

Tumour-Infiltrating Lymphocyte Subsets, PD-L1 Expression, HPV/p16 Status, and Neutrophil-to-Lymphocyte Ratio in Oral Squamous Cell Carcinoma: A Longitudinal Stratified Study of Non-Recurrent and Recurrent Disease

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Abstract

Oral squamous cell carcinoma (OSCC) imposes a disproportionate epidemiological burden in India, yet lacks validated predictive biomarkers for immune-based therapies. We characterised the tumour immune microenvironment (TME) by evaluating tumour-infiltrating lymphocyte (TIL) subsets (CD3+, CD4+, CD8+, FOXP3+ and CD57+), PD-L1 expression, HPV/p16 status and the systemic peripheral neutrophil-to-lymphocyte ratio (NLR) in resection specimens from 299 patients (stratified by disease outcome: non-recurrent, n=106; recurrent, n=193) treated at two tertiary oncology centres in Bangalore and Cuttack, India, between 2020 and 2023. TIL subsets were assessed by immunohistochemistry; PD-L1 was scored using the Dako 22C3 assay with a cut-off of, and p16 was interpreted per College of American Pathologists guidelines. FOXP3+ regulatory T cells showed the broadest association with adverse histopathology, including lymphovascular invasion (LVI; $P < 0.001$), worst pattern of invasion (WPOI; $P = 0.038$), and lymph node metastasis ($P = 0.003$). PD-L1 expression correlated positively with all five TIL subsets ($P < 0.001$ for each), defining an “inflamed but suppressed” TME. p16-positive tumours (4.0%, associated with recurrent patients) showed markedly reduced FOXP3+ infiltration ($P < 0.001$). In multivariate regression, only LVI and WPOI were associated with elevated pre-treatment log-NLR. The immune microenvironment was statistically indistinguishable between non-recurrent and recurrent tumours across all immune parameters. This integrated multi-biomarker framework – combining intratumoral TIL subset profiling, PD-L1 scoring, HPV/p16 status, and systemic NLR – demonstrates practical utility for immunotherapy patient stratification in South Indian OSCC. Notably, in our study, this utility operates independently of recurrence prediction, suggesting immunotherapy eligibility is a distinct biological dimension from recurrence risk

Introduction

Oral squamous cell carcinoma (OSCC) is one of the most prevalent and therapeutically challenging malignancies worldwide, with a pronounced epidemiological concentration in South and South-East Asia. GLOBOCAN 2022 estimated 389,846 new cases of lip and oral cavity cancer globally, with Asia accounting for over 66% of cases and 75% of deaths.¹ India bears an exceptional burden: oral cavity cancer ranks first among all cancer sites in Indian males, with an age-standardised incidence rate of 14.67 per 100,000, and the country contributes approximately one-third of the global oral cancer case burden.^{2,3} Unlike Western counterparts—where the epidemiology is shifting towards HPV-driven oropharyngeal cancers—Indian OSCC is overwhelmingly attributable to smokeless tobacco, areca nut and smoked tobacco, with HPV playing a negligible aetiological role in oral cavity primaries.^{4,5,6}

Despite decades of refinement in surgery, radiotherapy and concurrent chemoradiotherapy, five-year overall survival for OSCC has remained stagnant at approximately 50–60%, and locoregional recurrence continues to be the dominant cause of treatment failure. The emergence of immune checkpoint inhibitors (ICIs) has fundamentally altered the therapeutic landscape: the KEYNOTE-048 trial demonstrated that pembrolizumab significantly improved overall survival over cetuximab-based chemotherapy in recurrent/metastatic head and neck squamous cell carcinoma (HNSCC), with a hazard

ratio of 0.61 among patients with a combined positive score (CPS) ≥ 20 .⁷ This development has elevated characterisation of the tumour immune microenvironment (TME) from an academic exercise to a clinical imperative.⁸

The TME of OSCC is populated by a heterogeneous assemblage of TILs—principally pan T cells (CD3+), helper T cells (CD4+), cytotoxic T cells (CD8+), regulatory T cells (FOXP3+/Tregs) and natural killer (NK) or senescent T cells (CD57+). The relative composition and distribution of these subsets determine whether a tumour is immunologically “hot” (inflamed), “cold” (immune-excluded) or an immunological desert, a classification with direct implications for ICI eligibility.^{9,10,11,12} A meta-analysis by Borsetto et al. found that high CD8+ TIL density is associated with a 36% reduction in mortality risk (HR 0.64), although significance was not demonstrated for the oral cavity subsite in isolation; in contrast, elevated FOXP3+ Tregs are consistently associated with adverse pathological features and poor prognosis through immunosuppressive attenuation of cytotoxic immunity.^{13,14,15,16}

PD-L1 overexpression in TIL-rich tumours can establish a paradox of “adaptive immune resistance,” in which active immune infiltration inadvertently drives its own suppression through upregulation of the PD-1/PD-L1 checkpoint axis.¹⁷ The CD8+/FOXP3+ ratio, representing the effector-to-suppressor balance, has emerged as a more powerful independent prognostic metric than individual TIL counts.^{18,19} Additionally, HPV/p16 positivity defines a biologically distinct tumour subset with a more immunogenic TME and better outcomes—well characterised in oropharyngeal cancers but comparatively understudied in Indian OSCC, where HPV prevalence is low.^{20,21,22}

The peripheral NLR provides a non-invasive, cost-effective surrogate for the systemic inflammatory milieu, and multiple large meta-analyses confirm that elevated pre-treatment NLR independently predicts poorer overall and disease-free survival in OSCC and HNSCC.^{23,24} However, the specific histopathological determinants that drive NLR elevation have not been rigorously characterised.

This study was designed to address these gaps through an integrated, multicentric characterisation of TIL subsets, PD-L1 expression, HPV/p16 status, and NLR in a large Indian cohort of patients with non-recurrent and recurrent OSCC. Our objectives were to: (i) correlate individual TIL subset densities with established adverse histopathological features; (ii) assess the relationship between PD-L1 expression and TIL composition; (iii) examine the immunological impact of HPV/p16 status; (iv) identify independent histopathological predictors of pre-treatment NLR using multivariate analysis; and (v) determine whether the immune microenvironment evolves significantly between non-recurrent and recurrent disease.

Methods

Study design and patient population

This was an exploratory, longitudinal, hospital-based study conducted at two tertiary oncology centres in India—HCG Cancer Centre, Bangalore, Karnataka, and HCG Cancer Centre, Cuttack, Odisha—between January 2020 and December 2023. Institutional Ethics Committee approval was obtained at both centres, all procedures conformed to the Declaration of Helsinki, and written informed consent was obtained from all participants. Three hundred consecutive patients with histologically confirmed OSCC who underwent primary surgical resection were enrolled; 299 (stratified by disease outcome: non-recurrent, n=106; recurrent, n=193) with complete clinicopathological and immunohistochemical datasets formed the final analytical cohort. Recurrence was defined as disease reappearing within 12 months of completing primary treatment. Inclusion criteria were histologically confirmed squamous cell carcinoma of the oral cavity (buccal mucosa, retromolar trigone, lip, alveolus, anterior palate or tongue), availability of a primary surgical resection specimen, and complete clinical, haematological and pathological data. Exclusion criteria were oropharyngeal, nasopharyngeal or laryngeal carcinoma; non-squamous histology; prior systemic immunotherapy; and neoadjuvant treatment preceding the resection specimen.

Histopathological assessment

Haematoxylin-and-eosin (H&E) sections were evaluated for tumour grade, AJCC 8th-edition pathological stage, LVI, PNI, WPOI, lymph node status, surgical margin status, and desmoplasia (mild, moderate, or dense). Tumour immune phenotyping used a stromal TIL threshold of >40% to classify tumours as immunologically “Hot” and $\leq 40\%$ as “Cold,” per the International Immuno-Oncology Biomarker Working Group framework.^{9,10} TIL assessment was performed by two independent pathologists on whole-slide images (Philips Ultrafast Scanner 300), with senior pathologist arbitration when inter-observer discordance exceeded 10%.

Immunohistochemical panel

Immunohistochemistry was performed on 4- μ m formalin-fixed paraffin-embedded sections. The TIL panel comprised CD3 (Biocare Medical, clone BC33), CD4 (clone 4B12), CD8 (clone SP16), FOXP3 (clone 236A/E7) and CD57 (clone BC56C04). TIL expression was graded semi-quantitatively by the percentage of positive stromal cells: Grade A (0–10%), Grade B (11–50%) and Grade C (51–90%). PD-L1 testing used the Dako 22C3 pharmDx clone on a Roche Ventana BenchMark Ultra; Tumour Proportion Score and Combined Positive Score were interpreted per pembrolizumab protocol guidelines and categorised as no expression, any expression (CPS ≥ 1) and high expression (CPS ≥ 10). p16 IHC used BioGenex clone G175-405, interpreted per CAP guidelines ($\geq 70\%$ strong, diffuse nuclear and cytoplasmic staining = p16-positive); equivocal cases were confirmed by PCR.²⁵

NLR assessment

Peripheral NLR was calculated from routine complete blood counts collected preoperatively and at 3 and 6 months post-surgery (NLR = absolute neutrophil count ÷ absolute lymphocyte count). Given the right-skewed distribution, log-transformed NLR was used as the dependent variable for multivariate modelling.

Statistical analysis

Categorical variables were compared using Pearson's chi-squared or Fisher's exact test; continuous variables using the Mann–Whitney U test (two groups) or Kruskal–Wallis test (≥ 3 groups), with rank-biserial correlation as the effect-size measure. Multivariate ordinary-least-squares linear regression identified independent predictors of pre-treatment log-NLR; model fit was assessed by R^2 , adjusted R^2 and the F-statistic, and multicollinearity by the variance inflation factor. Receiver-operating-characteristic analysis evaluated the discriminative capacity of pre-treatment NLR for LVI status. A two-tailed $P < 0.05$ was considered significant. Analyses used IBM SPSS Statistics v26.0 and Python (scipy, statsmodels).

Results

Non-recurrent versus recurrent comparison

A comprehensive comparison of non-recurrent ($n = 106$) and recurrent ($n = 193$) tumours across all demographic, histopathological, immunological and NLR variables is presented in Table 1. The two groups were statistically indistinguishable across the vast majority of parameters: age ($P = 0.750$), sex ($P = 0.691$), immune phenotype (Hot/Cold; $P = 0.856$), PD-L1 distribution ($P = 0.420$), all five CD-marker expressions (all $P > 0.36$) and NLR at all time points (all $P > 0.36$) showed no significant differences.

Only two variables achieved statistical significance: desmoplasia (87.0% in recurrent versus 77.4% in non-recurrent; $P = 0.045$) and p16 positivity (4.1% recurrent versus 0.0% non-recurrent; $P = 0.049$). No statistically significant correlation was identified between any individual TIL subset density and recurrence status (all $P > 0.167$), although recurrence was numerically least frequent in patients with the highest CD3+ (14% recurrence) and FOXP3+ (13% recurrence) densities.

Table 1. Comparison of clinicopathological and immune characteristics between non-recurrent and recurrent OSCC.

Variable	Non-recurrent (n = 106)	Recurrent (n = 193)	P value
Mean age (years)	53.3 ± 11.8	53.9 ± 12.5	0.750 (NS)
Male sex	84/106 (79.2%)	158/193 (81.9%)	0.691 (NS)
Early stage (I/II)	65 (61.3%)	140 (72.5%)	0.062 (†)
Lymph node involved	38 (35.8%)	50 (25.9%)	0.095 (†)
LVI present	27 (25.5%)	41 (21.2%)	0.432 (NS)
WPOI present	8 (7.5%)	19 (9.8%)	0.593 (NS)
Desmoplasia present	82 (77.4%)	168 (87.0%)	0.045*
p16 positive	0 (0.0%)	8 (4.1%)	0.049*
Hot immune phenotype (>40% TIL)	64 (60.4%)	120 (62.2%)	0.856 (NS)
PD-L1 distribution	Similar	Similar	0.420 (NS)
CD3+ expression	Similar	Similar	>0.36 (NS)
CD8+ expression	Similar	Similar	>0.36 (NS)
FOXP3+ expression	Similar	Similar	>0.36 (NS)
Pre-treatment NLR (mean)	5.01 ± 4.9	4.65 ± 4.3	0.626 (NS)

NS, not significant; † borderline trend. * $P < 0.05$. LVI, lymphovascular invasion; WPOI, worst pattern of invasion; TIL, tumour-infiltrating lymphocyte; NLR, neutrophil-to-lymphocyte ratio.

Patient demographics and clinicopathological characteristics

Excluding desmoplasia and p16 status, the remaining variables were analysed across the entire cohort of 299 patients. The cohort comprised 106 patients with non-recurrent OSCC (35.5%) and 193 with recurrent disease (64.5%) (Table 2). Mean age was 53.7 ± 12.2 years (range 24–89; median 53; IQR 45–62). A pronounced male predominance was observed (242 males, 80.9%; 57 females, 19.1%; male-to-female ratio 4.2:1), consistent with the tobacco/areca-nut-associated aetiology. The oral cavity was the dominant subsite (n = 215; 71.7%), followed by tongue (n = 73; 24.3%) and larynx (n = 12; 4.0%). Moderately differentiated SCC was the most frequent grade (n = 185; 61.9%), and early-stage disease (AJCC stage I/II) predominated (n = 205; 68.6%).

Adverse pathological features were identified as follows: LVI in 68 patients (22.7%), perineural invasion (PNI) in 114 (38.1%), lymph node involvement in 88 (29.4%), WPOI in 27 (9.0%), and moderate-to-dense

desmoplasia in 250 (83.6%). Involved surgical margins were rare (n = 4; 1.3%). On H&E-based immune phenotyping, 62.2% of tumours were classified as immunologically Hot (>40% stromal TIL infiltration).

Table 2. Clinicopathological characteristics of the study cohort (n = 299).

Variable	Category	n	%
Sex	Male	242	80.9
	Female	57	19.1
Age	Mean $\pm$ SD (years): 53.7 $\pm$ 12.2		
	Range (years): 24–89; Median (IQR): 53 (45–62)		
Primary site	Oral cavity (BM, RMT, lip, alveolus, palate)	215	71.7
	Tongue	73	24.3
	Larynx	12	4.0
Tumour grade	WDSCC	90	30.1
	MDSCC	185	61.9
	PDSCC	24	8.0
Clinical stage	Early (I/II)	205	68.6
	Late (III/IV)	94	31.4
Disease type	Non-recurrent	106	35.5
	Recurrent	193	64.5
LVI	Present	68	22.7
	Absent	231	77.3
PNI	Present	114	38.1
	Absent	185	61.9
Lymph-node status	Involved	88	29.4
	Free	211	70.6
WPOI	Present	27	9.0
	Absent	272	91.0
Desmoplasia	Mild	49	16.4
	Moderate	199	66.6
	Dense	51	17.1
Surgical margins	Involved	4	1.3
	Free	295	98.7
Immune phenotype	Hot (>40% TIL)	186	62.2

Variable	Category	n	%
	Cold ($\leq 40\%$ TIL)	113	37.8
p16 status	Positive	12	4.0
	Negative / low	287	96.0

WDSCC/MDSCC/PDSCC, well/moderately/poorly differentiated squamous cell carcinoma; BM, buccal mucosa; RMT, retromolar trigone; LVI, lymphovascular invasion; PNI, perineural invasion; WPOI, worst pattern of invasion; TIL, tumour-infiltrating lymphocyte.

Correlation of TIL subsets with adverse histopathological features

Correlations of the five TIL subsets with key adverse histopathological parameters are presented in Table 3. FOXP3+ regulatory T cells demonstrated the broadest pattern of associations, being significantly associated with LVI ($P < 0.001$), lymph-node metastasis ($P = 0.003$) and WPOI ($P = 0.038$)—the three major routes of tumour dissemination—with higher-grade FOXP3+ infiltration (Grade B, 11–50%) concentrated in LVI-positive and node-positive cases. CD57+ expression was significantly associated with lymph-node metastasis ($P = 0.014$) and desmoplasia ($P < 0.001$).

CD3+ pan-T-cell expression was significantly associated with desmoplasia ($P < 0.001$), with Grade C (51–90%) tumours showing substantially denser desmoplastic stroma (57%) than Grade B (3%). CD4+ expression was significantly associated with both desmoplasia ($P < 0.001$) and WPOI ($P < 0.001$), with higher CD4 (Grade C) associated with greater rates of lymph-node involvement (46% versus 28% in Grade B; $P = 0.028$). CD8+ expression was significantly associated with desmoplasia ($P < 0.001$) but not with LVI, PNI or lymph-node status individually. The CD57/FOXP3 ratio was significantly associated with PNI ($P = 0.024$), a higher ratio correlating with the absence of PNI; CD3/CD8 and CD3/CD4 ratios showed positive linear correlations with PD-L1 expression levels.

Table 3. Correlation of TIL subsets with adverse histopathological features (P values from chi-squared analysis).

Histopathological feature	CD3+	CD4+	CD8+	FOXP3+	CD57+
Lymphovascular invasion (LVI)	P = 0.361	NS	NS	P < 0.001***	NS
Perineural invasion (PNI)	P = 0.471	NS	NS	NS	NS
Lymph-node involvement (LN)	P = 0.079†	P = 0.028*	NS	P = 0.003**	P = 0.014*
Worst pattern of invasion (WPOI)	P = 0.055†	P < 0.001***	NS	P = 0.038*	NS
Desmoplasia	P < 0.001***	P < 0.001***	P < 0.001***	P < 0.001***	P < 0.001***
Tumour grade (differentiation)	P = 0.583	P = 0.314	P = 0.411	P = 0.420	P = 0.265

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; † borderline trend ($P = 0.055-0.095$); NS, not significant.

Correlation of PD-L1 expression with TIL subsets

PD-L1 expression (TPS/CPS), assessed by Dako 22C3, showed a strong, statistically significant positive correlation with all five TIL subsets ($P < 0.001$ for each; Table 4). High PD-L1 expression was associated with high-grade infiltration of CD3+ (89% of cases with high CPS in Grade C CD3+), CD4+, CD8+, FOXP3+ and CD57+ in a consistent, graded fashion.

Table 4. Correlation of PD-L1 expression with TIL subsets (n = 299).

TIL subset	No PD-L1	Any PD-L1 (CPS ≥ 1)	High PD-L1 (CPS ≥ 10)	P value
CD3+ Grade B (11–50%)	32 (50%)	153 (90%)	5 (8%)	<0.001***
CD3+ Grade C (51–90%)	4 (2%)	14 (8%)	59 (89%)	
CD4+ Grade A (0–10%)	33 (52%)	18 (11%)	0 (0%)	<0.001***
CD4+ Grade C (51–90%)	2 (3%)	5 (3%)	41 (62%)	
CD8+ Grade B (11–50%)	16 (25%)	82 (48%)	61 (92%)	<0.001***
CD57+ Grade A (0–10%)	63 (98%)	153 (90%)	28 (42%)	<0.001***
CD57+ Grade B (11–50%)	1 (2%)	17 (10%)	38 (58%)	
FOXP3+ Grade A (0–10%)	62 (97%)	155 (91%)	31 (47%)	<0.001***
FOXP3+ Grade B (11–50%)	2 (3%)	15 (9%)	35 (53%)	

****P* < 0.001 for all TIL subset comparisons. CPS, Combined Positive Score. PD-L1 categories: no expression (CPS < 1), any expression (CPS ≥ 1), high expression (CPS ≥ 10).

Correlation of p16/HPV status with TIL subsets

Strict p16 positivity per CAP guidelines was identified in 12 cases (4.0%), all confined exclusively to the recurrent tumour group (0.0% non-recurrent versus 4.1% recurrent; *P* = 0.049). p16 status showed significant immunological correlations with CD3+ (*P* < 0.001), CD8+ (*P* < 0.001), CD57+ (*P* < 0.001) and FOXP3+ (*P* < 0.001), but not with CD4+ (*P* = 0.230; Table 5). The most striking finding was the profoundly reduced FOXP3+ Treg infiltration in p16-positive tumours: 90% of p16-positive cases had low Treg density (Grade A, 0–10%), compared with 65% of p16-negative tumours.

Table 5. Correlation of p16/HPV status with TIL subsets (n = 299).

TIL subset	p16-negative (n ≈ 287)	p16-positive (n = 12)	P value
CD3+ high (51–90%)	44% in Grade C	18% Grade C; 70% Grade B	<0.001***
CD4+ (all grades)	Similar distribution	Similar distribution	0.230 (NS)
CD8+ high (51–90%)	46% in high-grade CD8	70% in high-grade CD8	<0.001***
CD57+ low (0–10%)	78% of p16-neg cases	83% of p16-pos cases	<0.001***
FOXP3+ low (0–10%)	65% of p16-neg cases	90% of p16-pos cases	<0.001***

NS, not significant. p16 positivity defined as ≥70% strong, diffuse nuclear and cytoplasmic staining (CAP criteria). All *P* < 0.001 except CD4+ (*P* = 0.230, NS).

Multivariate predictors of pre-treatment NLR

Pre-treatment NLR data were available for 282 patients; log-transformed NLR was the dependent variable. The multivariate ordinary-least-squares regression model was statistically significant (*F* = 3.518; *df* = 4, 277; *P* = 0.008) with *R*² = 0.048 and adjusted *R*² = 0.035 (Table 6). All variance-inflation-factor values were between 1.01 and 1.17, confirming no multicollinearity. LVI was a significant independent predictor of elevated log-NLR (β = +0.257, *SE* = 0.090, *P* = 0.004; 95% *CI* 0.082–0.433), corresponding to an approximately 29% higher NLR when present ($e^{0.257}$ = 1.29×). WPOI was also independent and significant (β = +0.278, *SE* = 0.136, *P* = 0.042; 95% *CI* 0.011–0.545), corresponding to approximately 32% higher NLR ($e^{0.278}$ = 1.32×). PNI (β = −0.030, *P* = 0.704) and desmoplasia (β = +0.012, *P* = 0.902) were not significant. ROC analysis showed that pre-treatment NLR predicted LVI status with an AUC of 0.619 (95% *CI* 0.543–0.695).

Univariate analysis of TIL subsets against pre-treatment NLR showed no significant correlations for any individual subset (CD3, CD4, CD8, CD57 or FOXP3; all $P > 0.05$); mean NLR across CD3 grades was 3.5, 3.5 and 3.6 for Grades A, B and C, respectively ($P = 0.924$).

Table 6. Multivariate linear regression: independent predictors of pre-treatment log-transformed NLR (n = 282; $R^2 = 0.048$, $F = 3.518$, $P = 0.008$).

Predictor	β	SE	t	P value	95% CI
Intercept	+1.258	0.093	+13.60	<0.001***	1.076, 1.439
Lymphovascular invasion (LVI)	+0.257	0.090	+2.87	0.004**	0.082, 0.433
Worst pattern of invasion (WPOI)	+0.278	0.136	+2.04	0.042*	0.011, 0.545
Perineural invasion (PNI)	-0.030	0.080	-0.38	0.704 (NS)	-0.187, 0.126
Desmoplasia (present vs mild)	+0.012	0.098	+0.12	0.902 (NS)	-0.180, 0.204

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant. β , unstandardised regression coefficient; SE, standard error. VIF range 1.01–1.17. LVI, ~29% higher NLR when present; WPOI, ~32% higher NLR.

Discussion

This two-centre longitudinal study from South India systematically profiles the OSCC TME, examining five intratumoural TIL subsets (CD3, CD4, CD8, CD57, and FOXP3), PD-L1 expression, and HPV/p16 status in tissue samples, alongside systemic NLR, in 299 patients (stratified by disease outcome: non-recurrent, n=106; recurrent, n=193). Our findings converge on five themes with direct clinical relevance: the primacy of FOXP3+ Tregs as mediators of immune evasion linked to aggressive histopathology; the universality of the “inflamed but suppressed” PD-L1/TIL relationship; the Treg-depleting effect of HPV/p16-positive oncogenesis; the identification of LVI and WPOI as independent histological drivers of systemic inflammation; and the observation that the primary-resection immune profile does not distinguish non-recurrent disease from the one destined for recurrence.

FOXP3+ regulatory T cells as dominant immunosuppressive effectors

Among all subsets assessed, FOXP3+ regulatory T cells showed the broadest and most clinically meaningful associations with adverse features, spanning the vascular and lymphatic routes of dissemination (LVI, WPOI, and lymph node metastasis). This pattern suggests that Treg-mediated immunosuppression creates a microenvironment permissive to tumour spread. The findings accord with Stasikowska-Kanicka et al., who reported elevated FOXP3+ infiltration in poor-prognosis OSCC with strong co-localisation alongside PD-L1 and CD4+ cells—consistent with a dual immunosuppressive role

for regulatory CD4⁺ subsets within the CD4⁺ pool.¹⁵ In 50 OSCC cases from AIIMS New Delhi, a high CD8⁺/FOXP3⁺ ratio independently predicted longer disease-free and overall survival, underscoring that the balance between cytotoxic and suppressive immunity—rather than absolute counts—governs outcome.¹⁹ Our CD57/FOXP3 ratio data extend this principle: a higher ratio, favouring NK/senescent effectors over Tregs, was associated with the absence of PNI, suggesting that effector-to-suppressor balance also helps maintain anti-perineural immunity. Together, these observations support prospective validation of FOXP3⁺ density and FOXP3-based ratios as clinically actionable biomarkers in Indian OSCC.

An inflamed but immunosuppressed microenvironment

The positive correlation between PD-L1 expression and all five TIL subsets is a defining finding of this study and operationalises the concept of adaptive immune resistance originally characterised by Lyford-Pike et al. in HNSCC: as T-cell infiltration intensifies and IFN- γ secretion increases, tumour cells and macrophages upregulate PD-L1 as a compensatory mechanism that neutralises the cytotoxic response.¹⁷ Our data show this is not limited to CD8⁺ effectors but encompasses CD4⁺, CD3⁺, FOXP3⁺ and CD57⁺ cells, indicating a broad, TME-wide adaptive response. The association of CD57⁺ cells with lymph-node metastasis and desmoplasia points to NK-cell dysfunction,²⁶ potentially through chemokine-mediated recruitment of non-cytotoxic rather than cytotoxic NK cells; engineered chimeric-antigen-receptor NK (CAR-NK) strategies are now being developed to overcome this dysfunction.²⁷ Critically, concurrent elevation of FOXP3⁺ Tregs and PD-L1 reflects synergistic, two-pronged immunosuppression—Tregs acting through IL-10 and TGF- β while PD-L1 exhausts surviving CD8⁺ effectors via PD-1. This combination of high immune density and functional inadequacy is precisely the phenotype in which anti-PD-1/PD-L1 therapy is most likely to be effective. The high prevalence of PD-L1 positivity ($\approx 90\%$) reported in Indian oral cavity cancers at Tata Memorial Hospital supports the premise that this “inflamed but suppressed” phenotype may be especially prevalent in smokeless-tobacco-induced Indian OSCC, with important immunotherapy implications.^{28,29}

HPV/p16 status and restructuring of the microenvironment

p16 positivity was rare (4.0%), confined to the recurrent group, and accompanied by markedly reduced FOXP3⁺ Treg density. The very low HPV prevalence in the oral cavity is consistent with extensive Indian data, confirming that HPV plays a minimal aetiological role in Indian oral cavity cancer and that p16 should not be used as an HPV surrogate at this subsite.^{22,30,31} In our cohort, p16 expression in recurrent cases is more plausibly attributable to an alternative, tobacco-related mechanism.³² The co-occurrence of p16 positivity—a tumour-suppressor marker—with low FOXP3 within recurrent disease appears paradoxical, but the literature reconciles it: p16, a reliable HPV and prognostic surrogate in the oropharynx, is unreliable in the oral cavity, where HPV rarely drives tumours and p16 changes instead

arise from tobacco-related epigenetic alterations in epithelial cells.^{33,34,35} Moreover, unlike many sites, higher FOXP3 can be prognostically favourable in head and neck cancer, with FOXP3 acting as both a tumour and an immune suppressor depending on context; the low FOXP3 levels observed in p16-positive recurrent patients are therefore concordant with reported associations and with the prognostic weight of the CD8+/FOXP3+ ratio.^{36,37,18} This complex, context-dependent relationship between p16, smokeless-tobacco exposure, desmoplasia and FOXP3 warrants further study in Asian populations.

Histopathological aggressiveness and systemic inflammation

The identification of LVI and WPOI as independent predictors of elevated pre-treatment log-NLR provides a histological mechanistic basis for NLR elevation in OSCC. Biologically, vascular invasion and non-cohesive invasion patterns plausibly trigger systemic pro-inflammatory signalling—mediated by tumour-derived cytokines and cancer-associated myelopoiesis—producing neutrophilia and relative lymphopenia. This adds mechanistic depth to a prognostic literature that has documented the adverse impact of elevated NLR in OSCC and HNSCC, but has not explained why it is elevated in specific patients.^{23,24} The modest ROC discrimination of NLR for LVI is consistent with prior data from Bengaluru and the Tata Memorial Hospital.^{38,39} The absence of significant univariate TIL–NLR correlations, despite the established inverse relationship between systemic inflammation and local immunity, likely reflects the comparatively modest range of NLR values in this aetiologically distinct cohort and the semi-quantitative resolution of TIL grading.^{40,41}

Clinical Perspectives/Hypothesis 1: The FOXP3–PD-L1–TGF- β axis and desmoplasia

Because immune profiles did not differ between non-recurrent and (subsequent) recurrent disease, the immune-landscape analysis pooled all 299 patients. The OSCC FOXP3–PD-L1–TGF- β axis involves complex, incompletely understood interactions that can drive progression jointly or independently. PD-1/PD-L1 blockade can reduce Treg expansion and revive exhausted T cells, but restoration is often partial, and PD-1+ Tregs amplified during blockade via an alternative axis can paradoxically promote hyperprogression.^{42,43,44} (Figure 1). TGF- β —produced by tumour cells — on the one hand upregulates PD-1/PD-L1 checkpoints leading to checkpoint inhibitor resistance and, on the other hand, expands peripheral Tregs while enhancing aberrant angiogenesis, epithelial–mesenchymal transition, cancer-associated fibroblast (CAF) activity, and extracellular matrix collagen deposition. CAFs, in turn, produce TGF- β , which becomes part of a self-reinforcing set of complex loops. These changes suppress CD8+, NK, and dendritic cells, constituting an interactive yet independent route to immune evasion. This underlies the rationale for use of bispecific TGF- β /PD-L1 agents, which outperform anti-PD-1/PD-L1 monotherapy in preclinical and clinical settings.⁴⁵ CAFs further facilitate Treg accumulation—for

example, via interleukin-1 β -driven CCL22 signalling—thereby yielding the high Treg burden characteristic of head and neck cancer and making Treg/CAF targeting rational.^{46,47,48}

Desmoplasia compounds these effects: the CAF-driven extracellular matrix forms a mechanotransduction barrier that impedes drug delivery, traps immune cells and promotes epithelial–mesenchymal transition, plasticity and immune escape, thereby reducing immunotherapy responsiveness, with TGF- β central to the desmoplastic reaction.^{49,50} Future combination protocols could therefore incorporate matrix-metalloproteinase inhibitors and antifibrotic or anti-stiffness agents (such as lysyl oxidase inhibitors or TGF- β inhibitors) to improve response and reduce recurrence. Metabolic and epigenetic modulation—for example, targeting HIF-1 α /lactate-driven acidification that supports both Tregs and TGF- β , or epigenetic reversion of p16—may further normalise the axis.^{51,52} Radiotherapy and stereotactic body radiotherapy, alongside chemotherapy, contribute cytotoxic cell killing and neoantigen presentation promoting immune enhancement complementary to immunotherapy effects. However, both radiotherapy and chemotherapy have concurrent immunosuppressive effects that can be mitigated by combination with immunotherapy. This synergism forms the basis for combining CT/RT/SBRT with immunotherapy.⁵³ Combining checkpoint inhibition with Treg/CAF depletion and TGF- β blockade (in combination with conventional CT/RT/SBRT-immunotherapy protocols) may improve current response rates of approximately 13–20% and enhance durability in responders while limiting acquired resistance.⁵⁴ Figure 1 summarises these interactions and the resistance pathways implicated by our findings and the supporting literature.

Clinical Perspectives/Hypothesis 2: Immune profiling and recurrence

Although the literature findings are mixed, a central observation in our study is that non-recurrent and recurrent OSCC were immunologically indistinguishable across virtually all parameters—Hot/Cold phenotype, all five TIL subsets and PD-L1 distribution. This carries an important implication: recurrence cannot be predicted from the primary-resection immune profile alone. The finding is concordant with Wongpattaraworakul et al., who likewise did not identify CD3, CD4, CD8 or FOXP3 TILs as independent prognostic markers, in contrast to conventional adverse histopathological features, which remain the primary drivers of recurrence.⁵⁵ Our data add to this point of view: TILs may serve mainly in early-stage risk stratification, and early TME characterisation is best viewed as a durable input for immunotherapy planning rather than a recurrence predictor. The only recurrence-associated features were greater desmoplasia and exclusive p16 positivity (Figure 2), implicating stromal remodelling rather than immune-phenotypic evolution as the distinguishing feature of recurrent disease.

Limitations and future directions

Several limitations merit acknowledgement. The exploratory design is not powered to validate survival endpoints. Semi-quantitative IHC grading does not capture the spatial and functional TIL distribution at the tumour–stromal interface or invasive front, a parameter of particular prognostic significance.¹¹ The very low HPV prevalence limits definitive conclusions about the HPV-positive subgroup, and the absence of post-recurrence survival data precludes direct prognostic validation. Future directions include digital pathology-based spatial TIL mapping, multiplexed immunofluorescence for TIL co-localisation, functional TIL profiling, longitudinal NLR tracking integrated with TIL dynamics, and prospective correlation of these biomarker profiles with ICI treatment outcomes in the Indian oral cancer population.

Conclusion

This multicentric longitudinal study characterises the immune landscape of Indian OSCC through the integrated lens of five TIL subsets (CD3+, CD4+, CD8+, FOXP3+ and CD57+), PD-L1 expression, HPV/p16 status and systemic NLR. FOXP3+ regulatory T cells emerged as the dominant immunosuppressive effectors, with associations across all three major routes of tumour dissemination. The universal positive correlation between PD-L1 and all TIL subsets operationalises adaptive immune resistance and defines the “inflamed but suppressed” OSCC phenotype that underpins the rationale for immune checkpoint therapy. HPV/p16 positivity, though rare in Indian oral cavity cancer, fundamentally reconfigures the TME by reducing FOXP3+ Treg density and thereby establishing a less immunosuppressive microenvironment. LVI and WPOI were identified as independent histological predictors of elevated systemic NLR, providing a mechanistic bridge between aggressive pathological behaviour and systemic inflammatory burden. Finally, the immune microenvironment did not differ significantly between non-recurrent and recurrent disease, supporting early TME characterisation as a durable strategy for immunotherapy planning. Collectively, these findings establish a multi-biomarker framework—integrating TIL subset profiling, PD-L1 scoring, p16 assessment and NLR—that may meaningfully advance personalised immunotherapy selection for Indian OSCC patients.

Declarations

Ethics approval and consent to participate

Institutional Ethics Committee approval was obtained from HCG Cancer Centre, Bangalore, and HCG Cancer Centre, Cuttack. All procedures conformed to the Declaration of Helsinki. Written informed consent was obtained from all participants.

Consent for publication

All authors have reviewed and approved the manuscript for submission. Consent for publication was obtained from all relevant institutional authorities.

Competing interests

The authors declare no competing interests.

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Authors' contributions

Author 1 – Study design, methodology and data acquisition, Author 2 – Manuscript preparation and review, Author 3 – Concept and review, Author 4 - Statistical analysis, Authors 5, 6 and 7 – Manuscript review.

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Data availability

The datasets analysed in this study are available from the corresponding author on reasonable request.

Generative AI statement

During the preparation of this work, the author(s) used Claude (Anthropic PBC; model Claude Opus 4.5; accessed July-August 2026) to appraise an author-written draft for internal inconsistencies, and concordance with the cited literature, and to restructure and language-edit. Also used Grammarly (<https://www.grammarly.com/>) to improve language quality/readability. After using these tools/services, the authors reviewed and edited the content as needed.

The tool was not used to generate, select, or interpret clinical data, and did not originate the clinical decisions or conclusions reported. The tool was not used to produce or modify any figure or image. Authors take full responsibility for the accuracy and integrity of the publication content.

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Figures

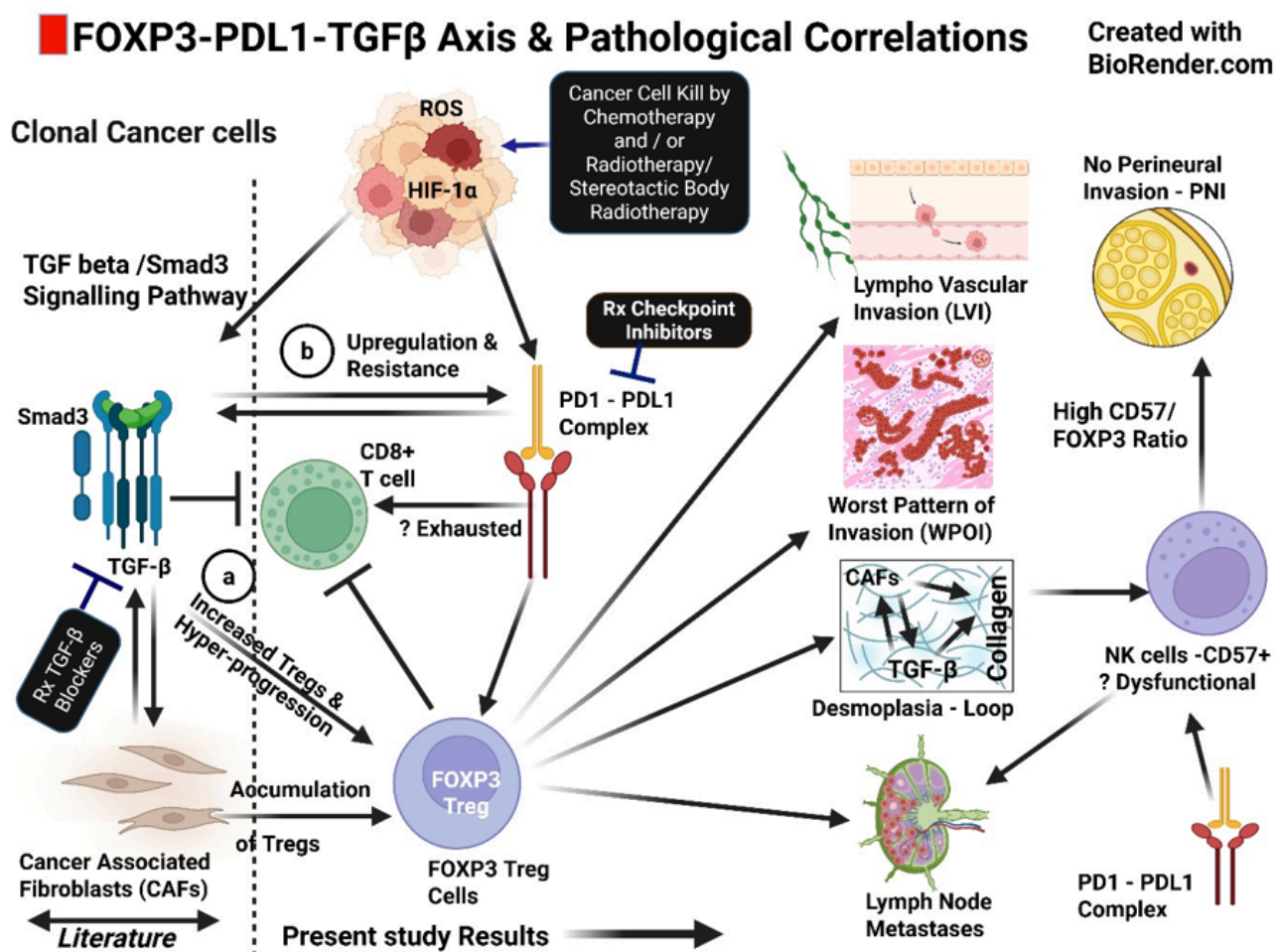


Figure 1

The FOXP3–PD-L1–TGF-β axis and its pathological correlations. →, positive correlation; −, inhibition/depletion. "Created in BioRender. Swamy, K. (2026) <https:// BioRender.com/br96ito>."

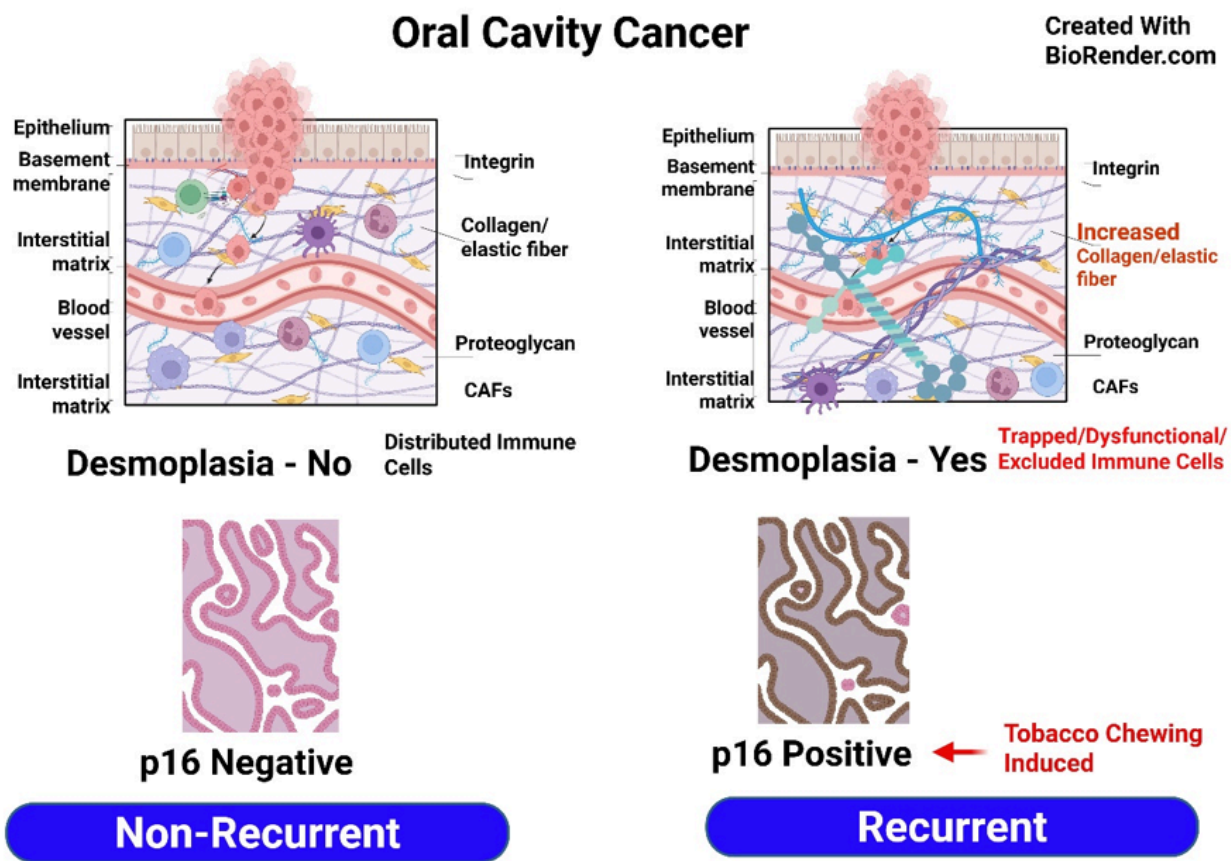


Figure 2

Histopathological features at primary surgery—desmoplasia and p16—the only two factors significantly associated with recurrence compared with non-recurrent disease. "Created in BioRender. Swamy, K. (2026) <https:// BioRender.com/bojn4oe>."