion, nodal and distant metastasis needs to be further studied in order to be used in clinical decision making. Moreover, there are several ways to analyze TB regarding staining techniques (Hematoxylin and Eosin [H&E] or immunohistochemistry [IHC]), qualitative or quantitative assessment methods, cutpoint values, and area of examination. However, an optimized and standardized evaluation method of TB still needs to be assessed.[26]

Diagnosis and management of HNSCC improved significantly over the last decades, but long-term overall survival (OS) did not experience similar progresses, especially in advanced cases. HNSCCs are diverse and complex diseases showing high levels of intertumoral and intratumoral heterogeneity as well as disparities in therapeutic response irrespective of clinical stage.[27] Investigating biomarkers beyond the conventional clinicopathological prognostic factors appears to be of paramount importance to identify the cases with unfavorable prognosis and individualize treatment. The main aim of this systematic review of the literature is to assess the current level of knowledge on the role of TB in the biology, behavior and prognosis of HNSCC, which could be crucial for the development of improved combined therapeutic approaches for HNSCC.

2 | METHODS

2.1 | Protocol registration

The protocol of this systematic review was registered on PROSPERO, an international database of prospectively registered systematic reviews in health and social care (Center for Reviews and Dissemination, University of York, York, UK), in May 2023 (registry number CRD42023417316).

2.2 | Search strategy

A systematic literature review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations.[28] The electronic databases Scopus, Pubmed, and Embase were searched from database inception to May 2, 2023. A combination of MeSH terms and free-text words were used (Table S1, Supporting Information). The reference lists of all the included articles were thoroughly screened to find other relevant articles. References were exported to Zotero bibliography manager (v6.0.10, Center for History and New Media, George Mason University, Fairfax, VA). After duplicates removal, two reviewers (Antonio Daloiso and Tiziana Mondello) independently screened all titles and abstracts and then evaluated the full texts of the eligible articles based on the inclusion criteria. Any disagreement between the reviewers involved in the literature search was resolved through discussion with all authors to reach a consensus.

2.3 | Selection criteria

Studies were deemed eligible when the following inclusion criteria were met: (i) confirmed pathological diagnosis of HNSCC; (ii) presenting data on TB in HNSCC; (iii) definition and cut-off point of TB; (iv) tissue specimen analysis performed through H&E staining and/or IHC; (v) TB assessment based on the magnification field and/or high-power field (HPF). Exclusion criteria were as follows: (i) retrospective series with less than 50 cases; (ii) lack of relevant data; (iii) non-original studies (i.e., reviews, recommendations, letters, editorials, conference paper and book chapters); (iv) animal model studies, (v) studies not described in English.

2.4 | Data extraction and quality assessment

Extracted data were collected in an electronic database including first author, year of publication, country of origin, study design, sample size, number of patients included, mean age of the patients, sex ratio, mean tumor size, investigated biomarkers, methods applied for biomarkers detection, study aim, key findings. The quality of the studies eligible for inclusion was categorized as Poor, Fair, and Good, in agreement with the National Institutes of Health quality assessment tool for Observational Cohorts and Cross-Sectional Studies (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools, accessed on 21 April 2023).[29] Two reviewers (Antonio Daloiso and Tiziana Mondello) independently evaluated the papers, and any disagreement was resolved by discussion.

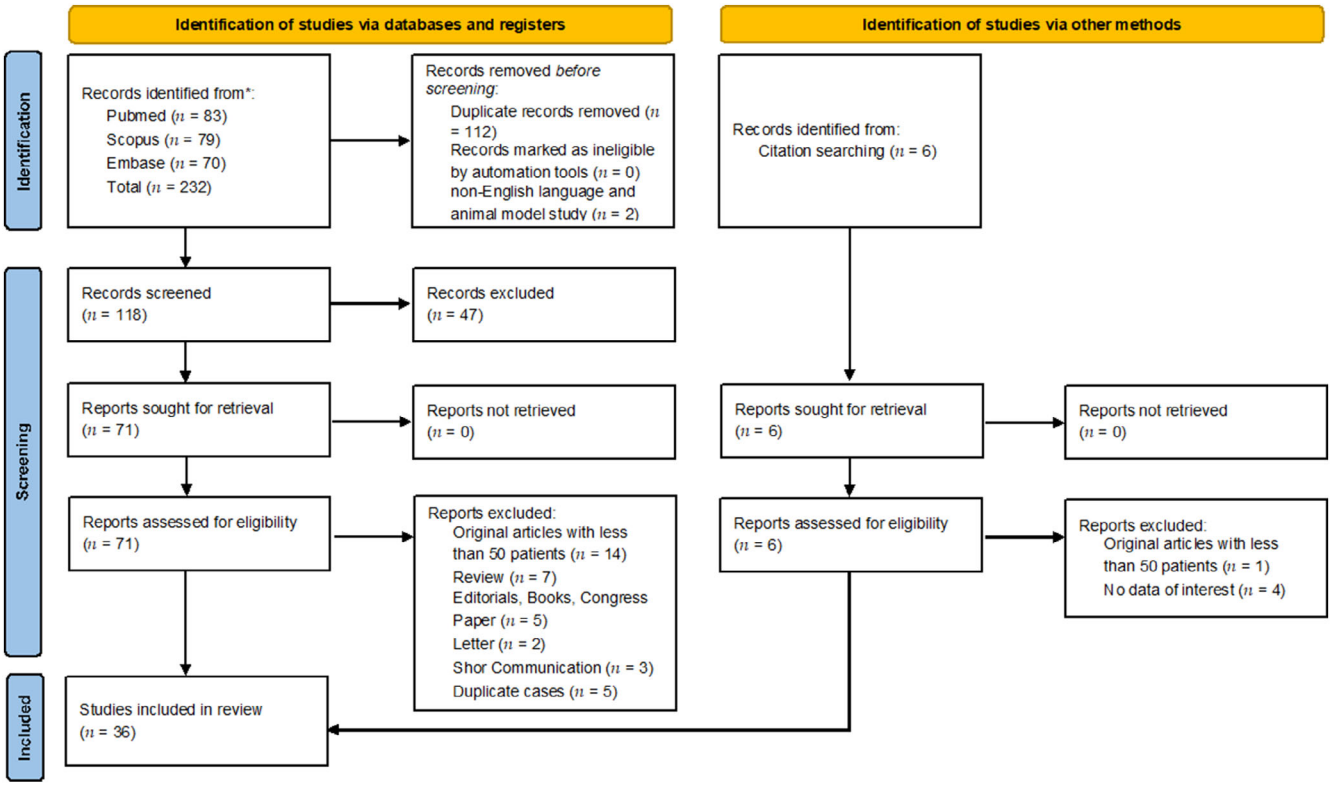

FIGURE 1 PRISMA diagram resembling electronic database search and inclusion/exclusion process of the review. Legend: date of last search May 2, 2023 [Color figure can be viewed at wileyonlinelibrary.com]

3 | RESULTS

3.1 | Search results and quality assessment

A total of 232 titles were collected from our literature search. After duplicates removal and exclusion of 47 records due to coherence with the inclusion/exclusion criteria, 71 articles relevant to the topic were examined. No records were unavailable for retrieving. Finally, 36 were included in the review.[25,30–64] A detailed flowchart of the search process is shown in Figure 1.

In accordance with the National Institutes of Health quality assessment tool for Observational Cohorts and Cross-Sectional Studies,[29] 15 studies (42%) were deemed of Good quality, 21 (58%) Fair, and none as Poor (Table S2).

3.2 | Included studies' characteristics

Among the 36 studies included in the qualitative analysis, 35 had an observational retrospective design and were ex vivo tissue investigations based on histopathological analysis of biopsies or surgical specimens,[25,30–32,33–64] while only 1 study had a prospective design.[33] These studies were published between 2010 and 2023. The median number of patients per study was 150 (range 53–254 cases). Histopathological analysis was based on biopsies in 4 studies (11.1%),[38,45–47] on surgical specimens in 30 (83.3%),[30–33,35–37,39–43,48–64] and mixed bioptic samples/surgical specimens in 2 (5.55%).[34,44]

Major findings of the retrieved articles are discussed in dedicated paragraphs of section 4. Data on patients' demographics, study design, tumor characteristics, and relevant conclusions of each included article are reported in Tables 1–3.

4 | DISCUSSION

TB represents the most non-cohesive pattern of neoplasm invasion.[65] In fact, studies have shown that TB is composed of cells exhibiting typical features of EMT, with increasing invasiveness.[66]

4.1 | Biological processes associated with tumor budding in HNSCC

To date, the mechanism by which the TB is induced and develops has not yet been fully clarified, but it has been

TABLE 1 Summary of studies dealing with prognostic performance of tumor budding in oral squamous cell carcinoma included in the systematic review

Author	Year	Country	Type of article	Cases (*n*)	Tumor site	Bioptic samples/ surgical specimens	TNM	Follow-up (months) mean ± SD (range)	Cut-off (*n* of buds)	TB assessment modality	High budding score (%)	TB staining	Remarks
Almangush et al.[30]	2013	Finland	ORS	233	OTSCC	Surgical specimens	T1-2 N0 M0	67 (1–267)	5	HM	34.8%	H&E	TB and DOI were strongly associated with increased OTSCC-related mortality. DOI, TB were associated to tumor mortality in univariate and multivariate analysis
Attramadal et al.[33]	2015	Norway	OPS	62	OSCC	Surgical specimens	T1-2 N0 M0	55 (3–151)	5	HM	52%	IHC for CK	A high number of buds (≥5) was associated with poor prognosis. High-budding cases showed a trend for fewer relapses after postoperative radiation. TB was associated with EMT-like changes
Boxberg et al.[35]	2017	Germany	ORS	157	OSCC	Surgical specimens	T1-4 N+ Mx	33.2 (1.4–105.3)	5 and 15	HM and MFE	26.1%/20.03%	H&E	High TB and small CNS were associated with advanced pT-, pN-, and UICC-stages. TB was a prognostic factor for OS, DSS, and DFS
Dourado et al.[39]	2020	Brazil	ORS	254	OSCC	Surgical specimens	T1-4 N+ M+	47 (1–178)	5	HM	41.5%	H&E	TB was significantly associated with reduced DSS. The TSR/TB model was independently associated with a high risk of cancer mortality and DFS
Hori et al.[42]	2021	Japan	ORS	62	OTSCC	Surgical specimens	T1-2 N+ M0	Median 68 (8–201)	5	NR	43.5%	H&E	A high density of Tumor CD163+ macrophages served as the poorest prognostic factor for regional control and DFS. Patients with both a high density of Tumor CD163+ macrophages and an intermediate- or a high-grade budding score had a poor prognosis for regional control
Mneimneh et al.[48]	2021	USA	ORS	150	OSCC	Surgical specimens	T1-4 N+ Mx	Median 41 (4–318)	10	HM	27%	H&E	High TB was associated with decreased OS and distant metastasis free survival

TABLE 1 (Continued)

Author	Year	Country	Type of article	Cases (*n*)	Tumor site	Bioptic samples/ surgical specimens	TNM	Follow-up (months) mean ± SD (range)	Cut-off (*n* of buds)	TB assessment modality	High budding score (%)	TB staining	Remarks
Shimizu et al.[55]	2018	Japan	ORS	91	OSCC	Surgical specimens	T1-2 N0 M0	Median 90 (6–164)	0–4; 5–9; ≥10	HM	14.28%	IHC for CK	The mode of invasion and TB were associated with regional metastasis and LVI in univariate analysis. TB was the only independent predictor of regional metastasis in logistic regression analysis. The mode of invasion, WPOI, and TB, were found to be predictors of 5-year DFS as well as LVI and PNI
Strieder et al.[57]	2017	Brazil	ORS	53	lip SCC	Surgical specimens	T1-4 N+ M+	Median 159.4 (T1/T2) 57.5 (T3/T4)	5	MFE	67.9%	H&E	In the BD model the higher histologic grade was associated with worse prognosis
Wang et al.[58]	2011	China	ORS	230	OTSCC	Surgical specimens	T1-4 N+ Mx	Median 65 (3–120)	5	HM	48.30%	H&E	TB, which was associates with EMT, resulted a frequent event and appeared to be an independent prognostic factor in OTSCC
Xie et al.[59]	2015	China	ORS	195	OTSCC	Surgical specimens	T1-2 N0 M0	58.76 (5–133)	5	HM	52.82%	H&E	Tumor budding independently predicted prognosis of patients with T1/2 stage OTSCC and may be used for routing pathological diagnosis and the decision for elective neck dissection
Xu et al.[60]	2021	USA	ORS	329	OTSCC	Surgical specimens	T1-3 N0 M0	73 (0.03–224)	0–4; 5–9; ≥10	HM	19.80%	H&E	WPOI 5 was an independent adverse prognostic factor for OS, high TB was associated with a high risk of nodal metastasis on multivariable logistic regression analysis
Yadav et al.[62]	2023	India	ORS	200	OSCC	Surgical specimens	T1-4 N+ Mx	NR	5	MFE	64.90%	IHC for CK	Evaluation of TB by IHC CK AE1/AE3 showed a higher reproducibility and replicability compared to H&E sections alone
Yu et al.[63]	2019	China	ORS	246	OTSCC	Surgical specimens	T1-4 N+ M+	Median 36 (2–60)	5	HM	24.80%	H&E	Both TB and inflammatory status were independent variables for predicting OS and DFS of OTSCC patients. The iBD scoring model was strongly associated with lymph node metastasis and recurrence in TSCC and could be a promising survival predictor for TSCC patients

(Continues)

TABLE 1 (Continued)

Author	Year	Country	Type of article	Cases (*n*)	Tumor site	Bioptic samples/ surgical specimens	TNM	Follow-up (months) mean ± SD (range)	Cut-off (*n* of buds)	TB assessment modality	High budding score (%)	TB staining	Remarks
Zhang et al.[64]	2019	China	ORS	80	OSCC	Surgical specimens	T1-4 N0-3 M+	(20–61)	5	HM	51.52%	IHC for CK	Enhanced expression of IL-17 in the tumor and tumor margins, which was positively associated with tumor budding in the TIF. IL-17 combined with TB was an independent predictor of prognosis for patients with OSCC

Abbreviations: CNS, cell nest size; DFS, disease-free survival; DSS, disease-specific survival; EMT, epithelial-mesenchymal transition; H&E, Hematoxylin and Eosin stain; HM, hotspot method = number of buds in a single 200× field (0.785 mm^2) at the tumor front of invasion area with the highest budding intensity); HNSCC, head and neck squamous cell carcinoma; HSCC, hyphopharyngeal squamous cell carcinoma; IHC for CK, immunohistochemistry for cytokeratin; LSCC, laryngeal squamous cell carcinoma; MFE, multiple field evaluation = number of buds in 10 HPF selected within the tissue area with highest budding density); NPC, nasopharyngeal carcinoma; NR, not reported; OPS, observational prospective study; ORS, observational retrospective study; OS, overall survival; OSCC, oral squamous cell carcinoma; OTSCC, oral tongue squamous cell carcinoma; TB, tumor budding.

shown that the interaction between the development of TB and the EMT is very close. These events, with anoikis resistance and exosome production, are a crucial point in the progression and invasiveness of solid tumors, indeed they are a consequence of transcriptional reprogramming leading to the migration of the cancer cell detached from the tumor site, which preludes metastasis, and lymph node invasion.[67] Anoikis is a programmed cell death process that manifests itself following the physiological detachment of epithelial cells; in tumors the system fails, a downregulation expression of E-cadherin and an upregulation of N-cadherin achieves the formation of TB, followed by the metastatic process.[68,69] Even if most of the evidence reported in the literature mainly comes from the study of colorectal tumors (much more frequent), TB has also been well studied in HNSCC.[67]

The EMT is a typical process of embryogenesis occurring when mesenchymal transition is required, characterized by high migratory capacity of stem cells, whose main features are resistance to apoptosis and high extracellular matrix production.[37,70] It is well known that the development of EMT is owing to an imbalance between the expression of epithelial and mesenchymal transcription factors. Among HNSCC, such dysregulation has been reported in oral squamous cell carcinoma (OSCC), where an increased expression was observed of zinc finger E-box binding homeobox 1 (ZEB1), paired related homeobox 1 (PRRX1), vimentin, nuclear β-catenin, as well as the activation of the transforming growth factor beta (TGFβ), and a decreased expression of mesenchymal-epithelial transcription factors, E-cadherin, and most members of the miR-200 family.[33,58,67,71] To confirm the involvement of EMT in OSCC patients, it has also been shown that tumor budding is related to the nuclear expression of Snail and Twist transcriptors, known as EMT regulators, which result directly associated with poor cancer prognosis.[72]

In budding cells, it has been seen that as a consequence of increased expression of TGFβ activators (thrombombospondine-1 and integrin β6), a downstream activation occurs of genes promoters of the matrix deposition mediated by the fibronectin gene 1 (FN1), and a down-regulation of miR-200 transcription, the latter plays an important role in establishing epithelial identity and tumor progression.[67] The FN1 gene encodes for fibronectin, a glycoprotein present in the plasma or cell surface and in the extracellular matrix, which is involved in the processes of both adhesion and cell migration, thus is implicated in anoikis resistance of cancer cells,[73] in cell aggregation promotion, and in tumor immune microenvironment. It is highly related with the activation of macrophages M0 and mast cells.[68] Macrophages surrounding the tumor tissue induce the loss of cellular tight junction

TABLE 2 Summary of studies dealing with predictive performance of tumor budding in oral squamous cell carcinoma included in the systematic review

Author	Year	Country	Type of article	Cases (*n*)	Tumor site	Bioptic samples/ surgical specimens	TNM	Follow-up (months) mean ± SD (range)	Cut-off (*n* of buds)	TB assessment modality	High budding score (%)	TB staining	Remarks
Angadi et al.[31]	2015	India	ORS	75	OSCC	Surgical specimens	T1-4 N+ M0	NR	10	HM[a]	45%	H&E	High budding index was associated with DOI and lymph node metastasis. Age, DOI, POI, LVI, PNI, and high TB index were associated with lymph node metastasis in univariate analysis. TB and DOI were strongly related to lymph node metastasis in multivariate analysis
Arora et al.[32]	2017	India	ORS	336	OSCC	Surgical specimens	T1-2 N+ M0	72.4 ± 11.5	5	HM	39.6%	H&E	T stage, grade, TB, DOI, shape of tumor nest, lymphoid response, POI, LVI, and PNI were associated with lymph node metastasis in univariate analysis. DOI, POI, PNI, grade, LVI, lymphoid response, and TV were associated with lymph node metastasis in multivariate analysis
Bjerkli et al.[34]	2020	Norway	ORS	150	OTSCC	84.7% surgical specimens 12% bioptic samples 3.3% no data	T1-3 N+ M0	≥5 year	5	HM	19.3%	H&E	TB was not a significant prognosticator for DSS when patients with low-stage and high stage disease were analyzed separately
de Assis et al.[38]	2023	Brazil	ORS	150	OSCC	Bioptic samples	T1-3 Nx Mx	NR	5	HM	55.4%	IHC for CK	LVD was greater in tumors with high-intensity TB and showed no difference in LVD between the budding area and the area outside the budding. Podoplanin was greater in neoplastic cells of OSCC, tumors with high-intensity budding and tumors with low-intensity or no tumor budding. TB was a biological phenomenon associated with the progression and biological OSCC behavior

(Continues)

TABLE 2 (Continued)

Author	Year	Country	Type of article	Cases (*n*)	Tumor site	Bioptic samples/ surgical specimens	TNM	Follow-up (months) mean ± SD (range)	Cut-off (*n* of buds)	TB assessment modality	High budding score (%)	TB staining	Remarks
Hamada et al.[41]	2020	Japan	ORS	99	OTSCC	Surgical specimens	T1-4 N+ M0	39 (6–121)	10	HM	22%	IHC for CK	Lymphatic vessel invasion and podoplanin expression were significantly correlated with NLM. Podoplanin expression in TB was an independent predictor of NLM in the tongue SCC with low TB grade
Ho et al.[43]	2019	Taiwan	ORS	200	OSCC	Surgical specimens	T1-4 N+ M0	Median 14.95 ± 8.30	NR	HM	52.5%	H&E	TB was predominant in the tongue and floor of the mouth in younger patients with OSCC and associated with PNI and LVI. Patients with TB had significantly lower recurrence-free survival. TB was significantly associated with lymph node metastasis in patients with early cancer stage. Multivariate analysis demonstrated extra-nodal extension and TB as independent predictors of lymph node recurrence
Marangon et al.[47]	2013	Brazil	ORS	57	OSCC	Bioptic samples	NR	NR	5	HM	75.4%	IHC	Higher laminin-5 c2 expression was associated with high-intensity TB and with higher density of stromal myofibroblasts, suggesting that this expression was related to the establishment of an invasive phenotype of neoplastic cells and a permissive environment for tumor's invasion in this neoplasia
Marangon et al.[46]	2019	Brazil	ORS	163	OSCC	Bioptic samples	NR	NR	5	HM	39.88%	IHC for CK	ALDH1 expression was higher in the budding area than in the area outside the budding. OSCC showed phenotypic characteristics of CSC-like cells in accordance with the CSC model of oral carcinogenesis

TABLE 2 (Continued)

Author	Year	Country	Type of article	Cases (*n*)	Tumor site	Bioptic samples/ surgical specimens	TNM	Follow-up (months) mean ± SD (range)	Cut-off (*n* of buds)	TB assessment modality	High budding score (%)	TB staining	Remarks
Noda et al.[49]	2022	Japan	ORS	186	OSCC	Surgical specimens	T1-4 N+ Mx	NR	10	HM	20.43%	H&E	Bivariate and multivariate analyses revealed that high TB and pathological DOI > 10 mm in resections were independent factors for the presence of ENE
Sakai et al.[51]	2022	Japan	ORS	70	OTSCC	Surgical specimens	T1-2 N0 M0	Median 47 (5–125)	10	HM	51%	IHC for CK	Lymph node metastasis correlated with the pathological depth of invasion, TB grade, and TSR in univariate analysis and pDOI and TSR in multivariate analysis
Sakata et al.[52]	2018	Japan	ORS	97	OTSCC	Surgical specimens	T2 N0 M0	Median 53	4	HM	34%	IHC for CK	TB score ≥4 was a significant independent prognostic factor in univariate and multivariate analyses
Seki-Soda et al.[54]	2019	Japan	ORS	248	OSCC	Surgical specimens	NR	52 (1–108)	5	HM	28.22%	IHC for CK	S-1 administration may be more effective in patients with high TB scores than in those with lower scores
Shimizu et al.[55]	2018	Japan	ORS	91	OSCC	Surgical specimens	T1-2 N0 M0	Median 90 (6–164)	0–4; 5–9; ≥10	HM	14.28%	IHC for CK	The mode of invasion and TB were associated with regional metastasis and LVI in univariate analysis. TB was the only independent predictor of regional metastasis in logistic regression analysis. The mode of invasion, WPOI, and TB were found to be predictors of 5-year DFS as well as LVI and PNI
Xie et al.[59]	2015	China	ORS	195	OTSCC	Surgical specimens	T1-2 N0 M0	58.76 (5–133)	5	HM	52.82%	H&E	Tumor budding independently predicted prognosis of patients with T1/2 stage OTSCC and may be used for routing pathological diagnosis and the decision for elective neck dissection

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Xu et al.[60]	2021	USA	ORS	329	OTSCC	Surgical specimens	T1-3 N0 M0	73 (0.03–224)	0–4; 5–9; ≥10	HM	19.80%	H&E	WPOI 5 was an independent adverse prognostic factor for OS, high TB was associated with a high risk of nodal metastasis on multivariable logistic regression analysis
Yamakawa et al.[61]	2019	Japan	ORS	337	OTSCC	Surgical specimens	T1-2 N0 M0	Median 58.2 (4–116)	5	HM	14.20%	H&E	The clinical T stage (T2), DOI (≥4 mm), tumor budding (≥5 buds/ field), and tumor-adjacent tissue (muscle tissue) correlated strongly with and were independent predictors of DNM
Yu et al.[63]	2019	China	ORS	246	OTSCC	Surgical specimens	T1-4 N+ M+	Median 36 (2–60)	5	HM	24.80%	H&E	Both TB and inflammatory status were independent variables for predicting OS and DFS of OTSCC patients. The iBD scoring model was strongly associated with lymph node metastasis and recurrence in TSCC and could be a promising survival predictor for TSCC patients

Abbreviations: CNS, cell nest size; DFS, disease-free survival; DSS, disease-specific survival; EMT, epithelial-mesenchymal transition; H&E, Hematoxylin and Eosin stain; HM, hotspot method = number of buds in a single 200× field (0.785 mm^2) at the tumor front of invasion area with the highest budding intensity); HNSCC, head and neck squamous cell carcinoma; HSCC, hyphopharyngeal squamous cell carcinoma; IHC for CK, immunohistochemistry for cytokeratin; LSCC, laryngeal squamous cell carcinoma; MFE, multiple field evaluation = number of buds in 10 HPF selected within the tissue area with highest budding density); NPC, nasopharyngeal carcinoma; NR, not reported; OPS, observational prospective study; ORS, observational retrospective study; OS, overall survival; OSCC, oral squamous cell carcinoma; OTSCC, oral tongue squamous cell carcinoma; TB, tumor budding.
[a]Budding assessed at 400×.

TABLE 3 Summary of studies on non-oral squamous cell carcinomas included in this systematic review

Author	Year	Country	Type of article	Cases (*n*)	Tumor site	Bioptic samples/ surgical specimens	TNM	Follow-up (months) mean ± SD (range)	Cut-off (*n* of buds)	TB assessment modality	High budding score (%)	TB staining	Remarks
Boxberg et al.[25]	2019	Germany	ORS	157	LSCC, HSCC	Surgical specimens	T1-4 N+ M0	62.6	15	MFE	31.2%	H&E	TB was prognostic for OS, DSS, and DFS
Caruntu et al.[36]	2021	Romania	ORS	100	Cutaneos and mucosal HN SCC	Surgical specimens	T1-4 N+ M0	37	5	NR	48%	H&E	TB and tumor necrosis were significantly correlated with tumor dimensions parameters. TSR, immune infiltration at the front of invasion, TB, and tumor necrosis were significantly associated with clinical TNM staging in SCC. PNI was strongly associated with tumor/ stroma ratio, immune infiltration, and TB. Tumor recurrence revealed significant association with the tumor/stroma ratio and TB
Cho et al.[37]	2019	Republic of Korea	ORS	121	Oropharyngeal SCC	Surgical specimens	T1-4 N+ M0	Median 37.1 (0.8–99.6)	5	HM[a]	45%	H&E	High TB seemed to be more frequently identified in p16-positive OPSCCs
Gupta et al.[40]	2022	India	ORS	50	LSCC	Surgical specimens	T1-4 Nx Mx	NR (12–32)	5	HM	42% by H&E 59% by CK/IHC	IHC for CK	Presence of lymphoplasmacytic infiltration was significantly associated with TB and higher grade. Transglottic location of tumor and pT stage were associated with high budding. CK-IHC was helpful to detect especially cases with low-grade TB
Karpathiou et al.[44]	2019	France	ORS	266	LSCC, pharyngeal SCC	Surgical specimens Bioptic samples	T1-4 N+ M0	NR	5 and 15	HM and MFE	27.5%/48.3% (TB pts) 12.04%/21.8% (total pts)	H&E	Stroma-rich tumors, TB, smaller CNS at core and front area, and fibroblastic stroma type, were all adverse prognostic factors. Stroma-poor tumors and with larger CNS showed good response to induction chemotherapy
Luo et al.[45]	2012	China	ORS	105	NPC	Bioptic samples	TNM 2002	NR	5	Number of buds in the single HPF with the highest budding intensity and selected among four HPF	49.5%	IHC for CK	The intensity of budding correlated strongly with T classification, lymphatic invasion, vascular invasion,

(Continues)

TABLE 3 (Continued)

Author	Year	Country	Type of article	Cases (*n*)	Tumor site	Bioptic samples/ surgical specimens	TNM	Follow-up (months) mean ± SD (range)	Cut-off (*n* of buds)	TB assessment modality	High budding score (%)	TB staining	Remarks
													lymph node metastasis, and clinical stage. High budding grade patients had poorer survival in univariate analysis. TB was an independent predictor of survival in multivariate analysis. Budding cells showed high-level expression of the cancer stem cell marker ALDH1 which contributed to aggressive behaviors and poor survival
Ozturk et al.[50]	2022	Turkey	ORS	130	LSCC	Surgical specimens	T1-4 N+ M+	NR (12–86)	15	MFE	16.15%	H&E	High TB grade significantly reduced the OS and DFS
Sarioglu et al.[53]	2010	Turkey	ORS	64	LSCC	Surgical specimens	T1-4 N+ Mx	Median 53 (6–181)	≥2/3 of the invasive margin	Budding extension along all the front of invasion, explored at 400× magnification	21.88%	H&E	High TB was identified as a prognostic factor by univariate analysis for distant metastasis-free survival. Multi-variate analysis revealed that only the number of metastatic lymph nodes and budding were significantly associated with distant metastasis
Stögbauer et al.[56]	2023	Germany	ORS	331	HNSCC	Surgical specimens	T1-4 N+ M+	NR	5 and 15	HM and MFE	22%	H&E	TB was able to stratify patients with HPV- positive HNSCC into low-grade and high-grade subgroups. CDG-high cases were more frequently associated with occult lymph-node metastases and inferior clinical outcome

Abbreviations: CNS, cell nest size; DFS, disease-free survival; DSS, disease-specific survival; EMT, epithelial-mesenchymal transition; H&E, Hematoxylin and Eosin stain; HM, hotspot method = number of buds in a single 200× field (0.785 mm^2) at the tumor front of invasion area with the highest budding intensity); HNSCC, head and neck squamous cell carcinoma; HSCC, hyphopharyngeal squamous cell carcinoma; IHC for CK, immunohistochemistry for cytokeratin; LSCC, laryngeal squamous cell carcinoma; MFE, multiple field evaluation = number of buds in 10 HPF selected within the tissue area with highest budding density); NPC, nasopharyngeal carcinoma; NR, not reported; OPS, observational prospective study; ORS, observational retrospective study; OS, overall survival; OSCC, oral squamous cell carcinoma; OTSCC, oral tongue squamous cell carcinoma; TB, tumor budding.

[a]Budding assessed at 400×.

and consequently cell detachment.[45] In tumor stroma an increased expression has been observed of the interleukin 17 (IL-17). This pro-inflammatory cytokine is primarily secreted by T-helper lymphocyte and neutrophils. The role of this protein in TB has not been well clarified, but some research groups suggested that it promoted the formation and progression of budding cells, other groups that it acted together with TB in tumor progression. Furthermore, it is well known that IL-17 promotes tumor dissemination through its effects on matrix metalloproteinases and angiogenesis.[64] The former act proteolyzing the laminin-5 gamma 2 chain (laminin-5 c2), whose function is endorsing the static adhesion between basal epithelial cells and the adjacent basement membrane. The release of laminin-5 c2 (peptide obtained by the laminin-5 gamma 2 chain proteolysis) leads to disruption of the cell-basement membrane adhesion through the intracytoplasmic phosphorylation of integrin brought about by its binding to the epidermal growth factor receptor.[47] Moreover, tumor microenvironments under hypoxic conditions induce the secretion of extracellular vesicles and the activity of fibroblast surrounding budding cells promoting cell invasion by reshaping the extracellular matrix and thus leading to angiogenesis and tumor progression.[45]

About the correlation between TB and angiogenesis, some research groups reported that the angiogenetic markers CD34 (pan-expression in vascular proliferation) and CD105 (angiogenic marker induced by hypoxia[74]) were both overexpressed in tumor stroma, promoting increased microvascular density but without any correlation with the number of budding cells, even if the CD34 resulted more highly represented in the budding area than outside, demonstrating high microvascular density in this area, even though the study examined biopsies.[38] Another relevant feature is the correlation between cancer stem cells and TB. High expression of aldehyde dehydrogenase 1 (ALDH1) in TB cells has been observed in nasopharyngeal carcinoma (NPC) and OSCC.[45,46] Luo et al.[45] reported that budding cells having fibroblast-like phenotype were barely identifiable within cancer stroma; thus, they tackled the issue by staining the tumor sections with this cytosolic enzyme. The ALDH1 is a cancer stem cells marker that promotes the oxidation of proteins involved in the early stages of cell differentiation (aldehyde and retinoic acid) and they show that it resulted directly correlated with tumor aggressiveness.[34,75] To confirm the role of cancer cells stemness, in OSCC patients, the expression of CD44 resulted closely related to the EMT process and therefore to tumor aggressiveness.[76]

In summary, budding cells show characteristics between EMT and cancer stem cells, such as morphology and lack of E-cadherin, high expression of β-catenin, vimentin, and matrix metalloproteinase.[58] Budding cells are influenced by fibroblastic and macrophage presence even if their prognostic value correlates independently. Overall, all these factors promote tumor aggressiveness enhancing lymphatic invasion and metastatic potential.[59,60]

4.2 | The pathological evaluation of tumor budding in HNSCC

As stated by the International Tumor Budding Consensus Conference (ITBCC), TB is defined as a single tumor cell or a cluster of up to four tumor cells.[18] This definition was primarily proposed for colorectal cancer and then adopted for other tumors including HNSCC.[20] In accordance with previous studies and with the ITBCC recommendations, TB should be assessed on H&E-stained slides (Figure 2), while the use of immunoreaction for cytokeratins should be limited to increase evaluation accuracy only in challenging cases.[77]

The ITBCC recommends assessing TB in a single field measuring 0.785 mm^2 on the invasive front, which should be selected where the tumor shows the highest density of tumor buds ("Hotspot method").[18] Different scoring systems for TB have been used in HNSCC, as highlighted in our review. The Hotspot method was the most frequently adopted (about 75% of the studies), whereas 22% of studies used a multiple fields evaluation method, meaning that examiners provided the mean value of buds counted in multiple fields along the tumor invasion front (e.g., "5 high power field" and "10 high power field" methods). Interestingly, one study applied a different scoring system that considered the spatial extension of the buds along the front of tumor invasion.[53] Despite the differences in TB detection methods, its clinical relevance was maintained across the studies, endorsing the concept that the prognostic significance of TB results independent of the scoring system used. However, the presence of a single adopted standard is crucial for reproducibility in routine diagnostic practice and for research purposes, and the definition of shared recommendations for TB evaluation in HNSCC is highly desirable. Moreover, despite ITBCC recommended adopting a three-tier score system (low risk ≤4 buds; intermediate risk 5–9 buds; high risk ≥10 buds) to perform risk stratification, most of the studies discussed herein adopted a two-tier score system with a cut-off point of five buds (low risk <5 buds vs. high risk ≥5 buds), while only four studies maintained the three-tier score system.[34,44,56,60] Generally, the 5 buds cut-off seemed to be an appropriate discriminator to stratify patients; however, further studies are needed to validate these data.[20]

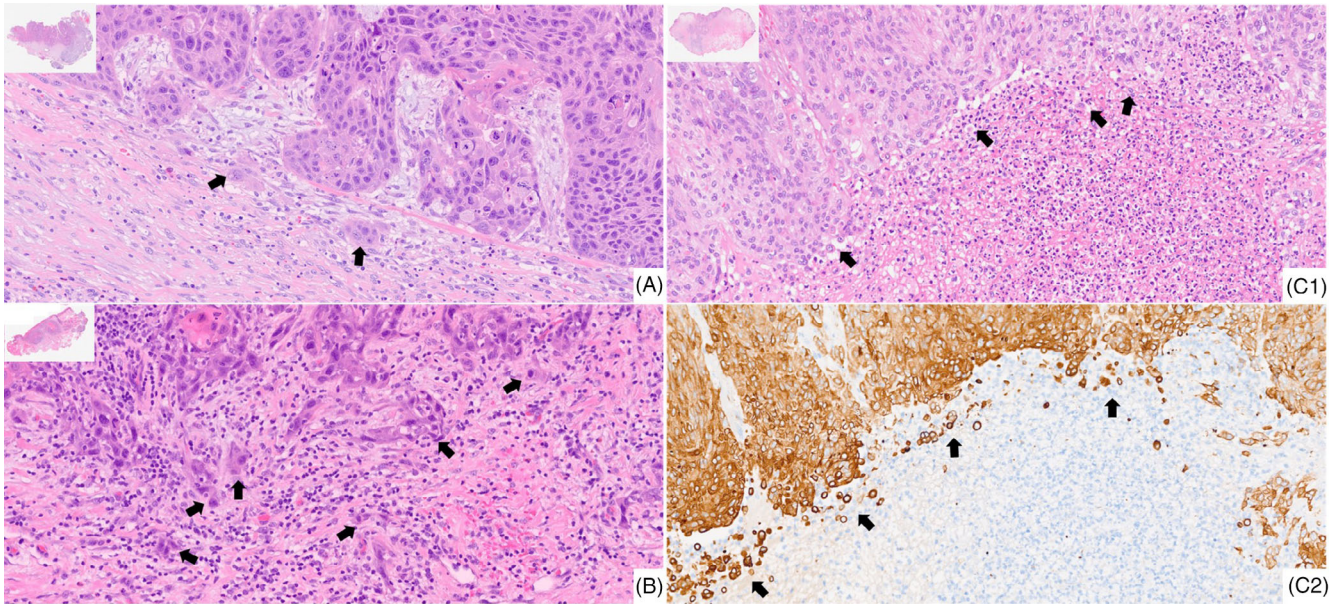

FIGURE 2 Representative Hematoxylin and Eosin images of the different degree of tumor budding in three different moderately differentiated squamous cell carcinomas of the oral cavity (panoramic overview of the corresponding digital slides in the upper-left corner of each box). Low tumor budding (A), intermediate tumor budding (B), and high tumor budding associated to high peritumoral inflammation (C1); black arrows indicate tumoral buds spreading into the peritumoral stroma. Due to the inflammatory context in C1, budding detection is challenging, and it is better recognized by cytokeratin stain (black arrows). Original magnification 200× (A, B, C1, C2); immunostaining with cytokeratin AE1-AE3 in C2 [Color figure can be viewed at wileyonlinelibrary.com]

The evaluation of TB in preoperative biopsies is an aspect less investigated in HNSCC. TB in biopsy material is better defined as intratumoral budding as it is not possible to consider a biopsy as representative of the entire tumor invasion front.[18] Few studies[24,78–80] assessed TB in preoperative biopsies, and in all the cases the TB resulted a reliable marker for tumor aggressiveness, suggesting that TB seemed to maintain in biopsies the same clinical relevance observed in surgical specimens. In any case, the real impact of intratumoral budding in preoperative biopsies deserves further investigations.

4.3 | Tumor budding in oral squamous cell carcinoma

4.3.1 | Prognostic role

For OSCCs with higher TB scores, the existing literature agrees in describing significantly less favorable outcomes compared to those with lower TB, in terms of OS,[48,57–60,62–64] disease-specific survival (DSS),[30,39] and disease-free survival (DFS).[33,42,48,55,57,63] The summary of findings of the included studies has been reported in Table 1. Generally speaking, such prognostic associations were significant regardless of the considered TB scoring system, mostly including either two-tier (<5 vs. ≥5 buds)[30,33,35,39,58,64] or three-tier (<5, 5–9, ≥10 buds)[34,42,48,55,60] classifications. Also, research groups who used other TB classification methods (e.g., TB absence vs. presence) obtained results in line with this general tendency. Ho et al.[43] found significantly lower locoregional recurrence-free survival in cases with the presence of TB at histopathological examination, compared to those with no evidence of TB. However, such an association did not reach statistical signification for DSS.

Recently, clinicopathological research has focused on the prognostic role of TB in the specific setting of early-stage OSCC. Retrospectively considering a Brazilian cohort of 254 OSCC patients, Dourado et al.[39] concluded that a TB count higher than 5 was independently associated with reduced DSS. Dividing such a cohort into two subgroups (early [I-II] vs. advanced [III-IV] stages), the authors found that TB was not significantly associated with prognosis in the advanced cases, whereas it was a significant predictor of DSS in the early stages.[39] In line with such results, also the Almangush et al.[30] data seemed to support the prognostic role of TB in early oral cancers. In fact, in their Finnish cohort of 233 T1-2N0 OSCC cases, this research group found that TB ≥5 was significantly associated with reduced DSS at both univariate and multivariate analysis.[30] Similar results were reported by Attramadal et al.[33] in early-stage OSCCs. In their retrospective analysis of cT1-2N0 OSCCs (all treated

with upfront surgery followed by adjuvant radiation therapy in the case of close or positive margins), these authors found that a high number of tumor buds (≥5) was significantly associated with a poor prognosis in terms of reduced DFS.[33] Moreover, in that investigation, a significantly higher recurrence rate (33%) was found among the high-budding cases, compared to the low-budding ones (14%).[33] Results in line with those described above were also found by Shimuzu et al.,[55] Xie et al.,[59] and Xu et al.[60]

The prognostic value of TB has also been tested also in OSCC occurring in young adults. In a retrospective study on 150 OSCC patients younger than 40 years, Mneimneh et al.[48] found that high-risk TB (≥10 buds) was associated with significantly reduced OS and distant metastasis-free survival, thus substantially confirming the prognostic value of such a histological feature also in this specific cohort.

4.3.2 | Predictive role

As TB has been widely established as an adverse prognostic factor for colorectal carcinoma,[81] there is growing interested in confirming such a predictive role in OSCC, as well. High-intensity TB is a strong independent predictive factor for neck lymph node metastasis and an important parameter for assessment of tumor behavior.[31,32,34,38,41,43,46,47,49,51,52,54,55,59–61,63] Table 2 reports the findings of the studies dealing with predictive role of TB in OSCC. In a retrospective study which included 186 surgically resected OSCC, Noda et al.[49] found in their bivariate and multivariate analyses that high TB and pathological depth of invasion >10 mm in resections were independent factors for the presence of extranodal extension (ENE+). Moreover, their combination showed high specificity (91%) and accuracy (83%) in predicting ENE+. In 2022, a clinicopathological research focused on the predictive role of TB in the specific setting of early-stage OSCC.[51] Retrospectively considering a cohort of 70 early-staged OSCC patients, Sakai et al.[51] concluded that a TB count higher than 5 was independently associated with neck node metastasis in a univariate setting, while in a multivariate one tumor-stroma ratio correlated more strongly with neck node metastasis than TB grade. Similar conclusions were proposed by Sakata et al.,[52] who demonstrated that the evaluation of TB was effective for pinpointing patients with cT2N0 OSCC at high risk of occult neck metastasis. Previously, Xie et al.[59] reported that TB independently predicted prognosis of patients with T1/2 classification oral tongue SCC and might be used for routing pathological diagnosis and the decision for elective lymph node dissection. Some patients with early-stage oral cancer have a poor prognosis due to delayed neck metastasis occurrence. In this setting, Yamakawa et al.[61] underlined the role of TB as additional to conventional features to predict delayed neck metastasis in early oral tongue SCC. Recently, Hamada et al.[41] analyzed a sample of 99 patients diagnosed with early-stage OSCC of the tongue identifying lymphatic vessel invasion and podoplanin expression as predictors for neck lymph node metastasis. These authors further defined a cluster of 77 patients with low-TB scores, where podoplanin expression in TB cells was an independent prognostic factor for neck metastasis. In conclusion, they suggested that patients with T1-2 tongue carcinoma, with low-grade TB and no podoplanin expression might be candidates for a less aggressive treatment of the neck.

In 2017, Arora et al.[32] developed a new outcome prediction model in early-stage OSCC based on histopathological parameters. At first, they found TB as an independent predictor of neck node metastasis, then they graded it with other significant independent factors (depth of invasion, pattern of invasion, perineural invasion, lympho-vascular invasion, lymphoid response) to design a scoring system that allowed accurate evaluation of the risk of metastasis with accuracy independent of the traditional TNM system.

The predictive role of TB could be of clinical importance for OSCC patients where the clinicians have refrained from neck dissection. A high degree of budding might indicate a higher risk of neck node metastases, implying that a tighter follow-up is warranted and elective neck dissection could probably be recommended.

4.4 | Tumor budding in non-oral HNSCC

The role of TB in non-oral HNSCCs has been investigated, too (Table 3). Considering the pathological assessment of TB, several studies showed IHC of cytokeratin to have higher detection rates of single cells at the invasive tumor front than the H&E method.[82,83] A retrospective case series by Gupta et al.[40] confirmed a high efficacy of IHC in TB assessment in LSCC. Indeed, IHC was able to detect scattered single tumoral cells mixed within the inflammatory cells at the invasive front.[40] On H&E sections, low-grade TB and high-grade TB were found in 30% and 42% of the specimens, respectively. On the other hand, IHC showed low-grade TB in 17% of tumors and high-grade TB in 59% of them. Therefore, Gupta et al.[40] defined IHC as a better method for TB assessment and confirmation in LSCC.

Several studies focused on the formulation of new grading systems based on easily reproducible morpho-

pathological findings with potential prognostic role.[30,84,85] In 2016, Weichert et al.[86] suggested that in pulmonary SCC, tumor cell nest size (CNS) and TB had an impact on the prognosis of patients and proposed a grading scheme. Similarly, for HNSCC, Boxberg et al.[35] suggested a grading system based on CNS and TB scores in OSCC, while Caruntu et al.[36] found significant correlations between TB and tumor necrosis and the clinical staging in cutaneous and mucosal HNSCC series. In addition, TB was significantly correlated with perineural invasion.[36] More recently, the same group adapted their grading system in patients with laryngeal and hypopharyngeal squamous cell carcinomas (LSCC, HSCC).[25] In addition to TB and CNS, other morphopathological parameters were taken into consideration, such as degree of keratinization, mitotic count, nuclear size, and stromal content.[25] TB resulted to be prognostic for OS, DSS, and DFS, in particular the OS of patients with high TB was the worst. The keratinizing histotype significantly correlated both with a higher frequency of TB and small CNS/single-cell infiltration compared with non-keratinizing SCC. High TB levels significantly correlated with moderate/high stromal content, intermediate/large nuclear size, and CNS. In addition, the presence of TB and small CNS/single-cell infiltration was both significantly associated with lymph-angio-invasion and perineural invasion and were also correlated with each other. Both TB and CNS, were significantly correlated with pT classification, pN classification, and UICC classification. Therefore, the new grading system proposed by Boxberg et al.[25] was able to successfully stratify patients from a prognostic viewpoint into three groups (nG1, nG2 and nG3) in both LSCC and HSCC as well as in OSCC.

A recent investigation by Stögbauer et al.[56] suggested an improved histopathological grading system that for the first time was comprehensive of both HPV positive and negative HNSCC. In particular, they analyzed the prognostic significance of TB and CNS and stratified tumors in two subgroups of cellular dissociation grading according to budding levels. The prognostic value of TB in HPV-positive HNSCCs was confirmed in this study.[56] Subsequently, they compared this new grading system to the well-established WHO classification and Brandwein–Gensler (BG) risk models.[5,87] In a multivariate analysis, the new grading system seemed to be the only one with a significant prognostic role.[56] Moreover, they studied the correlation between TB and neck lymph node metastasis in cN0 patients, thus suggesting the role of TB in identifying lymph nodes with an elevated risk of micro-metastasis and highlighting the importance of this finding in the decision making for surgical treatment planning. In addition, they hypothesized that small tissue areas that can be analyzed for TB under 1 HPF could be transferable to an excisional biopsy sample.[56]

The prognostic role of TB in LSCC was initially studied in 2010 by Sarioglu et al.[53] who analyzed a sample of 64 patients with LSCC. In this series, N+ status and higher TB scores were significantly associated with distant metastasis in LSCC.[53] Analogously, in the Ozturk et al.[50] series, higher TB levels were associated with reduced OS and DFS. Moreover, the lymphocytic host response was found to be significantly reduced in high-grade TB tumors, thus resulting in a poorer prognosis.[50] The prognostic role of TB was further evaluated by Karpathiou et al.[44] who focused their attention on tumor stroma parameters in a large series of LSCC and pharyngeal SCC biopsies and surgical specimens. In particular, TB and abundant stroma tumors were associated with a worse prognosis and a more aggressive tumor behavior. Indeed, TB levels, stroma-rich tumors, and fibroblastic stroma type were all associated with advanced T classification, whereas abundant stroma tumors were only marginally correlated with N classification. Response to neoadjuvant chemotherapy was evaluated in association with tumor related variables; low TB, high CNS, and stroma-poor tumors were predicting factors for a good response to chemotherapy. On the other hand, high TB, stroma rich tumors, and a low CNS seemed to be negatively correlated with a poorer lymphocytic host response, thus highlighting the fundamental role of immune surveillance to reduce tumor aggressiveness.[44] Similarly, a Romanian group studied the prognostic potential of tumor/stroma ratio, local immune response, TB activity and tumor necrosis using standard (H&E) staining, in SCCs involving distinct areas of the head and neck. They suggested that TB could provide additional information on the aggressiveness of SCCs. In particular, both TB and tumor necrosis were significantly related with tumor dimensions and advanced clinical stages of the disease. Moreover, perineural invasion was more frequently present in tumors with high TB activity.

EMT markers such as Twist and Snail are known to be associated with tumor size, distant metastasis, patient survival, and tumor bud formation in the context of lymph node metastasis in several tumors, for instance breast cancer and colorectal cancer.[88,89] Some studies suggest that TB may play a role in EMT in several types of cancer as well as in HNSCCs.[2,33,58,79] A recent study by a Korean research group investigated the relationship between TB and both p16 expression and EMT markers, namely Twist and Snail, in a cohort of 121 oropharyngeal SCC and 270 cases of non-oropharyngeal HNSCC as a control group.[37] A marked expression of Twist and Snail/Slug was observed in p16-positive cases among all HNSCCs. Focusing on oropharyngeal SCC, p16-positive

tumors showed significantly higher Twist and Snail/Slug expression than the p16-negative ones. Moreover, high TB count was significantly associated with p16 positivity in oropharyngeal SCCs, whereas at IHC, p16 expression was not related to TB in the control group. Compared with non-oropharyngeal SCCs, oropharyngeal SCCs were significantly associated with lympho-vascular invasion, lymph node metastasis, and larger maximal diameter of metastatic foci in lymph nodes, as well as with p16 positivity. These findings suggested that EMT markers changes, as well as morphological changes, can be related to the lymph node metastasis in oropharyngeal SCCs. Cho et al.[37] reported that p16 expression in oropharyngeal SCC was correlated with EMT markers expression and TB formation, thus suggesting the important role HPV infection played in altering such markers, leading to an increase in tumor aggressiveness and consequently to metastasis formation, despite the relatively small tumor size.

NPC is an uncommon and aggressive tumor, also because it tends to have cervical lymph node metastases even at an early stage.[90] In 2012, Luo et al.[45] studied the intensity of TB levels and its prognostic significance in NPC biopsy samples. Furthermore, they investigated similar features to cancer stem cells in TB cells at the invasive tumor front using IHC for ALDH1. A total of 122 NPC samples were analyzed and TB was stained with pan-cytokeratin antibodies. They suggested that TB could play a significant role in NPC invasion and malignant progression. Indeed, a significant association was found between the degree of budding and advanced stage tumors, highlighting a possible correlation between TB and tumor invasion's depth, as well as an increase in metastatic potential when a higher degree of TB is present. Moreover, Luo et al.[45] reported high TB levels were contributing for lymphatic and vascular tumor invasion. Subsequently, they found a positive correlation between ALDH1 expression and the grade of budding, in terms of staging, lymph node metastasis and both lymphatic and vascular invasion. TB with a high level of ALDH1 expression were reported to be negatively associated with OS, thus suggesting the prognostic relevance of this factor in NPC.[45]

4.5 | Future perspectives

It is necessary to investigate the role of TB in SCC arising from different anatomic sites of the head and neck region, as well as HPV-negative and HPV-positive tumors.[23] There is a paucity of data specifically relating to predisposing factors for TB, which could contribute to our understanding of TB. From a pathological viewpoint, TB has been framed as a marker that could reflect EMT in different malignancies including HNSCC. A clearer understanding of the molecular background of TB in HNSCC necessarily requires more studies.

Moreover, an issue exists in current literature regarding the relationship between TB and the worst pattern of invasion (WPOI). WPOI was firstly proposed by Brandwein-Gensler et al.[5] Unlike the TB, the WOPI grading system is focused on the pattern of invasion, and provides a qualitative assessment stratified in five invasion patterns: Type 1, pushing borders; Type 2, finger-like growth; Type 3, large separate islands, >15 cells per island; Type 4, small tumor islands, ≤15 cells per island; and Type 5, tumor satellites, ≥1 mm from the main tumor or next closest satellite.[5] The last AJCC staging system endorsed the use of WPOI method for OSCC risk stratification[91]; however, the complementary role of TB and WOPI in stratify patients with HNSCC is an aspect still under investigation.[5,60,92]

It has been proposed that in the future routine reporting of surgical pathological specimens, "TB intensity" will have an essential role.[20,93] In HNSCC, the prognostic and predictive role of TB need not necessarily be considered in isolation but also in combination or in panels of other clinicopathological variables as the WOPI score. Routine assessment of TB on biopsy should be considered with caution and further investigated, as it can be affected by section effects and compression artifacts.

5 | CONCLUSIONS

TB represents groups of cancer cells that are more invasive than that in the tumor core, leading to an increased risk of lymphovascular spread. There is a substantial body of data indicating that TB may be an important sign of invasive growth and metastasis in HNSCC. Consequently, several studies supported the clinicopathological relevance and prognostic role of TB.

Evidence suggests that TB may play a role in future staging systems for HNSCC,[94] potentially influencing treatment decisions.[87] However, to facilitate this, further research (particularly prospective studies) is needed on biopsy and surgical material to standardize TB quantification and implement it into the pathology reports. Although TB is not included as a negative prognostic factor in the current guidelines for the management of HNSCC, it is reasonable to assume that cases with high histopathological evidence of TB can be addressed for closer follow-up.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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