

MOLECULAR PORTRAIT OF ORAL SQUAMOUS CELL CARCINOMA: GENETIC DRIVERS, BIOMARKERS, AND THERAPEUTIC TARGETS

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ABSTRACT

Oral squamous cell carcinoma (OSCC) remains one of the most burdensome malignancies of the head and neck region, with roughly 390,000 new cases and nearly 190,000 deaths estimated annually worldwide. Its five-year survival has stagnated near 50% despite refinements in surgery and radiotherapy. Histological grading and TNM staging alone fail to capture the molecular heterogeneity that governs tumor behavior and treatment response. This review synthesizes current evidence on the genomic architecture of OSCC, integrating data on somatic driver alterations, human papillomavirus (HPV)-defined molecular subtypes, and emerging biomarkers that inform diagnosis, prognosis, and therapeutic selection. We summarize the roles of TP53, PIK3CA, EGFR, NOTCH1, CDKN2A, and FAT1 as the principal genomic drivers, discuss how HPV status bifurcates OSCC into biologically distinct entities with divergent mutational burdens and clinical trajectories, and examine the evidence for tissue-based next-generation sequencing panels, tumor mutational burden, microsatellite status, and circulating tumor DNA as complementary diagnostic and monitoring tools. We further review translation of this knowledge into practice through EGFR-directed therapy, PI3K/AKT/mTOR inhibition, and immune checkpoint blockade, highlighting advances alongside the persistent gap between genomic rationale and clinical benefit, and critically appraise biomarker reproducibility, the limited actionability of tumor-suppressor-dominated genomes, and barriers to implementing genomic and liquid-biopsy testing in routine care. Molecular profiling of OSCC has matured from a descriptive to an increasingly actionable discipline, but validated, minimally invasive panels for early detection and recurrence monitoring, together with rationally designed combination regimens, are needed before genomically guided care becomes standard for this disease.

KEYWORDS: oral squamous cell carcinoma; molecular biomarkers; TP53; human papillomavirus; circulating tumor DNA; targeted therapy

1. INTRODUCTION

Oral squamous cell carcinoma (OSCC) arises from the mucosal epithelium of the oral cavity and constitutes roughly 90% of all oral malignancies [1]. Global estimates place the annual burden at approximately 390,000 new cases and 188,000 deaths, with the highest absolute incidence recorded in Asia, reflecting the regional prevalence of tobacco, alcohol, and betel-quid use [2,3]. Projections based on the Global Cancer Observatory suggest that incidence will rise by roughly 40% by 2040 as populations age and risk-factor exposure persists in many regions [1]. Despite decades of progress in ablative surgery, reconstructive techniques, and conformal radiotherapy, five-year overall survival for locally advanced disease has remained close to 50%, a figure essentially unchanged across the past two decades [3,4].

This therapeutic plateau is not primarily a failure of local control but a reflection of the biological heterogeneity that conventional staging cannot resolve. Two tumors of identical size, nodal status, and histologic grade may carry entirely different mutational repertoires, proliferative drivers, and immune microenvironments, and consequently respond very differently to identical treatment. The TNM system, while indispensable for anatomic planning, was never designed to capture this molecular diversity, and its persistence as the sole basis for treatment stratification is increasingly recognized as a limiting factor in outcome improvement [1].

Large-scale sequencing efforts, beginning with The Cancer Genome Atlas (TCGA) analysis of 279 head and neck squamous cell carcinomas (HNSCC), reshaped the understanding of this disease by revealing a genome dominated by inactivation of tumor-suppressor genes rather than by classical oncogene activation, and by formally distinguishing HPV-associated from HPV-independent disease as biologically separate entities with divergent driver profiles [5]. These insights have since been refined and extended by numerous smaller cohorts, meta-

analyses, and functional studies focused specifically on the oral cavity subsite, which behaves somewhat differently from the oropharynx with respect to HPV biology and prognosis [6,7].

The purpose of this review is threefold. First, we systematize current knowledge of the principal somatic genetic drivers of OSCC – the tumor suppressor TP53, the oncogenic PI3K/AKT/mTOR axis (chiefly through PIK3CA), the growth-factor receptor EGFR, the context-dependent tumor suppressor NOTCH1, and the frequently altered genes CDKN2A and FAT1 – describing both their mutation frequencies and their documented prognostic associations. Second, we characterize the principal molecular biomarkers now under clinical or translational investigation for diagnosis and prognosis, spanning tissue-based genomic panels, tumor mutational burden (TMB), microsatellite instability (MSI), and the rapidly expanding field of liquid biopsy through circulating tumor DNA (ctDNA). Third, we discuss how this biomarker landscape is beginning to inform, and in some cases already determines, therapeutic decision-making through EGFR-targeted antibodies, PI3K/mTOR pathway inhibitors, and PD-1/PD-L1 immune checkpoint blockade. The review proceeds from driver mutations, through molecular classification and biomarkers, to their clinical translation, closing with a critical discussion of the gaps that currently separate genomic characterization from routine, actionable precision oncology in OSCC.

2. Key Genetic Drivers of OSCC Carcinogenesis

2.1 The Tumor Suppressor TP53: "Guardian of the Genome"

TP53 is the single most frequently altered gene in OSCC, with reported mutation frequencies ranging widely across cohorts from approximately 40% to 80%, among the highest rates recorded for any solid tumor type [8]. This variability reflects differences in sequencing methodology, population risk-factor profiles, and inclusion of subclinical or truncating variants; large surgical cohorts from Brazil and the United States have reported frequencies in the 47-60% range, while TCGA and other next-generation sequencing series report figures at the upper end of this spectrum [9]. Under physiological conditions, p53 governs cell-cycle checkpoints, DNA-damage repair, and apoptosis; loss-of-function mutations abrogate these safeguards, allowing genomically unstable clones to survive and expand, which is thought to represent an early, potentially field-wide event in the transition from oral potentially malignant disorders to invasive carcinoma [8].

The clinical relevance of TP53 status extends well beyond its role as an early driver. Truncating and other functionally disruptive mutations are consistently associated with a more aggressive phenotype, including lymph-node metastasis, extranodal extension, and shortened disease-specific and overall survival, whereas wild-type or missense-preserved p53 correlates with more favorable outcomes [9,10]. In cohorts with extranodal extension specifically, TP53 DNA-binding-domain missense mutations independently predicted worse disease-specific and overall survival on multivariable analysis [11]. Beyond prognostication, TP53 mutation status is increasingly explored as a predictive marker: mutated tumors tend to display a distinct immune microenvironment and altered response to platinum-based chemotherapy and immunotherapy, positioning TP53 sequencing as a candidate stratification tool for future trial design [10,12].

2.2 Oncogenic Drivers: PIK3CA, EGFR, and the Dual Role of NOTCH1

PIK3CA and the PI3K/AKT/mTOR axis. Activating alterations of the PI3K/AKT/mTOR signaling cascade represent the most frequently dysregulated oncogenic pathway in HNSCC. A systematic review and meta-analysis of 105 studies (8,630 patients) estimated pooled mutation prevalence at 13% for PIK3CA, 4% for PTEN, 3% for MTOR, and 2% for AKT1, with PIK3CA alterations significantly associated with advanced TNM stage and, notably, with HPV-positive oropharyngeal disease [55]. In cohorts restricted to the oral cavity, reported PIK3CA mutation frequencies range from roughly 10% to 13%, occurring predominantly as missense hotspot substitutions in exons 9 and 20 [13,14,15]. Functionally, these alterations converge on unchecked activation of AKT and mTOR signaling, driving proliferation, metabolic reprogramming, and resistance to apoptosis, which has made this axis one of the most actively pursued therapeutic targets in the disease [14].

EGFR. Epidermal growth factor receptor overexpression is detected immunohistochemically in 70-90% of OSCC specimens, chiefly through transcriptional upregulation and, less commonly, gene amplification, with activating point mutations remaining rare in this tumor type [16,17]. A meta-analysis of fifty studies comprising 4,631 patients confirmed that EGFR overexpression is significantly associated with worse overall survival (hazard ratio 1.38), nodal positivity, and poorer histologic differentiation, cementing its status as a robust, if imperfect, prognostic biomarker [18]. The near-universal overexpression of EGFR across OSCC, contrasted with its comparatively low mutation rate, explains both the biological rationale for anti-EGFR antibody therapy and the modest, heterogeneous responses observed in practice, discussed further in Section 4.

NOTCH1: an unusual tumor suppressor. Unlike its oncogenic role in T-cell malignancies, NOTCH1 behaves predominantly as a tumor suppressor in squamous epithelia, with loss-of-function mutations disrupting terminal keratinocyte differentiation. NOTCH1 is mutated in approximately 10-20% of HNSCC cases, making it the second most frequently altered driver gene after TP53 in most large series, though activating alterations have also been reported in Asian cohorts, suggesting a more context-dependent role than initially appreciated [19,20]. Functional work indicates that NOTCH1 loss-of-function promotes proliferation and migration while paradoxically increasing tumor infiltration by naïve CD8-positive T cells and modulating PD-L1 localization, an observation that has motivated interest in NOTCH1 status as a biomarker for immunotherapy response and for genomically selected PI3K/mTOR inhibition, discussed in Section 5 [21,22].

2.3 Additional Recurrently Altered Genes: *CDKN2A* and *FAT1*

CDKN2A (p16). This cyclin-dependent kinase inhibitor gene is inactivated through mutation, homozygous deletion, or promoter hypermethylation in a large proportion of OSCC, with some series reporting functional inactivation in over 80% of cases when all mechanisms are combined [23]. In oral tongue SCC specifically, direct mutation was documented in roughly 20% of tumors, with additional cases attributable to copy-number loss or methylation [24]. The relationship between CDKN2A disruption and survival has been inconsistent across cohorts – some report a trend toward worse overall survival in p16-negative tumors, others find no significant association – likely reflecting the heterogeneity of inactivating mechanisms and the confounding influence of HPV status, since HPV-driven p16 overexpression operates through an entirely different, E7-mediated mechanism discussed in Section 3 [23,24]. Rare germline CDKN2A mutations have also been described in familial HNSCC, underscoring a hereditary predisposition axis that warrants consideration in atypical presentations, such as young, non-smoking patients [25].

FAT1. This atypical cadherin, involved in cell-cell adhesion, Hippo-pathway regulation, and Wnt signaling, is among the most frequently mutated genes in OSCC, with loss-of-function alterations reported in approximately one-fifth to one-quarter of cases in TCGA and related cohorts [26,27]. FAT1 inactivation reduces intercellular adhesion and, through loss of its cytoplasmic domain, stabilizes the transcriptional co-activators YAP and TAZ by impairing their MIB2-mediated degradation, thereby activating a pro-proliferative transcriptional program [28]. FAT1 loss-of-function mutations frequently co-occur with CASP8 inactivation in the same tumors, and their combined disruption promotes migration, clonal growth, and resistance to apoptosis in experimental models, suggesting a cooperative rather than independent contribution to carcinogenesis [26].

Table 1. Principal somatic genetic drivers in oral squamous cell carcinoma

Gene	Alteration type	Reported frequency	Functional role	Prognostic association
TP53	Missense / truncating mutation (loss-of-function)	~40-80% (cohort-dependent)	Loss of cell-cycle checkpoint, DNA-damage repair and apoptosis control	Poor prognosis; nodal metastasis; extranodal extension [8,9,11]
PIK3CA	Activating missense mutation (helical/kinase domain)	~10-13% oral cavity; ~13% pooled HNSCC	Constitutive PI3K/AKT/mTOR pathway activation	Associated with advanced stage; actionable therapeutic target [13,14,15,55]
EGFR	Protein overexpression (transcriptional); rare amplification/mutation	~70-90% (overexpression)	Enhanced proliferative and survival signaling	Worse overall survival; nodal positivity [16,17,18]
NOTCH1	Loss-of-function mutation (context-dependent)	~10-20%	Disrupted terminal keratinocyte differentiation (tumor suppressor)	Second most mutated driver after TP53; candidate immunotherapy/PI3K-inhibitor biomarker [19,20,21,22]
CDKN2A (p16)	Mutation, homozygous deletion, promoter methylation	Up to ~80% (all mechanisms combined)	Loss of Rb-pathway cell-cycle control	Inconsistent survival association; must be distinguished from HPV-driven p16 overexpression [23,24,25]
FAT1	Loss-of-function mutation	~20-25%	Reduced intercellular adhesion; YAP/TAZ stabilization (Hippo pathway)	Co-occurs with CASP8 loss; promotes migration and chemoresistance [26,27,28]

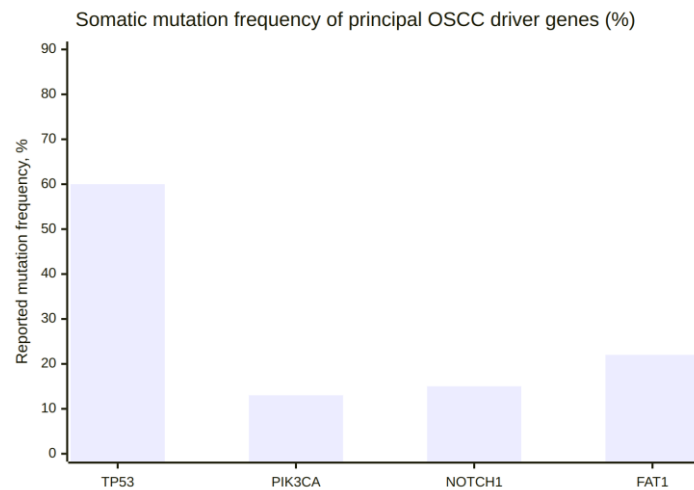


Figure 1a. Somatic mutation frequency of principal OSCC driver genes (representative values from cited sources; see Table 1 for ranges).

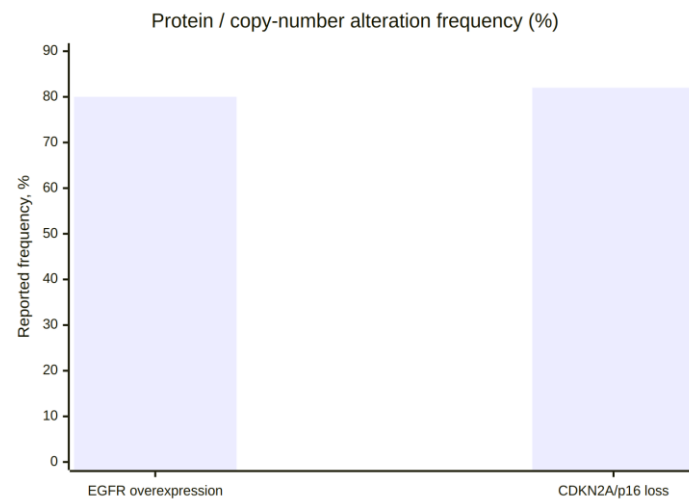


Figure 1b. Frequency of protein-level overexpression (EGFR) and combined-mechanism gene inactivation (CDKN2A/p16) – metrics distinct from somatic mutation frequency and shown separately from Figure 1a to avoid conflating alteration types.

3. Molecular Classification: HPV-Positive versus HPV-Negative Disease

3.1 A Bimodal Disease Entity

Genomic profiling has firmly established that OSCC – and HNSCC more broadly – comprises two biologically distinct subtypes distinguished by HPV status, each with a characteristic mutational signature, immune contexture, and clinical trajectory [5,29]. HPV-negative tumors, which remain the dominant subtype in the oral cavity proper, are driven principally by carcinogen exposure (tobacco, alcohol, betel quid) and display a comparatively high mutational burden dominated by near-universal TP53 loss-of-function mutations, frequent CDKN2A inactivation, and recurrent copy-number alterations affecting 3q26/28 and 11q13/22 [5]. HPV-positive tumors, by contrast, arise through viral oncoprotein-mediated degradation of p53 and retinoblastoma protein rather than through somatic TP53 mutation, and are characterized instead by helical-domain activating mutations in PIK3CA, loss of TRAF3, and amplification of the cell-cycle gene E2F1, alongside an overall lower mutational burden [5,29]. A comprehensive comparison of secondary genetic alterations further shows that HPV-positive OSCC carries a distinctive copy-number signature not shared with HPV-negative disease, including recurrent loss of chromosome 11q (encompassing CCND1) and 14q (encompassing DICER1 and AKT1), regions that are instead gained in HPV-negative tumors; this argues that HPV-positive oral cavity cancer is not merely "HPV-negative disease with a virus," but carries a partially distinct genomic identity of its own [59].

Comparative genomic analyses have quantified this difference directly: in one integrative cohort, the average somatic exonic mutation burden in targeted cancer-gene panels was similar between HPV-positive and HPV-negative tumors overall (14.4 versus 15.2 mutations), but stratification by smoking history revealed that smoking, rather than HPV status per se, was the dominant determinant of mutational load, with smokers carrying roughly twice the mutation burden of never-smokers regardless of viral status [29]. This finding is important because it complicates a purely dichotomous HPV-based model and suggests that a subset of HPV-positive tumors arising

in smokers may behave more aggressively and carry higher mutational complexity than the "classic" HPV-positive, non-smoking phenotype [30,31].

An important nuance for the oral cavity specifically is that the favorable prognostic effect of HPV positivity, well established for oropharyngeal carcinoma, is less consistently observed for OSCC arising in the oral cavity proper. In a systematic comparison of oral and oropharyngeal cohorts, HPV/p16 positivity conferred a significant survival advantage in oropharyngeal SCC but showed no statistically significant association with overall survival in OSCC of the oral cavity [6]. This finding has since been reinforced by a large, nucleic-acid-based validation cohort of 1016 OSCC cases, which found a low prevalence of transcriptionally active HPV in the oral cavity and no survival difference between HPV-positive and HPV-negative tumors, arguing against extrapolating the oropharyngeal HPV paradigm to this subsite [56]. This distinction is frequently blurred in the literature and warrants explicit acknowledgment when translating HPV/p16 biomarker data from oropharyngeal to oral-cavity practice.

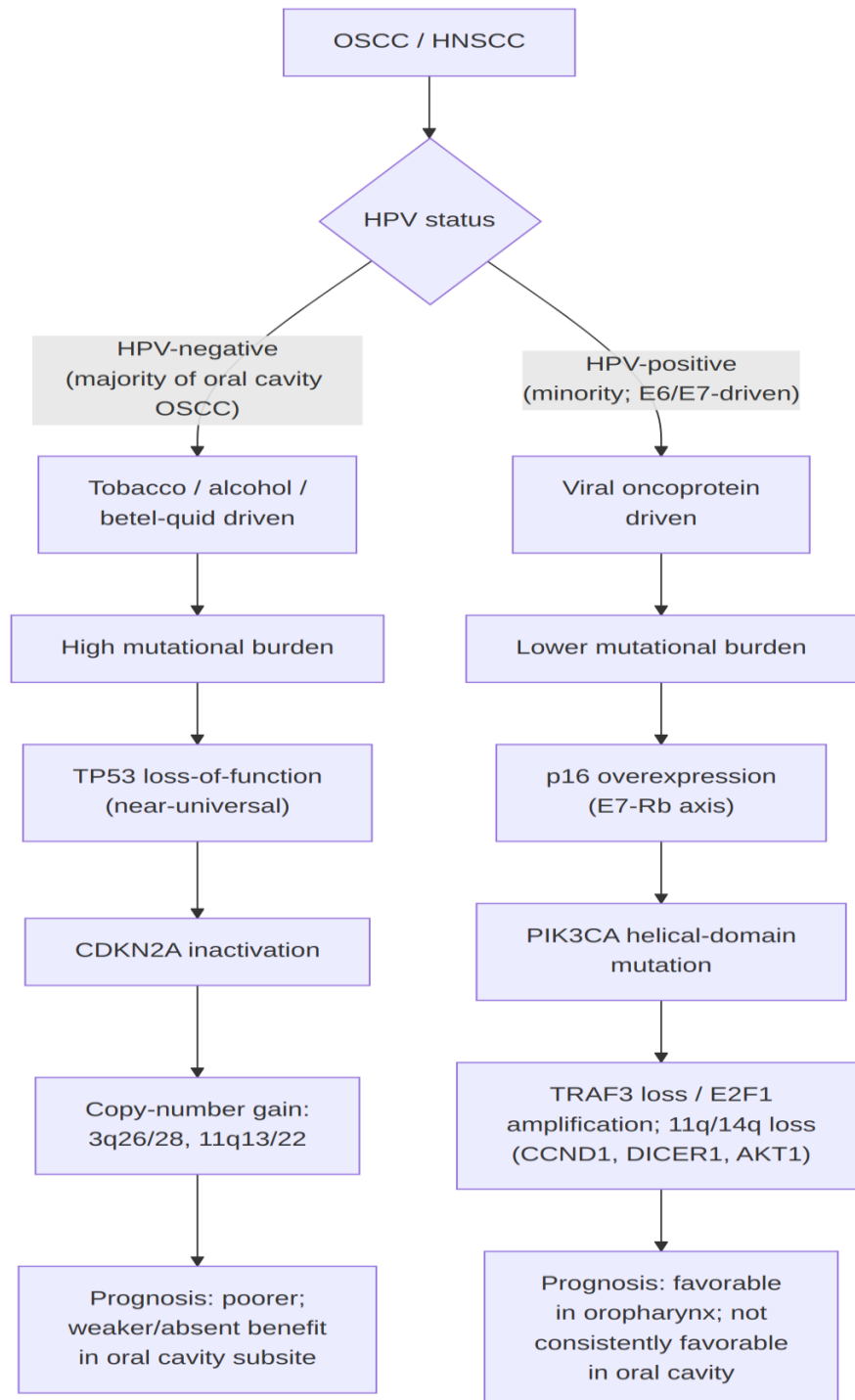


Figure 2. Bimodal molecular classification of OSCC/HNSCC by HPV status, showing the principal genomic alterations, copy-number features, and prognostic differences between HPV-negative and HPV-positive disease.

Table 2. Molecular comparison of HPV-negative and HPV-positive oral/head-and-neck squamous cell carcinoma

Feature	HPV-negative	HPV-positive
Etiology	Tobacco, alcohol, betel quid	High-risk HPV (mainly type 16); E6/E7 viral oncoproteins
Mutational burden	High	Lower overall, but elevated in smokers [29]
TP53 status	Near-universal somatic loss-of-function mutation	Rare somatic mutation (p53 degraded via E6) [5]
Key genomic alterations	CDKN2A inactivation; copy-number gain at 3q26/28 and 11q13/22	PIK3CA helical-domain mutation; TRAF3 loss; E2F1 amplification [5]
p16 immunostaining	Variable / typically negative (genomic loss)	Overexpressed (E7-Rb axis; clinical surrogate marker) [6,7]
Prognosis, oropharynx	Poorer	Favorable [6,7]
Prognosis, oral cavity	Poorer	Not consistently favorable [6]

3.2 p16 as a Surrogate Immunohistochemical Marker

Because direct HPV genotyping is not universally available, p16^{INK4a} immunohistochemistry – reflecting E7-mediated retinoblastoma protein inactivation and consequent CDKN2A transcriptional upregulation – is widely used as a practical surrogate for HPV-driven carcinogenesis, particularly in the oropharynx, where it has been incorporated into the American Joint Committee on Cancer staging system [6,7]. p16 overexpression by this viral mechanism must be distinguished from the CDKN2A gene inactivation discussed in Section 2.3: the former reflects a transcriptionally active, HPV-driven state associated with favorable oropharyngeal biology, while the latter reflects genomic loss of tumor-suppressor function and is generally associated with an unfavorable, HPV-independent phenotype. Because these two processes produce opposite immunohistochemical readouts through entirely different mechanisms, correct clinical interpretation of p16 staining requires contextualizing it with anatomic subsite and, where feasible, direct viral testing [7].

4. Biomarkers: From Diagnosis to Personalized Management

4.1 Genomic and Mutation-Based Biomarkers

Targeted next-generation sequencing (NGS) panels covering the recurrently mutated genes described above (TP53, PIK3CA, CDKN2A, NOTCH1, FAT1, HRAS, and others) are increasingly applied to formalin-fixed paraffin-embedded tumor tissue for combined diagnostic confirmation, prognostic stratification, and identification of actionable alterations. In a cohort of 201 resected OSCC patients with extranodal extension profiled using a 45-gene ultra-deep NGS panel, HRAS mutation and TP53 DNA-binding-domain missense mutation independently predicted distant failure and disease-specific survival even after adjustment for conventional clinicopathologic prognosticators, demonstrating that genomic data can meaningfully refine risk stratification beyond TNM-based staging alone [11]. Such findings support the incorporation of hotspot NGS panels into the diagnostic workup of high-risk resected disease, although prospective validation across independent cohorts remains limited.

Tumor mutational burden and microsatellite status. TMB, quantifying the number of somatic mutations per megabase of sequenced genome, has emerged as a biomarker of interest for predicting response to immune checkpoint inhibition, based on the rationale that a higher mutation load generates more neoantigens and thus greater potential immunogenicity. A meta-analysis of eleven HNSCC cohorts (1,200 patients) found that high TMB was associated with a significantly superior objective response rate (odds ratio 2.62) and survival benefit (hazard ratio 0.53) following immune checkpoint inhibitor therapy [32]. A subsequent meta-analysis confirmed the predictive value of both tissue- and blood-based TMB for objective response, although blood-based assays showed more limited and heterogeneous evidence, and no consensus cutoff has yet been established across platforms [33]. In practice, TMB in HNSCC is generally low to intermediate compared with tumor types classically responsive to immunotherapy, and PD-L1 expression and TMB frequently show no significant correlation with one another, implying that the two biomarkers may capture complementary rather than redundant biological information [34]. Microsatellite instability, by contrast, is a rare event in OSCC and has not achieved the predictive relevance it holds in colorectal or endometrial cancer, though sporadic MSI-high cases may still derive benefit from checkpoint blockade where identified.

4.2 Liquid Biopsy: Circulating Tumor DNA

Liquid biopsy through analysis of circulating tumor DNA (ctDNA) has emerged as one of the most promising non-invasive technologies in OSCC and HNSCC management, offering the possibility of longitudinal, repeatable sampling that tissue biopsy cannot provide [35].

Detection of minimal residual disease and recurrence. The LIONESS study applied a personalized, tumor-informed ctDNA assay to seventeen patients with p16-negative HNSCC undergoing curative surgery, detecting ctDNA in baseline plasma from all seventeen patients and, critically, identifying molecular relapse ahead of

clinically confirmed recurrence in all five patients who subsequently progressed, with lead times ranging from 108 to 253 days and no false-positive detections among patients who remained disease-free [36]. Complementary work using a next-generation sequencing-based minimal-residual-disease assay in the immediate postoperative period (preprint) similarly demonstrated that early ctDNA positivity predicted subsequent recurrence and survival, reinforcing the potential of ctDNA as a dynamic, real-time surveillance tool that could in principle guide the intensity and timing of adjuvant therapy [37]. This clinical-utility question was addressed directly in a prospective cohort of oral cavity squamous cell carcinoma patients profiled with a 769-gene tumor-informed ctDNA assay: postoperative MRD positivity independently predicted shorter progression-free survival (hazard ratio 6.07; negative predictive value 85%), and, notably, adjuvant treatment intensification appeared to improve outcomes specifically in the MRD-positive subgroup, providing early prospective evidence that ctDNA-guided treatment stratification, rather than simple recurrence prediction, may be clinically actionable in OSCC [57].

Comparison of plasma and salivary compartments. Because the oral cavity is anatomically proximate to saliva, several studies have compared plasma- and saliva-derived ctDNA as complementary or competing analytes. A systematic review of eighteen studies assessing ctDNA and cell-free DNA in oral cancer found that elevated levels in both compartments correlated with lymph-node metastasis, larger tumor size, and advanced stage, but noted that plasma-derived ctDNA has generally shown a stronger and more consistent association with metastatic spread and survival outcomes than salivary DNA, whose diagnostic performance remains more variable across studies [38]. This is consistent with earlier work demonstrating that PIK3CA hotspot mutations detectable in tumor tissue could also be identified in matched saliva samples in a subset of patients, supporting the biological plausibility of salivary sampling while underscoring its comparatively lower sensitivity relative to plasma [14]. Taken together, the current evidence favors plasma ctDNA as the more clinically mature liquid-biopsy compartment for prognostic stratification and recurrence monitoring, while salivary analytes remain an attractive, fully non-invasive complement warranting further prospective validation, particularly for local surveillance given their anatomic proximity to the primary tumor bed [39].

The overall trajectory of this field points toward ctDNA-guided adaptive management – using postoperative molecular residual disease status to escalate or de-escalate adjuvant treatment, and using serial ctDNA kinetics during systemic therapy to detect emerging resistance before it becomes clinically or radiographically apparent [35,40]. Realizing this potential in OSCC specifically, as opposed to virus-associated HNSCC where circulating viral DNA offers an even more sensitive analyte, will require larger, prospectively designed validation cohorts and standardization of assay design and reporting thresholds across centers [37].

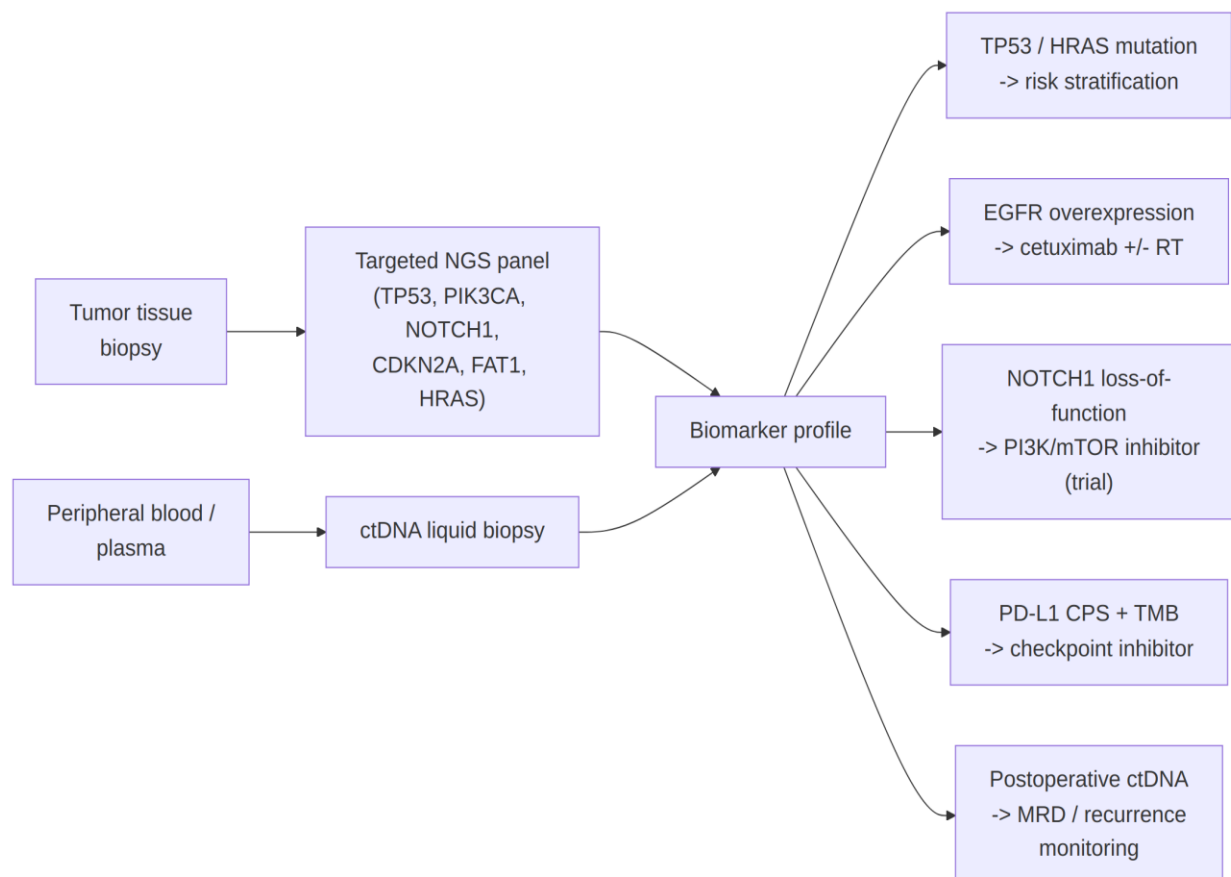


Figure 3. Simplified workflow linking tissue- and plasma-based biomarker profiling to therapeutic and monitoring decisions in OSCC.

5. From Biomarkers to Targeted Therapy and Immunotherapy

5.1 Targeted Therapy

EGFR inhibition. Cetuximab, a chimeric IgG1 monoclonal antibody against the EGFR extracellular domain, remains the only EGFR-targeted agent with regulatory approval in HNSCC, used in combination with radiotherapy for locally advanced disease and with platinum-fluorouracil chemotherapy for recurrent or metastatic disease [41]. Despite the near-universal overexpression of EGFR in OSCC, single-agent response rates to cetuximab are modest, in the range of 10-13%, reflecting multiple intrinsic and acquired resistance mechanisms, including compensatory activation of HER family members, AXL, and downstream AKT signaling that can persist despite adequate receptor blockade [42,43]. Efforts to overcome this resistance have included dual EGFR blockade (cetuximab combined with the tyrosine kinase inhibitor afatinib), which demonstrated encouraging clinical activity, particularly in p16-negative disease, in a recently reported phase 2 trial [44]. Biomarker-driven approaches, including protein-expression signatures predictive of cetuximab sensitivity, are under investigation but have not yet reached routine clinical application [45].

PI3K/AKT/mTOR pathway inhibition. Given the frequent dysregulation of this pathway, particularly through PIK3CA activation, considerable effort has been directed toward pan-PI3K and dual PI3K/mTOR inhibitors such as buparlisib and bimiralisib. Contrary to a simplistic expectation that PIK3CA-mutant tumors would be the ideal candidates for these agents, preclinical work demonstrated that HNSCC cell lines harboring NOTCH1 loss-of-function mutations undergo apoptosis upon PI3K/mTOR inhibition, whereas PIK3CA-mutant lines predominantly undergo reversible cell-cycle arrest without cell death [22]. This mechanistic insight informed a genomic biomarker-driven phase 2 trial of the dual PI3K/mTOR inhibitor bimiralisib specifically in NOTCH1 loss-of-function-mutant recurrent or metastatic HNSCC, illustrating a shift from pathway-based to genotype-and-mechanism-based patient selection [46,47]. More broadly, clinical experience with PI3K pathway inhibitors in HNSCC has shown a manageable but non-negligible toxicity profile and modest, heterogeneous single-agent activity, reflecting both compensatory pathway reactivation (including a paradoxical increase in cytoprotective autophagy) and the biological complexity of tumor-suppressor-driven, rather than classically oncogene-addicted, disease [48,49].

5.2 Immunotherapy: A New Standard for Advanced Disease

Immune checkpoint inhibition targeting the PD-1/PD-L1 axis has transformed the treatment landscape for recurrent or metastatic HNSCC over the past decade. The pivotal KEYNOTE-048 trial established pembrolizumab, alone or combined with platinum and fluorouracil, as a standard first-line option, demonstrating non-inferiority to the prior standard of cetuximab-platinum-fluorouracil and enabling regulatory approval of pembrolizumab monotherapy in patients with a PD-L1 combined positive score (CPS) of 1 or greater [50]. Nivolumab, established through the CheckMate 141 trial in the platinum-refractory setting, is approved irrespective of PD-L1 status, and more recent data from CheckMate 651 and related trials suggest that dual checkpoint blockade with nivolumab plus ipilimumab may offer additional benefit in patients with higher CPS, though results across trials remain heterogeneous [51,52].

Predictive biomarkers of immunotherapy response. PD-L1 CPS remains the most widely used predictive biomarker, with higher CPS generally associated with greater likelihood of response, although clinically meaningful responses are also observed in CPS-low and CPS-negative populations, underscoring the imperfect discriminatory power of this single marker [53]. As discussed in Section 4.1, TMB provides complementary, partially independent predictive information, and combined or multimodal biomarker strategies incorporating T-cell-inflamed gene expression signatures alongside TMB and PD-L1 are under active investigation to improve patient selection beyond what any single marker can achieve [54]. HPV-associated tumors, and NOTCH1-mutant tumors in particular given their distinct immune-cell infiltration pattern, represent additional biologically plausible strata for future prospective biomarker-stratified trials [21].

Table 3. Selected targeted and immunotherapy agents evaluated in OSCC/HNSCC

Agent	Target / mechanism	Clinical setting / evidence	Key outcome
Cetuximab	EGFR (chimeric IgG1 monoclonal antibody)	+ radiotherapy (locally advanced); + platinum/5-FU (recurrent/metastatic)	ORR ~10-13% monotherapy; sole approved targeted agent [41,42,43]
Cetuximab + afatinib	Dual EGFR blockade	Phase 2, recurrent/metastatic HNSCC	Encouraging activity, particularly in p16-negative disease [44]
Bimiralisib	Dual PI3K/mTOR inhibitor	Phase 2, NOTCH1-LOF-mutant recurrent/metastatic HNSCC (biomarker-selected)	Tolerable; activity signal restricted to NOTCH1-LOF tumors [22,46,47]

Buparlisib / other PI3K inhibitors	Pan-PI3K or PI3K α inhibition	Phase 2 combinations (e.g. + paclitaxel), recurrent/metastatic HNSCC	Manageable safety; modest activity, >50% without substantial remission [48,49]
Pembrolizumab	PD-1 blockade	KEYNOTE-048 (1st line, CPS ≥ 1); KEYNOTE-055 (platinum/cetuximab-refractory)	Non-inferior to cetuximab-platinum-5FU; ORR ~16-17% [50,53]
Nivolumab $\pm$ ipilimumab	PD-1 $\pm$ CTLA-4 blockade	CheckMate 141 (platinum-refractory); CheckMate 651 (1st line, dual blockade)	Survival benefit vs standard of care; possible added benefit at high CPS [51,52]

6. DISCUSSION

The past decade has transformed OSCC from a disease defined almost exclusively by anatomic staging into one increasingly amenable to molecular characterization. Several points deserve explicit critical appraisal.

First, the genomic architecture of OSCC is dominated by loss-of-function alterations in tumor suppressors – TP53, CDKN2A, NOTCH1, FAT1 – rather than by classically druggable, gain-of-function oncogenes. This structural feature explains much of the translational difficulty in the field: restoring the function of an inactivated tumor suppressor is intrinsically harder than inhibiting an overactive kinase, and it is a central reason why, more than a decade after the original TCGA analysis defined this landscape, cetuximab remains the sole targeted agent with regulatory approval [5,41]. The bimiralisib experience in NOTCH1-mutant disease is instructive here: rational drug development in this genomic context increasingly depends on exploiting synthetic-lethal or context-dependent vulnerabilities created by tumor-suppressor loss, rather than on direct pathway inhibition of an activated oncogene [22].

Second, the HPV-positive/HPV-negative dichotomy, while a genuinely useful organizing framework, requires more nuanced application in the oral cavity than in the oropharynx. The literature reviewed here indicates that the strong prognostic benefit of HPV positivity, well replicated in oropharyngeal carcinoma, is considerably weaker and less consistent for oral cavity disease specifically [6]. Clinicians and researchers should therefore avoid uncritically extrapolating oropharyngeal HPV biomarker data to OSCC, and future studies should report oral-cavity outcomes separately from pooled HNSCC cohorts wherever possible.

Third, liquid biopsy currently represents the area of most rapid technical progress but also the widest gap between proof-of-concept data and clinical implementation. The LIONESS and related minimal-residual-disease studies are compelling, with lead times of several months between molecular and clinical relapse, but they derive from small, single-center cohorts using bespoke, patient-specific assays that are not yet standardized or widely accessible [36,37]. Whether plasma ctDNA-guided escalation or de-escalation of adjuvant therapy improves hard outcomes such as overall survival, rather than simply advancing the timing of recurrence detection, remains to be demonstrated in randomized trials. Salivary ctDNA, while conceptually attractive for a disease anatomically bathed in an accessible biofluid, currently trails plasma in analytical maturity and reproducibility and should be viewed as a complementary rather than a replacement analyte [38,39].

Fourth, the predictive biomarker landscape for immunotherapy remains incomplete. PD-L1 CPS and TMB each provide real but partial and largely non-overlapping information, response rates to single-agent checkpoint blockade remain modest even in biomarker-selected populations, and no biomarker to date reliably identifies which patients will fail to benefit and might be spared exposure to a costly, potentially toxic therapy [32,34,53]. Composite or multimodal biomarker panels are conceptually appealing but require large, adequately powered prospective validation before they can be recommended for routine use.

Fifth, most of the biomarkers reviewed here remain, in practice, far from routine clinical deployment. A recent appraisal of the OSCC biomarker field concluded that although a wide range of molecular and histopathological candidates has been explored, few have reached the level of validation required for routine clinical application, with methodological heterogeneity across studies and a limited number of adequately powered validation cohorts continuing to slow translation from discovery to the clinic [58]. Beyond validation itself, practical barriers include the absence of standardized protocols for NGS panel content and liquid-biopsy assay design, restricted access to targeted molecular testing outside high-volume academic centers, and the cost of genomic and ctDNA assays in health systems without established reimbursement pathways for these tests. These barriers apply unevenly across settings and are likely to disproportionately limit access to genomically guided care in lower-resource environments, even as the underlying evidence base matures.

Finally, a broader methodological caveat applies across this literature: reported mutation frequencies for nearly every gene discussed here vary substantially between studies, reflecting differences in sequencing depth and platform, tumor purity, population risk-factor exposure, and variant-calling thresholds. Readers should interpret point estimates as approximate ranges characteristic of the disease rather than fixed, universally reproducible figures, and should weight findings from larger, multi-institutional or meta-analytic sources more heavily than isolated single-center reports.

CONCLUSION

Genomic profiling of oral squamous cell carcinoma has matured substantially, moving from the descriptive cataloguing of driver mutations toward biomarkers with genuine, if still partial, clinical actionability. TP53, PIK3CA, EGFR, NOTCH1, CDKN2A, and FAT1 collectively define a genomic landscape dominated by tumor-suppressor loss, while HPV status continues to serve as the principal axis of molecular and clinical classification, albeit one that behaves differently in the oral cavity than in the oropharynx. Clinically, this molecular knowledge already shapes practice through EGFR-targeted antibody therapy and PD-1/PD-L1 checkpoint blockade, and is beginning to inform genotype-selected pathway inhibition, as exemplified by NOTCH1-directed use of PI3K/mTOR inhibitors. The integration of tissue-based next-generation sequencing panels into routine diagnostic workflows is becoming increasingly feasible and clinically justified, particularly for patients with high-risk features such as extranodal extension, where genomic data have been shown to refine prognosis beyond conventional staging. Looking forward, the most consequential unmet need is the development and prospective validation of minimal, standardized genomic and liquid-biopsy panels capable of supporting early detection, dynamic recurrence monitoring, and adaptive treatment escalation or de-escalation, together with rationally designed combination strategies – pairing targeted agents with immunotherapy, or exploiting tumor-suppressor-created vulnerabilities – capable of overcoming the resistance mechanisms that currently limit the durability of single-agent molecular therapies in this disease.

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