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EGFR-PI3K-AKT-mTOR Signaling in Head and Neck Squamous Cell Carcinomas - Attractive Targets for Molecular-Oriented Therapy

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Abstract

Importance of the field—Recent advances in understanding the oncogenesis of head and neck squamous cell carcinomas (HNSCC) have revealed multiple dysregulated signaling pathways. One frequently altered axis is the EGFR/PI3K/Akt/mTOR pathway. This pathway plays a central role in numerous cellular processes including metabolism, cell growth, apoptosis, survival and differentiation, which ultimately contributes to HNSCC progression.

What the reader will gain—This article reviews the current understanding of EGFR/PI3K/Akt/mTOR signaling in HNSCC, including the impact of both genetic and epigenetic alterations. This review further highlights the potential of targeting this signaling cascade as a promising therapeutic approach in the treatment of HNSCC.

Areas covered in this review—Books, journals, databases and websites have been searched to provide a current review on the subject.

Take home message—Genetic alterations of several nodes within this pathway, including both genetic and epigenetic changes, leading to either oncogene activation or inactivation of tumor suppressors, have frequently been implicated in HNSCC. Consequently, drugs that target the central nodes of this pathway have become attractive for molecular oriented cancer therapies. Numerous preclinical and clinical studies are being performed in HNSCC, however, more studies are still needed to better understand the biology of this pathway.

Keywords

Akt; EGFR; Head and neck squamous cell carcinoma; mTOR; PI3K; targeted therapy

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common type of cancer with an annual incidence of approximately 600,000 worldwide and a five year survival rate

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Declaration of interest

Gefitinib (Astra Zeneca) and PI3K-mTOR inhibitors (Pfizer) have been provided for research and clinical trials through material transfer and clinical trials agreements between these companies and the National Cancer Institute or National Institute on Deafness and Other Communication Disorders. Dr. Van Waes and the other authors declare no financial conflict of interest and have received no payment in preparation of this manuscript.

of 50%, showing only little improvement over the last 20 years ¹. HNSCC comprises cancers arising from five major anatomical sites: oral cavity, oropharynx, nasopharynx, hypopharynx, and larynx. Recent studies have focused on the genetic and epigenetic alterations of HNSCC ², providing better understanding of the molecular events underlying the pathogenesis of HNSCC. One of the most frequently altered signaling pathways in HNSCC is the EGFR/PI3K/Akt/mTOR cascade (Figure 1). Several genetic and epigenetic mechanisms contribute to these alterations, providing multiple targets for molecular oriented therapy in HNSCC (Table 1). This review focuses on central nodes within this pathway including EGFR, PI3K, PTEN, PDK1, Akt and mTOR, revealing their common alterations in HNSCC and their potential role for targeted molecular therapy.

2. EGFR/PI3K/Akt/mTOR signaling

Multiple ligands activate epidermal growth factor receptor (EGFR), including epidermal growth factor (EGF), transforming growth factor- α (TGF- α), amphiregulin, heregulin, and heparin binding-EGF ³, leading to the activation of Class I phosphoinositide – 3 kinases (PI3K). Once activated, PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P₂] generating phosphatidylinositol-3,4,5-trisphosphate [PtdIns(3,4,5)P₃] ⁴. PTEN (phosphate and tensin homolog deleted on chromosome 10), an important tumor suppressor, antagonizes PI3K function by dephosphorylating PtdIns(3,4,5)P₃ to PtdIns(4,5)P₂ ⁵. PtdIns(3,4,5)P₃ initiates Akt activation by its translocation to the plasma membrane, leading to a conformational change in Akt. Subsequently, Akt is phosphorylated at three regulatory sites: Thr³⁰⁸ in the activation loop of the catalytic domain by 3-phosphoinositide-dependent protein kinase 1 (PDK1) ⁶, Ser⁴⁷³ in the COOH-terminal domain by mTOR-RICTOR (mammalian target of rapamycin-rapamycin insensitive companion of mTOR) complex ⁷ and a recently discovered Ser¹²⁹ by casein kinase 2 (Di Maira et al. 2005). Once activated, Akt phosphorylates multiple downstream targets involved in key cellular processes including apoptosis, metabolism, cell proliferation and cell growth. Akt promotes cell survival by blocking the function of pro-apoptotic proteins and promoting the induction of cell survival proteins ⁸. Akt can also activate the anti-apoptotic NF- κ B pathway through phosphorylation and activation of IKK α which with IKK β then phosphorylates the NF- κ B inhibitor I κ B α ⁹. Furthermore, Akt phosphorylates the FOXO family members: FOXO, FOXO3a and FOXO4, inducing their export from the nucleus preventing FOXO-mediated transcription of pro-apoptotic targets ¹⁰. Another major downstream effector of Akt is mTOR complex 1 (mTORC1). Activation of mTORC1 is initiated by Akt-mediated phosphorylation and sequential inactivation of the tuberous sclerosis complex. The tuberous sclerosis complex consists of tumor-suppressor protein TSC2 (tuberin) associated with another tumor-suppressor protein TSC1 (hamartin) ¹¹. Once phosphorylated, the tuberous sclerosis complex loses its ability to suppress Rheb1, which subsequently activates mTORC1 ¹².

2.1 Targeting EGFR

One extensively studied receptor tyrosine kinase upstream of PI3K/Akt signaling is the epidermal growth factor receptor (EGFR, also known as HER-1 or ErbB-1). Overexpression of EGFR has been identified in many cancers from epithelial origins, including HNSCC, where overexpression of EGFR is found in over 95% of all tumors ¹³¹⁴¹⁵¹⁶. Overexpression and mutation of EGFR have been associated with a more aggressive malignant phenotype, including increased resistance to treatment, and poorer clinical outcome ³¹⁷. One reason for this overexpression is chromosomal amplification. Cytogenetic analysis of oral squamous cell carcinomas showed increased copy numbers of 7p12 (the locus for EGFR) in 30–47% of samples ¹⁸¹⁹ and this EGFR amplification was associated with poor survival ²⁰. In addition, truncation of the complete, wild-type EGFR gene is a common event in HNSCC, leading to a truncated 150 kDa protein lacking exons 2–7 (EGFRvIII). This in-frame

deletion is within the ligand-binding domain, causing constitutive phosphorylation and activation of EGFR, independent of ligand presence²¹. This constitutively active mutation is reportedly found in ~42% of HNSCC tumors, and has been shown through *in vitro* and mouse xenograft models to enhance tumor growth and confer resistance to EGFR-blocking antibodies^{21,22}. Another common alteration of EGFR in HNSCC is a variable deletion of a CA dinucleotide repeat within intron 1. This region varies from 9–21 repeats, and correlations have been made between length of repeats and decreased mRNA and protein expression^{23,24}.

Therapeutic approaches for interrupting EGFR signaling include monoclonal antibodies targeting the ligand binding domain of the receptor and small-molecule tyrosine kinase inhibitors (TKIs)²⁵. The most studied monoclonal antibody is cetuximab (ErbixTM, ImClone Systems, New York, NY, USA), which received FDA approval in 2006 for the treatment of locally or regionally advanced HNSCC in combination with radiotherapy²⁶, based on a phase III trial where the median duration of overall survival was 49.0 month among patients treated with combined therapy and 29.3 month among those treated with radiotherapy alone. Overall, 5-year overall survival was 45.6% in the cetuximab-plus-radiotherapy group and 36.4% in the radiotherapy-alone group.²⁷ However, the improvement in response rate attributable to cetuximab was primarily seen in patients with oropharyngeal carcinoma, a subsite associated with Human Papilloma Virus etiology and better prognosis among HNSCCs²⁸. In addition, treatment of platinum-refractory recurrent or metastatic HNSCC with cetuximab as a single-agent showed response rates of 13% and disease control rates (complete response/partial response/stable disease) of 46%, which led to the FDA approval of cetuximab as a single-agent for the treatment of platinum-refractory recurrent/metastatic HNSCC²⁹. Thus far, no study showed that combination of cetuximab and radiation is as or more effective than concurrent cisplatin-based chemoradiation, which is currently a standard of care for locally advanced HNSCC. An ongoing multi-institutional Phase III trial comparing cisplatin-based chemoradiation with or without cetuximab will elucidate whether cetuximab combined with chemoradiation is equivalent to concurrent cisplatin-based chemoradiation.

Several tyrosine kinase inhibitors (TKIs) have been developed that competitively bind to the ATP pocket of EGFR leading to the inhibition of phosphorylation and the subsequent activation of the receptor's tyrosine kinase activity³⁰. The most clinically advanced EGFR TKIs are gefitinib and erlotinib which both selectively and reversibly inhibit tyrosine kinase activity. Gefitinib (IressaTM, AstraZeneca, London, UK), the first of such inhibitors with oral bioavailability, has been studied as monotherapy for patients with recurrent HNSCC, with an observed response rate of 10.6% and a disease control rate of 53%³¹. The median time to progression and survival were 3.4 and 8.1 months, respectively. In addition, Gefitinib has been used in combination with paclitaxel (TaxolTM) and radiation in patients with local-regionally advanced HNSCC by our group, demonstrating molecular inhibition of the EGFR-AKT axis in only 1/7 on-treatment tumors sampled³². For both, gefitinib and erlotinib, combination therapies with other chemotherapeutic agents or radiation showed better results than in monotherapy^{33,34,35}. Recently, Hughes et al. showed that Cyp1A1 and 1A2 induction by tobacco smoking reduced erlotinib exposure in non-small-cell lung cancer (NSCLC) patients, as steady-state trough plasma concentrations in smokers treated with 300 mg erlotinib were comparable to non-smokers at 150mg³⁶. As tobacco consumption is common in HNSCC patients, dose escalations of erlotinib may need to be considered in current smokers. Interestingly, resistance to EGFR inhibitors seems to be associated with over-activation of PI3K-Akt-mTOR signaling³⁷; Gefitinib-resistant MDA-468 breast cancer cells showed increased Akt activation due to loss of PTEN, however, when PTEN was reintroduced, gefitinib treatment reduced Akt activity, induced apoptosis and promoted cell cycle delay³⁸. In addition, loss of PTEN might be predictive of resistance to cetuximab plus

irinotecan in Colorectal Cancer³⁹. Further studies are needed to confirm if activation of Akt-PI3K-mTOR signaling predicts resistance to EGFR inhibitors.

2.2 Targeting PI3K

So far eight PI3K proteins have been identified and grouped into three subclasses (I–III) of which Class I is implicated to play the most important role in cancer biology⁴⁰. Class I PI3Ks are comprised of an 85-kDa regulatory subunit, which mediates receptor binding, activation and localization of the enzyme, and one of four 110-kDa catalytic subunits (α , β , δ : class IA; γ : class IB)⁶. Common mutations of the PIK3CA gene, which encodes p110 α , are E542K, E545K and H1047R⁴¹. These mutations, as well as another novel mutation (Y343C) within exon 4 are found in HNSCC, with an overall mutation-rate for the PIK3CA gene in HNSCC of 11–40%^{42,43,44}. In addition, 3q26 copy number gain has been reported as a common and early oncogenic event in ~40–50% of HNSCC^{18,45}, which has been correlated with transition to a more invasive phenotype⁴⁶, vascular invasion⁴⁷, and a higher probability of lymph node metastasis⁴⁵.

The two well studied first-generation PI3K inhibitors are wortmannin, a natural compound isolated from *Talaromyces (Penicillium) wortmannii*, and LY294002, the first synthetic drug-like small molecule inhibitor⁴. While wortmannin irreversibly inhibits PI3K by forming a covalent bond with a conserved lysine residue in the ATP binding pocket of PI3K⁴⁸, LY294002 is an ATP-competitive PI3K inhibitor⁴⁹. Due to toxic side effects, poor pharmacological properties and lack of selectivity for Class I PI3K isoforms⁵⁰, their application has been primarily restricted to preclinical studies.

A novel Arg-Gly-Asp-Ser-(RGDS)-conjugated LY294002 prodrug, named SF1126 (Semafore Pharmaceuticals Inc.) with favorable pharmacokinetics, has already shown both antitumor and antiangiogenic activity in preclinical studies⁵¹ and has recently entered phase I clinical trials. In addition, the wortmannin analog, PX-866, with improved stability and decreased hepatotoxicity, compared to wortmannin, exhibits significant antitumor activity in human ovarian cancer, colon cancer, and non-small cell lung cancer xenografts^{52,53,54}. In contrast to these pan-PI3K inhibitors, current research has already focused on developing inhibitors that preferentially target Class I isoforms. Those selective Class I PI3K inhibitors, including ZSTK474 (Japanese Foundation for Cancer Research) and XL147 (Exelixis Inc.), demonstrated potent antitumor effects in human xenograft models⁵⁵ and have recently entered clinical trials for patients with solid tumors.

2.3 Targeting PTEN Inactivation

Some reports indicate a decreased PTEN function in HNSCC due to several genetic and epigenetic alterations, including Loss of Heterozygosity (LOH), microsatellite instability (MSI), and gene inactivation by promoter hypermethylation. LOH at chromosome 10q, the chromosomal region of PTEN, has been correlated with poor prognosis in HNSCC⁵⁶. Shin et al. showed mutations within codon 222–267 of the PTEN gene in three of five samples with MSI and MSI has been reported in the 10q region in about 10–15% of HNSCC samples⁵⁷. More recently, promoter hypermethylation has been implicated in the downregulation of PTEN in HNSCC cell lines⁵⁸. These studies were carried out using 5-aza-2'-deoxycytidine (a known demethylating agent) to restore protein expression in cell lines lacking PTEN.

2.4 Targeting PDK1

One agent reported to inhibit PDK1 is UCN-01 (7-hydroxystaurosporine). UCN-01 was isolated from the broth of *Streptomyces* species N-126⁵⁹ and exhibited potent anti-tumor effects in both *in vitro* and *in vivo* tumor models^{60,61,62}. In addition, Amorphomoltham et

al. showed that UCN-01 inhibited the AKT pathway in HNSCC cell lines⁶³ and the growth of HNSCC xenografts⁶⁴. Several phase I and II clinical trials have already investigated effects of UCN-01 in advanced cancer patients, both as single agent and combined with conventional chemotherapeutic agents. However, significant antitumor activities have not yet been reported⁶⁵.

Another potent drug, which may exert its cytotoxic effects through inhibition of PDK1, is topotecan. Topotecan [10-hydroxy-9-dimethylaminomethyl-(*S*)-camptothecin], a water-soluble camptothecin analogue, is a novel topoisomerase I inhibitor that shows activity in a number of solid tumors and hematological malignancies⁶⁶. Interestingly, Nakshio et al. showed that topotecan inhibited PDK1 kinase activity in lung cancer cell lines in a dose-dependent manner⁶⁷. Irinotecan, another topoisomerase I inhibitor, demonstrated single-agent activity in patients with metastatic or recurrent HNSCC⁶⁸. However, further elucidation is needed to ascertain if these effects were due to inhibition of AKT signaling.

In addition, Hsp90 inhibitors were shown to decrease Akt signaling by affecting PDK1. Heat shock protein 90 (Hsp90) is a molecular chaperone that interacts with and stabilizes many client substrates involved in modulating cell proliferation, survival, and apoptosis. When Hsp90 is inhibited, client proteins are degraded through a proteasomal-dependent pathway⁶⁹. Hsp90 inhibitors can also antagonize EGFR.

Recently, several reports documented the down-regulation of phospho-Akt and Akt signaling by HSP90 inhibitors^{70,71}. Fujita et al. showed that PDK1 is bound to Hsp90 via its kinase domain, thus maintaining PDK1 in a soluble and active conformational state. Hsp90 inhibitors abrogated that binding, leading to proteasomal degradation of PDK1⁷². The first Hsp90 inhibitors identified were benzoquinone ansamycins (i.e., geldanamycin) isolated from fermentation broth of *Streptomyces hygroscopicus*⁷³. Preclinical evaluation of geldanamycin showed severe hepatotoxicity in animals at doses producing concentrations that were active in vitro doses⁷⁴, preventing further clinical development. Additional screening of derivatives of geldanamycin led to the identification of 17-allylamino-17-demethoxygeldanamycin (17-AAG). In several animal models, 17-AAG has similar anti-tumor activity to that of geldanamycin with less toxicity^{75–77}. 17-AAG has entered several phase I and II clinical trials^{78,79}, and has been generally well-tolerated with hepatotoxicity occurring at more aggressive dosing schedules⁸⁰. Wu et al. showed that Hsp90 is abundantly expressed in human esophageal cancer and that 17-AAG effectively inhibited cell proliferation of esophageal carcinoma cell lines and increased radiosensitivity⁸¹. These effects of 17-AAG were correlated with its ability to downregulate signal transduction, notably in Akt-signaling⁸¹. One limitation with 17-AAG is its poor water solubility and formulation issues, which have led to the development of 17-(Dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG) and IPI-504. Both compounds are more water soluble in comparison to 17-AAG, and have entered into clinical trials^{78,79}. In 2001, Chiosis et al.⁸² described the first-generation synthetic Hsp90 inhibitor, PU3, a purine-based analog with a relative binding affinity of 15–20 μM . More recently, synthetic small molecule inhibitors AUY922A⁸³ and SNX-2112⁸⁴ are being investigated. SNX-2112 which has a binding affinity for Hsp90 at 30 nM , caused degradation of Hsp90 client proteins such as HER2 and Akt in the BT-474 breast cancer line in a fashion similar to that of 17-AAG. Pharmacodynamic studies with a single dose of a SNX-2112 prodrug (SNX-5422) revealed substantial decline of HER2, p-Akt, and p-Erk levels along with increased levels of cleaved PARP⁸⁴.

2.5 Targeting CK2

Casein kinase II (CK2) can activate Akt by phosphorylating Ser¹²⁹ located in the linker region between the pleckstrin homolog (PH) and catalytic domains CK2 is a highly

conserved serine/threonine kinase that exists as a tetramer consisting of two catalytic subunits (α and α') and two regulatory subunits (β). Although CK2 is constitutively active and located in both the nucleus and cytoplasm of eukaryotic cells, elevated levels have been associated with a variety of solid tumors including HNSCC⁸⁵⁸⁶. Of note, increased CK2 activity is associated with the malignant transformation of normal mucosa to HNSCC, and furthermore, the increased activity is associated with poorer clinical outcomes in patients with HNSCC⁸⁷⁸⁸ which makes CK2 a prime candidate for targeted therapy. Although there are commercially available cell permeable CK2 inhibitors such as TBB (4,5,6,7-Tetrabromobenzotriazole) and DMAT (2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole), more selective inhibitors are needed for clinical investigation. One example is Emodin, an anthraquinone derivative (1,3,8-trihydroxy-6-methylantraquinone) extracted from the rhizomes of *Rheum palmatum*. *In vitro*, Emodin treatment resulted in downregulation of Akt kinase activity through dephosphorylation of the Ser⁴⁷³ and Thr³⁰⁸ residues⁸⁹. Although Emodin is as effective in inhibiting CK2 as DMAT, cells treated with DMAT did not decrease Akt phosphorylation to the extent of Emodin treatment. Further analysis showed that Emodin also directly inhibits mTOR, selectively inhibits PI3K by influencing PDK1 phosphorylation, and inhibits the phosphorylation of PTEN. A more selective CK2 inhibitor is hematein and it has been demonstrated in A548 lung cancer cells treated with this compound that CK2 kinase activity as well as Akt phosphorylation at Ser¹²⁹ decrease in a dose dependent manner, ultimately leading to cell apoptosis⁹⁰.

2.6 Targeting Akt

Three isoforms of the serine-threonine kinase Akt have been described – Akt1, Akt2 and Akt3, which are encoded by the genes PKB α , PKB β and PKB γ , respectively⁶. Recent studies showed an overall increased activity of Akt signaling, including gene amplification and other alterations in HNSCC tumor specimens and cell lines, respectively⁶³⁴³⁹¹⁹². Activation of Akt is an integral step in the progression of alterations occurring in skin carcinogenesis⁹³. Furthermore, overexpression of Akt leads to a more aggressive phenotype with decreased apoptosis and differentiation⁹³. The activation status of Akt correlates well with disease progression, showing significant differences between dysplasia, carcinoma in situ and HNSCC tissue⁶³. As the most crucial node downstream of PI3K, Akt presents an attractive therapeutic target. A well established lipid-based Akt inhibitor is perifosine, which interacts with the pleckstrin homology domain of Akt preventing Akt from binding to PtdIns(3,4,5)P₃, and consequently, from activation⁴. In preclinical studies, perifosine displayed potent antiproliferative activity against several in vitro and in vivo human tumor models⁹⁴, and an antiproliferative effect of perifosine in HNSCC cell lines was observed by blocking cell cycle progression at G1-S and G2-M⁹⁵. However, in a Phase II study, Perifosine lacked antitumor activity as a single agent in recurrent or metastatic HNSCC⁹⁶.

2.7 Targeting mTOR

One major downstream effector of Akt is the atypical serine/threonine kinase, mammalian target of rapamycin (mTOR), which regulates cell growth by coordinating growth factor and nutrient signaling⁹⁷. Two mTOR-containing complexes have been described: mTOR complex 1 (mTORC1), a rapamycin-sensitive complex which interacts with the protein regulatory-associated protein of mTOR (RAPTOR); and mTOR complex 2 (mTORC2), a rapamycin-insensitive complex which interacts with rapamycin-insensitive companion of mTOR (RICTOR)⁹⁸, which phosphorylates Akt on Ser⁴⁷³⁹⁹. mTORC1 phosphorylates key eukaryotic translation regulators, including S6 kinase 1 (S6K1, also known as p70^{S6K}) and the eukaryotic translation initiation factor 4E (eIF-4E)-binding protein 1 (4E-BP1), which plays an important role in cell growth¹⁰⁰. In HNSCC, eIF-4E facilitated tumor progression through the expression of angiogenic factors b-FGF (basic fibroblast growth factor) and

VEGF (vascular endothelial growth factor)¹⁰¹ and can be used as an independent predictor of recurrence in histologically “tumor-free” surgical margins of HNSCC¹⁰¹.

Amornphimoltham et al. showed that the mTOR pathway plays a central role in HNSCC, as the phosphorylated active form of S6K1 is frequently accumulated in clinical specimens from HNSCC patients and in HNSCC-derived cell lines^{102,103}. Hence, mTOR has become an attractive target for molecular-oriented drug development. The first mTOR inhibitor Rapamycin (sirolimus; Rapamune; Wyeth Pharmaceuticals), a natural product, was originally used because of its antifungal properties¹⁰⁴ before its immunosuppressive and, more recently, antineoplastic effects were discovered⁹⁸. Rapamycin associates with FK506-binding protein, which subsequently binds directly to mTORC1 suppressing the phosphorylation of its downstream substrates S6K1 and 4E-BP1⁹⁸. No other proteins beside mTOR have been identified as rapamycin targets, making this drug unique. Thus far, three rapamycin analogues (rapalogues) have been developed which exhibit desirable pharmacological characteristics, including oral or IV bioavailability, prolonged half-life, and reduced immunosuppressive effects compared to rapamycin. These include temsirolimus (CCI-779; Torisel, Wyeth Pharmaceuticals), everolimus (RAD001, Novartis) and deforolimus (AP23573, ARIAD Pharmaceuticals, Inc. and Merck & Co., Inc.).

In preclinical models, rapamycin and its derivatives have shown antitumor activity in a variety of malignancies including HNSCC both *in vitro* and *in vivo*. Amornphimoltham et al. showed that rapamycin reduced level of activated S6 protein, a downstream effector of mTOR in HNSCC cell lines and in HNSCC xenograft models at clinically relevant doses¹⁰². A pilot study of neoadjuvant rapamycin for evaluation of clinical and molecular activity prior to surgery has received IRB approval at the NIH.

Nathan et al. showed that CCI-779, a more water-soluble analogue of rapamycin, inhibited growth of HNSCC cell lines and xenograft models through inactivation of mTOR, as shown by decreased levels of the mTOR downstream targets S6K1 and 4E-BP1, leading to reduced production of VEGF and FGF-2¹⁰⁵. Preliminary reports from a phase I trial of neoadjuvant CCI-779 with radiation therapy provided evidence for tumor reduction or stabilization. In addition, Jimeno et al. studied the effect of temsirolimus alone and in combination with the EGFR inhibitor erlotinib in two HNSCC xenograft models, one resistant and the other sensitive to EGFR inhibitors¹⁰⁶. The growth inhibitory effect of temsirolimus monotherapy superseded erlotinib monotherapy due to abrogation of the mTOR pathway. As a negative-feedback loop exists in which S6K1 directly phosphorylates IRS1 (insulin receptor substrate protein 1) and blocks IGF-1 (insulin-like growth factor 1) signaling to PI3K¹⁰⁷, a major drawback of selectively inhibiting mTOR is the consequent activation of PI3K, which can ultimately enhance tumor growth¹⁰⁸. Thus, it is reasonable to develop compounds targeting both PI3K and mTOR. PI-103, a selective dual PI3K/mTOR inhibitor showed antitumor activity in a broad range of human tumor xenografts¹⁰⁹, however, application of PI-103 was restricted to preclinical studies because of adverse pharmacological characteristics. Other dual PI3K/mTOR inhibitors including BEZ235 (Novartis) and BGT226 (Novartis), with promising results from preclinical studies¹¹⁰, have recently entered clinical trials and may prove useful towards treatment of HNSCC.

3. Expert opinion

EGFR-PI3K-AKT-mTOR signaling plays a crucial role in the tumorigenesis of various human malignancies including HNSCC. Many up- and downstream components of this pathway including EGFR, PI3K and mTOR have been shown to be highly activated in a majority of HNSCC samples, due to genetic and epigenetic alterations, which makes this pathway very attractive for molecular oriented drug therapies. While targeting EGFR has

become an integral part in the therapy of HNSCC and cetuximab is used in clinical practice for HNSCC patients with locally advanced tumors and recurrent or metastatic disease, many preclinical and clinical studies are continuing to evaluate the role of other specific inhibitors of this pathway in the treatment of HNSCC patients (Table 1). Despite promising preclinical results, some of them did not show the expected effects in clinical trials, which might be due to the complexity of this pathway with multiple nodes, feedback loops, crosstalk and redundancy with other pathways. So, it seems reasonable that targeting this pathway at several points simultaneously may be more effective and may also decrease the possibility of chemotherapy resistance.

A major challenge in targeted therapies is matching the correct therapy to the patient. Hence, efforts have already been made in the search for biomarkers to identify those patients who may benefit from certain inhibitors. In several cases, patients with activated EGFR-PI3K-AKT-mTOR signaling have been reported to be more sensitive to selective pathway inhibitors. Three relevant examples include: loss of PTEN, which may predict sensitivity to everolimus in patients with endometrial cancer ¹¹¹; pretreatment p-AKT Ser⁴⁷³ and total AKT levels towards predicting response to Gefitinib treatment ³²; and, EGFR CA dinucleotide repeat (based on *In vitro data*) towards predicting sensitivity toward erlotinib, a specific RTK inhibitor ¹¹².

In order to evaluate the efficacy of molecular oriented therapy, it is important to develop biomarkers which can be used to verify that a specific inhibitor is reaching its desired target. Commonly used biomarkers for measuring the efficacy of PI3K inhibition are levels of phosphorylated Akt and phosphorylated S6K1. A common side effect of EGFR targeted agents, used as a correlative biomarker is a distinctive skin rash, as studies have revealed that rash severity was significantly associated with survival in HNSCC ¹¹³. Phospho-protein biomarkers for EGFR and downstream signal pathway components modulated by these inhibitors have been defined in HNSCC by immunohistochemistry and reverse phase protein microarray ^{32, 114}.

In conclusion, future molecular oriented therapy for HNSCC should take into account the activation status of the desired target found in each individual tumor. Furthermore, the chosen agent should alter the activation of the target(s) and the altered activation should be associated with clinical benefit for the patient.

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Highlights

- HNSCC is the sixth most common cancer worldwide
- 5 year survival rate at 50%, with little to no improvement in the last 20 years
- recent studies have focused on the molecular interactions within the EGFR/PI3K/Akt/mTOR pathway
- many genetic alterations in this pathway are found to be subsets of different oncogenic populations in HNSCC
- studies have shown increased chemo- and radiosensitivity with treatment of specific genetic alterations
- there are relatively few specific targets being exploited in the treatment of HNSCC, with many promising possibilities either being explored, or yet to be explored
- the ultimate goal of targeted therapy is to correlate the correct course of treatment with the patient whose oncogenesis is most susceptible to therapy

Figure 1.
Current Understanding of EGFR-PI3K-AKT-mTOR signaling in Head and Neck Squamous Cell Carcinomas and potential drugs for Molecular-Oriented Therapy.

Overview of clinical trials with molecular oriented therapies targeting key nodes in the EGFR-PI3K-Akt-mTor pathway. Due to multiple ongoing studies using cetuximab, only two key clinical trials are listed, which led to the FDA approval for cetuximab as single-agent therapy for platinumum-refractory recurrent/metastatic HNSCC and for the combination of cetuximab and RT for the treatment of unresectable HNSCC.

Table 1

EGFR inhibitors	Cetuximab	Combination with radiation therapy	Phase III	Bonner et al. ²⁶	Stage III/IV HNSCC
		Single agent	Phase III	Vermorken et al. ²⁸	Recurrent or metastatic HNSCC
		Combination with radiation therapy and cisplatin	Phase III	NCT00265941	Stage III/IV HNSCC
	Gefitinib	Combination therapy with cyclooxygenase-2 inhibitor celecoxib	Phase I	Wirth et al. ¹¹⁵	Recurrent or metastatic HNSCC
		Combination therapy with radiation therapy ± cisplatin	Phase I	Chen et al. ¹¹⁶	Locally advanced HNSCC
		Single agent	Phase II	Cohen et al. ³¹	Recurrent or metastatic HNSCC
mTor inhibitors					
	Tenisirolimus (CCI-779)	Combination therapy with EGFR inhibitor erlotinib	Phase II	NCT01009203	Platinum-Refractory or -Ineligible, advanced, HNSCC
		Combination therapy with paclitaxel and carboplatin	Phase I/II	NCT01016769	Recurrent or metastatic HNSCC
		Combination therapy with cisplatin and cetuximab	Phase I/II	NCT01015664	Recurrent or metastatic HNSCC
	Everolimus (RAD001)	Combination therapy with docetaxel and cisplatin	Phase I	NCT00935961	Local-regional advanced HNSCC
		Combination therapy with intensity modulated radiation therapy and cisplatin	Phase I	NCT00858663	Head and Neck Cancer
Akt inhibitors	Perifosine	Single agent	Phase II	Argiris et al. ⁹⁶	Recurrent or metastatic HNSCC
HSP90 inhibitors	17-DMAG	Single agent	Phase I	NCT00089362	Metastatic or unresectable Head and Neck cancer