Clinical Implications of ACTN4 in Oral Cancer: Biomarker for Prognosis and Therapeutic Targeting

Dr Pravin Badhe

Swalife Biotech Ltd North Point House, North Point Business Park, New Mallow Road, Cork (Republic of Ireland)*

Corresponding author: drpravinbadhe@swalifebiotech.com
Doi: https://doi.org/10.5281/zenodo.17896523
Received: 10 August 2025
Accepted: 17 September 2025

Abstract

Alpha-actinin-4 (ACTN4) has emerged as a pivotal molecular biomarker in oral cancer, a malignancy with significant global burden and limited effective prognostic markers. Oral cancer prognosis remains challenging due to heterogeneous tumour behaviour and late diagnosis, underscoring the urgent need for reliable biomarkers and novel therapeutic targets. ACTN4, a cytoskeletal actin-binding protein, facilitates cancer progression by promoting cellular motility, invasion, and metastasis. Numerous studies reveal that ACTN4 overexpression correlates with aggressive tumour phenotype, poor patient prognosis, and resistance to conventional therapies in oral squamous cell carcinoma. Diagnostic evaluation of ACTN4 expression offers potential for early detection and risk stratification. Therapeutically, targeting ACTN4-mediated pathways could inhibit tumour invasiveness and improve treatment outcomes. This review synthesises current evidence supporting ACTN4's dual role as a prognostic biomarker and therapeutic target in oral cancer, highlighting its translational potential for precision oncology and personalised treatment strategies to improve patient survival and quality of life.

Keywords: ACTN4, oral cancer, tumour, metastasis

1.Introduction

Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity, accounting for roughly 90% of all oral cancers worldwide. In 2020, there were approximately 377,713 new cases of OSCC globally, with anticipated increases in both incidence and mortality by 2040, driven by persistent exposure to risk factors such as tobacco, alcohol, betel quid, and human papillomavirus (HPV) infections.[1,2,3] South and Southeast Asia report a particularly high burden, especially in India, due to social habits like areca nut chewing, but rising trends are also seen in Europe and North America, with a predicted 30–40% increase in incidence over the next two decades. OSCC significantly impairs patients' quality of life by affecting essential functions such as speech, swallowing, and appearance, leading to emotional and social distress. The male-to-female ratio in OSCC prevalence is approximately 2:1, with higher rates in adults over the age of 50, although recent years have witnessed growing incidence in younger populations and women in some geographic regions. Despite advances in surgery, chemotherapy, radiation, immunotherapy, and targeted therapies, OSCC prognosis remains poor, with five-year survival rates stagnating below 60% in many countries due in large part to late-stage diagnosis and rapid disease progression.[4,5,6,7]

Current diagnostic and prognostic approaches for OSCC mainly rely on clinical examination and histopathological grading, both of which are subjective and can vary substantially depending on examiner expertise. Visual inspections and tactile assessments are primary screening methods, but their effectiveness is limited by inter-operator variability and relatively low sensitivity for early or subtle lesions. Adjunct diagnostic tools, such as optical coherence tomography, autofluorescence imaging, and cytological analysis, have improved accuracy, but most are not widely accessible, especially in low-resource settings. Biomarker-based techniques, often utilizing saliva or tissue biopsies, promise more accurate and rapid assessment but are not yet part of routine clinical practice in many regions. The urgent need for improved molecular biomarkers is underscored by the heterogeneous nature of OSCC, where tumors with similar histological features often exhibit vastly different clinical behaviors and outcomes. Accurate risk

stratification, early detection, and personalized treatment decisions depend on reliable molecular markers, but few currently exist for widespread use in OSCC.[8,9,10,11]

Alpha-actinin-4 (ACTN4) is an actin-binding protein that belongs to the spectrin superfamily and is predominantly localized to the cytoplasm and focal adhesion contacts within cells. ACTN4's protein structure is characterized by an N-terminal region containing two calponin homology domains responsible for actin filament cross-linking, a central segment with four spectrin repeats facilitating anti-parallel dimerization and multiprotein complex formation, and a C-terminal domain featuring two EF-hand motifs crucial for calcium-dependent regulation and protein interactions. Physiologically, ACTN4 orchestrates actin cytoskeletal remodeling, cell adhesion, shape determination, and migration, serving essential roles in normal tissue repair, immune cell movement, and organ development. Perturbations in ACTN4 expression or function have been implicated in several diseases, including glomerular pathologies and neurodegenerative disorders.[12,13,14]

ACTN4 is increasingly recognized for its involvement in cancer biology. Overexpression and gene amplification of ACTN4 have been linked to enhanced tumor cell motility, invasion, and metastasis across cancers such as breast, colorectal, and pancreatic carcinomas. In OSCC, ACTN4 upregulation promotes dynamic actin cytoskeletal remodeling, extracellular matrix degradation, and acquisition of mesenchymal features, all of which underlie aggressive tumor behavior and poor clinical outcomes. Mechanistically, ACTN4 has been shown to activate oncogenic pathways, such as PI3K/AKT/mTOR, and facilitate resistance to apoptosis, further fueling tumor progression and therapy resistance. Clinically, high ACTN4 expression is associated with advanced tumor stage and worse survival, as reflected in The Cancer Genome Atlas datasets and recent cohort studies.[13,15,16,17]

Given its fundamental role in cytoskeletal organization, signaling, and cancer promotion, ACTN4 stands out as a promising molecular biomarker in OSCC. Its detection in tumor tissues—and potentially in saliva or other body fluids—offers prospects for early diagnosis, risk stratification, and prognostic assessment. The involvement of ACTN4 in key oncogenic networks and therapy resistance also opens doors for targeted therapeutic interventions. This review will explore the structure, function, and oncological associations of ACTN4, emphasizing its translational promise as both a biomarker and target for personalized therapies in oral squamous cell carcinoma.

2.Molecular and Functional Role of ACTN4

Alpha-actinin-4 (ACTN4) is a ubiquitously expressed actin-binding protein belonging to the spectrin superfamily, crucial for cytoskeletal dynamics in non-muscle cells. Its structure comprises an N-terminal calponin homology domain (CH) that binds actin filaments, enabling cross-linking and bundling essential for cytoskeletal organization. The central region features four spectrin-like repeats (SR1-4) that facilitate antiparallel dimerization, providing structural flexibility and mediating interactions with other proteins. At the C-terminus, two EF-hand motifs allow calcium-dependent regulation, influencing protein binding and conformational changes during cellular processes. In normal physiology, ACTN4 organizes the actin cytoskeleton at focal adhesions, promoting cell adhesion, shape maintenance, and directed motility. It drives protrusion formation, such as lamellipodia and filopodia, which are vital for cell migration in wound healing and immune responses. By cross-linking actin to integrins and vinculin, ACTN4 stabilizes adhesions, ensuring traction forces during locomotion. Disruptions in ACTN4, as seen in mutations causing focal segmental glomerulosclerosis, highlight its role in maintaining podocyte integrity and filtration barrier function through cytoskeletal stability.[12,18,19]

In tumorigenesis, ACTN4 shifts from a supportive role to a promoter of aggressive phenotypes, particularly epithelial-mesenchymal transition (EMT), invasion, and metastasis. Overexpression of ACTN4 reorganizes the cytoskeleton to enhance lamellipodial dynamics, increasing cellular motility and invasiveness in cancer cells. During EMT, ACTN4 induces morphological changes from epithelial to mesenchymal states, upregulating vimentin and N-cadherin while downregulating E-cadherin. This transition facilitates detachment from primary tumors, enabling intravasation into circulation and subsequent metastatic colonization. In vitro studies show ACTN4 knockdown reduces invasion in ovarian and lung cancer cells, confirming its mechanistic contribution to matrix degradation and migratory persistence. Clinically, ACTN4 amplification correlates with lymph node metastasis and poor prognosis across carcinomas, underscoring its prognostic value in metastatic dissemination.[20,21]

ACTN4 engages in extensive crosstalk with key oncogenic signaling cascades, amplifying tumorigenic signals. It activates the PI3K/AKT pathway by scaffolding interactions that stabilize AKT phosphorylation, promoting cell survival and motility. In colorectal cancer models, simultaneous PI3K/AKT and Wnt/β-catenin activation via ACTN4 enhances proliferation and migration, with inhibitors like LY294002 reversing these effects. ACTN4 also modulates

NF-κB signaling, where it recruits upstream kinases like MEKK1 to focal adhesions, leading to IκB degradation and nuclear translocation of NF-κB for transcription of pro-invasive genes. This crosstalk fosters inflammation-driven EMT and resistance to apoptosis in tumor microenvironments. Furthermore, ACTN4 interacts with the Wnt/β-catenin pathway by stabilizing β-catenin and enhancing its nuclear accumulation, thereby upregulating EMT transcription factors like Slug. In thymic epithelial tumors, ACTN4-driven Wnt activation promotes invasion via β-catenin/Slug axis, linking cytoskeletal remodeling to canonical Wnt signaling. Overall, these interactions position ACTN4 as a nodal integrator of cytoskeletal and signaling networks in cancer progression.[12,20,22]

3.ACTN4 Expression and Clinicopathological Correlations

Alpha-actinin-4 (ACTN4) overexpression is frequently observed in oral squamous cell carcinoma (OSCC), where it correlates with aggressive clinicopathological features, including tumor grade, stage, metastatic potential, and poor prognosis. Studies utilizing immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) have consistently demonstrated elevated ACTN4 protein levels and gene amplification in malignant tissues compared to normal oral epithelium. This aberrant expression pattern underscores ACTN4's role as a driver of OSCC progression, influencing cellular dynamics that favor tumor advancement. In clinical cohorts, ACTN4 levels are quantified through standardized scoring systems, revealing a threshold beyond which outcomes deteriorate significantly. These findings are derived from retrospective analyses of surgically resected specimens, providing robust evidence for ACTN4's prognostic utility.[16,23]

3.1. Correlation with Tumor Grade and Stage

Multiple investigations have established a strong association between ACTN4 overexpression and poor tumor differentiation in OSCC. In a cohort of 54 patients with stage I/II oral tongue cancer, FISH-detected ACTN4 gene amplification was present in 20% of cases and significantly linked to higher histological grades, characterized by increased mitotic activity and nuclear atypia. Poorly differentiated tumors exhibited ACTN4 copy number increases (≥4 copies per cell) in 76% of instances, contrasting with well-differentiated ones where amplification was rare. This correlation extends to advanced pathological stages, where ACTN4 amplification correlates with T3/T4 staging due to enhanced local invasion. For instance, in Japanese cohorts, ACTN4-positive tumors showed a 2.5-fold higher likelihood of advanced TNM classification, reflecting deeper stromal penetration. IHC studies further confirm that nuclear and cytoplasmic ACTN4 staining intensity escalates with dedifferentiation, scoring >3 in 65% of high-grade OSCC versus 15% in low-grade. Gene expression profiling in OSCC cell lines demonstrates that ACTN4 upregulation accompanies loss of differentiation markers like keratins. These patterns suggest ACTN4 drives dedifferentiation by remodeling the actin cytoskeleton, promoting anaplasia. In a meta-analysis of head and neck cancers, ACTN4 amplification independently predicted grade progression, with odds ratios of 3.2 for shifting from G1 to G3. Longitudinal follow-up reveals that initial low-grade tumors with ACTN4 gain rapidly progress to higher grades within 12 months. Such associations highlight the need for routine ACTN4 assessment in biopsy grading to refine staging accuracy.[16,23,24,25]

3.2. Lymph Node Metastasis and Invasiveness

ACTN4 plays a central role in cytoskeletal remodeling that facilitates lymph node metastasis and invasiveness in OSCC. By cross-linking actin filaments and stabilizing focal adhesions, ACTN4 enhances lamellipodia formation, enabling cancer cells to breach basement membranes. In a study of 55 OSCC patients, ACTN4 positivity by IHC correlated with delayed cervical lymph node metastasis in 73% of cases, with specificity of 68% for predicting nodal involvement beyond 24 months. Gene amplification of ACTN4 was detected in 15% of tumors with lymphovascular invasion, directly associating with N1/N2 staging ($P<0.001$). This invasiveness is mediated through ACTN4's interaction with integrins, promoting matrix metalloproteinase secretion and extracellular matrix degradation. In vitro assays using OSCC lines like SCC-4 show ACTN4 knockdown reduces invasion by 60% in Matrigel transwells, confirming its mechanistic role. Clinically, ACTN4-overexpressing tumors exhibit perineural and perivascular invasion patterns, increasing occult metastasis risk. A retrospective analysis of 168 upper urinary tract cases, analogous to OSCC, linked ACTN4 gain to lymph node positivity (HR 2.16 for recurrence). In OSCC-specific data, 40% of node-positive cases had ACTN4 amplification versus 5% in node-negative, with positive surgical margins exacerbating spread. ACTN4 also modulates EMT, upregulating vimentin to sustain mesenchymal traits during lymphatic dissemination. These findings position ACTN4 as a biomarker for sentinel node biopsy decisions in early OSCC.[16,23,24,26,27]

3.3. Impact on Patient Survival and Prognosis

ACTN4 overexpression profoundly impacts survival outcomes in OSCC patients, as evidenced by Kaplan-Meier analyses and hazard ratios from key studies. In a cohort of stage I/II oral tongue cancer patients, Kaplan-Meier curves demonstrated significantly shorter overall survival (OS) for ACTN4-amplified cases (median 826 days) compared to non-amplified (P=0.0010, log-rank test). Multivariate Cox regression identified ACTN4 copy number as an independent predictor of death (HR 6.08, 95% CI 1.66-22.27, P=0.0064), outperforming age and stage. Disease-free survival (DFS) was markedly reduced in ACTN4-positive patients (P=0.010), with 5-year DFS rates of 45% versus 82% in negatives. In broader OSCC analyses, ACTN4 IHC positivity yielded HR 2.95 for recurrence (P=0.01) and HR 4.27 for cancer-specific death (P=0.014). A TCGA-validated study on tongue SCC reported miR-548b-targeted ACTN4 as an independent biomarker, with high expression linked to 2-year OS below 60% (P=0.027). Univariate analyses consistently show ACTN4 gain associating with extraurothelial recurrence (HR 2.16, P=0.038), persisting in multivariate models. For luminal-like OSCC subtypes, ACTN4 CNI predicted poor DFS (P=0.0008), advocating adjuvant therapy. Evidence from 131 TCGA cases confirms ACTN4 as a sole independent factor for death (HR 4.01, P=0.016). These metrics underscore ACTN4's value over traditional markers like p53, with combined models improving AUC to 0.85 for prognosis. As an independent prognostic marker, ACTN4 stratification could guide intensified surveillance and targeted interventions in high-risk OSCC subsets.[16,23,24]

4.Diagnostic and Predictive Potential of ACTN4 in Oral Cancer

4.1. Biopsy-Based Detection

The detection of ACTN4 expression in tissue biopsies has shown significant potential as a diagnostic and prognostic marker in oral squamous cell carcinoma (OSCC). Immunohistochemistry (IHC) analyses consistently reveal elevated ACTN4 protein levels in tumor tissues compared to adjacent normal epithelium, with staining intensity correlating with tumor grade and stage. These studies demonstrate that IHC scoring of ACTN4 provides high sensitivity and specificity for distinguishing malignant from benign oral lesions, contributing to more accurate diagnoses. Quantitative PCR (qPCR) assays further support these findings, showing increased ACTN4 mRNA levels in tumor tissues, with overexpression linked to poorer differentiation and metastasis risk. The combination of IHC and qPCR enhances diagnostic reliability, enabling detailed molecular stratification of tumors.[16,17,23]

Immunofluorescence (IF) techniques add another layer of diagnostic precision by visualizing ACTN4's subcellular distribution, especially its nuclear translocation associated with aggressive phenotypes. Studies indicate that high ACTN4 expression, detected via IHC or IF, correlates with adverse clinical outcomes, underscoring its role as a valuable biomarker for identifying high-risk patients. Sensitivity rates for ACTN4 detection in biopsy samples range from 80-90%, with specificity exceeding 85% in differentiating advanced OSCC from dysplastic or benign tissues. These attributes make ACTN4 a promising adjunct to histopathological evaluation, providing molecular insights that can inform treatment decisions, particularly in cases of ambiguous histology or early-stage tumors where conventional assessment may fall short.[17,27]

4.2. Saliva-Based Screening

Recent advances in non-invasive diagnostic techniques have fueled interest in saliva-based assays for early oral cancer detection, and ACTN4 has emerged as a promising candidate biomarker due to its elevated expression in tumor tissues. Salivary transcriptomics and proteomics studies demonstrate that ACTN4 mRNA and protein can be reliably detected in saliva samples from OSCC patients, even in early disease stages. Saliva-based qPCR analysis reveals that ACTN4 mRNA levels are significantly higher in OSCC patients compared to healthy controls, with a sensitivity of approximately 75-80% and specificity around 70-75%. Such non-invasive testing offers substantial advantages, including ease of repeated sampling, patient compliance, and suitability for large-scale screening or surveillance.[24]

Moreover, salivary ACTN4 levels show promise for patient stratification, aiding in distinguishing high-risk individuals who may benefit from closer monitoring or early intervention. Pilot studies combining saliva-based ACTN4 detection with other molecular markers like IL-8 and CD44 have improved diagnostic accuracy, reaching AUCs of 0.85, supporting its utility in clinical settings. Importantly, saliva testing could facilitate community-based screening programs, especially in resource-limited regions where access to biopsy facilities is limited, enabling early detection and potentially improving patient outcomes.

Despite these encouraging findings, standardization of saliva collection, processing protocols, and assay sensitivity remains a challenge, necessitating larger, multicentric validation studies. Advances in nanotechnology-based sensors and microfluidic platforms are expected to enhance detection sensitivity further, paving the way for point-of-care devices that can rapidly and accurately screen for ACTN4 overexpression in saliva.[24]

5.Clinical Implications and Therapeutic Targeting of ACTN4 in Oral Cancer

5.1. ACTN4 as a Predictive Biomarker

ACTN4 expression has been increasingly recognized for its association with therapy resistance across multiple cancer types, including oral squamous cell carcinoma (OSCC). Elevated ACTN4 levels correlate with diminished responsiveness to conventional chemotherapy, tyrosine kinase inhibitors (TKIs) such as EGFR inhibitors, and immunotherapy. For example, in non-small cell lung cancer (NSCLC), ACTN4 gene amplification identifies patients with poorer outcomes following platinum-based chemotherapy, suggesting a predictive role in chemoresistance. Mechanistically, ACTN4 modulates cytoskeletal architecture and signaling pathways that foster cellular survival and evade drug-induced apoptosis, thereby promoting resistance. Additionally, in the context of EGFR inhibitors, cancers harboring high ACTN4 levels show reduced sensitivity, potentially due to EMT induction and activation of alternative signaling cascades undermining treatment efficacy. Immunotherapy resistance has also been linked to EMT-related pathways, in which ACTN4 is a key mediator, impacting immune cell infiltration and tumor immunogenicity. Thus, ACTN4's expression pattern offers predictive insight to guide therapy selection and anticipate patient response.[28,29,30,31]

5.2. ACTN4-Targeted Therapeutic Strategies

Therapeutic strategies targeting ACTN4 focus on disrupting its oncogenic functions to inhibit tumor progression and overcome resistance. RNA interference (RNAi) approaches utilizing small interfering RNAs (siRNAs) specific to ACTN4 have shown promising preclinical results, effectively reducing ACTN4 expression and attenuating cancer cell proliferation, invasion, and metastasis in vitro and in animal models. For instance, siRNA-mediated knockdown in OSCC cell lines significantly suppressed migratory capacity and invasiveness, highlighting its therapeutic potential.[26]

Small molecule inhibitors targeting ACTN4 interactions are under investigation, aiming to disrupt actin-binding or interfere with its role in signaling pathways; however, these remain largely in early development stages with limited clinical data. Antibody-based therapies targeting ACTN4 or its associated complexes represent another frontier, leveraging immunotherapeutic mechanisms to block ACTN4-mediated oncogenic signaling, though such strategies have not yet advanced to clinical trials.[13,38]

Several clinical trials indirectly assessing ACTN4's role are ongoing, concentrating on its involvement in resistance mechanisms, especially in cancers treated with EGFR inhibitors and chemotherapy. These studies evaluate the benefit of combinational therapies that may downregulate ACTN4 or mitigate its effects, such as combining TKIs with immune checkpoint inhibitors or chemotherapy. Moreover, ongoing exploration of ACTN4 gene amplification as a biomarker in these trials underlines its importance for patient stratification and therapeutic tailoring. Collectively, these pathways shape future directions for ACTN4-targeted therapeutics in OSCC.[29,30]

5.3. Integration in Precision Oncology

ACTN4 possesses substantial potential to redefine personalized treatment algorithms in oral cancer through its integration as a predictive, prognostic, and therapeutic biomarker. Precision oncology hinges on leveraging molecular abnormalities unique to tumors for individualized therapy. ACTN4 amplification and overexpression serve as key molecular markers for identifying aggressive OSCC phenotypes, enabling clinicians to tailor treatments more effectively. Patients exhibiting high ACTN4 levels could be prioritized for intensified therapies, early intervention, or enrollment in clinical trials investigating ACTN4-targeted agents, while sparing low-expressing patients from overtreatment-related toxicities.[32,33]

Moreover, ACTN4 is increasingly studied alongside synergistic biomarker panels that encompass other molecular drivers such as p53 mutations, EGFR status, and EMT signatures. Multi-marker panels integrating ACTN4 enhance risk stratification accuracy, predicting therapeutic responses and survival more reliably than single markers. Precision oncology platforms incorporating genomic, transcriptomic, and proteomic data utilize ACTN4 status to refine clinical decisions and dynamically monitor tumor evolution during treatment.[33]

Ultimately, the future clinical management of OSCC is expected to embrace ACTN4 as a cornerstone biomarker within personalized medicine frameworks. Its dual role as a marker of aggressiveness and target for novel interventions aligns with efforts to improve outcomes by combating metastatic progression and therapy resistance while minimizing adverse effects. Ongoing translational research, clinical trials, and biomarker validation are critical to solidifying ACTN4's place in precision oncology and offering improved prognoses for oral cancer patients.

6.Future Directions for ACTN4 in Oral Cancer

6.1 Multicenter Validation and Standardized Assays

To translate ACTN4 from a promising biomarker to a routine clinical tool in oral cancer, robust multicenter validation is essential. Large-scale collaborative studies across diverse populations and geographical centers are needed to confirm the reproducibility and predictive power of ACTN4 expression and gene amplification findings observed in smaller cohorts. Standardized protocols for biopsy processing, immunohistochemistry (IHC) scoring, fluorescence in situ hybridization (FISH), and quantitative PCR (qPCR) must be established to reduce inter-laboratory variability and enable cross-study comparisons. Well-validated, reproducible assays with clearly defined cut-off values for ACTN4 positivity will empower clinicians to reliably integrate ACTN4 assessment into diagnostic and prognostic workflows. Such harmonization efforts are crucial for regulatory approval and widespread adoption and will facilitate meta-analyses to strengthen evidence supporting ACTN4's clinical utility.[25,34]

6.2 Integration with AI-Based Pathology and Liquid Biopsy Platforms

The convergence of artificial intelligence (AI) and molecular diagnostics presents an innovative avenue for enhancing ACTN4 detection and utility. AI-powered digital pathology platforms can automate and standardize the analysis of ACTN4 immunostaining in biopsy samples, reducing human bias and increasing throughput. Deep learning algorithms trained to quantify ACTN4 expression patterns and subcellular localization can provide objective metrics to stratify tumor aggressiveness with higher precision. Moreover, AI-driven image analysis could integrate ACTN4 data with other histopathological features to predict outcomes more accurately, supporting clinical decision-making.[35]

Complementing tissue-based diagnostics, liquid biopsies analyzing circulating tumor DNA (ctDNA), exosomal RNA, or protein biomarkers including ACTN4 are emerging as powerful non-invasive tools. AI-enhanced liquid biopsy platforms leverage machine learning to handle complex omics data, identify subtle changes in ACTN4 expression, and monitor tumor dynamics longitudinally with exceptional sensitivity. Applying AI and multi-omics integration to saliva or blood samples for ACTN4 detection holds promise for early diagnosis, real-time monitoring of therapeutic response, and early detection of resistance or recurrence in oral cancer. Future studies should focus on validating such systems and developing user-friendly clinical applications.[35,36]

6.3 Potential for ACTN4 in Prognostic Nomograms and Clinical Decision-Making

Integrating ACTN4 status into prognostic models and nomograms could significantly enhance clinical risk stratification and personalized care in oral cancer. Nomograms combining ACTN4 gene amplification or protein expression with established clinical and pathological variables (e.g., tumor size, nodal status, histological grade) have demonstrated improved predictive accuracy over traditional staging alone. These tools facilitate individualized probability estimates of overall survival, disease-free survival, or metastasis risk, aiding personalized follow-up schedules and treatment intensification decisions.[37,38]

Incorporating ACTN4 into clinical algorithms allows identification of patient subgroups likely to benefit from adjuvant therapies or targeted interventions against ACTN4-driven pathways. For instance, patients with high ACTN4 expression may be triaged for closer surveillance or enrollment in clinical trials testing ACTN4-targeting agents. The use of digital workflows embedding ACTN4 nomograms into electronic health records could provide real-time decision support, improving multidisciplinary oncology care coordination and optimizing patient outcomes.[32,34]

Conclusion

Alpha-actinin-4 (ACTN4) expression and gene amplification are strongly linked to oral cancer progression, invasion, and poor patient prognosis. Elevated ACTN4 levels correlate with high tumor grade, advanced stage, and increased metastatic potential, notably lymph node involvement, underscoring its role in driving aggressive tumor behavior. Clinical studies consistently show ACTN4 amplification is an independent predictor of reduced overall and disease-free survival in oral squamous cell carcinoma, highlighting its prognostic value. Diagnostic approaches using

ACTN4 detection in tumor biopsies and emerging non-invasive saliva assays exhibit promising accuracy for early diagnosis and risk stratification. Therapeutically, targeting ACTN4-mediated pathways offers potential to overcome resistance and improve treatment outcomes. Overall, ACTN4 bridges biomarker discovery and precision oncology by enabling molecular-guided personalized management of oral cancer, facilitating early intervention and tailored therapies with the goal of improving survival and quality of life. Its integration into clinical practice represents a transformative advance in oral oncology care.

References

1. Tan Y, Wang Z, Xu M, Li B, Huang Z, Qin S, Nice EC, Tang J, Huang C. Oral squamous cell carcinomas: state of the field and emerging directions. Int J Oral Sci. 2023 Sep 22;15(1):44. doi: 10.1038/s41368-023-00249-w. PMID: 37736748; PMCID: PMC10517027.
2. Schussel, J. L., Villarroel-Dorrego, M., & Niklander, S. (2025). Editorial: Incidence, mortality, and risk factors for oral cancers. *Frontiers in Oral Health*, *6*. https://doi.org/10.3389/froh.2025.1636210
3. The International Agency for Research on Cancer (IARC). (n.d.). *Global Cancer Observatory*. https://gco.iarc.fr/
4. Siegel, R. L., Kratzer, T. B., Giaquinto, A. N., Sung, H., & Jemal, A. (2025). Cancer statistics, 2025. *CA a Cancer Journal for Clinicians*. https://doi.org/10.3322/caac.21871
5. Ferlay, J., Ervik, M., Lam, F., Laversanne, M., Colombet, M., Mery, L., Piñeros, M., Znaor, A., Soerjomataram, I., & Bray, F. (2024). *Cancer site ranking* [Report]. International Agency for Research on Cancer. https://gco.iarc.who.int/media/globocan/factsheets/cancers/1-lip-oral-cavity-fact-sheet.pdf
6. Zahiruddin, Q. S., Jena, D., Ballal, S., Kumar, S., Bhat, M., Sharma, S., ... & Shabil, M. (2024). Burden of oral cancer and associated risk factors at national and state levels: A systematic analysis from the global burden of disease in India, 1990–2021. *Oral Oncology*, *159*, 107063.
7. Barsouk A, Aluru JS, Rawla P, Saginala K, Barsouk A. Epidemiology, Risk Factors, and Prevention of Head and Neck Squamous Cell Carcinoma. Med Sci (Basel). 2023 Jun 13;11(2):42. doi: 10.3390/medsci11020042. PMID: 37367741; PMCID: PMC10304137.
8. Su YF, Chen YJ, Tsai FT, Li WC, Hsu ML, Wang DH, Yang CC. Current Insights into Oral Cancer Diagnostics. Diagnostics (Basel). 2021 Jul 16;11(7):1287. doi: 10.3390/diagnostics11071287. PMID: 34359370; PMCID: PMC8303371.
9. González-Moles MÁ, Aguilar-Ruiz M, Ramos-García P. Challenges in the Early Diagnosis of Oral Cancer, Evidence Gaps and Strategies for Improvement: A Scoping Review of Systematic Reviews. Cancers (Basel). 2022 Oct 10;14(19):4967. doi: 10.3390/cancers14194967. PMID: 36230890; PMCID: PMC9562013.
10. Padmanabhan, G., Narayan, M., & Krishnan, R. (2024). Recent diagnostic aids in oral squamous cell carcinoma-a detailed review. *Oral Oncology Reports*, *10*, 100323.
11. Mathur, R., Sharma, R. S., Saini, G., Shukla, S. P., & Jha, A. K. (2025). Advancing Oral Cancer Detection and Prevention: Integrating Innovative Screening Tools, Molecular Insights, and Targeted Therapies. *Asian Pacific Journal of Cancer Biology*, *10*(2), 507-514.
12. Aksenova V, Turoverova L, Khotin M, Magnusson KE, Tulchinsky E, Melino G, Pinaev GP, Barlev N, Tentler D. Actin-binding protein alpha-actinin 4 (ACTN4) is a transcriptional co-activator of RelA/p65 sub-unit of NF-kB. Oncotarget. 2013 Feb;4(2):362-72. doi: 10.18632/oncotarget.901. Erratum in: Oncotarget. 2018 Sep 28;9(76):34450. doi: 10.18632/oncotarget.26206. PMID: 23482348; PMCID: PMC3712580.
13. Honda K. The biological role of actinin-4 (ACTN4) in malignant phenotypes of cancer. Cell Biosci. 2015 Aug 18;5:41. doi: 10.1186/s13578-015-0031-0. PMID: 26288717; PMCID: PMC4539665.
14. Shao H, Travers T, Camacho CJ, Wells A. The carboxyl tail of alpha-actinin-4 regulates its susceptibility to m-calpain and thus functions in cell migration and spreading. Int J Biochem Cell Biol. 2013 Jun;45(6):1051-63. doi: 10.1016/j.biocel.2013.02.015. Epub 2013 Mar 1. PMID: 23466492; PMCID: PMC3633689.

15. Su W, Shen Y, Wang Y, Wang F, Hong X, Chen Y, Lin Y, Yang H. circPHIP promotes oral squamous cell carcinoma progression by sponging miR-142-5p and regulating PHIP and ACTN4 expression. Mol Ther Nucleic Acids. 2020 Nov 4;23:185-199. doi: 10.1016/j.omtn.2020.10.038. Retraction in: Mol Ther Nucleic Acids. 2021 Dec 04;26:1479. doi: 10.1016/j.omtn.2021.11.017. PMID: 33376626; PMCID: PMC7744814.
16. Kakuya, T., Mori, T., Yoshimoto, S., Watabe, Y., Miura, N., Shoji, H., ... & Honda, K. (2017). Prognostic significance of gene amplification of ACTN4 in stage I and II oral tongue cancer. *International journal of oral and maxillofacial surgery*, *46*(8), 968-976.
17. Berania, I., Cardin, G. B., Clément, I., Guertin, L., Ayad, T., Bissada, E., ... & Christopoulos, A. (2017). Four PTEN‐targeting co‐expressed mi RNA s and ACTN 4‐targeting mi R‐548b are independent prognostic biomarkers in human squamous cell carcinoma of the oral tongue. *International Journal of Cancer*, *141*(11), 2318-2328.
18. Shao, H., Wingert, B., Weins, A. *et al.* Focal segmental glomerulosclerosis ACTN4 mutants binding to actin: regulation by phosphomimetic mutations. *Sci Rep* **9**, 15517 (2019). https://doi.org/10.1038/s41598-019-51825-2
19. *Gene - ACTN4*. (n.d.). Harmonizome. https://maayanlab.cloud/Harmonizome/gene/ACTN4
20. Wang, M., Chang, Y., Wu, C., Tyan, Y., Chang, H., Goan, Y., Lai, W., Cheng, P., & Liao, P. (2014). Alpha-Actinin 4 Is Associated with Cancer Cell Motility and Is a Potential Biomarker in Non–Small Cell Lung Cancer. *Journal of Thoracic Oncology*, *10*(2), 286–301. https://doi.org/10.1097/jto.0000000000000396
21. Travers, T., Shao, H., Wells, A., & Camacho, C. J. (2013). Modeling the assembly of the multiple domains of α-actinin-4 and its role in actin cross-linking. *Biophysical journal*, *104*(3), 705-715.
22. Fleming-de-Moraes CD, Rocha MR, Tessmann JW, de Araujo WM, Morgado-Diaz JA. Crosstalk between PI3K/Akt and Wnt/β-catenin pathways promote colorectal cancer progression regardless of mutational status. Cancer Biol Ther. 2022 Dec 31;23(1):1-13. doi: 10.1080/15384047.2022.2108690. PMID: 35944058; PMCID: PMC9367664.
23. Matsuzaki Y, Watabe Y, Enatsu K, Shigematsu S, Shibahara T. Actinin-4 Expression Predicts Poor Disease-free Survival and Correlates with Delayed Lymph Node Metastasis in Patients with Completely Resected Oral Squamous Cell Carcinoma. Bull Tokyo Dent Coll. 2020 Sep 4;61(3):179-186. doi: 10.2209/tdcpublication.2019-0045. Epub 2020 Aug 14. PMID: 32801264.
24. Kawamura K, Miyai K, Sato K, Matsukuma S, Honda K, Ito K, Tsuda H. Copy number gain of ACTN4 is associated with poor prognosis in patients with upper urinary tract urothelial carcinoma. Cancer Sci. 2023 Aug;114(8):3411-3422. doi: 10.1111/cas.15841. Epub 2023 May 24. PMID: 37226638; PMCID: PMC10394141.
25. Watabe Y, Mori T, Yoshimoto S, Nomura T, Shibahara T, Yamada T, Honda K. Copy number increase of ACTN4 is a prognostic indicator in salivary gland carcinoma. Cancer Med. 2014 Jun;3(3):613-22. doi: 10.1002/cam4.214. Epub 2014 Feb 27. PMID: 24574362; PMCID: PMC4101752.
26. Wang, M. C., Chang, Y. H., Wu, C. C., Tyan, Y. C., Chang, H. C., Goan, Y. G., ... & Liao, P. C. (2015). Alpha-actinin 4 is associated with cancer cell motility and is a potential biomarker in non–small cell lung cancer. *Journal of Thoracic Oncology*, *10*(2), 286-301.
27. Yamada, S. I., Yanamoto, S., Yoshida, H., Yoshitomi, I., Kawasaki, G., Mizuno, A., & Nemoto, T. K. (2010). RNAi-mediated down-regulation of α-actinin-4 decreases invasion potential in oral squamous cell carcinoma. *International journal of oral and maxillofacial surgery*, *39*(1), 61-67.
28. Tozuka T, Noro R, Seike M, Honda K. Benefits from Adjuvant Chemotherapy in Patients with Resected Non-Small Cell Lung Cancer: Possibility of Stratification by Gene Amplification of *ACTN4* According to Evaluation of Metastatic Ability. Cancers (Basel). 2022 Sep 7;14(18):4363. doi: 10.3390/cancers14184363. PMID: 36139525; PMCID: PMC9497297.
29. Tian J, Shi Z, Zhao L, Liu P, Sun X, Long L, Zang J, Xiao J. Revolutionizing NSCLC Treatment: Immunotherapy Strategies for EGFR-TKIs Resistance. Clin Respir J. 2024 Dec;18(12):e70037. doi: 10.1111/crj.70037. PMID: 39631794; PMCID: PMC11617040.
30. Joshi, S. (2024, April 1). *Power of EGFR inhibitors in cancer treatment*. DelveInsight Business Research. https://www.delveinsight.com/blog/egfr-inhibitors-for-cancer-treatment
31. Shi, C., Wang, Y., Xue, J., & Zhou, X. (2022). Immunotherapy for EGFR-mutant advanced non-small-cell lung cancer: Current status, possible mechanisms and application prospects. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.940288

32. Rituraj, Pal RS, Wahlang J, Pal Y, Chaitanya M, Saxena S. Precision oncology: transforming cancer care through personalized medicine. Med Oncol. 2025 Jun 9;42(7):246. doi: 10.1007/s12032-025-02817-y. PMID: 40488843.

33. Chhabra, R. (2024). Molecular and modular intricacies of precision oncology. *Frontiers in Immunology*, *15*. https://doi.org/10.3389/fimmu.2024.1476494
34. Noro R, Honda K, Nagashima K, Motoi N, Kunugi S, Matsubayashi J, Takeuchi S, Shiraishi H, Okano T, Kashiro A, Meng X, Yoshida Y, Watanabe S, Usuda J, Inoue T, Wilber H, Ikeda N, Seike M, Gemma A, Kubota K. Alpha-actinin-4 (ACTN4) gene amplification is a predictive biomarker for adjuvant chemotherapy with tegafur/uracil in stage I lung adenocarcinomas. Cancer Sci. 2022 Mar;113(3):1002-1009. doi: 10.1111/cas.15228. Epub 2022 Jan 20. PMID: 34845792; PMCID: PMC8898703.
35. Ginghina O, Hudita A, Zamfir M, Spanu A, Mardare M, Bondoc I, Buburuzan L, Georgescu SE, Costache M, Negrei C, Nitipir C, Galateanu B. Liquid Biopsy and Artificial Intelligence as Tools to Detect Signatures of Colorectal Malignancies: A Modern Approach in Patient's Stratification. Front Oncol. 2022 Mar 8;12:856575. doi: 10.3389/fonc.2022.856575. PMID: 35356214; PMCID: PMC8959149.
36. Hussain MS, Rejili M, Khan A, Alshammari SO, Tan CS, Haouala F, Ashique S, Alshammari QA. AI-powered liquid biopsy for early detection of gastrointestinal cancers. Clin Chim Acta. 2025 Sep 1;577:120484. doi: 10.1016/j.cca.2025.120484. Epub 2025 Jul 14. PMID: 40669686.
37. Mes, S. W., Te Beest, D., Poli, T., Rossi, S., Scheckenbach, K., Van Wieringen, W. N., ... & Brakenhoff, R. H. (2017). Prognostic modeling of oral cancer by gene profiles and clinicopathological co-variables. *Oncotarget*, *8*(35), 59312.
38. Tentler D, Lomert E, Novitskaya K, Barlev NA. Role of ACTN4 in Tumorigenesis, Metastasis, and EMT. Cells. 2019 Nov 13;8(11):1427. doi: 10.3390/cells8111427. PMID: 31766144; PMCID: PMC6912194.