

Chorus line in oral squamous cell carcinoma: How stromal and immune players orchestrate tumor progression (Review)

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Abstract. The tumor microenvironment (TME) in oral squamous cell carcinoma (OSCC) represents a dynamic and heterogeneous ecosystem in which non-immune stromal cells play important roles in tumor progression, invasion and therapeutic resistance. Among these, cancer-associated fibroblasts (CAFs), derived mainly from normal oral fibroblasts under the influence of tumor-derived cytokines such as transforming growth factor β (TGF- β), angiopoietin-like 3 and platelet-derived growth factor-BB, are the most abundant. CAFs exhibit a myofibroblastic phenotype characterized by α -smooth muscle actin, fibroblast activation protein and integrin $\alpha 6$ expression and their presence correlates with aggressive tumor behavior and poor prognosis. Functionally, CAFs contribute to the ‘reverse Warburg effect’, remodeling of the extracellular matrix via matrix metalloproteinases and lysyl oxidase, promotion of angiogenesis and immunosuppression through cytokines such as TGF- β , interleukin (IL) 6 and IL-10. Programmed death-ligand 1 (PD-L1), a key immune checkpoint molecule, suppresses T-cell activation by binding programmed

death-1 (PD-1) on lymphocytes while also exerting intrinsic oncogenic functions, including enhancement of epithelial-mesenchymal transition, proliferation and resistance to radiotherapy and chemotherapy. PD-L1-enriched extracellular vesicles released by CAFs and tumor cells further propagate immune evasion and metastasis. Although PD-1/PD-L1 blockade with pembrolizumab or nivolumab has improved outcomes in advanced OSCC, variability in PD-L1 expression and intratumoral heterogeneity challenge predictive accuracy. The present review integrated stromal and immune perspectives, emphasizing the dual oncogenic and immunomodulatory roles of CAFs and PD-L1 in shaping the OSCC TME and identifying future therapeutic opportunities targeting both compartments.

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1. Introduction

The oral cavity comprises a large and heterogeneous group of malignancies with distinctive histological features, among which oral squamous cell carcinoma (OSCC) is the most common (1). Such heterogeneity is also evident within OSCC at different levels: starting from its etiopathogenesis, where associations with lifestyle habits (smoking, alcohol

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consumption, exposure to wood and leather dust) and viral infections [human papillomavirus (HPV) and Epstein-Barr virus] have been demonstrated, continuing with its various histopathological variants (such as verrucous carcinoma, basaloid carcinoma, papillary squamous carcinoma, spindle cell carcinoma, acantholytic, or lymphoepithelial carcinoma) and ending with the degree of tumor-associated inflammation (2,3). Although tumor-infiltrating immune cells are recognized as important biomarkers in several malignancies, such as breast, colorectal and lung cancers, their prognostic role in OSCC remains controversial (4,5). Currently, based on the distribution of inflammatory cells within the tumor microenvironment (TME), 'hot' tumors (characterized by a significant infiltrate of tumor-associated immune cells) can be distinguished from 'cold' tumors (showing an almost complete absence of immune cells; Fig. 1) (6).

These findings have markedly influenced oncological decision-making in patients with OSCC, particularly in advanced and metastatic disease (4,7). Similar awareness is increasingly emerging across other therapeutic modalities, emphasizing that the cellular constituents of TME exert a substantial effect on patient prognosis and may even serve as therapeutic response predictors (5,7).

The present review provided a comprehensive overview of the principal cellular components of the OSCC TME and discussed their intrinsic involvement in mechanisms of immune evasion, immunosuppression and response to immunotherapy.

2. Tumor-infiltrating lymphocytes (TILs)

The concept of TILs was first described in 1986 to denote immune cells [mainly T, B and natural killer (NK) lymphocytes] within the TME, where they play a central role in anti-tumor immunity (8). TILs are established prognostic biomarkers in several malignancies, however, their prognostic relevance in OSCC remains controversial (4).

According to the International Immuno-Oncology Biomarker Working Group guidelines (5), TIL density should be reported as the percentage of the stromal area occupied by lymphocytes, a method originally developed for breast cancer and later extended to other tumor types (4). Nonetheless, the literature shows considerable variability in the cut-off values used to distinguish high from low TIL infiltration, with 20 and 30% thresholds being the most frequently adopted in the absence of formal consensus (7).

Furthermore, based on TILs spatial distribution within the TME, solid tumors can be classified into three major immune phenotypes: Immune-inflamed, immune-excluded and immune-desert (Fig. 1). Immune-inflamed tumors are characterized by a dense infiltration of immune cells within the tumor parenchyma. By contrast, immune-excluded tumors display immune cells confined to the peritumoral stroma, without effective infiltration of the tumor core. The immune-desert phenotype ('cold' tumor), exhibits an almost complete absence of immune infiltration. This may result from several mechanisms, including defective recruitment of antigen-presenting cells, impaired T-cell activation or migration and altered cytokine production (5,9). Low Human Leukocyte Antigen (HLA) and interferon- γ (IFN- γ) expression levels have also been reported in such tumors (10). Notably, in a study by Troiano *et al* (9), all OSCC cases with

an immune-desert phenotype were classified as stage IV and exhibited markedly poorer overall survival compared with the other immune profiles.

T lymphocytes. The main component of TILs is represented by T lymphocytes, which migrate from the bloodstream into the tumor as part of the body's immune response to cancer. These cells can be divided into three primary subpopulations: cytotoxic T cells, T helper (Th) cells and regulatory T cells (Treg) (6).

Cytotoxic T-cells, detected by the CD8 marker, can directly kill cancer cells and secrete tumoricidal cytokines (11). In OSCC, CD8+T cells outnumber CD4+T cells, meaning the CD4+/CD8+T ratio is reversed in the TME, compared with the matched peripheral blood (12). A large meta-analysis conducted by Huang *et al* (13) in 2019 on 2,698 OSCC cases demonstrated that high cytotoxic T-cells infiltration is a strong prognostic factor for improved overall survival (OS), independent of their spatial distribution. Wongpattaraworakul *et al* (11) also demonstrated that CD3, CD4 and CD8 were associated with OS and progression-free survival (PFS) in univariate analysis, however, none of these markers demonstrate significance in a multivariate analysis.

A higher CD8+/CD4+ T-cell ratio confers superior disease-specific (DSS) and disease-free survival (DFS) and a high CD8+/Forkhead box protein P3 (FOXP3)+ T-cell ratio correlates with OS and DFS (10,14,15). Although effector T cells are abundant in TILs, a number of display an exhausted phenotype [PD-1+, T-cell Immunoglobulin and Mucin-domain containing-3 (TIM-3)+] in tumor tissues, suggesting a partial inability to mount effective immune responses (12).

Th cells, identified by the CD4 marker, play a crucial role in modulating the adaptive immune response against cancer, particularly by promoting cytotoxic T-cell and B-cell proliferation and differentiation (16). However, Huang *et al* (13) in their meta-analysis demonstrate that most studies report contrasting results regarding their relationship with prognosis in OSCC, probably because CD4+ T cells represent a heterogeneous class divided into multiple subgroups (such as T1, T2, T9, T17, T22, Tregs and Tfh), with distinct and occasionally opposing roles that remain incompletely understood (16).

Tregs are a unique subset of CD4+ T lymphocytes characterized by expression of CD25 and Foxp3 molecules, representing up to 20-30% of the total CD4+ population within the TME. Tregs cells have an anti-inflammatory function through the production of immune-suppressive molecules such as interleukin (IL) 10, IL-35 and transforming growth factor- β (TGF- β), as well as by inhibiting proliferation and activation of other T cell subsets, NK cells, B cells and macrophages, inducing lymphocyte exhaustion via the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) protein (16,17).

Treg levels are associated with prognosis in different malignancies; however, their prognostic significance in OSCC remains inconsistent (18-20). Song *et al* (19) demonstrated in their study that Th17/Treg ratio was an independent prognostic factor for patient survival with greater predictive accuracy for OS. In contrast. By contrast, Ni *et al* demonstrates that tumor-infiltrating CD1a+ dendritic cells and CD8+/FOXP3+ ratios are not independent prognostic factors for tongue squamous cell carcinoma patients (14).

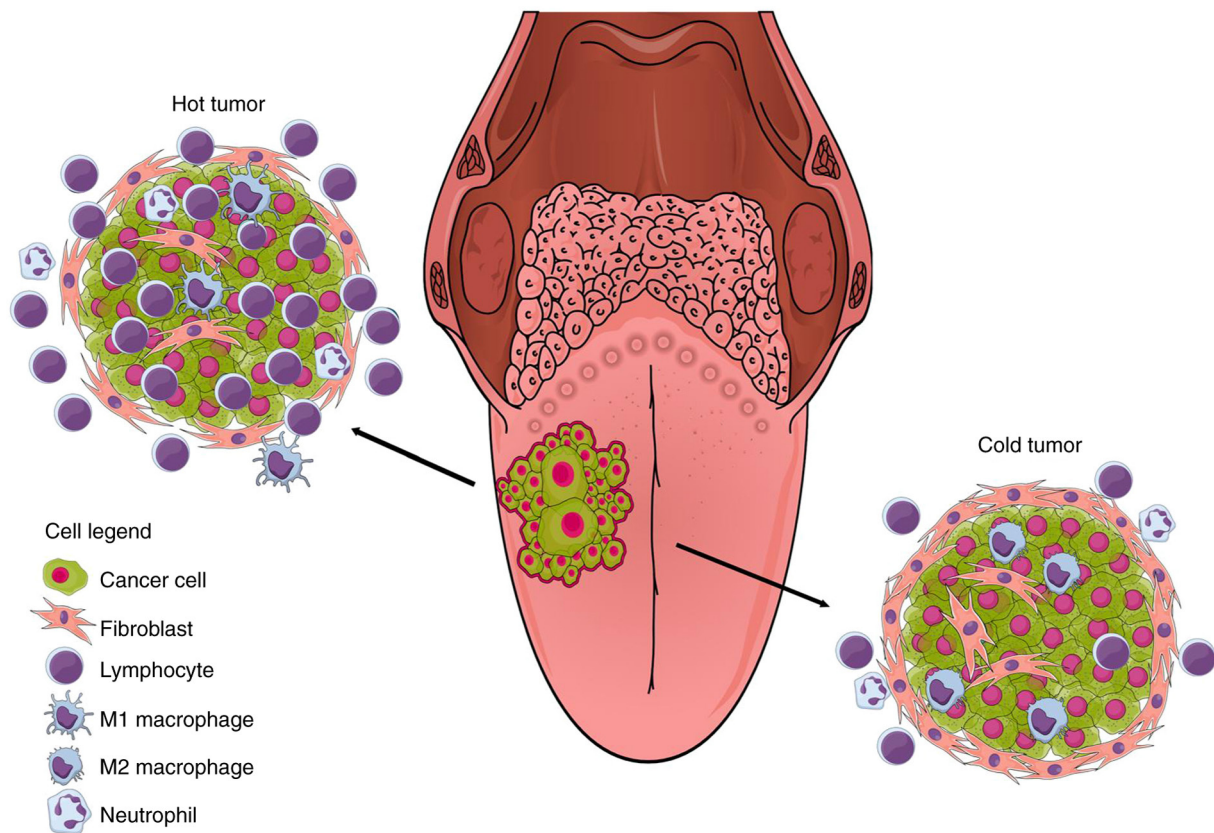


Figure 1. Contrasting immune landscapes in oral squamous cell carcinoma: the 'hot' and 'cold' tumor paradigms. The figure illustrates two distinct cancer immune profiles based on the composition of the TME: On the upper left the so-called 'hot tumor' (upper left), while on the bottom right the so-called 'cold tumor' (bottom right). Hot tumors are characterized by a substantial number of immune cells within the TME, primarily consisting of lymphocytes (particularly CD8+ T cells) and M1 macrophages, with low levels of CAFs. This profile includes activated and excluded immunophenotypes and is associated with a improved prognosis, indicating an active role of the immune system in coping cancer growth and spreading. In such cases, IC therapy may yield favorable outcomes, as it can unleash the lymphocytes activity already present around and/or within the tumor. By contrast, cold tumors are characterized by very low levels of lymphocytes, variable number of M2 macrophages and substantial fibrosis. This immuno-desert phenotype highlights the tumor's capacity to evade immune surveillance and correlates with a poorer prognosis. The 'choristers' that shape the TME toward either of these profiles are multiple and not yet fully known, though cytokines and chemokines secreted by cancer cells and other TME components (first of all transforming growth factor β) play a significant role. TME, tumor microenvironment; CAFs, cancer-associated fibroblasts; IC, immune checkpoint.

Notably, other investigations reported that high FOXP3+ levels are associated with improved prognosis (10,21,22). Several hypotheses have been proposed to explain how high FOXP3+ T-cell numbers may associate with improved outcomes despite their immunosuppressive activity. One possibility is that elevated FOXP3+ TIL expression reflects a generally higher T-cell infiltration, where the cytotoxic effects of CD8+ T cells outweigh Treg-mediated suppression, as suggested by the association between a high CD8+/FOXP3+ ratio and favorable prognosis (22).

Boxberg *et al* (10) further demonstrated that stromal Tregs (CD4+/FOXP3+) constitute an independent prognostic factor in OSCC. Patients with low stromal Treg density ('FOXP3-non-inflamed' OSCC) had poorer OS, DSS and DFS than those with high-density infiltrates ('FOXP3-inflamed' OSCC). A high FOXP3+/CD4+ ratio, indicating a greater proportion of Tregs among Th cells, was markedly associated with improved outcomes.

Two main hypotheses may justify this finding. First, Tregs may accumulate in a T-cell-inflamed environment with a functional effector T-cell infiltrate driven by IFN type I and II expression, linking to improved prognosis. Alternatively, Tregs

might have dual effects: Detrimental when inhibiting effector T cells, but beneficial when reducing protumor chronic inflammation, especially in inflammation-dependent cancers such as OSCC (10). This latter view is indirectly supported by a murine model of carcinogen-induced OSCC, where late-stage Treg depletion did not induce tumor regression but instead promoted rapid tumor growth; early depletion produced no measurable change (23). These observations indicate that Tregs play a complex, context-dependent role in malignancies, varying with tumor type, site, stage and TME factors.

A minor subset of T lymphocytes (<5%) within the TME is represented by $\gamma\delta$ T cells (unconventional lymphocytes with a heterodimeric T-cell receptor (TCR) composed of γ and δ chains). Their anti-tumor capabilities are increasingly supported by evidence. In this regard, Lu *et al* (24) confirmed that $\gamma\delta$ T-cell infiltration in tumor tissue can serve as an independent prognostic factor in head and neck squamous cell carcinoma (HNSCC); however, their prognostic role in OSCC remains unproven.

An inverse relationship exists between high T-cell infiltration and low expression of cancer stem cell (CSC) markers (NANOG, SOX2 and nestin) in OSCC, supporting

the association between high TIL counts and favorable prognosis (25).

Finally, studies demonstrated that TILs—particularly CD8+ T lymphocytes are abundant in oral premalignant disorders (OPMDs), suggesting that antitumor immune responses develop progressively during carcinogenesis. A significant positive association exists between TIL abundance and dysplasia grade (26,27), due to an increase in CD8+/CD57+ cells during OSCC development (28). Likewise, B-lymphocyte infiltration increases in tongue preneoplastic and neoplastic lesions according to transformation stage (29).

NK lymphocytes. NK cells are a subgroup of lymphocytes that exert anti-tumor activity directly and indirectly. Directly through degranulation of cytotoxic molecules such as perforin and granzyme and also by induction of tumor cell apoptosis via FasL or TNF-related apoptosis-inducing ligand (TRAIL) and indirectly via antibody-dependent cytotoxicity. They also secrete IFN- γ , activating other immune cells within the TME in a proinflammatory and immunostimulatory manner. NK cells constitute only a minor fraction of viable TILs and are identified by expression of CD56 and/or CD57 antigens.

Few studies have assessed their prognostic relevance in HNSCC and OSCC; however, NK cell infiltration consistently correlates with improved survival in both HPV-positive and HPV-negative tumors (13,20,30,31).

B lymphocytes. B cells infiltrating tumor tissues can be classified into three functional categories: Antigen-presenting, antibody-secreting and regulatory B cells. Upon antigen activation and clonal expansion, B cells exert antitumor effects through antigen presentation and antibody secretion; they are also abundant in tertiary lymphoid structures (TLSs). Conversely, regulatory B cells can suppress immune responses and promote tumor progression (32).

In OSCC, high expression of CD20+ B cells is generally associated with improved OS (12,33,34). Notably, a significant reduction in infiltrating CD20+ B cells has been observed in T3-T4 tumors, suggesting that decreased B-cell infiltration may accompany tumor progression. An inverse association between B-lymphocyte count and CSC markers has also been reported (34).

TLSs. TLSs represent ectopic lymphoid aggregates forming in nonlymphoid tissues, including the TME, in response to chronic inflammation and persistent immune stimulation. They have been described in multiple solid tumors, where their presence typically correlates with longer survival. TLSs facilitate germinal center reactions, anti-tumor immune responses mediated by T and B cells and promote lymphocyte recruitment through high endothelial venules (HEVs) (35).

In OSCC, TLS has been proposed as an independent prognostic factor, associated with improved clinical outcomes (32,35,36). TLS abundance follows a gradual increase across immune-desert, immune-excluded and immune-inflamed phenotypes and appears predictive of immunotherapy response (35). The combined presence of high CD8+ and CD57+ TIL densities further enhances prognostic accuracy (36).

TILs in tumor draining lymph nodes (TDLNs). A few studies have explored the role of T lymphocytes in TDLNs, the natural sites of primary immune response against the OSCC, with noteworthy findings. In a study by Kågedal *et al* (37), patients with low proportion of CD4+/CD69+ and high proportion of CD8+HLA-DR+ T cells in sentinel lymph nodes showed markedly increased recurrence and decreased DFS compared with those displaying the opposite pattern. CD69 marks recently activated T cells (hours post-activation), while HLA-DR marks longer-activated, potentially exhausted T cells (days post-activation). These data suggest that T-cell exhaustion or impaired activation occurs not only within the tumor but also in TDLNs, potentially contributing to disease progression. Consequently, such patients might benefit from closer clinical monitoring and/or immunotherapy with checkpoint inhibitors.

Similarly, Piersiala *et al* (38) demonstrated that patients with higher levels of Tregs and immune checkpoints expression (PD-1, TIGIT, CTLA-4 and TIM-3) in TDLNs had the highest recurrence risk, regardless of TNM stage. The authors proposed that these patients might derive the greatest benefit from neoadjuvant immunotherapy.

These findings offer a foundation for investigating the pathobiological mechanisms underlying locoregional metastasis and carcinomas of unknown primary origin, which in the head and neck region frequently localize to laterocervical lymph nodes (39,40).

3. Macrophages

Macrophages are innate immune cells characterized by remarkable functional plasticity. They can exhibit two main phenotypes: M1 (classically activated) and M2 (alternatively activated). M1 macrophages produce type I proinflammatory cytokines such as IL-1 β , IL-1 α , IL-12 and TNF- α . They contribute to tumor suppression by presenting tumor-specific antigen and eliminating cancer cells through reactive oxygen species production or antibody dependent cellular cytotoxicity (41).

Conversely, M2 macrophages secrete type II cytokines, including IL-4, IL-6 and IL-10, thereby promoting anti-inflammatory responses. They stimulate tumorigenesis, angiogenesis, matrix remodeling and metastasis and can be identified by surface markers CD163, CD204 and CD206. Macrophages are abundant in the TME of solid tumors, where they are termed tumor-associated macrophages (TAMs); most localize in the tumor stroma and are polarized toward the M2 phenotype (42).

Tumor cells drive M2 polarization of TAMs, while M2 TAMs in turn enhance tumor progression, establishing a positive feedback loop that reinforces malignancy. OSCC cells actively recruit peripheral monocytes to the TME via chemokines such as CCR7 (43) and promote M2 polarization through cytokines such as TGF- β (38), PAI-1 and IL-8 (44), as well as through tumor-derived extracellular vesicles (EVs) (43,45).

TAMs promote cancer progression directly and indirectly, they secrete growth factors (such as EGF, TGF- β and CCL2) that stimulate cancer cell proliferation and invasion, aided by metalloproteinases (MMPs) (46-48). M2 macrophage-derived EVs enhance OSCC malignancy by delivering miR-23a-3p, which promotes tumor progression

via phosphatase and tensin homolog (PTEN) targeting (46). Indirectly, TAMs shape an immunosuppressive milieu by expressing PD-1 ligands (PD-L1 and PD-L2) and immunosuppressive cytokines, thereby inhibiting lymphocyte function, promoting FOXP3+ Treg expansion and reducing immunotherapy efficacy (17).

TAM infiltration is also linked to increased microvessel density (MVD), facilitating angiogenesis, tumor growth and metastasis. TAMs secrete vascular endothelial growth factor (VEGF) and other molecules that promote endothelial cell proliferation, basement membrane degradation and migration, resulting in neovascularization (49,50). Furthermore, TAMs are enriched within lymph node metastasis (LNM), where they remodel the TME by activating fibroblasts and inducing T-cell exhaustion through SPP1-CD44 and CD155-CD226 interactions, aiding metastatic colonization (51).

Fusobacterium nucleatum (Fn), an oral commensal bacterium implicated in chronic periodontitis, also influences macrophage-tumor interactions. Nie *et al* (52) demonstrated that Fn activates the NF κ B pathway in OSCC cells and macrophages, inducing C-X-C motif chemokine ligand 2 secretion that enhances OSCC proliferation, migration and macrophage M2 polarization. Weber *et al* (53) reported increased macrophage infiltration and M2 polarization in transforming oral leukoplakia (OLP) compared with non-transforming OLP and normal mucosa, suggesting that M2 macrophages facilitate malignant progression by secreting growth factors. Smoking appears to promote this process by inducing M2 polarization (54).

TAMs also contribute to therapy resistance. HPV-negative HNSCC exhibits greater M2 macrophage infiltration than HPV-positive tumors. In this context, TAMs confer radioresistance by releasing heparin-binding EGF-like growth factor (HB-EGF), which activates the non-homologous end-joining (NHEJ) DNA repair pathway, promoting post-radiation survival (55). Similarly, EVs from M2 macrophages can decrease tumor sensitivity to fluorouracil and cisplatin by activating AKT/GSK-3 β signaling in cancer cells, leading to apoptosis resistance and enhanced proliferation (56).

Studies have shown that TAM density, particularly CD163+ M2 macrophages, is higher in HNSCC than in normal mucosa, associated with LNM and poor survival (20). A meta-analysis by Chohan *et al* (57) confirmed that elevated CD163+ TAM levels markedly associate with poor prognosis in OSCC, consistent with several other studies (42,58).

4. Neutrophils

Neutrophils are innate immune cells frequently found in solid cancers as tumor associated neutrophils (TANs). Similarly to macrophages, TANs may acquire either antitumor (N1), or pro-tumor (N2) activity depending on the cytokines profile of TME. N1 TANs express high levels of IL-12, tumor necrosis factor- α (TNF- α), TRAIL and chemokines CCL3, CXCL9 and CXCL10. Their antitumor effects occur directly, via phagocytosis, degranulation of antimicrobial peptides, generation of reactive oxygen species, or indirectly, through antibody-dependent cytotoxicity and activation of other immune cells (T cells, NK cells, B cells and dendritic cells) in a pro-inflammatory and immunostimulatory manner (59,60).

By contrast, N2 TANs promote tumor growth and dissemination by secreting extracellular matrix (ECM) remodeling enzymes, MMPs and pro-angiogenic factors such as VEGF and immunosuppressive cytokines (IL-10, TGF- β and IL-2). These mediators recruit Tregs, myeloid-derived suppressor cells (MDSCs) and cancer-associated fibroblasts (CAFs), enhancing tumor immune evasion (17,59,60).

TANs typically display an antitumor N1 phenotype at early stages but progressively shift toward a tumor-promoting N2 phenotype as cancer advances, consistent with the immunoeating theory. TGF- β plays a central role in this transition (61). This polarization model, initially identified in mice, has also been demonstrated in human peripheral blood. In cancer patients, high-density neutrophils (HDNs) and low-density neutrophils (LDNs) reflect N1 and N2 phenotypes, respectively. LDNs comprising immature MDSCs and mature HDN-derived cells in a TGF- β -dependent manner, appear transiently during self-limiting inflammation but accumulate persistently in cancer, exhibiting immunosuppressive functions unlike mature HDNs (62).

In OSCC, tumor cells and the TME regulate TAN recruitment, which reciprocally influences tumor growth and TME composition. Chemerin, a multifunctional adipokine secreted by OSCC cells, promotes neutrophil chemotaxis in a concentration-dependent manner. High chemerin expression correlates with increased TAN density, LNM, higher clinical stage, recurrence and reduced OS and DFS, thus serving as an independent prognostic factor (63).

Yu *et al* (64) demonstrated that TGF- β 1 and IL-17A synergistically induce N2 polarization, characterized by high MMP-9 and low CCL3 expression. The main sources of these cytokines are tumor and immune cells within the TME. N2 TANs facilitate OSCC progression by inducing epithelial-mesenchymal transition (EMT) via NF- κ B activation and releasing immunosuppressive cytokines. Circulating neutrophils in OSCC patients exhibit a phenotype analogous to TAMs (MMP-9 high/CCL3 low).

Another mechanism contributing to neutrophil-mediated tumor progression is the formation of neutrophil extracellular traps (NETs), web-like DNA structures decorated with histones and enzymes such as myeloperoxidase (MPO), neutrophil elastase (NE) and MMP-9. While MPO may exert cytotoxic effects and histones induce thrombosis (reducing tumor perfusion), NET proteases degrade ECM components, facilitating invasion and metastasis (65,66).

NETs have been detected in OSCC tissues by Garley *et al* (65,67) and Zhai *et al* (68) using multiplex immunohistochemistry (mIHC) co-localization of citrullinated histone H3 (citH3) and MPO. NET-rich tumors were associated with larger size and LNM, suggesting a role in disease progression. Also, *in vitro*, OSCC cells exposed to NET-containing conditioned media exhibited EMT-related gene upregulation, pyroptosis inhibition and increased proliferation and invasion, effects primarily attributed to NE (68).

The oral microbiota also modulates neutrophil-tumor interactions. *Streptococcus mutans*, which relocates from the mucosal surface to tumor tissue during OSCC development, stimulates cancer cells to produce the oncometabolite kynurenic acid (KYNA). KYNA reprograms the TME by expanding N2 TANs, which release IL-1 β , leading to CD8+

T-cell exhaustion and FOXP3+ Treg induction, thereby fostering tumor progression (69).

Across several solid cancers, a high TAN density in histologic sections and an elevated neutrophil-to-lymphocyte ratio (NLR) in peripheral blood are associated with advanced stage, LNM and poor prognosis, making them potential prognostic biomarkers (59,70). Studies in OSCC confirm that increased TAN infiltration and elevated NLR are markedly associated with worse clinical outcomes and reduced OS and PFS (63,70-72).

5. Mast cells

Mast cells (MCs) are tissue-resident myeloid cells of the innate immune system whose cytoplasm contains basophilic granules rich in histamine, leukotrienes, tryptase, chymase, heparin and inflammatory mediators. MCs are well known for their roles in allergic and autoimmune diseases but are also present in various solid tumors, where they exert tumor-promoting or tumor-suppressive effects depending on tumor type and activation state (73,74).

In OSCC, their role remains controversial. This inconsistency probably arises from variations in case selection, detection methods (such as toluidine blue, Alcian blue-safranin, or anti-tryptase antibodies) (75), MC subtypes (chymase+ vs. chymase-) and tumor site. Some authors reported a positive association between MC density and histological grade, lymphovascular invasion and depth of invasion (76), although its prognostic significance remains uncertain (77,78).

Cai *et al* (74) demonstrated in their findings that MCs are involved in oral cancer progression and may serve as a potential diagnostic and prognostic marker. Current evidence suggests that MCs are most active in the early stages of OSCC tumorigenesis. Shrestha *et al* (79) observed the lowest MC count in normal mucosa and significant increases in OPMDs with dysplasia and OSCC, indicating a role in malignant transformation. IL-1 released by MCs promotes epithelial proliferation in OLP, while histamine increases mucosal permeability, facilitating irritant infiltration and enhancing the risk of dysplastic transformation (79,80). Degranulated MCs also release TNF- α , which activates additional MCs, sustaining chronic inflammation and promoting carcinogenesis (81). By contrast, in a systematic review, Tzorakoleftheraki and Koletsa (78) described that some studies propose that mast cells may exert anti-tumor effects by enhancing immune responses against the cancer cells.

Several studies have reported an increase in MC density in OPMDs compared with OSCC, probably due to TME remodeling and impaired MC migration (79,82). Conversely, Iamaroon *et al* (83) and Michailidou *et al* (84) observed persistent or increased MC infiltration in OSCC, particularly within the lamina propria and invasive front, as well as in high MVD areas, supporting their role in invasion and angiogenesis.

MCs facilitate these processes by releasing proteases (tryptase, chymase) that activate MMPs, leading to ECM degradation and metastasis. They also promote angiogenesis through VEGF, tryptase, IL-8 and histamine release (81,85,86), explaining the observed increase in MVD during disease progression (83,87).

6. Fibroblasts

Fibroblasts are the most abundant non-immune cell type within the TME of solid neoplasms, where they are referred to as CAFs. In OSCC, as in other solid tumors, CAFs predominantly originate from quiescent oral mucosal fibroblasts activated by a variety of tumor-derived cytokines, notably TGF- β , angiopoietin-like protein 3 (ANGPTL3) (88) and platelet-derived growth factor-BB (PDGF-BB) (89). However, pericytes, adipocytes, endothelial cells and bone marrow-derived mesenchymal stem cells have also been identified as potential CAF progenitors in specific contexts (90,91). Most CAFs display a myofibroblastic phenotype, virtually absent in normal oral mucosa (92), similar to that seen in healing wounds and fibrotic disorders. The most commonly used markers for their identification include α -smooth muscle actin (α -SMA), integrin α 6 and fibroblast activation protein (FAP) (20). However, these markers lack complete specificity (being expressed by pericytes and smooth muscle cells) and sensitivity (as not all CAF subsets express them) (91).

Attempts to subclassify fibroblasts according to their phenotype, secretory profile, or gene expression have been made, but characterization of CAF heterogeneity in OSCC remains incomplete. CAFs probably represent a subpopulations spectrum with partially overlapping functions and the ability to interconvert (88). In OSCC, CAFs are generally tumor-promoting, with the following main roles: i) Production of cytokines and growth factors that support cancer growth and stimulate fibroblast activation; ii) provision of metabolic support to cancer cells; iii) promotion of tumor invasion; iv) stimulation of angiogenesis; v) facilitation of immune suppression; and vi) induction of therapy resistance (90-92).

Tumor support via soluble factors and extracellular vesicles (EVs). CAFs promote tumor growth through the secretion of soluble factors such as IL-1 β (93), activin A (94), hepatocyte growth factor (HGF) (95), epiregulin (96) and insulin-like growth factor-1 (IGF-1) (97), as well as by releasing EVs that enhance cancer progression. IL-1 secreted by OSCC cells increases CAF proliferation and the expression of secreted cytokines such as CCL7, CXCL1 and IL-8, all of which stimulate tumor cell proliferation *in vitro* (91). CAFs also produce abundant TGF- β , which drives cancer cell proliferation, invasion and metastasis while inducing the transdifferentiation of normal fibroblasts into CAFs, creating a positive feedback loop (98).

Metabolic reprogramming. OSCC cells profoundly reprogram CAF metabolism, converting them into factories that generate intermediate metabolites for tumor growth. A metabolic coupling arises between tumor cells and CAFs: The latter transfer mitochondria to cancer cells through exosome fusion or cytoplasmic bridges, enhancing oxidative phosphorylation (OXPHOS) in OSCC cells. Concurrently, CAFs undergo aerobic glycolysis ('reverse Warburg effect'), producing pyruvate and lactate that fuel OXPHOS and biosynthetic pathways in cancer cells (91,99-101). Elevated lactate levels induce pseudo-hypoxia in CAFs, leading to caveolin-1 (CAV-1) loss, hypoxia-inducible factor-1 α upregulation and secretion of pro-angiogenic cytokines. Indeed, a glycolytic CAF/tumor-stroma

phenotype correlates with higher MVD in OSCC (101). Regarding lipid metabolism, IL-8 produced by cancer cells activates AKT signaling in fibroblasts, leading to ATP citrate lyase phosphorylation and lipid synthesis. These lipids are released and taken up by OSCC cells via CD36, contributing to their metabolic flexibility (102).

Promotion of invasion and ECM remodeling. CAFs facilitate OSCC invasion through ECM remodeling and deposition. They secrete matrix MMPs that degrade type I collagen, enabling cancer cell invasion and produce laminin while reducing fibrillar protein synthesis, thereby strengthening tumor-ECM adhesion via integrins. CAFs also express high levels of hyaluronan synthase 2, which promotes invasion through hyaluronan production (91,103,104). Additionally, they increase ECM stiffness via lysyl oxidase (LOX), which cross-links collagen and elastin. Tumor cells sense this stiffened matrix, activating integrin-FAK signaling and downstream Wnt/ β -catenin (105) and YAP pathways (106), promoting EMT and migration. CAFs also contribute to bone invasion by producing receptor activator of NF- κ B ligand and promoting osteoclastogenesis (91,107).

Angiogenesis. CAFs are key mediators of neoangiogenesis through VEGF production, Notch pathway activation and recruitment of proangiogenic immune cells such as macrophages (91).

Immune suppression. CAFs promote immune evasion by producing immunosuppressive cytokines (TGF- β , IL-10, IL-6) (20), expressing PD-L1/PD-L2 and creating dense ECM barriers rich in collagen, fibronectin and proteoglycans (hyaluronan, versican) that physically restrict T-cell infiltration. They also induce macrophage polarization toward an M2 phenotype (91,108,109). A subset of OSCC-associated CAFs express tryptophan 2,3-dioxygenase (TDO2), which degrades tryptophan, essential for T-cell proliferation, thereby suppressing CD4⁺ and CD8⁺ T-cell activity. TDO2⁺ CAFs recruit these T cells via CXCL9/10/11 and subsequently induce CD4⁺ T-cell conversion to Tregs and CD8⁺ T-cell dysfunction (110).

Therapy resistance. CAFs contribute to cisplatin resistance in HNSCC by forming a collagen barrier that limits drug diffusion (91), sequestering drugs intracellularly (111) and secreting IL-11 (112) and EVs carrying miRNAs that induce chemoresistance-related gene expression in cancer cells (113).

High CAF density is consistently associated with poor prognosis in multiple cancers, including OSCC. Morphologic and immunohistochemistry (IHC) studies show that a high density of α -SMA-positive CAFs correlates with reduced patient survival. Although different cut-offs are used, high CAF density is typically defined by CAF predominance within tumor tissue. A recent meta-analysis by Graizel *et al* (114) integrating data from 11 studies confirmed high CAF density as an independent negative prognostic factor for OSCC, with particularly adverse effects in younger and female patients. Moreover, Li *et al* (115) demonstrated that podoplanin-positive CAFs are associated with poorer prognosis, possibly due to

enhanced secretion of TGF- β , MMP-2, MMP-9 and exosomal lncRNA FTX, which promotes apoptosis resistance.

7. Immunotherapy in OSCC

Inflammatory cells represent a principal component of the TME (116) and their interactions with cancer cells constitute a major mechanism enabling carcinomas to evade immune surveillance (5,117). Immunotherapy can be divided into two categories: Active, in which the immune system directly targets tumor cells, and passive, in which costimulatory mechanisms enhance immune activation through cellular receptors, conferring cytotoxic capacity. The discovery of immune checkpoints (ICs), which modulate inflammatory cell activity and consequently tumor progression, has profoundly transformed cancer therapy (118-120).

Among ICs, PD-1 and its ligand PD-L1 are key regulators of T-cell suppression and immune escape (121,122). PD-L1 is a transmembrane protein expressed on tumor and immune cells, including macrophages, dendritic cells and T cells, where it binds to PD-1 on activated lymphocytes to attenuate immune activity. This interaction downregulates TCR signaling, decreases cytokine production and induces T-cell apoptosis or exhaustion, thereby promoting immune tolerance within the TME (123-128). In OSCC, PD-L1 expression has been associated with tumor aggressiveness, advanced clinical stage, LNM and poor prognosis. Hypoxia, oncogenic pathways (such as PI3K/AKT and MAPK) and proinflammatory cytokines like IFN- γ are major inducers of PD-L1 transcription in tumor cells (129).

Importantly, PD-L1 also exhibits non-immune functions that contribute to oncogenesis. Its cytoplasmic domain interacts with downstream signaling molecules that promote proliferation, EMT and resistance to apoptosis. Elevated PD-L1 levels have been associated with increased resistance to radiotherapy and chemotherapy, highlighting its multifaceted role in therapeutic evasion (122). Moreover, PD-L1 expression on exosomes released by tumor cells can suppress immune surveillance at distant sites, enabling systemic immunosuppression and facilitating metastatic dissemination. Thus, PD-L1 serves not only as a predictive biomarker for immune checkpoint blockade but also as a dynamic effector of tumor progression and immune modulation in OSCC (129).

Over the years, in order to identify patients who could benefit from this treatment, researchers have investigated possible cut-offs of PD-L1 expression showing a significant association between their levels and response to treatment (128-131). However, such studies revealed different cut-offs of IHC expression in tumor cells and/or in TME (130-132). Considering the different scoring systems used for the quantification of PD-L1 expression, which represents the gold standard in a number of solid tumors, the combined positive score (CPS), that evaluates through a mathematical formula its expression in tumor cells and immune cells (125), currently is recommended for the suitability for immunotherapy of patients with OSCC (125,126).

The PD-1 blockade agents, pembrolizumab (133) and nivolumab (134), have been approved for metastatic OSCC following important clinical trials. The KEYNOTE-048 phase III trial enrolled 882 patients with recurrent or metastatic

HNSCC (339 oral primaries) and showed that pembrolizumab, either alone or combined with platinum and 5-fluorouracil, achieved a statistically significant survival advantage compared with standard therapy (EXTREME regimen) in PD-L1-positive cases (CPS ≥ 1) (133). After a median follow-up of 45 months, OS improved with pembrolizumab monotherapy in the PD-L1 CPS ≥ 20 [hazard ratio (HR) 0.61; 95% CI 0.46-0.81] and CPS ≥ 1 (HR 0.74; 95% CI 0.61-0.89) groups and was noninferior in the overall population (HR 0.81; 95% CI 0.68-0.97). OS also increased with pembrolizumab-chemotherapy in all CPS categories (≥ 20 , ≥ 1 and total), with HRs ranging from 0.62-0.71 (135). The CheckMate-141 trial enrolled 361 patients (175 oral cavity primaries) with recurrent HNSCC progressing within six months of platinum-based therapy. Nivolumab markedly improved OS compared to standard agents (HR 0.68; 95% CI 0.54-0.86) after 24.4 months of follow-up (136).

Expanding immunotherapy to earlier disease stages is a current focus. In a multicenter phase II study, pembrolizumab administered preoperatively to 36 patients with locally advanced, non-HPV-related HNSCC improve DFS to 17% at 12 months in high-risk patients. An association between partial tumor response (pTR1-2) and PD-L1 expression was observed, suggesting that pTR could predict favorable outcomes even with a single neoadjuvant dose (137). Another phase II study using two preoperative pembrolizumab cycles reported 1-year DFS rates of 97% (95% CI 71-90%) in intermediate-risk and 66% (95% CI 55-84%) in high-risk groups (138). These findings were confirmed in a subsequent phase III trial (139) and align with results from other solid malignancies (140,141).

Ongoing studies continue to explore combination strategies. Cemiplimab, another PD-1 inhibitor, is being tested with platinum-doublet chemotherapy and cetuximab in locally advanced HNSCC (142). A phase II trial evaluated camrelizumab (anti-PD-1) with nab-paclitaxel and cisplatin as neoadjuvant therapy in 48 patients (16 oral cavity primaries), achieving an overall response rate (ORR) of 89.6% (95% CI 80.9-98.2) (143).

Following the success of PD-1/PD-L1 blockade, novel immune checkpoints are under investigation to further enhance anti-tumor immunity (144,145). Tumor necrosis factor receptor superfamily member 4 (OX40), a T-cell costimulatory receptor, has shown promise: in a phase Ib study, Duhon *et al* (146) reported increased CD4⁺ and CD8⁺ lymphocyte infiltration in peripheral blood and post-surgical specimens following neoadjuvant anti-OX40 (MEDI6469) therapy in locally advanced HNSCC.

Other checkpoint inhibitors under study include antibodies targeting CTLA-4, TIM-3, lymphocyte-activation gene 3 (LAG-3) and IDO, although clinical evidence in OSCC remains limited. CTLA-4 (CD152), expressed on CD4⁺ and CD8⁺ T cells, regulates immune homeostasis by inhibiting T-cell activation; its blockade may enhance recognition and elimination of neoplastic cells (146-148). Combination regimens such as nivolumab plus ipilimumab (anti-CTLA-4) and nivolumab plus relatlimab (anti-LAG-3) are currently being evaluated (NCT04080804) (149).

In the context of precision oncology, further modulation of IC pathways, including LAG-3 and TIM-3 blockade, has demonstrated in murine models the capacity to restore

CD8⁺ T-cell activity and suppress tumor growth (146,149). Additional immunostimulatory approaches include cytokine-based therapies using IL-2, IL-1 β , IFN- γ and TNF- α , which enhance immune activation and inhibit tumor-induced T-cell apoptosis (150). Monoclonal antibodies targeting Tregs (151) or TAMs (152) also hold potential. These findings are supported by the antitumor activity of IL-2 and IFN- α in enhancing T and NK cell responses within the TME, as demonstrated in other solid tumors (153,154).

Genetic susceptibility may influence immune modulation. For instance, Chien *et al* (155) found that the IL-23R rs10889677 polymorphism (C allele) conferred a 1.5-fold higher risk of oral cancer and associated with LNM. Furthermore, decreased IFN- γ protein and mRNA expression were shown to negatively be associated with OSCC development (156).

Emerging approaches include adoptive cellular immunotherapy, involving large-scale engineering of tumor-reactive immune cells and therapeutic vaccines. A phase II study evaluating cemiplimab with ISA-101b (targeting HPV16 E6/E7 oncoproteins) in 198 patients demonstrated an ORR of 25.3%, compared with 22.9% in controls. Patients with CPS ≥ 20 treated with the combination achieved markedly higher response rates and overall survival (157).

8. Future directions

The growing understanding of the TME in OSCC has transformed the perception of this malignancy from a purely epithelial disorder to a complex ecosystem of cellular and molecular interactions: CAFs, immune cells and ECM components form a dynamic and interdependent network that governs tumor progression, therapeutic resistance and patient outcomes.

Emerging evidence highlights that immune phenotypes ranging from 'immune-inflamed' to 'immune-desert' micro-environments strongly determine prognosis and response to immunotherapy. Patients with high densities of CD8⁺ T cells, TLSs and NK cells, tend to experience improved OS and favorable responses to PD-1/PD-L1 blockade. Conversely, tumors enriched with M2-polarized macrophages, NETs and CAF-driven fibrosis exhibit immune exclusion, metabolic stress and therapy resistance. Prospective studies on composite biomarkers, should validate integrated scores that combine CPS (PD-L1), immune contexture (CD8⁺/FOXP3⁺ ratios, TLS density), stromal load (α -SMA⁺ CAF index) and systemic inflammation (NLR) to stratify patients for immunotherapy and combination regimens (primary endpoints: OS/DFS; secondary: On-treatment immune remodeling signatures). It may be also possible to pair PD-1/PD-L1 inhibitors with agents that counter macrophage-mediated radioresistance (such as HB-EGF/NHEJ pathway modulators) and with ECM-softening strategies to improve T-cell trafficking. In CAF-directed stroma modulation research, early-phase trials should test inhibitors of TGF- β /IL-6 signaling, LOX-mediated stiffness, or FAP-targeted agents, alone or layered onto PD-1 blockade, to convert immune-excluded tumors into inflamed responders (biopsy-paired spatial readouts recommended). Furthermore, apply single-cell and spatial transcriptomics/proteomics on longitudinal (pre-/post-therapy) specimens to map niches of immune exclusion, CAF heterogeneity (such as

Table I. Key mechanisms, immune phenotypes and prognostic associations in OSCC tumor microenvironment.

Component	Main mechanisms/functions	Key molecules/markers	Prognostic/clinical association
Tumor cells	<ul style="list-style-type: none"> - PD-L1-mediated immune evasion - Secretion of TGF-β, IL-6, IL-8 promoting CAF activation, EMT, therapy resistance - Release of EVs carrying PD-L1, miRNAs 	PD-L1, TGF- β , IL-6, IL-8, EVs	High PD-L1 CPS (≥ 1 or ≥ 20) \rightarrow improved response to pembrolizumab/nivolumab; high PD-L1 heterogeneity limits predictive accuracy
TILs	<ul style="list-style-type: none"> - CD8⁺ cytotoxic killing - CD4⁺ Th cell modulation - Tregs suppress immune responses via IL-10, TGF-β, CTLA-4 	CD3, CD4, CD8, PD-1, CTLA-4	High CD8 ⁺ and CD8 ^{+/+} ratio \rightarrow \uparrow survival Immuneinflamed phenotype \rightarrow good prognosis Immune-desert phenotype \rightarrow poor prognosis (Stage IV)
B cells/TLSs	<ul style="list-style-type: none"> - Antigen presentation, antibody production - Formation of tertiary lymphoid structures (TLSs) 	CD20, TCL1A	High CD20 ⁺ B cells and TLS abundance \rightarrow \uparrow survival and immunotherapy response
NK cells	<ul style="list-style-type: none"> - Direct tumor killing (perforin, granzyme) - Indirect activation of immune cells 	CD56, CD57	High NK infiltration \rightarrow improved OS, both HPV+ and HPV- OSCC
Macrophages (TAMs)	<ul style="list-style-type: none"> - M1 (IL-12, TNF-α): antitumor - M2 (IL-10, TGF-β, VEGF): pro-tumor - Promote EMT, angiogenesis, immune suppression 	CD68, CD163, CD204, CD206	High CD163 ⁺ M2 TAMs \rightarrow poor OS, LNM, radioresistance
Neutrophils (TANs)	<ul style="list-style-type: none"> - N1: cytotoxic and proinflammatory - N2: immunosuppressive (via TGF-β, IL-17A) - Form NETs promoting invasion 	MPO, NE, MMP-9, CXCL2	High TANs or NLR \rightarrow poor OS and PFS Chemerin expression \rightarrow independent poor prognostic factor
Mast cells	<ul style="list-style-type: none"> - Release histamine, tryptase, VEGF - Promote angiogenesis and ECM degradation 	Tryptase, Chymase, VEGF	Controversial: May promote invasion (\uparrow density in OSCC) or early immune activation in OPMDs
Cancer-associated fibroblasts (CAFs)	<ul style="list-style-type: none"> - Activated by tumor cytokines (TGF-β, PDGF-BB, ANGPTL3) - ECM remodeling (MMPs, LOX) - Reverse Warburg effect (metabolic support) - Immunosuppression and drug resistance 	α -SMA, FAP, integrin $\alpha 6$, LOX, MMPs, IL-6, TDO2	High α -SMA ⁺ CAF density \rightarrow independent poor prognostic factor Podoplanin ⁺ CAFs \rightarrow worse OS
Immune checkpoints and therapy	<ul style="list-style-type: none"> - PD-1/PD-L1 interaction \rightarrow T-cell exhaustion - PD-L1 cytoplasmic signaling promotes EMT, proliferation - New targets: CTLA-4, OX40, LAG-3, TIM-3 	PD-1, PD-L1, CTLA-4, LAG-3, OX40	Pembrolizumab/Nivolumab improve OS in CPS ≥ 1 tumors (KEYNOTE-048, CheckMate-141) combination regimens under trial

PD-L1, programmed death-ligand 1; TGF- β , transforming growth factor β ; IL, interleukin; CAFs, cancer-associated fibroblasts; EMT, epithelial-mesenchymal transition; EVs, extracellular vesicles; miRNA, microRNA; CPS, combined positive score; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; FOXP3, Forkhead box protein P3 TLS, tumor-infiltrating lymphocytes; TCL1A, T-cell leukemia/lymphoma 1A; OS, overall survival; NK, natural killer; HPV, human papillomavirus; OSCC, oral squamous cell carcinoma; TAMs, tumor-associated macrophages; LNM, lymph node metastasis; MPO, myeloperoxidase; NE, neutrophil elastase; MMP, metalloproteinase; CXCL2, C-X-C motif chemokine ligand 2; NLR, neutrophil-to-lymphocyte ratio; PFS, progression-free survival; VEGF, vascular endothelial growth factor; ECM, extracellular matrix; OPMDs, oral premalignant disorders; α -SMA, α -smooth muscle actin; FAP, fibroblast activation protein; LOX, lysyl oxidase; OX40, tumor necrosis factor receptor superfamily member 4; LAG-3, lymphocyte-activation gene 3; TIM-3, T-cell Immunoglobulin and mucin-domain containing-3.

TDO2⁺ subsets) and treatment-induced state shifts that predict durable benefit. In addition, mechanistic and interventional studies on microbiome-immune intervention (such as targeted oral microbiome modulation) should test whether reshaping dysbiosis attenuates N2-skewing and T-cell exhaustion in OSCC.

The summarized data presented in Table I provided an integrated overview of these cellular players, their functional mediators and their prognostic associations within the OSCC microenvironment.

Together, these data position the CAF-myeloid-PD-L1-oral microbiome-axis as a unifying framework for prognosis and therapeutic design in OSCC.

9. Conclusions

Translating TME biology into practice will require composite diagnostics and mechanism-based combinations that are tested in biomarker-anchored, biopsy-paired trials. Aligning multi-omics, digital pathology and immuno-oncology within these designs offers the most direct path to durable benefit for patients with OSCC. In conclusion, the OSCC microenvironment represents both a challenge and an opportunity for precision oncology. Targeting the interplay between tumor cells, stromal components and immune effectors holds the potential to overcome therapeutic resistance and improve long-term outcomes. Future studies integrating multi-omics, digital pathology and immuno-oncology will be essential to translate these biological insights into actionable clinical strategies.

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