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Targeting the Epidermal Growth Factor Receptor for Head and Neck Cancer Chemoprevention

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Abstract

The epidermal growth factor receptor (EGFR) has been implicated in head and neck squamous cell carcinoma (HNSCC) carcinogenesis. It is currently the only molecular target in head and neck cancers for which there are pharmacologic therapeutic interventions approved by regulatory agencies worldwide to treat advanced disease. Oral pre-malignant lesions have increased EGFR protein expression and increased *egfr* gene copy number compared to normal mucosa. Oral pre-malignant lesions with overexpression of EGFR or *egfr* gene copy number gain are at higher risk for malignant transformation. Inhibition of EGFR in pre-clinical models of oral pre-malignancies validates this approach as an effective way to reduce the incidence of oral cancer, and supports investigation of this strategy in the clinic. Clinical trials with EGFR targeted agents, including cetuximab, erlotinib, and vandetanib, are currently under way, some with promising preliminary results. If ultimately shown to reduce the risk of oral cancer, chemoprevention with EGFR inhibitors may significantly reduce morbidity and possibly mortality from HNSCC.

Keywords

oral premalignant lesions; leukoplakia; chemoprevention; epidermal growth factor receptor; erlotinib; cetuximab

Introduction

The epidermal growth factor receptor (EGFR) is a cytoplasmic transmembrane protein belonging to the HER (ErbB) family of receptor tyrosine kinases and encoded by the *egfr* gene located on chromosome 7p12–13. The HER family is comprised of four distinct receptors: EGFR (also known as HER1 or ErbB-1), HER2 (ErbB-2, Neu), HER3 (ErbB-3)

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and HER4 (ErbB-4). The EGFR is a 170-kd protein encoded by a 110-kb-long gene localized in the short arm of chromosome 7. Its structure consists of an extracellular ligand-binding domain, a single transmembrane hydrophobic helix, and a cytoplasmic carboxy-terminal domain, to which tyrosine kinase activity is confined.¹

HER receptors are usually located in the basolateral membrane of the epithelial cells, where they can interact with their ligands present in the stroma, thus mediating signaling between the epithelium and the extra-cellular matrix. The ligands to HER receptors (also known as epidermal growth factor [EGF] family of growth factors) are characterized by the presence of an EGF-like domain (composed of three disulfide-bonded intramolecular groups, which confer binding specificity) and additional structural motifs (such as immunoglobulin-like domains, heparin-binding sites and glycosylation sites). They are produced as transmembrane precursors and may be subdivided into three groups according to their affinity for one or more HER receptors: the first group includes ligands that bind exclusively to EGFR (e.g. EGF, transforming growth factor- α [TGF- α], amphiregulin, CRIPTO); the second group includes ligands to both EGFR and HER4 (e.g. betacellulin, heparin-binding epidermal growth factor, epiregulin); the third group includes ligands to HER3 and HER4 (tomoregulin, neuregulins/hereregulins, neu differentiation factor). HER2 has no identified ligand, a fact explained by the structure of the extracellular region of the receptor, which is already in an activated conformation and does not allow ligand docking. Once the ligand binds to the extracellular domain, the receptor undergoes a conformational change of this region, which allows homodimerization or heterodimerization with another activated receptor of the HER family. Following dimerization, there is increased intracellular kinase activity of the receptor through a proximity effect, resulting in phosphorylation of critical tyrosine residues on the cytoplasmic domain, which then triggers the signal transduction cascade. Three major intracellular signaling pathways linked to EGFR activation have been identified: the Ras-Raf-mitogen-activated protein (MAP) kinase pathway, the phosphatidylinositol 3-kinase (PI-3 K)/Akt pathway and the Janus-kinase/signal transducer and activator of transcription (Jak2/STAT3) pathway. Once activated, these pathways contribute to the development of a malignant cellular phenotype, including resistance to apoptosis, increased proliferation, invasion, metastasis, and stimulation of angiogenesis (Figure 1).¹

The EGFR has been implicated in head and neck squamous cell carcinoma (HNSCC) carcinogenesis. It is currently the only molecular target in head and neck cancers for which there are pharmacologic therapeutic interventions approved by regulatory agencies worldwide to treat advanced disease. The purpose of this review is to discuss the importance of EGFR in oral pre-malignant lesions and the possible role of EGFR-targeted therapies for head and neck cancer chemoprevention.

EGFR-targeted agents for treatment of HNSCC

Pharmacologic strategies targeting the EGFR that have been approved for cancer therapy include the use of antibodies and small molecule receptor tyrosine kinase inhibitors (TKIs).

The monoclonal antibody cetuximab has been demonstrated to improve overall survival when added to radiation therapy for treatment of locally advanced disease,² and when added to chemotherapy for recurrent/metastatic disease.³ As a single agent, cetuximab elicited a response rate of 13% in patients with platinum-refractory HNSCC.⁴ The humanized monoclonal antibody panitumumab has also been studied for management of patients with recurrent/metastatic HNSCC in a large phase 3 trial. The primary endpoint of overall survival was not met, but the panitumumab plus chemotherapy arm had improved progression-free survival and response rates compared to the chemotherapy only arm.⁵ The third EGFR antibody studied in the phase 3 setting was zalutumumab. In patients with recurrent/metastatic disease, the drug failed to improve survival over best supportive care, although progression-free survival and response rates were higher in the zalutumumab-treated patients.⁶

The EGFR TKI gefitinib 250 or 500 mg/day was evaluated as monotherapy in phase 2 and 3 studies in recurrent/metastatic disease. Response rates ranged from 1.4% to 10.6%,⁷⁻⁹ but in the larger phase 3 trial, gefitinib failed to improve the primary endpoint of overall survival when compared to methotrexate.⁹ In a phase 2 study involving 115 patients with recurrent/metastatic HNSCC, treatment with erlotinib was associated with a response rate of 4.3%, with disease stabilization in 38.3% of patients, maintained for a median of 16.1 weeks.¹⁰ Newer generation of EGFR TKIs are being evaluated, including for example, the irreversible dual EGFR/HER2 inhibitor afatinib – preliminary results of a randomized phase II study demonstrated comparable efficacy with cetuximab in terms of response rates in previously treated patients with incurable HNSCC.¹¹

Taken together, the clinical trials completed to date demonstrate that targeting the EGFR for head and neck cancer therapy may improve survival, particularly with strategies using the monoclonal antibody cetuximab. EGFR TKIs also have activity against HNSCCs, but the majority of studies were performed in heavily pre-treated patients with recurrent/metastatic disease; in this setting, response rates were modest and regulatory approval of EGFR TKIs for HNSCC therapy has not been granted.

EGFR-targeted agents are, in general, well tolerated. Low grade skin toxicities (including acneiform rash, pruritus, dry skin) and diarrhea are the most common adverse events associated with EGFR TKIs. EGFR antibodies are also associated with similar skin toxicities, in addition to hypomagnesemia and risk for uncommon, albeit potentially severe, hypersensitivity reactions (especially with the non-humanized antibody cetuximab).^{2, 3, 9} The favorable toxicity profile and activity in advanced disease allow EGFR-targeted drugs to be considered for chemoprevention. Antibodies are administered intravenously as frequently as once per week, which could represent a disadvantage over oral TKIs to be used long term in the prevention setting. As discussed elsewhere,¹² using well tolerated drugs with ease of administration, and activity in established cancers may be a successful avenue for development of chemopreventive agents. Nonetheless, demonstration that a targeted agent (whether antibody or TKI) can interrupt the process of malignant transformation in pre-clinical models specific to prevention may increase the chances for successful clinical development of such agent to reduce cancer risk.¹² In the next sections, observational and

pre-clinical data will be reviewed, that support clinical investigation of EGFR inhibitors for oral cancer prevention.

Role of EGFR in oral pre-malignant lesions

In squamous cell carcinoma of the H&N, overexpression of EGFR^{13–16} as well as TGF-alpha^{16, 17} have consistently been reported to negatively affect survival. Patterns of expression of these proteins and their association with malignant transformation have been evaluated in oral pre-malignant lesions.

Levels of TGF-alpha and EGFR mRNA and protein have been found to be elevated in histologically normal mucosa several centimeters away from the primary tumor site, in patients with HNSCC.^{18, 19} Nondysplastic, histologically normal tissue adjacent to head and neck cancerous lesions also present moderately higher expression of EGFR compared to control tissues.^{20, 21} Shin et al. observed that, in this population, EGFR expression remains elevated as the tissue progresses from normal mucosa to hyperplasia to dysplasia; once the tissue changes to squamous cell carcinoma, there is a dramatic increase in EGFR expression. Additionally, as lesions progress to hyperplasia and dysplasia, the thickness of the epithelium that exhibits EGFR overexpression also increases, to include not only the basal layers, but also the parabasal and superficial layers.²⁰ Grandis et al. also observed a stepwise increase in EGFR protein expression in oral pre-malignant lesions with increasing degrees of dysplasia, both at a single time point in individual patients with multiple lesions, as well as over the course of time at a single site in patients with initial mild dysplasia progressing to invasive cancer. In contrast, TGF-alpha protein levels seem to be elevated in mildly dysplastic lesions, with no further increase with progressive degrees of cellular atypia.²² Increased protein expression of EGFR^{23, 24} and TGF-alpha²⁴ in oral pre-malignant lesions compared to normal mucosa was also observed by Rautava et al. and Beenken et al. The prognostic significance of EGFR protein expression was recently examined in 145 oral pre-malignant lesions of patients enrolled in a chemoprevention study of 13-cis-retinoic acid, versus beta-carotene + retinyl palmitate, versus retinyl palmitate alone. Using quantitative evaluation by automated analysis, high EGFR protein expression scores (transformed composite score > 7) were associated with higher oral cancer risk and shorter time to oral cancer in multivariate analysis.²⁵

Increased *egfr* gene copy number has also been demonstrated to be associated with worse survival in patients with HNSCC,^{26, 27} and such alterations have been evaluated in oral pre-malignant lesions as well. Early studies have demonstrated increased frequency of chromosomes 7 and 17 polysomy in the oral epithelium of patients with HNSCC, as tissues progress from histologically normal mucosa to hyperplasia to dysplasia to invasive cancer.²⁸ These results were corroborated by whole-genome analysis of high grade oral lesions identifying chromosomal loci 7p11.2 and 11q13 as the most frequently occurring regions of amplification.²⁹ Several groups have demonstrated *egfr* gene copy number gain in oral dysplastic lesions compared to normal mucosa assessed by competitive polymerase chain reaction³⁰ or fluorescence in situ hybridization (FISH).³¹ In the small cohort of patients evaluated by Poh et al. (N=35 dysplastic lesions), *egfr* gene gain (low or high) occurred more frequently in pre-malignant lesions that subsequently progressed to carcinoma *in situ*

or invasive carcinoma compared to non-progressing lesions.³¹ The prognostic significance of *egfr* gene copy number in oral pre-malignant lesions was also evaluated in the same cohort of patients enrolled in the aforementioned retinoid chemoprevention study. For this analysis, only a subgroup of patients with increased EGFR protein expression by immunohistochemistry were included (N=49). FISH positivity (increased *egfr* gene copy number) was defined as any increase in *egfr* gene and/or chromosome 7. Patients with FISH-positive lesions had a statistically significant higher incidence of oral cancer compared to FISH-negative lesions (oral cancer-free rate of 16% versus 67%, respectively, after 10 years, $P=0.0007$).²⁵

These observational studies strongly suggest a role for EGFR dysregulation in the carcinogenic process of HNSCC. Mechanistic studies in pre-clinical models described below further support the hypothesis that targeting EGFR may delay and/or interrupt the progression of oral pre-malignant lesions to invasive cancer.

Pre-clinical studies of EGFR-targeted agents for HNSCC chemoprevention

Oral epithelium of smokers exhibit increased proliferation index, cyclooxygenase 2, as well as increased expression of EGFR ligands such as TGF- α and amphiregulin.^{32, 33} Using an *in vitro* model of oral leukoplakia (MSK-Leuk1 cell line established from a dysplastic leukoplakia lesion adjacent to a squamous cell carcinoma of the tongue),³⁴ Moraitis et al. have demonstrated that a saline extract of tobacco smoke induced cyclooxygenase-2 expression via a mechanism involving upregulation of TGF- α and amphiregulin leading to activation of EGFR. EGFR blockade with antibodies or TKIs prevented tobacco smoke-induced cyclooxygenase-2 expression.³³ In the same cell line model, tobacco smoke-induced amphiregulin led to increased DNA synthesis, as well as invasiveness of leukoplakia cell lines, all of which could be inhibited by EGFR antibodies or TKIs.^{35, 36} An in depth analysis of gene expression profiles induced by tobacco smoke in MSK-Leuk1 cells revealed upregulation of EGFR and its ligands, forming an EGFR-centered network.³⁷ Taken together, these *in vitro* data suggest that EGFR blockade could be a useful strategy for chemoprevention of tobacco-induced oral cancers.

Indeed, pre-clinical models of oral carcinogenesis corroborate a central role of EGFR in the development of HNSCC. In the 7,12-dimethylbenz(a)anthracene-induced hamster buccal pouch carcinogenesis model, EGFR protein expression gradually increased with development of hyperplasia and dysplasia/squamous cell carcinoma.³⁸ Using the 4-nitroquinoline 1-oxide (4-NQO) oral carcinogenesis mouse model, Sheu et al. demonstrated that the EGFR inhibitor AG1748 reduced the incidence of invasive cancer and increased the fraction of lesions that remained at the low-grade dysplasia stage compared to placebo, when given to animals that had developed leukoplakia in their tongues after exposure to the carcinogen.³⁹ Likewise, erlotinib also prevented the development of higher grade dysplasia in experiments conducted by another group using a similar 4-NQO mouse model.⁴⁰ As such, these *in vitro* and *in vivo* pre-clinical data justify the ongoing clinical investigations of EGFR inhibitors for chemoprevention.

Clinical trials of EGFR-targeted agents for oral cancer chemoprevention

At least four clinical trials have recently been completed or are currently under way evaluating the efficacy of EGFR inhibitors for oral cancer chemoprevention.

In 2012, Califano et al. presented, in abstract form, the results of a phase 2 trial of cetuximab in patients with head and neck pre-malignant lesions. Inclusion criteria included presence of loss of heterozygosity at 3p, 9p21, or 17p; surgically unresectable high grade lesion; or development of high grade premalignant lesion after curative therapy for a prior HNSCC. Patients were randomized to observation versus active treatment. Cetuximab was given at standard doses for 8 weeks. Patients randomized to observation had the option to cross over to cetuximab. Out of 12 treated patients, 3 had complete resolution of dysplasia (2 patients without any recurrence of pre-malignancy at years 1.5 and 2, 1 patient without recurrence of premalignancy in the oral cavity, but developed hypopharyngeal squamous cell carcinoma 1.5 years after completion of treatment). None of the 5 patients randomized to observation had complete resolution of dysplasia.⁴¹ Strengths of this trial include the use of an approved agent, with established activity in advanced disease. Additionally, the eligibility criteria only allowed for inclusion of patients considered to be at high risk for development of invasive cancer, either because of molecular (loss of heterozygosity at defined chromosomal sites), histological (degree of dysplasia), or clinical (prior history of HNSCC) poor prognostic features. As discussed elsewhere,¹² focusing interventions on high risk groups improves the chances of success of a chemoprevention clinical trial, as this strategy optimizes the therapeutic index (i.e., potential reduction in the risk of cancer versus risk for therapy-related adverse events), especially in a cancer-free population perceived as “healthy” and for which acceptance of treatment-induced toxicities may be low. It remains to be determined, however, whether the inclusion criteria selected by Califano et al. are optimal for selection of patients for EGFR-targeted chemoprevention. The study also raises the question whether short-term interventions may be effective in eliminating pre-malignant cell clones, thus translating into long-term benefits. It is notable that histological responses seen in this trial could be durable, perhaps obviating the need for the unfeasible, costly, weekly, cetuximab intravenous therapy given for a prolonged period of time. On the other hand, the clinical trial by Califano et al. has limitations often associated with many chemoprevention studies. Accrual was slow, resulting in a small sample size that precludes any definitive conclusions if cetuximab can prevent oral cancers in this population. Furthermore, the short-term endpoint of histological response may not correlate with the more clinically meaningful, long-term endpoint of development of invasive cancer. Indeed, previous chemoprevention trials with retinoids have demonstrated that even though these drugs could reverse histological abnormalities of oral pre-malignant lesions, molecular aberrations associated with increased cancer risk still persisted in the histologically normal-appearing mucosa after treatment.⁴² Likewise, clinical response of leukoplakias was only marginally correlated with oral cancer development in the largest, longest-term retinoid chemoprevention trial performed in this setting.⁴³ These data illustrate that histological or clinical responses may not be the optimal endpoints for chemoprevention trials in oral pre-malignancies, and that assessment of incidence of oral cancer after a chemoprevention intervention may be required to ascertain the true value of any pharmacologic agent developed in this setting.

The largest EGFR-targeted chemoprevention clinical trial in HNSCC activated to date is the Erlotinib Prevention of Oral Cancer (EPOC) study [NCT00402779]. This is a multi-institutional effort that attempts to address several common limitations of clinical trials in the prevention setting. The primary hypothesis of this study is that erlotinib given for 1 year to patients with high-risk oral pre-malignant lesions will reduce the incidence of invasive oral cancer compared to placebo. The two key features of EPOC are: molecular risk assessment of oral cancer, and long-term use of an EGFR-targeted agent for chemoprevention.

The eligibility criteria for EPOC require the presence of a histologically defined oral pre-malignant lesion in patients without or with a prior history of oral cancer after curative therapy. Presence of dysplasia is not mandatory, nor is presence of a clinically visible lesion, as long as intra-epithelial neoplasia can be identified on histological evaluation of the mucosa. Lesions in patients without a prior history of oral cancer must have loss of heterozygosity at 3p14 and/or 9p21 plus at least one additional chromosomal site (17p, 8p, 11p, 4q, 13q). Lesions in patients with a prior history of oral cancer must have loss of heterozygosity at 3p14 and/or 9p21. These selection criteria evolved from retrospective studies demonstrating that the aforementioned pattern of loss of heterozygosity is associated with increased cancer risk in patients with oral pre-malignant lesions. Mao et al. reported that 7 out of 19 patients (37%) with an oral pre-malignant lesion and loss of heterozygosity at 3p14 and/or 9p21 developed HNSCC, while only 1 of 18 patients (6%) with an oral pre-malignant lesion without loss of heterozygosity at 3p14 and/or 9p21 developed HNSCC.⁴⁴ Retrospective analysis of an independent cohort of leukoplakia patients by another group corroborated these findings. Loss of heterozygosity at 3p and/or 9p but not at any of the other chromosomal arms evaluated had a slight increase in the relative risk for developing cancer of 3.8 fold. Loss of heterozygosity at 3p and/or 9p plus additional losses (on 4q, 8p, 11q, or 17p) was associated with a 22-fold increase in the relative risk of cancer.⁴⁵ More recently, loss of heterozygosity at 3p and/or 9p in low grade oral premalignant lesions was validated as a risk marker of cancer in the first prospectively collected cohort.⁴⁶ In patients with oral leukoplakia at a former cancer site, loss of heterozygosity at 3p and/or 9p was associated with a 26.3-fold increase in the risk of developing invasive cancer. In contrast, degree of dysplasia was only marginally associated with cancer risk (1.7-fold increase for moderate/severe dysplasia versus hyperplasia/mild dysplasia).⁴⁷ Taken together, these data justify the use of loss of heterozygosity as a preferred marker of cancer risk and selection criterion for chemopreventive studies in patients with oral pre-malignant lesions (as opposed to other clinical criteria), in line with the concept of molecular intra-epithelial neoplasia as an entity to be targeted for cancer prevention, as recently proposed by a panel of experts.⁴⁸

Patients on EPOC that meet the molecular eligibility criteria are randomized to receive erlotinib 150 mg/day versus placebo for 12 months and are followed for a minimum of 2 years after completion of treatment. Patients whose lesions are negative for loss of heterozygosity do not receive active intervention. Treated patients are biopsied at months 3 and 12 and specimens are assessed for histological changes and stored for future biomarker evaluation. The primary endpoint of the study is development of oral cancer. The planned sample size for EPOC is 150 patients, based on the following assumptions: 50 without and 100 patients with a prior history of oral cancer are expected to be randomized; incidence of

oral cancer at 3 years is estimated at 35% and 65% in patients without and with a prior history of oral cancer. With these statistical properties, EPOC has 85% power to detect a 40% reduction in the risk of oral cancer with a 2-sided type I error rate of 5%. From November 2006 until July 2012, 398 patients were screened for loss of heterozygosity, 150 patients were randomized, and the trial is now closed to new patient accrual. Results are expected in early 2014. The clinical trial design is an example of how chemoprevention clinical studies can be streamlined. By focusing on high risk groups (in which the rate of events will be high), and by inhibiting a pathway that is a validated target in advanced disease (a strategy that is expected to have improved efficacy), this study allows for the definitive endpoint of invasive cancer to be assessed in a relatively short time and with a relatively small sample size. EPOC is also an example of a convergent trial design,⁴⁹ in which candidates for secondary prevention (i.e., individuals with pre-malignant lesions but without prior cancer) and candidates for tertiary prevention/adjuvant treatment (i.e., individuals with a prior history of oral cancer) are grouped together in one study, given that they are considered to have related deregulations of molecular pathways leading to carcinogenesis, and are predicted to have a similarly high cancer risk. Nonetheless, in EPOC there is a dissociation of the chosen molecular marker of cancer risk and the therapeutic intervention – it is possible that EGFR targeting may not be critical for inhibiting the development of invasive cancer in the specific population of patients with loss of heterozygosity at 3p and/or 9p, and that the latter is a predictive marker of cancer risk, but not a predictive marker of benefit from EGFR inhibitors. Assessment of correlations between presence of loss of heterozygosity and EGFR overexpression or gene copy number gain (now demonstrated markers of cancer risk in patients with oral pre-malignant lesions)²⁵ will help elucidate potential connections between these molecular abnormalities, with further mechanistic and therapeutic implications. These correlative studies are planned as part of the EPOC biomarker efforts. Additionally, biospecimens collected during the course of the trial and careful clinical and histological characterizations of pre-malignant lesions will allow discovery of new prognostic biomarkers of cancer risk, potential predictive markers of benefit from erlotinib, assessment of modulation of pathways by EGFR TKIs, and determination whether modulation of these pharmacodynamic markers, as well as clinical and histological responses to EGFR TKIs may serve as surrogate endpoints for development of invasive cancers.

Another EGFR-focused chemoprevention strategy that has been evaluated in clinical trials is dual targeting. Shin et al. presented the results of a phase I study of erlotinib plus the cyclooxygenase-2 inhibitor celecoxib in patients with moderate to severe dysplasia. Out of 11 patients treated, 3 had a histological complete response, 2 had a histological partial response (defined as improvement of dysplasia by at least 2 degrees), 2 had histological disease progression (defined by worsening of dysplasia by at least 2 degrees or development of invasive cancer), and 4 had no post-treatment biopsies to assess for response. Downregulation of EGFR and pERK expression on biopsy specimens at the time of the last clinical response was associated with clinical response, in line with pre-clinical studies performed with this drug combination. Screening for biomarkers of response with antibody arrays demonstrated a panel of 6 proteins that could differentiate patients with disease progression versus non-progressors.⁵⁰ Another clinical trial with vandetanib, a dual EGFR

and vascular endothelial growth factor receptor inhibitor is currently under way in patients with oral pre-malignant lesions [NCT01414426], based on results from animal studies (4-NQO mouse model) demonstrating a reduction in the incidence of oral cancer from 71% in the placebo group to 12% in the vandetanib-treated mice.⁵¹

Conclusions

EGFR inhibition represents a unique opportunity to advance relatively well tolerated molecular-targeted agents already in use for advanced HNSCC treatment to the chemoprevention setting. Observational data from patients with oral pre-malignant lesions support the role of EGFR dysregulation as a molecular marker of cancer risk and a key player in the process of malignant transformation. Pre-clinical experiments confirm the efficacy of targeting this pathway to interrupt oral carcinogenesis. Clinical trials with EGFR inhibitors have been launched, some with unique designs that may help streamline development of the chemoprevention field. If positive, these studies may significantly reduce morbidity and possibly mortality from HNSCC.

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Conflicts of interest

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Figure 1.

Epidermal growth factor receptor (EGFR) pathway activation during HNSCC carcinogenic process. Loss of heterozygosity (LOH), EGFR overexpression/amplification and cyclooxygenase-2 (COX2) dysregulation in pre-malignant lesions have been associated with invasive cancer risk. EGFR activation contributes to acquisition of cancer hallmarks represented in the figure. EGFR can be inhibited by the use of monoclonal antibodies (cetuximab, panitumumab, zalatumumab) and tyrosine kinase inhibitors (erlotinib, gefitinib, vandetanib and afatinib). Vandetanib and afatinib exhibit multikinase activity against other

targets such as the vascular endothelial growth factor (VEGF) receptor (vandetanib) and HER-2 (afatinib).