Review Article

# Tumour Microenvironment as a Potential Immune Therapeutic Target for Tongue Cancer Management

Mahija Janardhanan[1], Smitha NV[2], Rajalakshmi G[3], Antony George[4], Manzoor Koyakutty[5], Subramania Iyer[3]

[1]Department of Oral and Maxillofacial Pathology, Amrita Vishwa Vidyapeetham, Amrita School of Dentistry, Kochi, Kerala, [2]Department of Pathology, Amrita Vishwa Vidyapeetham, Amrita Institute of Medical Sciences Kochi, Kerala, [3]Head and Neck Oncology, Amrita Vishwa Vidyapeetham, Amrita Institute of Medical Sciences, Kochi, Kerala, [4]Department of Oral Pathology, MES Dental College, Malappuram, Kerala, [5]Amrita Centre for Nanosciences and Molecular Medicine, Amrita Vishwa Vidyapeetham, Amrita Institute of Medical Sciences, Kochi, Kerala, India

## Abstract

Immunotherapy is a promising approach in the management of human cancers and has been proven to provide a durable response in many cancers. It is helpful as an adjuvant therapy for cancers and at present is considered as a fourth pillar supporting surgery, chemotherapy and radiotherapy. In the treatment of oral cancer, immunotherapy is approved in late-stage diseases where surgical resection cannot be carried out or fails, leading to recurrences and metastasis. Evidences suggest that when given as a first-line treatment, it can elicit an immune response that shrinks tumours, which could provide long-term benefit for patients. But unlike the traditional approach which follows the uniform protocol for all oral cancer patients, effective immunotherapy requires a more site-specific personalized approach. The aim of this paper is to review the various immune evasive mechanisms adopted by tumour cells and their relevance as potential targets for immunotherapy in oral tongue squamous cell carcinoma.

**Keywords:** Immune modulation, MDSCs, PD-1/PD-L1, TAM, TIM-3, tongue cancer, Tregs

**Address for correspondence:** Dr. Subramania Iyer, Department, Head and Neck Oncology, Amrita Vishwa Vidyapeetham, Amrita Institute of Medical Sciences, Kochi, Kerala - 682 041, India.
E-mail: subu.amrita@gmail.com

**Submitted:** 16-Mar-2023, **Revised:** 30-Mar-2023, **Accepted:** 13-Apr-2023, **Published:** 13-Jul-2023

## INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the eighth common cancer worldwide.[1] The most commonly affected sub-site is oral tongue[2] with oral tongue squamous cell carcinomas (OTSCC) making up around 22–49% of all oral cancers diagnosed. Data from across the world suggests that a progressively increasing incidence of OTSCC noticed in among young adults who are never smokers or drinkers is now emerging as a serious health concern. An alarming increase in the incidence reported across the world, occurrence at a younger age and lack of a specific etiological factor which precludes the scope for prevention in a subset of population affected, stress the need for a durable treatment protocol that may offer a potential cure, an utmost priority in the management of tongue cancers.

The introduction of immune checkpoint blockades which induces a long-term durable response was a major breakthrough in cancer immunotherapy. US Food and Drug Administration-approved monoclonal antibodies targeting immune checkpoint receptors, anti-CTLA-4

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.jomfp_134_23



**How to cite this article:** Janardhanan M, Smitha NV, Rajalakshmi G, George A, Koyakutty M, Iyer S. Tumour microenvironment as a potential immune therapeutic target for tongue cancer management. J Oral Maxillofac Pathol 2023;27:382-9.

and anti-programmed death-1 (anti-PD-1) are evolving as a promising therapeutic approach in many cancers including head and neck cancers. Other co-inhibitory receptors are also being evaluated as potential targets for cancer immunotherapy. Emerging data from various clinical trials demonstrating unprecedented response rate and survival advantage is very encouraging, but at the same time suggests that a personalized combination immunotherapy targeting multiple pathways may be needed to provide more effective management. In this review, we discuss the various immune evasive mechanisms adopted by tumour cells and their relevance as potential targets for immunotherapy in OTSCC.

## Immune modulation—a promising cancer treatment modality

The knowledge that cancer progression is not controlled by intrinsic genetic changes of cancer cells alone and a complex dynamic interaction between the components of tumour microenvironment (TME) and cancer cells are critical for the initiation and maintenance of tumorigenesis[3] had resulted in a recent shift of approach from attacking the tumour cells directly to targeting the TME. The TME is composed of multipotent stromal cells/mesenchymal stem cells, fibroblasts, blood vessels, endothelial cell precursors, immune cells and secreted factors such as cytokines. The immune cells form an essential component of TME, and the immune status of the TME has a decisive role in determining the behaviour of a tumour. The tumour cells, which are the altered normal cells, harbour surface non-self-proteins, induce anti-tumour response, apart from the self-proteins which are capable of inducing immune tolerance. Hence, the inflammatory cells in the TME may evoke anti-tumorigenic response by killing cancer cells or protumourigenic activities keeping an immunosuppressive TME which favours tumour progression. Those cells which survive the anti-tumour response are not recognized by the immune cells as they undergo immunoediting, losing the expression of antigens and also by interfering with the antigen-presenting machinery. Thus, they become more 'self' than 'non-self' and hence induce immune tolerance that helps them evade elimination by host immune system by creating an immunosuppressive TME.

A major focus of tumour immunology is to understand the immune evasion mechanism with the goal of developing therapeutic approaches that target immune evasion. Cytotoxic lymphocytes (CTLs) are the most important contributors to host immune defence against tumours. The primary function of immunosurveillance is carried out by the cytotoxic T lymphocytes by recognizing and killing potentially malignant cells. In a normally functioning immune system, the antigen is processed and presented to the lymphocytes by antigen-presenting cells in the context of major histocompatibility complex (MHC). As the lymphocytes do not possess the inherent capacity to distinguish between foreign and self-antigens, self-recognition is established by incorporating the molecular self-antigen system into the antigen recognition phase. The binding of antigenic peptide to the T cell receptors (TCR) initiates antigen-specific signals which results in clonal T cell proliferation. But for T cells to respond effectively, a second signal provided by co-stimulatory molecules is required and the co-stimulatory signals are principally delivered by the engagement of CD28 receptor on T cells by the ligands CD 80/86 (B7.1/B7.2) on antigen presenting cells (APCs).[4] T cell clonal expansion, recruitment of cytotoxic T cell response, generation of humoral response and cytokine release for effector cell proliferation follow and maintain the overall immune activation [Figure 1]. The co-stimulatory pathway is balanced by numerous co-inhibitory pathways which operate through negative immune regulatory molecules dampening T cell activation and controlling unnecessary tissue damage.[5,6] Together this co-signalling pathway which either potentiates or dampens the resultant immune response is known as immune checkpoints and activation of co-stimulatory or blockade of co-inhibitory pathways result in enhanced immune response. As stimulation of effector mechanism leads to severe side effects due to over stimulation of immune cells in non-tumoural organs, targeting the inhibitory mechanisms which evade host immunity seems to be a more promising approach for treating cancers. Also, as the failure of tumour antigen-specific or non-specific immunotherapies had been mainly attributed to immunosuppression induced by cancer cells, reversing the immunosuppression becomes crucial for better responses to immune-based treatment modalities. The manipulation of immune checkpoints and immune signals from the tumour-induced immunosuppressive cells in the TME seem to offer promising benefits. Till recently squamous cell carcinoma arising from various oral sub-sites had been considered as a single entity. But evidences suggest that there exists a molecular heterogeneity within the anatomic sub-sites highlighting the need to approach OTSCC as a distinct entity. Though more clarity is being added to the mechanisms of immunosuppression and immune evasion in head and neck carcinomas in general, studies pertaining to a specific intraoral sub-site are limited.

## The immune checkpoints of relevance in oral tongue squamous cell carcinoma

### *PD-1/PD-L1*

PD-1 is a type I transmembrane protein of the CD28 receptor family encoded by the PDCD1 gene, located on

chromosome 2q37. It is expressed on activated T and B cells, monocytes and a subset of thymocytes. PD-1 is expressed on a T lymphocyte upon activation, and T cell activation is regulated through the interaction with its ligands PD-L1 and PD-L2 which are expressed widely in non-lymphoid tissue.[7] PD-L1, located on human chromosome 9 p24.2, belongs to the B7 family and is expressed on lung, vascular endothelium, reticular fibroblasts, non-parenchymal liver cells, mesenchymal stem cells, islet cells, astrocytes, neuronal cells and keratinocytes. PD-L2 expression on the other hand in line with its function of regulating T cell priming is restricted to dendritic cells. On engagement with its ligands, PD-1 can activate intracellular signalling pathway which delivers inhibitory signals capable of decreasing cytokine production and inducing T cell anergy or apoptosis thereby dampening the T cell activation [Figure 2]. Immune regulation by PD-1/PD-L1 signalling pathway is carried out by several distinct mechanisms. The binding of PD-1 with PD-L1/L2 inhibits the PI3K/AKT pathway which downregulates expression of anti-apoptotic gene Bcl-xl lowering the threshold for T cell apoptosis.[8] It also restricts naive T cell migration and accumulation in APCs and downregulates TCR signalling preventing effective antigen presentation. PD-1–PD-L1/2 ligation also upregulates expression of gene PTEN causing blockade of AKT/mTOR/S6 pathway and converts Th1+ CD4+ T cells to become FOXP3+ Tregs that suppress the effector immune response.[9] In tumours like head and neck cancers, PD-L1 expression in tumour cells gets upregulated allowing the cancer cells to escape from host immune system by inactivating T cell immune surveillance. The upregulation of PD-L1 expression in cancers is believed to be occurring by two different mechanisms termed intrinsic and adaptive immune resistance which may co-exist within the same TME. Intrinsic mechanism refers to the induction of PD-L1 expression resulting from genetic mutation or activation of certain signalling pathways like AKT pathways and STAT3. Adaptive immune resistance on the other hand refers to the induction of PD-L1 on tumour cells by cytokines like IFN $^{\gamma}$ as an adaptive response of tumour cell on sensing inflammatory immune microenvironment that threatens the tumour.[10]

In the clinical setting, the high tumour expression of PD-L1 and/or tumour immune infiltration by PD-1-positive T lymphocytes had been considered as an indicator of tumour evasion. Preliminary analyses indicate that PD-L1 is expressed in 50% to 60% of HNSCCs.[11]

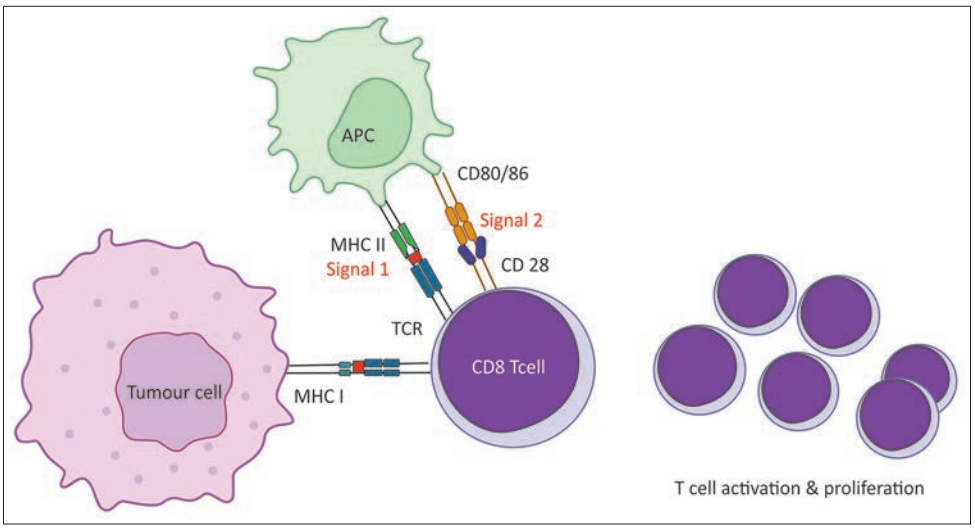


**Figure 1:** Activation of cytotoxic lymphocytes on antigenic challenge.

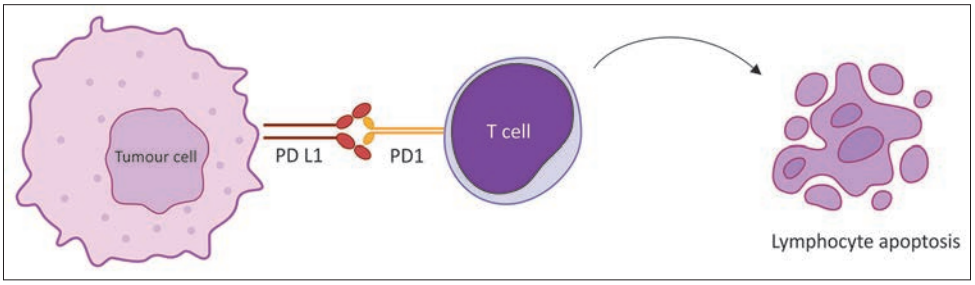


**Figure 2:** Dampening of lymphocyte activation on PD-1/PD-L1 binding.

Increased expression of both PD-1 and PD-L1 had been reported in most of the studies conducted in OSCC. Overexpression of PD-L1 in tumour cells which showed positive correlation with PT1 and pT2, and at the same time, no association with the overall survival suggested their role during the initial phases in OTSCCs.[12] An early time limited but beneficial response to PD-1 antibody treatment that failed with continued lesion progression had also been observed in a carcinogen-induced premalignant oral lesion animal model that progressed to oral cancer[13] suggesting their potential utility in preventing malignant transformation. But contrary to these findings, a significant association of PD-1 and PD-L1 expression with local recurrence and a significant decrease in 5-year disease-specific survival rate for patients with combined PD-1+/PD-L1+ expressions had also been documented in OTSCC.[14] A close correlation of PD-L1 expression of tumour cells with moderate and high levels of CD4+ & CD8+ tumour infiltrating lymphocytes in the TME was observed in OTSCC and the abundance of CD4+ TIL which co-localized with PD-1/PD-L1/CD68 more frequently than CD8+ TIL noted in the study, suggested their importance as pivotal regulators of PD-L1 levels and in determining the responsiveness of OTSCC to PD1-based immune checkpoint therapy.[15] Downregulation of PD-L1 expression by tumour cells responding to curcumin therapy indicating reversal of immune inhibition was observed in OTSCC cell lines and animal models.[16] It was also noted that PD-1/PD-L1 immune inhibition can be reversed by improving cytokine-induced killer cells (ICIKs) transfer which may be used as an effective therapy for tongue cancers.[17]

*T cell immunoglobulin and Mucin 3 (TIM-3)*

T cell immunoglobulin mucin 3 is a negative regulator of Th1 immunity and plays an important role in maintaining peripheral tolerance. TIM-3 is expressed on Th1 CD4+ lymphocytes, Tc1 CD8+ lymphocytes, Tregs, dendritic cells, NK cells and monocytes. The extracellular portion of TIM-3 has an immunoglobulin domain and mucin domain which acts as an immune checkpoint molecule. However, the mechanism of TIM-3 in regulating immunosuppression in head and neck squamous cell carcinoma (HNSCC) is still not quite clear. Four ligands binding to TIM-3, namely Galectin-9, PtdSer, HMGB1 and CEACAM1, had been identified. Upon interaction with Galectin-9 or other undefined ligands, TIM-3-expressing T cells undergo apoptosis and lose effector functions. Experimental studies had shown that administration of Galectin-9 *in vitro* causes cell death of Th1 cells in a TIM-3-dependent manner.[18] Attempts to assess the role of TIM-3/Galectin pathway in oropharyngeal carcinomas revealed increased expression of Gal-9 by CD4+ T cells in HPV positive cases. Further, it was found that co-culturing monocytes with high Gal-9-expressing CD4+ T cells resulted in the expansion of TIM-3+ monocytes, which suppressed interferon gamma production by activated CD8+ T cells and secretion of both interleukin 10 and interleukin 12 by monocytes which could be reversed by blocking TIM-3 and/or Gal-9.[19] TIM-3 nearly universally co-expressed with PD-1 on majority of tumour infiltrating lymphocytes and co-expression of both checkpoints in T cells were associated with reduced ability to proliferate, secrete IFN $\gamma$, IL-2 and TNF $\infty$ reflecting a more exhausted phenotype.[20] Blockade of TIM-3 increases production of IFN $\gamma$ and TNF$\alpha$ and acts synergistically when combined with PD-1 blockades and combined blockade had been found to be more effective in controlling tumour growth in preclinical experimental models.[18] High expression of TIM-3 which could be reversed on treatment with anti-TIM-3 was noticed on TILs in anti-PD-1-resistant murine tumour models, and a significant increase in median survival time was noticed in tumour-bearing mice following the combined therapy.[21] Targeting TIM-3 has been found to induce anti-tumour immune response by depleting MDSCs in murine models.[22] Previous studies had also shown a close association between TIM-Galectin pathway and blockade of TIM-3 by the anti-TIM-3 monoclonal antibody enhanced anti-tumour immune response by reducing Tregs.[23]

**Immunosuppressor cells of TME as potential targets in OTSCC**

In OSCC, the invasion of the underlying connective stroma by malignant epithelial cells invokes an inflammatory response leading to a heavy infiltration of the area by various inflammatory cells. Among the tumours of head and neck, more inflammatory response is noticed in OTSCC.[24] The tumour cells induce the recruitment of immunosuppressive cells which include Tregs, MDSCs and TAM which accumulate in the TME and promote tumour growth, and downregulate antitumor responses.

*Regulatory T cells (Tregs)*

Tregs are regulatory T cells, a subpopulation of CD4+ T lymphocytes that suppress the expansion of effector cells against self and maintain self-tolerance. There are accumulating evidences that Tregs play a substantial role in inducing and maintaining an immunosuppressive TME in various human cancers, including gastric, lung, breast, colorectal and HNSCC.[25] Tregs carry out immunosuppressive function by causing anergy, apoptosis and cell cycle arrest of activated T cells through inhibitory receptor cell contact (PD-1/PD-L1

interaction) or secreted factors such as IL-10, TGF-β, IL-27 and IL-35.[26] Two Tregs population had been identified so far naturally occurring CD4+ CD25+ Tregs and antigen-induced IL-10-secreting Tregs. In TME, these two types are thought to be opting two different ways to suppress the host immune response. Naturally occurring CD4+ CD25+ Tregs specifically expresses transcription factor, forkhead box P3 (FOXP3) which is the 'master regulator' of Tregs regulatory functions. They can inhibit proliferation and cytokine production by effector cells in an antigen-nonspecific, cytokine-independent, but cell–cell contact-dependent manner.[27] Antigen-induced IL-10 secreting Tregs on the other hand are induced by IL-10, and they produce high levels of IL-10 and suppress proliferation and cytokine production of effector cells in an IL-10-dependent manner.[28] Previous studies in various cancers including head and neck cancers had shown that Tregs help in tumour progression and metastasis and increase in Tregs predict worse survival. But improved prognosis associated with high level of Tregs had also been reported. The role of Tregs may vary with sub-site, and it has been suggested that OSCC being more closely associated with chronic inflammation than other HNSCCs, infiltration of the TME by Tregs may play a role in preventing tumour cell invasion and metastasis through the inhibition of inflammatory processes.[29] Though most of the studies conducted in OSCC showed a consistent increase in the number of infiltrating Tregs, the significance of this increase on the prognosis remains controversial. It has been observed that an elevated number of tumours infiltrating CD4+ T cells expressing FOXP3 in the cytoplasm are indicative of a favourable prognosis, whereas a high concentration of CD4+ T cells expressing nuclear FOXP3 is strongly associated with recurrence suggesting that the ratio between nuclear and cytoplasmic FOXP3+ CD4+ T cells may be a better prognostic indicator for OSCC.[30] Three functionally and phenotypically distinct population of immune suppressive and non-suppressive CD4+ FOXP3+ T cells exhibiting varied expression of FOXP3 and the cell surface molecules CD45RA and CD25 had been identified recently. The inconsistency observed regarding the association of Tregs with prognostic parameters hence may also be attributed to the difference in the composition of Tregs population in TME. Dense infiltration of FOXP3+ Tregs cell which are phenotypically distinct from those of lymph node or spleen was observed in normal oral mucosa identifying them as a critical component of the immune landscape[31] essential to control local tissue immunity. The available data from experimental mice models as well as in patient cases suggest an important role of Tregs in progression of tongue cancers and as potential predictors of significantly worse prognosis. A greater participation of Tregs cells in immunoinflammatory responses had been reported in older male patients, particularly during the early stages of OTSCC.[32] A sequential increase in the proportion of Tregs in both peripheral blood and lymph node which correlated with the transition from moderate dysplasia to severe dysplasia and SCC[33] and a significant increase in their number during the premalignant phase[34] and early stages of well to moderately differentiated tongue SCC[35] been demonstrated in 4NQO-treated mice experimental mice models. High-level infiltration of Tregs into both cancer nests and stroma has also been detected in early-stage OTSCC cases (stage I/II), which correlated significantly with poor disease-free survival rate.[22] An increase in the number of Tregs and Th17 cells along with an increase in the levels of the chemokines secreted by these cells in the peripheral blood of patients with tongue cancers had been reported earlier. The expression of the chemokines, IL-10 secreted by Tregs and IL 17 secreted by Th17 cells were significantly higher in the advanced stages of cancer compared with the early stages[36] suggesting that altered Tregs/Th17 balance may promote the disease progression in TSCC. It has been found that regulatory B cells (Bregs) induced by TSCC cells could convert CD4+ CD25 T cells into Tregs through secretion of IL-10.[37] In tongue carcinoma apart from Tregs, tumour cells were also found to be expressing FOXP3 and a significant association was noted with pathological differentiation, T stage and poorer patient survival.[38] It has been observed that FOXP3 in TSCC has distinct biological functions compared with that in Tregs and cancer-derived FOXP3 directly regulates the transcription of genes that affect certain internal biological processes of TSCC cells and indirectly influences the extracellular microenvironment.[39] Further investigation in this regard revealed that cancer cell-derived FOXP3 contributed to Tregs expansion in TSCC microenvironment with positive and negative feedbacks mediated by TGFβ and IL-17.[40]

*Tumour-associated macrophages (TAM)*

Macrophages are a heterogeneous population of myeloid cells derived from monocytic precursors in the blood and undergo specific differentiation depending on the signalling in the tissue. Tumour cells recruit macrophages to the tumour site by secreting the colony-stimulating factor (CSF-1), the chemokine ligands 2, 3, 4, 5 and 8 (CCL2, 3, 4, 5 and 8) and the vascular endothelial growth factor (VEGF).[41] Tumour-associated macrophages in TME exist as two functionally distinct subpopulation, M1 and M2. The activation and differentiation of TAM into M1 or M2 are induced by the various cytokines present in the TME.

M1 phenotype, the differentiation of which is induced by cytokines like TNF α and IFN γ releases pro-inflammatory mediators such as IL-12, IL-6 and TNF-α and hence are tumoricidal in nature. But the prevalent phenotype in most cancer TMEs is the M2 phenotype which often have poor antigen-presenting capacity and secrete immunosuppressive factors which inactivate cytotoxic CD8+ lymphocytes and recruit immune suppressing Tregs, thereby aiding the tumour cells in evading immune mechanism.[26] They also release proteins and cytokines like VEGF, EGR and MMPs which favours tumour invasion, tumour cell proliferation, angiogenesis and metastasis. It was observed that HSC-3 cell lines induced expression of epidermal growth factor and transforming growth factor beta in co-cultures with M2 macrophages and direct cell–cell contact between M2 macrophages and HSC cells induced migration and invasion of HSC cells suggesting that M2/TAMs have an important role in OTSCC regulating adhesion, migration, invasion and cytokine production of carcinoma cells favouring tumour growth.[42] It has also been observed that infiltration of Tregs and M2 TAMs is significantly associated with the progression of premalignant lesions to SCC involving various intraoral sub-sites including tongue suggesting that these cells represent prognostic biomarkers for premalignant lesions and could be potential immunotargets to prevent their progression to malignancy.[43] Increased number of IL17-positive macrophages, recently classified as M2 phenotype reported in high-grade OTSCC,[44] accumulation of Tregs and Th2 cells expressing CCR4, the receptor for CC motif chemokine ligand 22, (CCL22) a M2 macrophage-derived cytokine belonging to CC family around CCL22-positive macrophages and a significant correlation of the expression of CCL22 with prognostic parameters observed in tongue cancer TME[45] also suggest the possible protumourigenic role of tumour-associated macrophages in tongue cancers. But contrary to these findings, 4-nitroquinoline-1-oxide-induced tongue squamous cell carcinoma in a mouse model showed a reduction in the numbers of both of M1 and M2 macrophages when compared to normal tongue mucosa.[46] Direct correlation of the cumulative density of the protumourigenic inflammatory infiltrate composed of regulatory T cells (Tregs, FOXP3+), tumour-associated macrophages (TAM2, CD163+), and potentially Tregs-inducing immune cells (CD80+), with the density of CAFs was reported in mobile tongue cancer patients. The reciprocal interrelations between different cytokines suggesting the presence of molecular crosstalk between cancer cells and TME components were demonstrated *in vitro* highlighting the emerging need of new therapies targeting this crosstalk.[47] The expression of the CCR5, the receptor of macrophage inflammatory protein-1β (MIP-1β) and both stimulatory and inhibitory gradient-dependent effect of MIP-1β had been demonstrated in tongue carcinoma cell lines.[48]

**Myeloid-derived suppressor cells (MDSCs)**

MDSCs are heterogeneous collection of cell types including precursors of dendritic cells, monocytes and neutrophils. They are potent immunosuppressor cells and play a vital role in tumour cell survival, angiogenesis, invasion of healthy tissue by tumour cells and metastases.[49] In the tumour-bearing host, the MDSCs generated in the bone marrow migrate to the peripheral lymphoid organ and the tumour, to contribute to the formation of the TME. Recent studies had revealed a difference in the function and fate of MDSCs at these two sites. Two different types of MDSCs had been identified: polymorphonuclear MDSC (PMN-MDSC) which are morphologically and phenotypically similar to neutrophils and monocytic MDSC (M-MDSC) which are similar to monocytes. The activation of MDSCs in tumour had been attributed to persistent stimulation of the myeloid compartment with relatively low-strength signals coming from tumours and is characterized by relatively poor phagocytic activity, continuous production of reactive oxygen species (ROS), nitric oxide (NO) and mostly anti-inflammatory cytokines.[50] A tumour is predominantly populated with M-MDSC which are more suppressive than PMN-MDSC, whereas MDSC in peripheral lymphoid organs is largely represented by PMN-MDSC with relatively modest suppressive activity and in tumour M-MDSC rapidly differentiate into tumour-associated macrophages. Recruited to the tumour site from the bone marrow through various tumour derived factors, MDSCs can suppress the anti-tumour response either directly through the production of arginase 1, ROS, NO, immunosuppressive cytokines or indirectly by inducing Tregs. MDMSC–Tregs interaction is found to be stimulating immunosuppressive pathway in various human malignancies. Extensive infiltration of the stroma by MDSCs along the tumour invasive front, associated with an increase in their frequency in the peripheral blood had been observed in experimental animal models with 4NQO-induced tongue squamous cell carcinoma. Both in the tissue and in the peripheral blood, the increase was progressive from normal through dysplasia to squamous cell carcinoma suggestive of their role in the progression of tongue cancers. A significantly higher ARG-1 mRNA levels indicative of immunosuppressive TME was also observed in the tumour site.[51]

Immunosuppression in OTSCC is a complex process which involves activation of immune tolerance and chemotaxis of immune suppressor cells to the TME. The studies

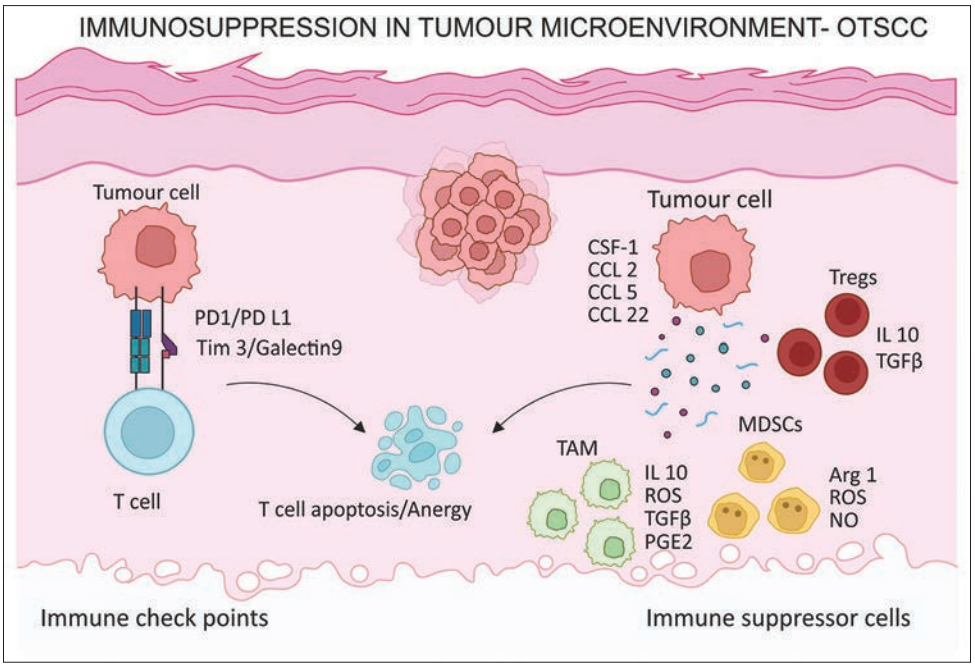


**Figure 3:** Immunosuppressive targets of relevance in the TME of OTSCC

conducted in human oral cancers as well as experimental animal models suggest that immune evasion by tumour cells plays an integral role in the development and progression of tongue cancer. Various immune evasive mechanisms adopted by the tumour cells of OTSCC based on available information are summarized in Figure 3.

## CONCLUSION

The interplay between the various immune components within the TME plays an integral role in the development and progression of tongue cancers. The heterogeneity and the dynamic nature of the immune cell population and the complex intercellular communications between them demand a more site-specific personalized approach targeting multiple pathways in their management. A better understanding of the underlying immune evasive mechanism involved may help in identifying the potential immune targets and in designing the immuno-therapeutic cocktail which may offer durable clinical benefits for the effective management of tongue cancer patients.

### Financial support and sponsorship
Nil.

### Conflicts of interest
There are no conflicts of interest.

## REFERENCES

1. Müller S. Update from the 4th Edition of the World Health Organization of head and neck tumours: Tumours of the oral cavity and mobile tongue. Head Neck Pathol 2017;11:33-40.
2. Almangush A, Heikkinen I, Mäkitie AA, Coletta RD, Läärä E, Leivo I, *et al*. Prognostic biomarkers for oral tongue squamous cell carcinoma: A systematic review and meta-analysis. Br J Cancer 2017;117:856-66.
3. Weinberg R. Dialogue replaces monologue. In: The Biology of Cancer. 2nd ed. New York: Garland Science, Taylor & Francis Group, LLC; 2014. p. 578-640.
4. Korman AJ, Peggs KS, Allison JP. Checkpoint blockade in cancer immunotherapy. Adv Immunol 2006;90:297-339.
5. Forster MD, Devlin MJ. Immune checkpoint inhibition in head and neck cancer. Front Oncol 2018;8:310.
6. Cogdill AP, Andrews MC, Wargo JA. Hallmarks of response to immune checkpoint blockade. Br J Cancer 2017;117:1-7.
7. Pitt JM, Vétizou M, Daillère R, Roberti MP, Yamazaki T, Routy B, *et al*. Resistance mechanisms to immune-checkpoint blockade in cancer: Tumor-intrinsic and -extrinsic factors. Immunity 2016;44:1255-69.
8. Maine CJ, Aziz NH, Chatterjee J, Hayford C, Brewig N, Whilding L, *et al*. Programmed death ligand-1 over-expression correlates with malignancy and contributes to immune regulation in ovarian cancer. Cancer Immunol Immunother 2014;63:215-24.
9. Amarnath S, Mangus CW, Wang JC, Wei F, He A, Kapoor V, *et al*. The PDL1-PD1 axis converts human TH1 cells into regulatory T cells. Sci Transl Med 2011;3:111ra120.
10. Lundqvist L, Stenlund H, Laurell G, Nylander K. The importance of stromal inflammation in squamous cell carcinoma of the tongue. J Oral Pathol Med 2012;41:379-83.
11. Qiao XW, Jiang J, Pang X, Huang MC, Tang YJ, Liang XH, *et al*. The evolving landscape of PD-1/PD-L1 pathway in head and neck cancer. Front Immunol 2020;11:1721.
12. Yoshida S, Nagatsuka H, Nakano K, Kogashiwa Y, Ebihara Y, Yano M, *et al*. Significance of PD-L1 expression in tongue cancer development. Int J Med Sci 2018;15:1723-30.
13. Levingston CA, Young MRI. Local immune responsiveness of mice bearing premalignant oral lesions to PD-1 antibody treatment. Cancers (Basel) 2017;9:62.
14. Naruse T, Yanamoto S, Okuyama K, Ohmori K, Tsuchihashi H, Furukawa K, *et al*. Immunohistochemical study of PD-1/PD-L1 axis expression in oral tongue squamous cell carcinomas: Effect of neoadjuvant chemotherapy on local recurrence. Pathol Oncol Res

2020;26:735-42.
15. Mattox AK, Lee J, Westra WH, Pierce RH, Ghossein R, Faquin WC, *et al*. PD-1 Expression in head and neck squamous cell carcinomas derives primarily from functionally anergic $CD4^+$ TILs in the presence of PD-$L1^+$ TAMs. Cancer Res 2017;77:6365-74.
16. Liao F, Liu L, Luo E, Hu J. Curcumin enhances anti-tumor immune response in tongue squamous cell carcinoma. Arch Oral Biol 2018;92:32-7.
17. Huang X, Zhang J, Li X, Huang H, Liu Y, Yu M, *et al*. Rescue of iCIKs transfer from PD-1/PD-L1 immune inhibition in patients with resectable tongue squamous cell carcinoma (TSCC). Int Immunopharmacol 2018;59:127-33.
18. Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. J Exp Med 2010;207:2187-94.
19. Dong J, Cheng L, Zhao M, Pan X, Feng Z, Wang D. Tim-3-expressing macrophages are functionally suppressed and expanded in oral squamous cell carcinoma due to virus-induced Gal-9 expression. Tumour Biol 2017;39:1010428317701651.
20. Fourcade J, Sun Z, Benallaoua M, Guillaume P, Luescher IF, Sander C, *et al*. Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients. J Exp Med 2010;207:2175-86.
21. Koyama S, Akbay EA, Li YY, Herter-Sprie GS, Buczkowski KA, Richards WG, *et al*. Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. Nat Commun 2016;7:10501.
22. Dardalhon V, Anderson AC, Karman J, Apetoh L, Chandwaskar R, Lee DH, *et al*. Tim-3/galectin-9 pathway: Regulation of Th1 immunity through promotion of CD11b+Ly-6G+ myeloid cells. J Immunol 2010;185:1383-92.
23. Liu JF, Wu L, Yang LL, Deng WW, Mao L, Wu H, *et al*. Blockade of TIM3 relieves immunosuppression through reducing regulatory T cells in head and neck cancer. J Exp Clin Cancer Res 2018;37:44.
24. Narayan G, Jha R, Srikant P, Sinha S, Swarnalata G, Raju KVVN. Carcinoma of the tongue in renal transplant recipients: An unusual spectrum of *De novo* malignancy at a tertiary care center in India over a period of 26 years. Indian J Nephrol 2018;28:119-26.
25. Al-Samadi A, Awad SA, Tuomainen K, Zhao Y, Salem A, Parikka M, *et al*. Crosstalk between tongue carcinoma cells, extracellular vesicles, and immune cells in *in vitro* and *in vivo* models. Oncotarget 2017;8:60123-34.
26. Barbi J, Pardoll D, Pan F. Treg functional stability and its responsiveness to the microenvironment. Immunol Rev 2014;259:115-39.
27. Raimondi G, Turner MS, Thomson AW, Morel PA. Naturally occurring regulatory T cells: Recent insights in health and disease. Crit Rev Immunol 2007;27:61-95.
28. Roncarolo MG, Gregori S, Battaglia M, Bacchetta R, Fleischhauer K, Levings MK. Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. Immunol Rev 2006;212:28-50.
29. Hanakawa H, Orita Y, Sato Y, Takeuchi M, Ohno K, Gion Y, *et al*. Regulatory T-cell infiltration in tongue squamous cell carcinoma. Acta Otolaryngol 2014;134:859-64.
30. Weed DT, Walker G, De La Fuente AC, Nazarian R, Vella JL, Gomez-Fernandez CR, *et al*. FOXP3 subcellular localization predicts recurrence in oral squamous cell carcinoma. PLoS One 2013;8:e71908.
31. Park JY, Chung H, DiPalma DT, Tai X, Park JH. Immune quiescence in the oral mucosa is maintained by a uniquely large population of highly activated $Foxp3^+$ regulatory T cells. Mucosal Immunol 2018;11:1092-102.
32. MendonÇa EF, GordÓn-NÚÑez MA, Alves PM, Nonaka CFW. FoxP3+ regulatory T cells in oral tongue squamous cell carcinoma in young and older patients. Braz Oral Res 2020;34:e096.
33. Zhao J, Wang Z, Han J, Qiu X, Pan J, Chen J. Increased frequency of CD4+ CD25+ FOXP3+ cells correlate with the progression of 4-nitroquinoline1-oxide-induced rat tongue carcinogenesis. Clin Oral Investig 2014;18:1725-30.
34. Yamaguchi H, Hiroi M, Mori K, Ushio R, Matsumoto A, Yamamoto N, *et al*. Simultaneous expression of Th1- and treg-associated chemokine genes and CD4+, CD8+, and Foxp3+ cells in the premalignant lesions of 4NQO-induced mouse tongue tumorigenesis. Cancers (Basel) 2021;13:1835.
35. Miki K, Orita Y, Gion Y, Takao S, Ohno K, Takeuchi M, *et al*. Regulatory T cells function at the early stage of tumor progression in a mouse model of tongue squamous cell carcinoma. Cancer Immunol Immunother 2016;65:1401-10.
36. Liu C, Tong Z, Tan J, Xin Z. Analysis of Treg/Th17 cells in patients with tongue squamous cell carcinoma. Exp Ther Med 2019;18:2187-93.
37. Zhou X, Su YX, Lao XM, Liang YJ, Liao GQ. CD19(+) IL-10(+) regulatory B cells affect survival of tongue squamous cell carcinoma patients and induce resting CD4(+) T cells to CD4(+) Foxp3(+) regulatory T cells. Oral Oncol 2016;53:27-35.
38. Liang YJ, Liu HC, Su YX, Zhang TH, Chu M, Liang LZ, *et al*. Foxp3 expressed by tongue squamous cell carcinoma cells correlates with clinicopathologic features and overall survival in tongue squamous cell carcinoma patients. Oral Oncol 2011;47:566-70.
39. Liang YJ, Lao XM, Liang LZ, Liao GQ. Genome-wide analysis of cancer cell-derived Foxp3 target genes in human tongue squamous cell carcinoma cells. Int J Oncol 2015;46:1935-43.
40. Li K, Huang SH, Lao XM, Yang L, Liao GQ, Liang YJ. Interaction of cancer cell-derived Foxp3 and tumor microenvironment in human tongue squamous cell carcinoma. Exp Cell Res 2018;370:643-52.
41. Coffelt SB, Hughes R, Lewis CE. Tumor-associated macrophages: Effectors of angiogenesis and tumor progression. Biochim Biophys Acta 2009;1796:11-8.
42. Pirilä E, Väyrynen O, Sundquist E, Päkkilä K, Nyberg P, Nurmenniemi S, *et al*. Macrophages modulate migration and invasion of human tongue squamous cell carcinoma. PLoS One 201526;10:e0120895.
43. A. Kouketsu A, Sato I, Oikawa M, Shimizu Y, Saito H, Tashiro K, *et al*. Regulatory T cells and M2-polarized tumour-associated macrophages are associated with the oncogenesis and progression of oral squamous cell carcinoma. Int J Oral Maxillofac Surg 2019;48:1279-88.
44. Bezerra TMM, Monteiro BVB, Pereira JDS, Silva LAB, Nonaka CFW, Silveira ÉJDD, *et al*. Assessment of the presence of interleukin $17^+$ macrophages and Th17 cells *in situ* in lip and oral tongue cancer. Hum Immunol 2021;82:945-9.
45. Kimura S, Nanbu U, Noguchi H, Harada Y, Kumamoto K, Sasaguri Y, *et al*. Macrophage CCL22 expression in the tumor microenvironment and implications for survival in patients with squamous cell carcinoma of the tongue. J Oral Pathol Med 2019;48:677-85.
46. Miki K, Orita Y, Gion Y, Takao S, Ohno K, Takeuchi M, *et al*. Tumor-associated macrophages in the development of 4-Nitroquinoline-1-Oxide-induced tongue squamous cell carcinoma in a mouse model. Oncology 2017;93:204-12.
47. Dayan D, Salo T, Salo S, Nyberg P, Nurmenniemi S, Costea DE, *et al*. Molecular crosstalk between cancer cells and tumor microenvironment components suggests potential targets for new therapeutic approaches in mobile tongue cancer. Cancer Med 2012;1:128-40.
48. Jia B, Qiu XL, Chu HX, Sun X, Pan J, Wang ZP, *et al*. Effect of macrophage inflammatory protein-1β on proliferation and apoptosis of human tongue squamous cell carcinoma CAL-27 cells in vitro. Nan Fang Yi Ke Da Xue Xue Bao 2017;37:1104-9.
49. Condamine T, Ramachandran I, Youn JI, Gabrilovich DI. Regulation of tumor metastasis by myeloid-derived suppressor cells. Annu Rev Med 2015;66:97-110.
50. Youn JI, Collazo M, Shalova IN, Biswas SK, Gabrilovich DI. Characterization of the nature of granulocytic myeloid-derived suppressor cells in tumor-bearing mice. J Leukoc Biol 2012;91:167-81.
51. Chu M, Su YX, Wang L, Zhang TH, Liang YJ, Liang LZ, *et al*. Myeloid-derived suppressor cells contribute to oral cancer progression in 4NQO-treated mice. Oral Dis 2012;18:67-73.