Magic bullets: Paving the way in oral cancer therapy
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Abstract
Head and neck squamous cell carcinoma (HNSCC) remains to be associated with high mortality and severe morbidity despite multimodality treatments. Advances in understanding the molecular mechanisms and pathways involved in the carcinogenesis of oral cancer have paved way to agents that target the tumor microenvironment and specifically inhibit tumor growth. This review provides insight into the novel therapies which target selective molecular pathways involved in carcinogenesis which might improve treatment outcomes and long-term survival in patients with this aggressive neoplasm. The molecular pathways discussed in the present article are epidermal growth factor receptor pathway, vascular endothelial growth factor receptor pathway, tumor suppressor gene p53/retinoblastoma pathway, phosphatase and tensin homologue/phosphatidylinositol 3-kinase/mammalian target of rapamycin pathway, the RAS/MEK/mitogen activated protein kinase pathway, notch pathway, etc., with special emphasis on HNSCC.

Keywords
Carcinogenesis, head and neck neoplasms, phosphatidylinositols, RAS proteins

Introduction
Despite recent advances in therapy, head, and neck squamous cell carcinoma (HNSCC) remains to be associated with high mortality and morbidity rates representing the sixth leading cause of cancer worldwide.¹ Clinical research in head and neck cancer (HNC) has yielded new therapies which have the potential to improve the outcomes of chemotherapy and radiation by targeting the cellular pathways associated with carcinogenesis.²³

HNSCC evolves through a multistage process involving the accumulation of genetic and epigenetic alterations. Several critical genes and pathways play a crucial role in the tumorigenesis of HNSCC. Few noted targets include TP53,⁴ cyclin D1,⁵ cyclin dependent kinases N2A,⁶ PIK3CA,⁷ HRAS, and epidermal growth factor receptor (EGFR).⁸

Dysregulation in the molecular pathways leads to uncontrolled cell proliferation and aberrant mitogenic signaling. A detailed understanding of the molecular basis of the disease and targeting them with specific agents could reverse or hinder oral carcinogenesis leading to favorable treatment outcomes.⁹ Targeted cancer therapies include drugs that prevent cell growth signaling, impede angiogenesis, and are cytotoxic to the cancer cells by stimulating the immune system. These agents have been successfully used in hematological malignancies as well as in solid tumors (eg., lung, colorectal cancer [CRC]) with promising results.³⁰

Cetuximab was approved by the US Food and Drug Administration (FDA) in 2006, making it the first and, currently, the only targeted biologic available for commercial use in HNC. Other drugs that target various pathways implicated in carcinogenesis still remain under active clinical investigation in HNC.³¹ In this review, we present the different molecular pathways that can be targeted, along with the various drugs that are under research. Blockage of growth factor based cellular signaling, angiogenesis related pathways, targeting apoptosis, and immunotherapy are the main strategies which have been evaluated in HNSCC.

EGFR Pathway
The EGFR belongs to the human epidermal receptor (HER)/ERBB family. These are a group of tyrosine kinases that convert extracellular signals into intracellular responses which influence cell proliferation, differentiation, survival, and migration. Ligand binding with growth factors (EGF, amphiregulin, heparin binding EGF-like growth factor, betacellulin, crypto, and epiregulin) results in a conformational change in the receptor that subsequently triggers the downstream signal transduction cascades like phosphatidylinositol 3-kinase (PI3K)/AKT, JAK/
STAT, mitogen activated protein kinase (MAPK) pathways. It can also translocate to the nucleus and act as a transcription factor. CCND1 that encodes cyclin D1 is one nuclear target of EGFR.[2,9,10]

**Rationale for EGFR targeted therapies**

1. EGFR overexpression has been estimated at 50-98% of all oral cancers.
2. EGFR overexpression is usually associated with poor prognosis, advanced tumor stage, and metastasis.[2,10]

**EGFR targeting therapies**

Primarily, two main categories of molecules have been studied:

**Monoclonal antibodies**

These bind to EGFR with a higher affinity than its ligands, preventing it from activation by dimerization and phosphorylation. Cetuximab is a chimeric human-murine IgG1 monoclonal antibody directed specifically against EGFR, which then interferes with cell cycle progression, obstructs angiogenesis, prevents metastasis, induces apoptosis and is synergistic with radiotherapy and chemotherapy. It remains the only FDA and EMA approved targeted therapy in HNSCC.

Two other anti-EGFR mAbs currently tested are panitumumab and zalutumumab. Use of these agents in HNSCC still remains questionable until study results are published.

**Tyrosine kinase inhibitors (TKI)**

These bind intracellularly to the tyrosine kinase of the EGFR and prevent activation of the downstream signaling pathways. Gefitinib and erlotinib are some advanced TKIs approved in non-small cell lung cancer. Lapatinib, a TKI with dual specificity against HER-2 and EGFR has been approved in breast cancers. Use of these drugs in HNSCC again remains experimental.[10-15]

**Vascular Endothelial Growth Factor (VEGF) Receptor Pathway**

Angiogenesis is fundamental to the growth of human tumors and development of metastasis. The VEGF signaling pathway has emerged as a crucial target in cancer therapeutics to curb angiogenesis. The mammalian VEGF family consists of 5 secreted glycoproteins: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PIGF). Of these, VEGF-A (commonly referred to as VEGF) plays a dominant role.[16] An overview of the VEGF signaling pathway has been depicted in Figure 1.

**Rationale for therapy**

i. Overexpression of VEGF has been associated with poor prognosis in several tumor types including CRC, breast cancer, prostate cancer, lung cancer, gastric cancer, pancreatic cancer, and melanoma.[16,17]

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![Diagram](image_url)

**Figure 1:** Receptor binding of vascular endothelial growth factor activates RAS and phosphatidylinositol 3-kinase pathways subsequently activating hypoxia inducible factor-1α, the mediator of hypoxic responses and driving transcription of many genes. ii. Intratumoral hypoxia and genetic alterations results in the expression of hypoxia inducible factor-1α which is overexpressed in human cancers, and is associated with treatment failure and increased mortality.[18]

**VEGF targeted therapy**

*Humanized VEGF-specific monoclonal antibody*

Bevacizumab, a humanized monoclonal antibody targeted against VEGF-A, has been approved and shows promising efficacy for the treatment of many cancers.[19]

**VEGF trap**

VEGF-A blockage can also be achieved by soluble receptor (VEGF-Trap, aflibercept) that monomerically “traps” the different isoforms of VEGF-A, in addition to VEGF-B and PIGF. It acts as a soluble decoy VEGF receptor, preventing VEGF-A, VEGF-B, and PIGF from interacting with their receptors.[16]

**Tyrosine kinase inhibitors**

TKIs are small molecules with the ability to bind specifically to the intracellular tyrosine kinases common to VEGFRs, platelet-derived growth factor receptors (PDGFRs), FGF receptors (FGFRs), EGFR, Raf kinases, and c-Kit (a receptor of the pluripotent cell growth factor, stem cell factor). This binding inhibits tyrosine kinase phosphorylation and impedes many downstream proangiogenic signaling pathways. These multi-targeted TKIs demonstrated efficacy against various malignancies in different clinical trials with sunitinib and sorafenib being FDA approved.[19]
Tumor Suppressor Gene P53/Retinoblastoma Pathway

Tumor suppressor p53 is a powerful transcription factor and plays a central role in the regulation of cell cycle, apoptosis, DNA repair, senescence, and angiogenesis. Figure 2 illustrates the mechanism of p53’s function as a tumor suppressor.[20]

Rationale for targeting

i. The frequency of TP53 mutation varies from ~10% (hematopoietic malignancies) to 50-70% (ovarian, colorectal, and head and neck malignancies).[21-23]

ii. Increased TP53 mutation is associated with tobacco and alcohol use and increased risk of transformation to malignancy.[21,24,25]

P53 targeting therapies

P53 targeted therapies have been summarized in Figure 3.[21,24,25]

Targeting wild type p53

In cancers with a wt p53, p53 is either degraded by overexpressed mouse double minute 2 homolog (Mdm2) or is excluded from the nucleus. Hence, it is nonfunctional. Activation of wt p53 can be done by means of:

Chemoradiation

It causes DNA damage and subsequent p53 activation and stabilization. Due to tumor heterogeneity, the cellular response may be variable.

Gene therapy

Use of replication-defective adenovirus (Ad-p53) to deliver p53 is currently being studied. It’s been reported to be well tolerated in the treatment of many cancers, particularly, HNC, either singly or in use with chemotherapy/radiation.[26]

Use of E1B-deleted virus (ONYX-015) is another p53 related gene therapy where these selectively replicate in p53 deficient cancer cells and lyse them.

Small molecules

 Reactivation of p53 and induction of tumor cell apoptosis binds to p53 with high affinity activating it, and inducing apoptosis and downregulates oncogenic proteins (c-Myc, cyclin E, β-catenin).[21]

Chemotherapy or radiation therapy

Along with the cancer cells also causes widespread damage to the normal cells, due to p53 activation and apoptosis induction. Temporary blockade of p53 induced apoptosis in normal cells by means of small molecules (pifithrin-α, pifithrin-μ) in p53 deficient tumors should minimize normal cell toxicity.[21,26]

Targeting mutant p53

In tumors with a mutant p53, the function may be restored by introducing specific small peptides or small molecules that create new contacts or stabilize the p53 conformation. These change the p53 conformation from mutant to wild type and transactivation of p53 target genes. Synthetic peptides (CDB3, p53 C-terminal peptide) and small molecules (CP-31398, PRIIMA-1, MIRA-1, ellipticine, p53R3) are few drugs under trial.[24]

Synthetic lethality

The p53 synthetic lethal drugs take advantage of the fact that cancer associated mutation itself is non-lethal but renders cancer cells susceptible to a second hit that then makes it a lethal phenotype. Therefore, mutant p53 containing cancer cells can be selectively killed and be used for chemoprevention to eliminate mutant p53 containing cancer-prone cells. Paclitaxel, metformin have been used in this regard.[21,26]

Targeting p53 regulators

Mdm2 inhibits p53 by binding to it and blocking its transcriptional activity and promotes its degradation via ubiquitination. Mdm2 has been found to be overexpressed in 7% of cancers (p53 Wang). Thus, disruption of Mdm2-p53 binding or Mdm2 E3 ubiquitin ligase activity can be targeted to reactivate p53. Small molecules (nutlins, benzodiazepinediones, spiro oxindoles derivatives, and Mdm2 E3 ubiquitin ligase inhibitors) can be further developed for clinical use.[21]

Phosphatase and tensin homologue/PI3K/mammalian target of rapamycin (PTEN/PI3K/mTOR) pathway

PI3 kinase and PTEN are the major regulators of the PI3 kinase pathway which regulates cell growth, survival and proliferation via activation of the mTOR downstream pathway.

In humans, mTOR primarily appears to be a nutrient sensing protein: mTOR is activated in the presence of growth factors and acts as a switch of cellular catabolism and anabolism.[27] Figure 4 recapitulates the PI3K-mTOR pathway.[24,29]
Therefore PI3K/mTOR inhibitors, used either singly or along with other therapies, may prove to be a novel targeted therapy for HNSCC tumors with mutated and altered PI3K signaling molecules.\(^{30}\)

**Rationale for targeting**

1. PIK3CA mutations are found 11-33% of HNC. The mutation sites include E542K, E545K, and H1047R/L\(^{31}\)
2. The PTEN somatic mutations are in 9-23% of HNSCC, and they are frequently seen in the phosphatase domain of the protein\(^{30}\)
3. AKT2 amplification was identified in 30% of HNSCC\(^{29}\)
4. mTOR downstream effectors ribosomal protein S6 kinase 1 (S6K1), 4E-binding protein and eukaryotic initiation factor are implicated in cellular transformation, and their overexpression has been linked to poor prognosis.\(^{24}\)

**PTEN/PI3K/mTOR targeting therapies**

Regulation of this pathway is questionable due to feedback mechanisms, hence multiple inhibitors for different components of the PI3K pathway may allow a tailored approach.

i. Numerous clinical trials have been conducted to evaluate the clinical efficacy of mTOR inhibitors in patients with HNSCC. The first generation (rapamycin, temsirolimus, everolimus, and ridaforolimus) and second generation (ATP-competitive Torin1, PP242, and PP30) mTOR inhibitors have been developed to interrupt mTOR. These inhibit mTOR preventing further phosphorylation of P70S6K, 4E-
BP1 and, indirectly, other proteins involved in transcription and translation and cell cycle control.\cite{32,33,34,35}

ii. Targeting the PI3K pathway at multiple checkpoints, such as p110α and mTOR might produce promising results. Compounds to inhibit many effectors within the PI3K pathway are currently under research.\cite{29}

**The RAS/MEK/MAPK pathway**

RAS genes comprised of the neuroblastoma cell line, Harvey murine sarcoma virus, and the Kirsten murine sarcoma virus, which is the most frequently activated type of RAS in human cancers.\cite{34,35} RAS proteins are primary intermediates in cell signaling and belongs to the family of G proteins. They switch between an active (guanosine triphosphate bound) or inactive (guanosine diphosphate bound) state. Upstream, RAS becomes activated on ligand binding to cell surface tyrosine kinase receptors such as EGFR, HER-2, VEGFR, PDGFR, and MET. Activated RAS then triggers a cascade of downstream phosphorylation events beginning with the most critical step-activation of Raf kinase. It culminates in the MAPK cascades which are involved in the regulation of normal cell proliferation, survival, and differentiation.\cite{36,37} Figure 5 gives an overview of the pathway.

**Rationale for therapy**

i. Human tumors very frequently express RAS proteins that have been activated by point mutation in 20% of all tumors have undergone an activating mutation in one of the RAS genes

ii. BRAF is also frequently activated by mutation in human tumors mainly in melanomas (~70%) and colon carcinoma (~15%).\cite{34,36}

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**RAS/MEK/MAPK targeted therapy**

**Farnesyltransferase inhibitors (FTIs)**

RAS proteins require post-translational modification to be biologically active. The farnesyl isoprenoid group is attached to the HRAS, KRAS, and NRAS proteins which localizes the RAS to the plasma membrane. This mechanism is essential for the biological activity of RAS and farnesyl transferase inhibitors target this pathway. The effects of FTIs have been reviewed extensively recently, but results are still controversial. The success of these trials will determine whether there is a future for the use of FTIs as cancer therapy, and for what kind of tumors they might be most suitable for.\cite{34,35}

**Inhibition of the expression of HRAS and c-RAF1**

Using short antisense synthetic oligonucleotides, which is specific for sequences in the mRNAs for these proteins. This method has reached clinical trials and is currently underway. Drugs that act on proteins downstream of the RAS pathway targeting the RAF–MAPK pathway at different points, are also being researched.\cite{36}

**Kinase inhibitors targeting pathways upstream of RAS**

RAS can be stimulated by aberrant activation even in tumors where RAS is not mutated and enable upstream signaling pathways. Therapeutic intervention by targeting the tyrosine kinases of growth factor receptors in particular EGFR and ERBB have proved to be effective. Small-molecule tyrosine kinase inhibitors and humanized antibodies against the receptor extracellular domains are being devised.\cite{36}

**NOTCH pathway**

The NOTCH signaling pathway regulates normal cell differentiation, lineage commitment, and embryonic development, cellular proliferation, tumor angiogenesis, and metastasis.\cite{34,35} The NOTCH pathway is so critical that 66% of HNSCC tumors carry some sort of genetic alteration to at least one member of the pathway.\cite{26} Figure 6 depicts the NOTCH signaling pathway.

**Rationale for therapy**

i. NOTCH1 mutations have been reported to be seen in 10-15% of HNSCC making NOTCH1 the second most frequently mutated gene after TP53.\cite{8}

**NOTCH based targeted therapy**

Pharmacodynamic studies have showed a γ secretase inhibitor producing modest NOTCH signaling inhibition. These are highly potent, with good oral bioavailability. They inhibit the cleavage of NOTCH, as well as other membrane proteins like CD44.\cite{38}

**Peroxisome Proliferator-activated Receptors (PPARs) Pathway**

PPARs are a family of a ligand-activated transcription factor that belong to the nuclear receptor superfamily. This family
The NOTCH pathway is activated when one cell expressing the NOTCH receptor engages the cell expressing the appropriate ligand (Jagged or Delta). Cleavage by metalloproteinases and γ-secretase releases the intracellular domain of NOTCH from the membrane translocating it to the nucleus where it binds to DNA binding factor CSL and recruits MAML family to turn on transcription.

With regard to anti-inflammatory effects in macrophages, PPARγ represses gene transcriptional responses that are mediated by other classes of signal-dependent transcription factors via a process called transrepression. Activation of PPARγ has been found to be associated with anti-proliferative, pro-apoptotic, pro-differentiation, anti-inflammatory, and anti-metastatic properties in a variety of cancer cell lines and rodent carcinogenesis model systems. Bren-Mattison et al. examined the mechanism responsible for suppression of carcinogenesis by activation of PPARγ and found that increased PPARγ resulted in a proportional decrease in cyclooxygenase-2 expression and protection from urethane-induced tumor formation.

Yoshida et al., using the carcinogen 4-nitroquinoline-1-oxide to induce tongue tumors, showed that increasing doses of troglitazone decreased the incidence of tumor compared with controls and severe dysplasia. A retrospective analysis of a database from 10 Veteran Affairs medical centers was performed by Govindrajan et al. to examine the effect of thiazolidinedione's on cancer risk in diabetic patients. The risk of oral squamous cell carcinoma decreased by 14-55% with the use of thiazolidinediones, either alone or with other anti-diabetic agents. More recently, a population based cohort study from France examined the association between pioglitazone and cancer risk in 1,491,060 diabetic patients. The risk of HNC was reduced by 15%.

### Future Directions

- Studies are needed to explore the interactions of HPV 16 and 18 oncoprotein interaction with aforementioned signaling pathways. Such interactions can modulate targeted drug response (favorably or unfavorably) to cancer cells.
- Since monotherapy is generally insufficient for treatment of cancer, the combined use of drug targets discussed in the present article and conventional cancer therapy is an interesting area of research for future.
- Inflammation can trigger activations of major carcinogenesis associated signaling pathways. Hence, studies are needed on an exploration of inflammation-mediated carcinogenesis pathways and possible therapeutic targets.
- More studies are needed on antiangiogenic targeted drugs in combination with radiotherapy to explore the possible interactions and implications.

### Conclusion

The discovery of molecular pathways that contribute to carcinogenesis promoting tumor growth and maintenance together with the drugs that are specific to these pathways and their constituents has heralded a new era in the treatment of cancer. In depth analysis of the mutational landscape in HNSCC might help us better understand the intricate signaling network of carcinogenesis and a personalized cancer therapy targeting these altered molecules and pathways can be effectively devised. Though several questions like their toxicity profile, dosing, combination remain unanswered, molecular targeted therapies might prove to be promising and effective in the near future with numerous clinical trials and research pivoted in this direction.

### References


