BIOPRESS JOURNAL OF COMPUTATIONAL LIFE SCIENCES, VOL. 1, ISSUE. 6 (2025)

Formulation Innovations for ACTN4 Inhibition: Targeting Metastatic Pathways in Oral Cancer

Dr Pravin Badhe, Ashwini 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.17898421
Received: 10 November 2025
Accepted:21 November 2025

Abstract

Oral cancer remains a major global health burden, with metastasis posing the most significant obstacle to curative management and survival improvement. ACTN4 (α-actinin-4), a cytoskeletal actin-binding protein, is overexpressed or amplified in aggressive oral squamous cell carcinomas and drives cytoskeletal remodeling, epithelial–mesenchymal transition, and metastatic invasion. Current chemotherapeutics poorly target ACTN4-mediated motility pathways and are limited by systemic toxicity and resistance. Emerging formulation innovations—such as nanoemulsions, liposomes, and herbal nanosystems—offer targeted delivery, enhanced intracellular uptake, and modulation of metastatic signaling. Liposomal and lipid-nanoparticle systems improve drug stability, circulation, and tumor selectivity, while herbal nanoformulations enable synergistic inhibition of ACTN4-linked oncogenic cascades. Artificial-intelligence-driven formulation design further enables predictive optimization of drug-excipient ratios, release kinetics, and targeting specificity. Integrating nanotechnology and AI presents a transformative avenue for next-generation ACTN4 inhibitors, paving the way toward precision therapeutics against metastatic oral cancer.

Keywords: oral cancer, ACTN4, Metastasis

Introduction

Epidemiology and Clinical Challenges in Oral Squamous Cell Carcinoma (OSCC)

Oral squamous cell carcinoma (OSCC), representing nearly 90% of all oral malignancies, is among the most prevalent cancers globally and constitutes a significant healthcare challenge, particularly in Asian countries with high tobacco usage. According to recent estimates, OSCC contributes to over 389,000 new cases annually worldwide, with projections suggesting a 65% rise by 2050, disproportionately affecting low- and middle-income countries due to limited healthcare infrastructure and preventive measures. Region-specific studies have revealed distinct demographic and site-based prevalence trends. For example, a 2025 institutional study in South India found that buccal mucosa and tongue were the most affected subsites, with over 60% of cases occurring in individuals aged between 51 and 75 years. Similarly, epidemiological data from Kerala, India showed a rising incidence among younger adults, indicating evolving risk factors and exposure patterns associated with lifestyle, tobacco, and betel nut habits.[1,2,3]

Clinically, OSCC poses persistent challenges due to late diagnosis, local invasion of vital structures, and high rates of locoregional recurrence and distant metastasis despite advances in surgery, radiotherapy, and chemotherapy. Standard multimodal treatments focus largely on cytotoxic eradication of tumor cells, frequently leading to significant morbidity, functional impairment, and diminished quality of life. Crucially, the biology of OSCC is defined not only by proliferation but also by its intrinsic capacity for invasion and metastasis—processes that are not adequately targeted by conventional chemotherapeutic strategies.[4,5]

Metastasis: The Critical Barrier to Cure

Metastasis remains the primary cause of OSCC-associated mortality. Even after complete resection of early-stage oral lesions, late cervical lymph node metastasis may develop, severely limiting survival outcomes. Tumor cell dissemination from primary lesions involves a multistep process encompassing cell detachment, extracellular matrix (ECM) degradation, migration, invasion, intravasation, and colonization of distant tissues. Molecularly, this process is driven by cytoskeletal remodeling, epithelial–mesenchymal transition (EMT), and altered adhesion signaling—hallmarks of aggressive OSCC phenotypes.[6,7,8]

Recent studies underscore that targeting metastatic signaling pathways may be more beneficial than focusing solely on cytotoxicity. Eliminating proliferative cells without addressing molecular drivers of motility or invasion often results in tumor relapse and dissemination. Thus, novel anti-metastatic strategies are being developed that emphasize suppressing migration, invasion, and EMT, aiming to prevent recurrence rather than merely reducing tumor mass.[4]

ACTN4: A Metastasis-Associated Actin-Binding Protein

Among the emerging biomarkers linked with OSCC progression, α-actinin-4 (ACTN4) has gained prominence as a key mediator of metastasis and poor clinical outcome. ACTN4, encoded by the ACTN4 gene on chromosome 19q13, is a cytoskeletal actin-binding protein originally characterized as a regulator of cell motility, adhesion, and cytoskeletal dynamics. It plays a pivotal role in crosslinking actin filaments and coordinating structural rearrangements essential for cell migration.[6,7]

Overexpression or gene amplification of ACTN4 has been documented in a broad spectrum of epithelial carcinomas, including breast, ovarian, colorectal, lung, and oral cancers. In OSCC, 70% of primary tumors have demonstrated elevated ACTN4 expression, correlating strongly with greater invasive potential as measured by Matrigel invasion assays. RNA interference experiments confirm that down-regulating ACTN4 expression reduces invasion and migration capacities in OSCC cell lines, providing direct evidence of its functional role in promoting metastatic behavior.[7,9]

At the clinical level, gene amplification of ACTN4 has been established as an independent prognostic marker in stage I/II oral tongue cancers, where it predicts significantly shorter overall survival (hazard ratio 6.08, $P = 0.001$). The study demonstrated that patients with ACTN4 copy number gain experience sixfold increased risk of mortality compared to non-amplified cases, highlighting its potential value for risk stratification and therapeutic targeting. Mechanistically, ACTN4 overexpression supports invadopodia formation, an essential step for basement membrane degradation and tissue invasion, linked to enhanced phosphorylation events (e.g., EGF-induced tyrosine 11 and 13 phosphorylation). This functional versatility underscores ACTN4's capacity to regulate multiple modes of cell motility—including both mesenchymal and amoeboid migration—making it a pivotal orchestrator of metastasis in OSCC.[6,7]

The Clinical Correlation Between ACTN4 Overexpression and Poor Prognosis

Clinical investigations consistently reveal a negative correlation between ACTN4 amplification and patient survival in OSCC. Both protein overexpression and gene amplification of ACTN4 serve as robust predictors of lymph node metastasis, local recurrence, and reduced disease-specific survival. These associations are reinforced by molecular evidence linking ACTN4 to EMT marker modulation—such as upregulation of vimentin and N-cadherin and downregulation of E-cadherin—which collectively facilitate the transition to an invasive phenotype. Moreover, in response to inflammatory signaling (e.g., TNFα/TNFR1 axis), ACTN4-mediated cytoskeletal rearrangements have been shown to amplify pro-metastatic signaling in OSCC microenvironments.[6,7,8]

Taken together, these findings position ACTN4 as not merely a biomarker but as an active driver of metastatic cascades. As therapeutic efforts shift from generalized cytotoxic approaches to molecularly defined targets, inhibiting ACTN4 expression or function presents a promising avenue for suppressing OSCC invasiveness.

Limitations of Conventional Chemotherapeutic Approaches

Despite available systemic treatments—comprising platinum-based drugs (cisplatin, carboplatin), fluorouracil (5-FU), and taxanes—metastatic OSCC remains poorly responsive due to multiple limitations: non-selective cytotoxicity, poor bioavailability, rapid systemic clearance, and tumor resistance mechanisms. These regimens primarily target dividing cells but fail to block cell migration and invasion pathways regulated by ACTN4. Moreover, systemic administration often leads to profound off-target toxicity, mucositis, and immunosuppression, limiting therapeutic compliance.[5,10]

Another challenge lies in the tumor microenvironment's heterogeneity, which restricts even drug distribution. Cytoskeletal and membrane-associated proteins like ACTN4 are intracellular and structurally protected, requiring efficient delivery of inhibitors across the cellular membrane. Therefore, novel nanoscale delivery platforms capable of penetrating tumor barriers and sustaining intracellular release of anti-metastatic drugs are urgently needed.

The Need for Targeted Delivery Systems to Suppress ACTN4-Driven Invasion

Advances in nanotechnology-enabled drug delivery present opportunities to circumvent the pharmacokinetic and pharmacodynamic challenges of conventional chemotherapeutics. Systems such as nanoemulsions, liposomes, polymeric nanoparticles, and herbal nanosystems have emerged as promising carriers for delivering small molecules, genes, or phytoconstituents directly to tumor tissues with enhanced selectivity. By improving tumor penetration, sustained release, and intracellular uptake, these systems enable more precise modulation of molecular targets like ACTN4.[2]

For example:

- Nanoemulsions provide high surface area and solubilization efficiency, improving delivery of hydrophobic natural compounds that inhibit cytoskeletal signaling.
- Liposomes protect bioactive molecules from enzymatic degradation and extend systemic circulation time, while surface modifications can facilitate ligand-directed targeting toward OSCC cells overexpressing specific receptors.
- Herbal nanosystems, incorporating phytochemicals such as curcumin, resveratrol, and quercetin, possess multi-targeting potential, capable of attenuating both EMT and ACTN4 expression through overlapping molecular cascades.

Moreover, RNAi-based nanosystems aiming to silence ACTN4 mRNA represent a feasible anti-metastatic strategy. When encapsulated in protective nanocarriers, siRNAs targeting ACTN4 evade degradation and achieve efficient cytoplasmic release, as demonstrated in cell-based studies reducing invasion potential by up to 60%.[9]

Integrating AI-Driven Formulation Optimization

The complexity of nanoscale systems and the vast parameter space of formulation design—composition ratios, particle size, surface charge, ligand types, and release kinetics—make artificial intelligence (AI) an invaluable tool for optimizing drug delivery vehicles. Machine learning (ML) algorithms and predictive modeling platforms can analyze high-dimensional experimental data to predict formulation stability, biodistribution, and target binding efficiency. Recent AI-assisted frameworks employ multi-objective optimization to simultaneously maximize encapsulation efficiency, minimize toxicity, and tailor drug release profiles specific to tumor biology.

In the context of ACTN4 inhibition, AI can facilitate computational screening of excipient–drug interactions, identification of optimal nanoparticle attributes for tumor tissue penetration, and prediction of intracellular trafficking patterns. Coupled with molecular dynamics simulations, AI-assisted formulation design holds the potential to accelerate translational nanotherapeutic development for ACTN4-targeted interventions.

ACTN4 Biology and Its Role in Metastatic Pathways

Structural Organization and Cytoskeletal Function of ACTN4

Alpha-actinin-4 (ACTN4) is an actin-binding protein belonging to the spectrin superfamily, encoded by the *ACTN4* gene on chromosome 19q13.1. Structurally, ACTN4 comprises three key regions: an N-terminal actin-binding domain (ABD) with two calponin homology subdomains (CH1–CH2), a central rod domain containing four spectrin repeats (SR1–SR4) responsible for antiparallel dimerization, and a C-terminal region with two EF-hand calcium-binding motifs. This architecture enables ACTN4 to cross-link actin filaments, regulate focal adhesion contacts, and orchestrate cytoskeletal remodeling.[12,13]

Functionally, ACTN4 localizes to the cytoplasm and focal adhesion plaques, where it maintains actin filament integrity and coordinates adhesion–migration dynamics. Through its binding to adhesion plaque proteins such as vinculin, paxillin, and α-integrins, ACTN4 transduces mechanical cues into cytoskeletal responses that promote cell spreading, motility, and mechanical resilience. The structural elasticity conferred by spectrin repeats facilitates dynamic reorganization of actin filaments, crucial for membrane protrusion and lamellipodia formation—key determinants of cancer cell migration.[12,14]

In addition to its structural role, ACTN4 functions as a signaling scaffold, interacting with protein kinases such as MEKK1, focal adhesion kinase (FAK), and phosphatidylinositol 3-kinase (PI3K), linking cytoskeletal rearrangements with intracellular signaling cascades that drive migration and invasion.

Mechanistic Pathways: EMT Induction and Invasiveness

1. ACTN4 and Epithelial–Mesenchymal Transition (EMT)

Epithelial–mesenchymal transition (EMT) is a fundamental process enabling epithelial cancer cells to acquire mesenchymal features, characterized by loss of E-cadherin, gain of vimentin, and increased motility. ACTN4 promotes EMT through multiple mechanisms involving PI3K/Akt, FAK, and NF-κB signaling pathways.[15,16,17]

- PI3K/Akt axis: Overexpression of ACTN4 enhances phosphorylation of Akt and downstream activation of GSK-3β/β-catenin signaling, leading to β-catenin nuclear accumulation and transcription of EMT-related genes such as *Snail* and *Twist*.[16,17]
- NF-κB signaling: ACTN4 upregulates NF-κB transcriptional activity, promoting expression of EMT transcription factors even in the absence of canonical TGF-β signals.[15]
- FAK–Src signaling: By interacting with FAK at focal adhesion sites, ACTN4 reinforces integrin-mediated cytoskeletal rearrangements, elevating cellular contractility and ECM degradation capacity essential for invasion.[14,18]

Silencing of ACTN4 expression through RNA interference has been shown to reverse EMT phenotypes, restore epithelial markers, and suppress invasion in multiple carcinoma models, including OSCC cell lines and xenografts.[7]

2. Crosstalk with Matrix Metalloproteinases (MMPs) and Integrins

ACTN4-mediated cell motility also depends on its regulatory interplay with matrix metalloproteinases (MMPs) and integrins. Upregulation of MMP-2 and MMP-9 through ACTN4-dependent PI3K/Akt/NF-κB activation facilitates extracellular matrix degradation, a prerequisite for tumor invasion and metastasis. Moreover, ACTN4 enhances β1-integrin clustering at focal adhesion sites, stabilizing adhesion turnover necessary for directional migration.[14,19,20]

Integrins, in tandem with ACTN4, mediate bidirectional signaling that couples ECM engagement to cytoskeletal reorganization. Integrin-linked kinase (ILK) activation by ACTN4 contributes to the mechanical feedback loop promoting traction force generation at the invasive front.[14,19]

3. Angiogenic and Invasion Signaling Integration

ACTN4's role extends beyond cytoskeletal remodeling to include the regulation of angiogenesis and tumor–stromal interactions. Evidence suggests that ACTN4 modulates the PI3K/Akt pathway and VEGF expression, thereby fostering endothelial migration and neovascularization within the tumor microenvironment. Furthermore, ACTN4 overexpression in tumors enhances secretion of pro-angiogenic factors and integrin-mediated endothelial activation, positioning ACTN4 as a multifunctional facilitator of the metastatic niche.[15,21,22]

Evidence from Oral Cancer Models and Clinical Biopsies

In oral squamous cell carcinoma (OSCC), ACTN4 is markedly overexpressed at both mRNA and protein levels compared with adjacent normal tissues. Functional assays using OSCC cell lines (e.g., HSC-3, SAS, and SCC-9) have demonstrated that ACTN4 knockdown decreases cellular motility, reduces invasion through Matrigel matrices, and suppresses formation of invadopodia—actin-rich protrusions facilitating ECM degradation. Conversely, overexpression of ACTN4 enhances invasive and migratory capacities by 3–5 fold, correlating with heightened integrin engagement and focal adhesion turnover.[6,7,12]

Clinical correlative studies confirm that ACTN4 gene amplification and protein overexpression are significantly associated with metastatic propensity and poor prognosis in oral cancer patients. A landmark retrospective study of 54 patients with stage I/II oral tongue carcinoma showed that ACTN4 gene amplification serves as an independent prognostic factor for overall survival, with a hazard ratio of 6.08 (95% CI: 1.66–22.27, P = 0.001). Immunohistochemical data reveal strong cytoplasmic and membranous localization of ACTN4 at the invasive front of OSCC lesions, frequently coinciding with lymphovascular infiltration. These findings substantiate a direct clinical link between ACTN4 activity and metastatic aggressiveness.[6]

Further evidence from high-throughput transcriptomic analyses (TCGA data) indicates that high ACTN4 expression corresponds with reduced progression-free and overall survival across epithelial cancers, including head-and-neck squamous cell carcinoma. In OSCC, ACTN4 amplification occurs early in tumorigenesis and is often retained during metastasis, underscoring its role as a driver gene rather than a secondary event.[14]

Mechanistic studies have revealed that ACTN4 promotes invadopodia maturation through regulation of myosin IIB expression and myosin IIA localization, promoting traction force asymmetry and directional invasion. These mechanobiological functions support invasive cell behavior under conditions of ECM stiffness and mechanical stress, both key features of the oral tumor microenvironment.[14]

Limitations of Current Therapeutic Approaches

Poor Targeting of Metastatic Cascades by Standard Chemotherapeutics

Cisplatin: Limitations in Metastatic Control

Cisplatin, a platinum-based alkylating agent, remains a cornerstone of OSCC treatment, particularly in locally advanced and recurrent/metastatic disease. Despite its efficacy in inducing DNA damage through platinum-DNA adduct formation and subsequent cell cycle arrest, cisplatin exhibits significant limitations in targeting metastatic cascades. The drug's primary mechanism involves DNA crosslink formation leading to apoptosis, but it fails to address the molecular drivers of invasion and metastasis—such as ACTN4-mediated cytoskeletal remodeling, EMT signaling, and integrin-dependent motility.

Clinical studies demonstrate that 33% of OSCC patients develop cisplatin resistance, often accompanied by enhanced metastatic behavior. The resistance mechanisms include upregulation of DNA repair pathways (e.g., nucleotide excision repair, homologous recombination), enhanced drug efflux via ATP-binding cassette transporters, and altered cellular metabolism that reduces platinum accumulation. Critically, cisplatin resistance correlates with activation of survival signaling pathways including PI3K/Akt, NF-κB, and Wnt/β-catenin—the same pathways that drive ACTN4-mediated metastasis.[23,24]

Moreover, cisplatin's systemic toxicity profile limits dose intensification. Nephrotoxicity, ototoxicity, peripheral neuropathy, and bone marrow suppression constrain therapeutic windows, preventing achievement of cytostatic

concentrations necessary to inhibit invasion-associated proteins like ACTN4. The drug's non-selective cytotoxicity also affects rapidly dividing normal cells while sparing slowly dividing, invasive cancer stem cells that drive metastatic seeding.

5-Fluorouracil (5-FU): Metabolic Targeting Without Anti-invasive Activity

5-Fluorouracil functions as a pyrimidine analog that disrupts DNA and RNA synthesis through thymidylate synthase inhibition and incorporation into nucleic acids. While effective against proliferating cells, 5-FU demonstrates 40.2% resistance frequency in OSCC patients. The predominant resistance mechanism involves thymidylate synthase overexpression and enhanced dihydropyrimidine dehydrogenase activity, which degrades 5-FU before it reaches therapeutic concentrations.[23]

Importantly, 5-FU's antimetabolite mechanism does not target cytoskeletal dynamics, focal adhesion signaling, or EMT regulatory networks that govern metastatic behavior. Consequently, surviving cancer cells may exhibit enhanced invasiveness due to selection pressure favoring motile, therapy-resistant phenotypes. Clinical observations confirm that OSCC patients treated with 5-FU-based regimens often experience local recurrence and distant metastasis despite initial tumor shrinkage, highlighting the need for anti-invasive adjuncts.[25]

Combination Therapy Limitations

Standard cisplatin plus 5-FU (PF) regimens achieve 30% overall response rates in recurrent/metastatic OSCC, with median progression-free survival of 3.0 months and overall survival of 9.8 months. These modest outcomes reflect the regimens' inability to prevent metastatic spread. The combination targets DNA replication and synthesis but leaves invasion-promoting pathways intact, allowing residual cancer cells to disseminate during treatment-induced stress responses.[25]

Furthermore, the additive toxicity of combination regimens—including severe stomatitis (6.6%), leukopenia (10%), and gastrointestinal toxicity (40%)—limits treatment completion and may compromise immune surveillance functions essential for controlling micro-metastases.

Bioavailability Issues of Anti-Metastatic Natural Compounds

Pharmacokinetic Challenges

Natural compounds with demonstrated anti-ACTN4 and anti-metastatic activities—including curcumin, resveratrol, quercetin, epigallocatechin gallate (EGCG), and ginsenosides—suffer from significant bioavailability limitations that restrict their clinical translation. These compounds face multiple pharmacokinetic barriers:

1. Poor aqueous solubility: Most bioactive phytochemicals exhibit hydrophobic characteristics limiting dissolution in biological fluids.

2. First-pass metabolism: Extensive hepatic metabolism via phase I (CYP450) and phase II (glucuronidation, sulfation) enzymes reduces systemic exposure.

3. Rapid clearance: Short plasma half-lives (typically <4 hours) prevent sustained target engagement.

4. Limited cellular uptake: Poor membrane permeability restricts intracellular accumulation necessary for ACTN4 modulation.

For instance, curcumin oral bioavailability is <1% due to rapid glucuronidation, resulting in therapeutic concentrations remaining unachievable through conventional dosing. Similarly, resveratrol undergoes extensive first-pass metabolism, with >90% conversion to inactive metabolites before reaching systemic circulation.[26]

Target Site Accessibility

Even when natural compounds achieve systemic levels, their distribution to tumor tissues remains limited by several factors:

- Plasma protein binding: High binding to albumin and α1-acid glycoprotein reduces free drug fractions available for tissue penetration.
- Vascular barriers: Abnormal tumor vasculature with discontinuous endothelium and irregular blood flow impairs compound delivery.
- Cellular efflux: Overexpression of P-glycoprotein and multidrug resistance proteins in cancer cells actively extrudes accumulated compounds.

These limitations necessitate supraphysiological concentrations for meaningful anti-ACTN4 activity, often associated with systemic toxicity that negates therapeutic benefits.[27]

Challenges in Delivering Drugs Across the Dense Tumor Microenvironment (TME)

Structural and Physiological Barriers

The oral tumor microenvironment presents unique obstacles to effective drug penetration, significantly limiting the efficacy of both conventional chemotherapeutics and targeted agents. Key barriers include:

1. Dense Extracellular Matrix (ECM)

OSCC tumors exhibit abundant collagen deposition, hyaluronic acid accumulation, and crosslinked fibrin networks that create physical impedance to drug diffusion. The dense ECM structure increases tortuous diffusion pathways and establishes concentration gradients that favor drug accumulation in peripheral tumor regions while limiting penetration to hypoxic, invasive cores where ACTN4 activity is highest.[28,29]

2. Elevated Interstitial Fluid Pressure (IFP)

Compromised lymphatic drainage combined with aberrant vascular architecture results in elevated interstitial fluid pressure (up to 40 mmHg in OSCC tumors versus 0-3 mmHg in normal tissues). This pressure gradient opposes convective drug transport from blood vessels into tumor parenchyma, significantly reducing therapeutic agent accumulation.[30]

3. Abnormal Vasculature

Oral tumors develop chaotic, leaky vasculature characterized by:

- Irregular vessel diameter and spacing
- Compromised pericyte coverage
- Heterogeneous blood flow patterns
- Vascular compression by proliferating cancer cells

These abnormalities result in heterogeneous drug distribution, hypoxic regions with limited perfusion, and inadequate drug exposure in areas of active invasion.[31]

Cellular and Molecular Resistance Mechanisms

1. Cancer-Associated Fibroblasts (CAFs)

CAFs constitute up to 50% of OSCC tumor mass and contribute to drug resistance through multiple mechanisms:

- ECM remodeling: Enhanced collagen synthesis and crosslinking
- Growth factor secretion: Production of TGF-β, PDGF, and FGF that promote drug efflux
- Metabolic competition: Consumption of glucose and amino acids, limiting drug uptake

2. Hypoxia-Induced Resistance

Hypoxic regions (oxygen tension <1%) within OSCC tumors exhibit:

- HIF-1α stabilization leading to MDR1 upregulation
- Reduced drug metabolism due to decreased enzymatic activity
- Enhanced DNA repair mechanisms that counteract platinum-induced damage
- ACTN4 upregulation via hypoxia-responsive elements, promoting invasion under therapeutic stress.[29]

3. Immunosuppressive Environment

The OSCC TME contains regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and M2 macrophages that:

- Suppress cytotoxic T cell function required for immune-mediated tumor clearance
- Secrete immunosuppressive cytokines (IL-10, TGF-β) that promote therapy resistance
- Support angiogenesis through VEGF and PDGF production.[26]

Oral Cavity-Specific Challenges

1. Saliva-Mediated Drug Dilution

The continuous saliva production (1-2 L/day) in the oral cavity poses unique challenges:

- Premature drug dilution reduces local therapeutic concentrations
- Enzymatic degradation by salivary amylases and proteases
- pH variations (5.5-7.5) affecting drug stability and absorption.[26]

2. Epithelial Barrier Function

Intact oral epithelium surrounding tumor lesions exhibits:

- Tight junction integrity limiting paracellular drug transport
- Active efflux pumps preventing drug accumulation
- Rapid cell turnover (7-14 days) diluting drug concentrations

Implications for ACTN4-Targeted Therapies

These TME-related barriers significantly impact ACTN4-targeted therapeutic strategies:

1. Inadequate target engagement: Poor drug penetration prevents effective ACTN4 inhibition in invasive tumor regions.

2. Subtherapeutic concentrations: Rapid clearance and limited bioavailability fail to achieve ACTN4 modulation thresholds.

3. Resistance development: Hypoxic stress and CAF signaling may upregulate ACTN4 expression, creating adaptive resistance.

Herbal Actives as ACTN4 Modulators

4.1 Curcumin

Mechanistic Basis for ACTN4 Suppression

Curcumin, a polyphenolic diketone derived from Curcuma longa, exerts potent anti-metastatic activity primarily through inhibition of NF-κB and PI3K/Akt signaling pathways, both of which transcriptionally regulate ACTN4 expression. In invasive oral squamous cell carcinoma (OSCC), ACTN4 overexpression parallels augmentation of NF-κB-dependent transcription of migration-associated genes (e.g., MMP-9, vimentin, Snail). Curcumin disrupts this signaling by preventing IKKβ-mediated IκB degradation, silencing NF-κB nuclear translocation and transcriptional activation of motility genes. Concurrently, curcumin inhibits PI3K/Akt/mTOR phosphorylation cascades, restoring PTEN activity and suppressing β-catenin nuclear accumulation—thereby attenuating EMT and cytoskeletal reorganization associated with ACTN4 upregulation.[32,33,34]

Nanoemulsion formulations of curcumin have illustrated enhanced suppression of PI3K/Akt/mTOR and upregulation of miR-199a, an established negative regulator of invasion-related signaling, which indirectly reduces ACTN4 transcription in OSCC models.[35]

Evidence from In Vitro and In Vivo OSCC Models

In vitro studies reveal that curcumin (10–20 μM) significantly downregulates ACTN4 expression in OSCC cell lines (HSC-3, SCC-9), coinciding with a reduction in lamellipodia formation, migratory distance, and matrigel invasion. The effect is synergistic with cisplatin, suggesting that curcumin sensitizes resistant cells by diminishing EMT regulators. In vivo xenograft models confirm reduced ACTN4 levels in tumor tissue following curcumin administration, accompanied by 55–70% decreases in tumor volume and reduction of pulmonary metastases compared to control groups. Mechanistically, curcumin reduces phosphorylated Akt (Ser473) and NF-κB p65 phosphorylation, thereby limiting transcription of cytoskeletal and adhesion genes linked to ACTN4 function.[35,36]

Collectively, these findings underscore curcumin's dual inhibitory action on upstream signaling nodes of ACTN4 transcription and on structural remodeling required for metastasis.

4.2 Neem (Azadirachta indica)

Phytochemical Profile and Anti-metastatic Potential

Neem (Azadirachta indica), a member of the Meliaceae family, contains over 140 bioactive constituents including nimbolide, azadirachtin, gedunin, and nimbin. These compounds demonstrate multi-targeted anticancer activities impacting proliferation, apoptosis, and metastasis. Limonoids such as nimbolide possess high cytotoxic selectivity toward malignant cells while sparing normal tissues. At the molecular level, neem constituents are known to suppress NF-κB, PI3K/Akt, Wnt/β-catenin, and GSK-3β pathways—critical mediators of ACTN4 activity and cytoskeletal modulation.[37,38]

Inhibition of EMT Markers and Cytoskeletal Remodeling

Preclinical data demonstrate that hydrophilic neem leaf extracts, including supercritical CO_2 Neem extract (SCNE), inhibit proliferation and migration of OSCC cell lines (SCC4, Cal27, and HSC3) by downregulating MMP-2, MMP-9, and vimentin expression while upregulating E-cadherin, indicative of EMT reversal. Neem-treated xenograft mice exhibited significant reductions in tumor volume and incidence of tongue carcinoma, confirming its systemic

antimetastatic effect. Moreover, these extracts attenuate focal adhesion kinase (FAK) activity and suppress actin stress fiber remodeling, thereby mimicking direct functional inhibition of ACTN4-related motility.[39]

Mechanistically, inhibiting NF-κB/PI3K pathways in neem-treated models curtails downstream transcription of ACTN4 and integrin-linked actin regulators. Proteomic profiling supports this observation, showing decreased ACTN4 and myosin IIA expression in neem-treated OSCC cohorts. These cumulative findings position neem phytochemicals as promising natural ACTN4 modulators with potential for inclusion in combinatorial nanoformulations to suppress invasion and metastasis.

4.3 Moringa (Moringa oleifera)

Bioactive Compounds and Invasion-Related Pathways

Moringa oleifera contains abundant bioactives—quercetin, kaempferol, niazimicin, and moringin—that target inflammation, oxidative stress, and invasion signaling. Among these, quercetin exhibits anti-metastatic and anti-cytoskeletal activity via downregulation of PI3K/Akt/mTOR and NF-κB pathways, both implicated in ACTN4 regulation. Niazimicin, derived from seed and leaf extracts, suppresses protein kinase C (PKC) and MMP-9, thereby preventing ECM degradation and cellular invasion. Moringa phytochemicals also enhance pro-apoptotic gene expression (Bax, p53, caspase-3/9), further limiting survival of migratory cancer phenotypes.[40,41]

Potential Synergy with ACTN4 Suppression

Evidence indicates that Moringa extracts inhibit nuclear translocation of NF-κB and reduce phosphorylation of Akt in multiple cancer lines, suggesting indirect transcriptional repression of ACTN4 and its associated motility cascade. In OSCC, Moringa oleifera leaf extract (50–100 μg/mL) causes a dose-dependent reduction in migration index and invadopodia formation, aligning with reduced ACTN4 and ezrin expression. Quercetin—a major flavonoid constituent—has independently demonstrated direct binding affinity to actin-binding regulatory domains, implying a capacity to interfere with ACTN4's cytoskeletal anchorage.

Furthermore, co-treatment of Moringa extract with chemotherapeutic agents amplifies apoptotic signaling while suppressing invasion markers, revealing synergistic therapeutic prospects. The inferable synergy between quercetin-driven PI3K/Akt inhibition and niazimicin-mediated MMP repression may yield dual anti-cytoskeletal and anti-metastatic effects, converging on ACTN4 downstream regulation.[41]

Advanced Formulation Approaches for ACTN4 Targeting

5.1 Nanoemulsions

Composition, Droplet Size Optimization, and Surfactant Selection

Nanoemulsions are kinetically stable colloidal dispersions of oil and water stabilized by surfactants with droplet sizes typically ranging from 20 to 200 nm. Their small size enhances physical stability and bioavailability of poorly soluble compounds like curcumin and neem phytochemicals. Optimal nanoemulsion formulations involve careful selection of:

- Oil phase: Medium-chain triglycerides (MCT), soybean oil, or castor oil for solubilizing hydrophobic actives.
- Surfactants: Non-ionic surfactants such as Tween 80 or Span 80 are preferred for biocompatibility and minimizing irritation.
- Co-surfactants: Ethanol or propylene glycol to reduce interfacial tension and stabilize droplet morphology.
- Droplet size targeting 50–200 nm with low polydispersity index (PDI $\leq$ 0.2) improves mucosal penetration and cellular uptake.

Enhancing Solubility and Mucosal Penetration of Herbal Actives

Nanoemulsions significantly improve the aqueous solubility and chemical stability of hydrophobic phytochemicals, protecting them from enzymatic degradation in the oral cavity and enhancing permeation through mucosal barriers. Their small droplet size facilitates close contact with mucosal surfaces, increasing residence time and absorption rates. The negative surface charge and surfactant coating reduce mucin interaction and mucus entrapment, favoring deeper tissue penetration.

Examples of Curcumin or Neem Nanoemulsions in Oral Cancer

Curcumin-loaded nanoemulsions have demonstrated potent anti-proliferative effects in OSCC models by inhibiting the PI3K/Akt/mTOR pathway and upregulating tumor-suppressing microRNAs (e.g., miR-199a). These formulations achieve high encapsulation efficiency (~95%) with particle sizes around 200 nm. Neem nanoemulsion formulations enriched with nimbolide and other limonoids also show significant suppression of EMT markers and metastatic signaling in oral cancer cells, although clinical research remains at preclinical stages.[35,38]

5.2 Liposomal Carriers

Advantages for Encapsulating Hydrophobic Compounds

Liposomes are spherical vesicles with phospholipid bilayers capable of encapsulating hydrophobic substances within their lipid membranes, protecting them from metabolic degradation and improving bioavailability. Their biocompatibility and biodegradability make them suitable carriers for delivering herbal actives such as curcumin and nimbolide.

Lipid Composition Tuning for Tumor Accumulation (EPR Effect)

Modification of lipid components, such as incorporation of hydrogenated phosphatidylcholine, cholesterol, and negatively charged phospholipids, optimizes liposome membrane rigidity and stability. This tuning enhances circulation half-life, promoting enhanced permeability and retention (EPR) effect-mediated accumulation in tumor microenvironments due to leaky vasculature.

PEGylation and Ligand-Targeting for ACTN4+ Tumor Sites

Polyethylene glycol (PEG) coating ("PEGylation") on liposome surfaces extends systemic circulation by reducing opsonization and recognition by the mononuclear phagocyte system. Targeting ligands (e.g., peptides, antibodies) directed against overexpressed receptors in ACTN4+ OSCC cells—such as α5β1 integrin—can be conjugated on PEG termini to facilitate receptor-mediated endocytosis and increase intracellular delivery of actin-modulatory drugs.[43,44]

5.3 Herbal-Based Nanosystems

Phytosome, Polymeric Nanoparticles, and Green-Synthesized Nanosystems

Phytosomes are complexes of phytochemicals with phospholipids, forming lipid-compatible molecular complexes that enhance absorption and bioavailability of herbal actives such as curcumin and quercetin. Polymeric nanoparticles using biodegradable polymers (e.g., PLGA, chitosan) provide sustained release and protect active constituents from degradation.

Green-synthesized metallic nanoparticles (e.g., gold, silver) using plant extracts combine natural biocompatibility with enhanced anticancer properties and drug delivery capabilities.[45,46]

Dual Delivery Strategies (Herbal + Chemotherapeutic)

Co-encapsulation strategies combining herbal actives (curcumin, neem extracts) with chemotherapeutics such as cisplatin or doxorubicin in nanosystems enable synergistic anticancer effects. These formulations reduce chemotherapeutic doses required, mitigate systemic toxicity, and improve targeting of metastatic pathways, particularly ACTN4-related invasion signaling.

Case Studies

- Curcumin–Cisplatin Liposomes: Curcumin-loaded liposomes co-delivering cisplatin demonstrate increased cytotoxicity in OSCC cell lines by enhancing apoptosis and suppressing metastatic signaling more effectively than individual agents.

- Neem–Doxorubicin Nanoformulations: Neem extract-enhanced doxorubicin nanocarriers exhibit superior targeting of OSCC cells, reduction in EMT, and inhibition of invasion markers compared to doxorubicin alone, suggesting improved therapeutic indices.[46]

AI-Driven Design and Optimization for ACTN4 Targeting

Machine Learning for Predicting Stability, Droplet Size, and Release Kinetics

Advanced machine learning (ML) algorithms have been increasingly applied to optimize nanoformulation parameters, including nanoemulsion and nanoparticle stability, droplet/particle size, and drug release profiles. By training models on extensive datasets incorporating variables such as emulsification energy, surfactant concentration, oil-to-water ratio, and processing conditions, ML methods like Gaussian Process Regression (GPR) and Kernel Ridge Regression (KRR) achieve highly accurate predictions with R^2 values > 0.9 for droplet size and zeta potential. These predictive models facilitate rational design by forecasting formulation stability over time and guiding parameter adjustments to achieve desired nanosystem characteristics vital for effective delivery of ACTN4-modulating agents.[47]

In Silico Screening for ACTN4—Ligand Interactions

Computational drug discovery leveraging molecular docking and molecular dynamics (MD) simulations accelerates identification of potential small-molecule inhibitors targeting ACTN4 functional domains. These techniques examine binding affinity, stability, and interaction dynamics between candidate ligands and ACTN4's actin-binding or regulatory sites. Docking provides initial rankings of compound effectiveness based on energy scores, while MD simulations confirm conformational stability and key intermolecular interactions under physiological conditions, improving confidence in hit selection for subsequent experimental validation.[48,49]

AI-Driven Optimization of Formulation Parameters

AI platforms extend beyond predicting formulation outcomes by enabling multi-objective optimization of parameters such as particle size, surfactant ratios, and zeta potential to maximize therapeutic efficacy and bioavailability. Through supervised learning and genetic algorithms, AI identifies optimal combinations that balance droplet stability, cellular uptake, and controlled release profiles. For ACTN4-targeted nanoformulations, such optimization ensures maximal intracellular delivery of inhibitors with minimal off-target toxicity, improving likelihood of inhibiting ACTN4-mediated metastatic pathways.[50]

Integrating Omics Data and Predictive Modeling for Personalized ACTN4-Targeted Therapy

The advent of multi-omics datasets encompassing genomics, transcriptomics, proteomics, and metabolomics paired with machine learning fosters precision medicine approaches for ACTN4-targeted therapy. Predictive models trained on omics profiles accurately classify patient subpopulations based on ACTN4 expression, metastatic risk, and drug sensitivity, enabling personalized treatment regimens. Hybrid models combining mechanistic insights from genome-scale metabolic models with AI-driven data integration improve interpretability and optimize therapeutic combinations on a patient-specific basis, paving the way for tailored nanomedicine formulations targeting ACTN4-driven oral cancers.[51,52,53]

Synergistic Therapy: Formulation–Drug Combinations

Combining Nanoformulations with Standard Chemotherapy

The combination of nanoformulations loaded with herbal actives alongside standard chemotherapeutics such as cisplatin and paclitaxel is emerging as a powerful strategy to enhance oral cancer therapy. Nanoformulations improve the solubility, stability, and targeted delivery of phytochemicals like curcumin and neem extracts while chemotherapy induces cytotoxicity, resulting in enhanced anti-tumor efficacy. Co-delivery systems exploit the complementary mechanisms of action, improving drug bioavailability in tumor tissues while potentially reducing systemic toxicity.

Mechanistic Synergy: Cytotoxic + Anti-Invasive Effects

Synergy arises as chemotherapy agents (cisplatin, paclitaxel) promote tumor cell apoptosis and proliferation arrest while herbal nanoactives suppress invasion, epithelial–mesenchymal transition (EMT), and metastatic signaling pathways, including ACTN4-mediated cytoskeletal remodeling. For example, curcumin nanoemulsions inhibit PI3K/Akt and NF-κB signaling — pathways implicated in chemoresistance and metastatic progression — thus restoring chemosensitivity and suppressing metastatic spread. This dual action targets both proliferative and migratory cancer cell compartments, overcoming limitations of monotherapy.[54]

Strategies for Co-Delivery Systems and Spatiotemporal Release

Advanced nano-carrier platforms employ co-encapsulation or combination-loading strategies facilitating spatiotemporally controlled release of chemotherapeutic and herbal agents. These systems are engineered to release anti-invasive herbal compounds at the tumor periphery concurrently or prior to chemotherapeutic drugs acting on proliferating cells. Such temporal coordination maximizes intracellular drug synergy and minimizes dosing frequency.

Examples include pH-responsive dual-drug nanocarriers that first release vascular-disrupting agents followed by cytotoxic chemotherapy, tailoring the drug gradient within heterogeneous tumor microenvironments. Scaffold designs allow sustained release over hours to days, ensuring prolonged target engagement and diminishing adverse effects.

Preclinical Outcomes and Translational Potential

Preclinical studies of cisplatin-curcumin co-loaded niosomes reported over 50% reduction in OSCC cell viability and enhanced apoptosis compared to single-agent treatments, illustrating synergistic cytotoxic and anti-migratory effects. Similarly, neem-doxorubicin nanoformulations demonstrated combined downregulation of EMT and invasion markers alongside DNA damage induction, reducing metastatic potential and tumor burden in xenograft models.

These promising outcomes support the translation of combinatorial nanoformulation therapies into clinical trials, aiming to improve therapeutic indices, reduce chemotherapeutic resistance, and enhance quality of life for oral cancer patients.[55,56]

Challenges and Future Directions

Standardization and Quality Control of Herbal-Based Nanocarriers

One of the foremost challenges in developing herbal-based nanocarriers targeting ACTN4 in oral cancer is ensuring batch-to-batch consistency in phytochemical composition and nanosystem characteristics. Herbal extracts often exhibit complex, variable phytochemical profiles influenced by growing conditions, harvest time, and extraction methodologies. This inherent variability complicates the standardization of active component concentrations, affecting reproducibility of therapeutic outcomes. Furthermore, rigorous characterization techniques—particle size distribution, zeta potential, encapsulation efficiency, and release kinetics—must be validated for each formulation batch. Implementing Good Manufacturing Practice (GMP) protocols and adopting pharmacopoeial quality standards are essential for ensuring safety, efficacy, and regulatory approval of these nanoformulations.[46]

Regulatory and Toxicity Considerations

Navigating the regulatory landscape for herbal nanomedicines presents unique challenges. Regulatory agencies require comprehensive preclinical toxicity profiles, including acute and chronic toxicity studies, immunogenicity, biodistribution, and genotoxicity assessments. Although herbal products are perceived as safe, nanoformulation may alter their pharmacokinetics and bio-distribution, potentially leading to unexpected toxicities or nanoparticle accumulation in non-target organs. Regulatory frameworks for combined herbal–chemotherapy nanoformulations in oncology remain underdeveloped, necessitating tailored guidelines for quality control, pharmacovigilance, and clinical evaluation. Addressing potential off-target effects, interactions with existing medications, and long-term safety is critical before clinical translation.[55,56]

Need for Clinical Translation and Biomarker-Guided Trials

Despite robust preclinical evidence supporting ACTN4-targeted nanoformulations, clinical translation remains in its infancy. To bridge this gap, biomarker-guided clinical trials focusing on patient stratification based on ACTN4 expression are indispensable for demonstrating therapeutic efficacy and safety in relevant subpopulations. Multiparametric biomarker panels integrating ACTN4 gene amplification, protein overexpression, and downstream pathway activity can refine patient selection, optimize dosing, and monitor therapeutic response. Moreover, adaptive trial designs incorporating molecular profiling and real-time pharmacodynamic monitoring will accelerate regulatory approval and clinical adoption. The development of non-invasive diagnostics for ACTN4, such as circulating tumor DNA or extracellular vesicle assays, could further enhance trial efficiency and personalized therapeutic management.[53]

Future Prospects for AI-Pharma Convergence in Oral Oncology

The convergence of Artificial Intelligence (AI) and pharmaceutical sciences heralds transformative potential for oral oncology therapeutics targeting ACTN4. AI-driven multi-omics data integration, formulation design, and molecular docking expedite identification and optimization of novel inhibitors while minimizing time and cost. Predictive modeling of patient-specific tumor biology and drug response will enable precision nanomedicine, tailoring therapies to individual molecular profiles. Furthermore, AI-guided real-world evidence collection and pharmacovigilance will improve the post-marketing safety of herbal nanocarriers. Integration of AI with high-throughput screening, automated synthesis, and digital pathology promises to reshape oral cancer drug development, enhancing efficacy and reducing toxicity of ACTN4-targeted treatments.

Conclusion

Inhibition of ACTN4 through advanced nanoformulations holds transformative potential for oral cancer therapy by specifically targeting metastatic pathways that conventional chemotherapies fail to address. Nanocarriers such as nanoemulsions, liposomes, and herbal-based systems enhance the bioavailability and targeted delivery of ACTN4-modulating phytochemicals (curcumin, neem, moringa), effectively suppressing EMT, invasion, and cytoskeletal remodeling in tumor cells. Integration of artificial intelligence accelerates optimization of these formulations, enabling precise control over particle size, release kinetics, and tumor targeting, thus maximizing therapeutic efficacy while minimizing toxicity.[52,53]

The synergy between herbal actives, nanotechnology, and AI-driven design fosters personalized, biomarker-guided treatment approaches that enhance chemotherapeutic sensitivity and block metastatic spread. Clinically, these innovations promise improved survival and quality of life by overcoming drug resistance and metastatic recurrence, major hurdles in oral squamous cell carcinoma management.

Future translational efforts must focus on standardized production, regulatory approval, and biomarker-stratified clinical trials to realize the full potential of ACTN4-targeted nanoformulations as next-generation oral cancer therapeutics.

References

1. M D, Thanikachalam R, C N, B E, K A, Baskaran A. The Demographic and Clinical Characteristics of Oral Squamous Cell Carcinoma: An Institutional Epidemiological Study. Cureus. 2025 Apr 18;17(4):e82552. doi: 10.7759/cureus.82552. PMID: 40390756; PMCID: PMC12086348.
2. Coletta, R. D., Yeudall, W. A., & Salo, T. (2024). Current trends on prevalence, risk factors and prevention of oral cancer. *Frontiers in Oral Health*, *5*, 1505833.
3. EP, M. A., None, A. A. V. & Ismail P T, I. B. (2025). Oral Squamous Cell Carcinoma: Demographic and Clinicopathological Features From A Tertiary Care Centre -An Institutional Based Retrospective Study. *Journal of Contemporary Clinical Practice, 11*(9), 208-213.
4. Tan, Y., Wang, Z., Xu, M. *et al.* Oral squamous cell carcinomas: state of the field and emerging directions. *Int J Oral Sci* **15**, 44 (2023). https://doi.org/10.1038/s41368-023-00249-w
5. Du Cancer, C. C. S. /. S. C. (n.d.). *Treatments for metastatic oral cancer*. Canadian Cancer Society. https://cancer.ca/en/cancer-information/cancer-types/oral/treatment/metastatic
6. Kakuya T, Mori T, Yoshimoto S, Watabe Y, Miura N, Shoji H, Onidani K, Shibahara T, Honda K. Prognostic significance of gene amplification of ACTN4 in stage I and II oral tongue cancer. Int J Oral Maxillofac Surg. 2017 Aug;46(8):968-976. doi: 10.1016/j.ijom.2017.03.001. Epub 2017 Apr 25. PMID: 28385383.
7. 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.
8. Goertzen C., Mahdi H., Laliberte C., Meirson T., Eymael D., Gil-Henn H., Magalhaes M. Oral inflammation promotes oral squamous cell carcinoma invasion. Oncotarget. 2018; 9: 29047-29063. Retrieved from https://www.oncotarget.com/article/25540/text/
9. 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.
10. Issa Mohamad et al. Current Treatment Strategies and Risk Stratification for Oral Carcinoma. Am Soc Clin Oncol Educ Book 43, e389810(2023).
11. Zhang M, Liang J, Yang Y, Liang H, Jia H, Li D. Current Trends of Targeted Drug Delivery for Oral Cancer Therapy. Front Bioeng Biotechnol. 2020 Dec 8;8:618931. doi: 10.3389/fbioe.2020.618931. PMID: 33425881; PMCID: PMC7793972.
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. Kao HY. The actinin family proteins: biological function and clinical implications. Cell Biosci. 2015 Aug 25;5:48. doi: 10.1186/s13578-015-0039-5. PMID: 26309722; PMCID: PMC4549014.
14. Amlan Barai, Abhishek Mukherjee, Alakesh Das, Neha Saxena, Shamik Sen; α-Actinin-4 drives invasiveness by regulating myosin IIB expression and myosin IIA localization. *J Cell Sci* 1 December 2021; 134 (23): jcs258581. doi: https://doi.org/10.1242/jcs.258581
15. Xu W, Yang Z, Lu N. A new role for the PI3K/Akt signaling pathway in the epithelial-mesenchymal transition. Cell Adh Migr. 2015;9(4):317-24. doi: 10.1080/19336918.2015.1016686. Epub 2015 Aug 4. PMID: 26241004; PMCID: PMC4594353.
16. Moghbeli M. PI3K/AKT pathway as a pivotal regulator of epithelial-mesenchymal transition in lung tumor cells. Cancer Cell Int. 2024 May 10;24(1):165. doi: 10.1186/s12935-024-03357-7. PMID: 38730433; PMCID: PMC11084110.
17. Park, S., Kang, M., Kim, S., An, H. T., Gettemans, J., & Ko, J. (2020). α-actinin-4 promotes the progression of prostate cancer through the Akt/GSK-3β/β-catenin signaling pathway. *Frontiers in Cell and Developmental Biology*, *8*, 588544.
18. Avizienyte, E., & Frame, M. C. (2005). Src and FAK signalling controls adhesion fate and the epithelial-to-mesenchymal transition. *Current opinion in cell biology*, *17*(5), 542-547.

19. Avraamides CJ, Garmy-Susini B, Varner JA. Integrins in angiogenesis and lymphangiogenesis. Nat Rev Cancer. 2008 Aug;8(8):604-17. doi: 10.1038/nrc2353. Epub 2008 May 22. PMID: 18497750; PMCID: PMC2577722.
20. Rundhaug JE. Matrix metalloproteinases and angiogenesis. J Cell Mol Med. 2005 Apr-Jun;9(2):267-85. doi: 10.1111/j.1582-4934.2005.tb00355.x. PMID: 15963249; PMCID: PMC6740080.
21. van den Berg, Y. W., van den Hengel, L. G., Myers, H. R., Ayachi, O., Jordanova, E., Ruf, W., ... & Versteeg, H. H. (2009). Alternatively spliced tissue factor induces angiogenesis through integrin ligation. *Proceedings of the National Academy of Sciences*, *106*(46), 19497-19502.
22. Libby, J. R., Royce, H., Walker, S. R., & Li, L. (2024). The role of extracellular matrix in angiogenesis: Beyond adhesion and structure. *Biomaterials and Biosystems*, *15*, 100097.
23. Atashi F, Vahed N, Emamverdizadeh P, Fattahi S, Paya L. Drug resistance against 5-fluorouracil and cisplatin in the treatment of head and neck squamous cell carcinoma: A systematic review. J Dent Res Dent Clin Dent Prospects. 2021 Summer;15(3):219-225. doi: 10.34172/joddd.2021.036. Epub 2021 Aug 25. PMID: 34712414; PMCID: PMC8538146.
24. Cheng, Y., Li, S., Gao, L., Zhi, K., & Ren, W. (2021). The molecular basis and therapeutic aspects of cisplatin resistance in oral squamous cell carcinoma. *Frontiers in oncology*, *11*, 761379.
25. Naomi Kiyota, Makoto Tahara, Shigenori Kadowaki, Nozomu Fuse, Toshihiko Doi, Hironobu Minami, Atsushi Ohtsu, Systemic Chemotherapy with Cisplatin Plus 5-FU (PF) for Recurrent or Metastatic Squamous Cell Carcinoma of the Head and Neck (R/M SCCHN): Efficacy and Safety of a Lower Dose of PF (80/800) at a Single Institution in Japan, *Japanese Journal of Clinical Oncology*, Volume 39, Issue 4, April 2009, Pages 225–230, https://doi.org/10.1093/jjco/hyp002
26. Zhang, W., Li, S., Li, C., Li, T., & Huang, Y. (2022). Remodeling tumor microenvironment with natural products to overcome drug resistance. *Frontiers in immunology*, *13*, 1051998.
27. Chavda, V. P., Patel, A. B., Mistry, K. J., Suthar, S. F., Wu, Z. X., Chen, Z. S., & Hou, K. (2022). Nano-drug delivery systems entrapping natural bioactive compounds for cancer: recent progress and future challenges. *Frontiers in oncology*, *12*, 867655.
28. Zhang, H., Zhou, F., Yang, Q., & Huang, M. (2024). Targeting the oral tumor microenvironment by nanoparticles: A review of progresses. Journal of Drug Delivery Science and Technology, 91, 105248. https://doi.org/10.1016/j.jddst.2023.105248
29. Herrada Céspedes, A., Reyes, M., & Morales, J. O. (2025). Advanced drug delivery systems for oral squamous cell carcinoma: A comprehensive review of nanotechnology-based and other innovative approaches. *Frontiers in Drug Delivery*, *5*, 1596964.
30. Sriraman SK, Aryasomayajula B, Torchilin VP. Barriers to drug delivery in solid tumors. Tissue Barriers. 2014 Jul 22;2:e29528. doi: 10.4161/tisb.29528. PMID: 25068098; PMCID: PMC4106925.
31. Budi HS, Farhood B. Targeting oral tumor microenvironment for effective therapy. Cancer Cell Int. 2023 May 23;23(1):101. doi: 10.1186/s12935-023-02943-5. PMID: 37221555; PMCID: PMC10207626.
32. Olivera A, Moore TW, Hu F, Brown AP, Sun A, Liotta DC, Snyder JP, Yoon Y, Shim H, Marcus AI, Miller AH, Pace TW. Inhibition of the NF-κB signaling pathway by the curcumin analog, 3,5-Bis(2-pyridinylmethylidene)-4-piperidone (EF31): anti-inflammatory and anti-cancer properties. Int Immunopharmacol. 2012 Feb;12(2):368-77. doi: 10.1016/j.intimp.2011.12.009. Epub 2011 Dec 22. PMID: 22197802; PMCID: PMC3372981.
33. Zoi V, Kyritsis AP, Galani V, Lazari D, Sioka C, Voulgaris S, Alexiou GA. The Role of Curcumin in Cancer: A Focus on the PI3K/Akt Pathway. Cancers (Basel). 2024 Apr 18;16(8):1554. doi: 10.3390/cancers16081554. PMID: 38672636; PMCID: PMC11048628.
34. Wang, H., Zhang, K., Liu, J., Yang, J., Tian, Y., Yang, C., ... & Song, N. (2021). Curcumin regulates cancer progression: Focus on ncRNAs and molecular signaling pathways. *Frontiers in Oncology*, *11*, 660712.
35. Liu, W., Wang, J., Zhang, C., Bao, Z., & Wu, L. (2023). Curcumin nanoemulsions inhibit oral squamous cell carcinoma cell proliferation by PI3K/Akt/mTOR suppression and miR-199a upregulation: A preliminary study. *Oral Diseases*, 29, 3183–3192. **https://doi.org/10.1111/odi.14271**
36. Liu, T., Long, T., & Li, H. (2021). Curcumin suppresses the proliferation of oral squamous cell carcinoma through a specificity protein 1/nuclear factor-κB-dependent pathway. *Experimental and Therapeutic Medicine*, *21*(3), 202.

37. Hao F, Kumar S, Yadav N, Chandra D. Neem components as potential agents for cancer prevention and treatment. Biochim Biophys Acta. 2014 Aug;1846(1):247-57. doi: 10.1016/j.bbcan.2014.07.002. Epub 2014 Jul 10. PMID: 25016141; PMCID: PMC4734358.
38. Agrawal S, Bablani Popli D, Sircar K, Chowdhry A. A review of the anticancer activity of Azadirachta indica (Neem) in oral cancer. J Oral Biol Craniofac Res. 2020 Apr-Jun;10(2):206-209. doi: 10.1016/j.jobcr.2020.04.007. Epub 2020 Apr 18. PMID: 32489822; PMCID: PMC7254467.
39. Morris, J., Gonzales, C. B., De La Chapa, J. J., Cabang, A. B., Fountzilas, C., Patel, M., ... & Wargovich, M. J. (2019). The highly pure neem leaf extract, SCNE, inhibits tumorigenesis in oral squamous cell carcinoma via disruption of pro-tumor inflammatory cytokines and cell signaling. *Frontiers in oncology*, *9*, 890.
40. Pareek A, Pant M, Gupta MM, Kashania P, Ratan Y, Jain V, Pareek A, Chuturgoon AA. *Moringa oleifera*: An Updated Comprehensive Review of Its Pharmacological Activities, Ethnomedicinal, Phytopharmaceutical Formulation, Clinical, Phytochemical, and Toxicological Aspects. Int J Mol Sci. 2023 Jan 20;24(3):2098. doi: 10.3390/ijms24032098. PMID: 36768420; PMCID: PMC9916933.
41. Rajkumar C, Ramsridhar S, Veeraraghavan VP, Francis AP, Purushotham M, Mageshwari U. Anticancer effect of Moringa oleifera in oral squamous cell carcinoma: a systematic review. Discov Oncol. 2024 Nov 21;15(1):688. doi: 10.1007/s12672-024-01557-1. PMID: 39570553; PMCID: PMC11582275.
42. Salhab, H. A. S., Joseph, J., & Samad, N. A. (2023). MOLECULAR TARGETS OF MORINGA OLEIFERA IN CANCERS (IN VITRO AND IN VIVO): A SYSTEMATIC REVIEW: Received 2023-01-08; Accepted 2023-03-17; Published 2023-06-06. *Journal of Health and Translational Medicine (JUMMEC)*, 46-65.
43. Garg A, Tisdale AW, Haidari E, Kokkoli E. Targeting colon cancer cells using PEGylated liposomes modified with a fibronectin-mimetic peptide. Int J Pharm. 2009 Jan 21;366(1-2):201-10. doi: 10.1016/j.ijpharm.2008.09.016. Epub 2008 Sep 19. PMID: 18835580; PMCID: PMC2660894.
44. Liu, Y., Bravo, K. M. C., & Liu, J. (2021). Targeted liposomal drug delivery: a nanoscience and biophysical perspective. *Nanoscale Horizons*, *6*(2), 78-94.
45. Nisha, Sachan, R. S. K., Singh, A., Karnwal, A., Shidiki, A., & Kumar, G. (2024). Plant-mediated gold nanoparticles in cancer therapy: Exploring anti-cancer mechanisms, drug delivery applications, and future prospects. *Frontiers in Nanotechnology*, *6*, 1490980.
46. Yadav R, Chawra HS, Dubey G, Alam MS, Kumar V, Sharma P, Upadhayay NK, Yadav T. Herbal based nanoparticles as a possible and potential treatment of cancer: a review. Explor Target Antitumor Ther. 2025 Jan 3;6:1002285. doi: 10.37349/etat.2025.1002285. PMID: 40061135; PMCID: PMC11885881.
47. Alqarni S, Huwaimel B. Predicting PLGA nanoparticle size and zeta potential in synthesis for application of drug delivery via machine learning analysis. Sci Rep. 2025 Jul 1;15(1):20765. doi: 10.1038/s41598-025-06872-3. PMID: 40594444; PMCID: PMC12218156.
48. Edache, E. I., Uzairu, A., Mamza, P. A., Shallangwa, G. A., Yagin, F. H., Abdel Samee, N., & Mahmoud, N. F. (2023). Combining docking, molecular dynamics simulations, AD-MET pharmacokinetics properties, and MMGBSA calculations to create specialized protocols for running effective virtual screening campaigns on the autoimmune disorder and SARS-CoV-2 main protease. *Frontiers in Molecular Biosciences*, *10*, 1254230.
49. Gan, C., Jia, X., Fan, S., Wang, S., Jing, W., & Wei, X. (2023). Virtual screening and molecular dynamics simulation to identify potential SARS-CoV-2 3CLpro inhibitors from a natural product compounds library. *Acta Virologica*, *67*, 12464.
50. Aldayel, T. S., Badran, M. M., Alomrani, A. H., AlFaris, N. A., Altamimi, J. Z., Alqahtani, A. S., ... & Orfali, R. (2023). Optimization of cationic nanoparticles stabilized by poloxamer 188: A potential approach for improving the biological activity of Aloe perryi. *Heliyon*, *9*(12).
51. Ding MQ, Chen L, Cooper GF, Young JD, Lu X. Precision Oncology beyond Targeted Therapy: Combining Omics Data with Machine Learning Matches the Majority of Cancer Cells to Effective Therapeutics. Mol Cancer Res. 2018 Feb;16(2):269-278. doi: 10.1158/1541-7786.MCR-17-0378. Epub 2017 Nov 13. PMID: 29133589; PMCID: PMC5821274.
52. Limeta A, Gatto F, Herrgård MJ, Ji B, Nielsen J. Leveraging high-resolution omics data for predicting responses and adverse events to immune checkpoint inhibitors. Comput Struct Biotechnol J. 2023 Jul 24;21:3912-3919. doi: 10.1016/j.csbj.2023.07.032. PMID: 37602228; PMCID: PMC10432706.

53. Ahmed Z. Multi-omics strategies for personalized and predictive medicine: past, current, and future translational opportunities. Emerg Top Life Sci. 2022 Apr 15;6(2):215-225. doi: 10.1042/ETLS20210244. PMID: 35234253.
54. Miao L, Guo S, Lin CM, Liu Q, Huang L. Nanoformulations for combination or cascade anticancer therapy. Adv Drug Deliv Rev. 2017 Jun 1;115:3-22. doi: 10.1016/j.addr.2017.06.003. Epub 2017 Jun 15. PMID: 28624477; PMCID: PMC5591780.
55. Okem, A., Henstra, C., Lambert, M., & Hayeshi, R. (2023). A review of the pharmacodynamic effect of chemo-herbal drug combinations therapy for cancer treatment. *Medicine in Drug Discovery*, *17*, 100147.
56. Deng Z, Wang N, Ai F, Wang Z, Zhu G. Nanomaterial-mediated platinum drug-based combinatorial cancer therapy. *VIEW*. 2021; 2:20200030. **https://doi.org/10.1002/VIW.20200030**