Erlotinib, Erlotinib–Sulindac versus Placebo: A Randomized, Double-Blind, Placebo-Controlled Window Trial in Operable Head and Neck Cancer


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Abstract

Purpose: The EGF receptor (EGFR) and COX2 pathways are upregulated in head and neck squamous cell carcinoma (HNSCC). Preclinical models indicate synergistic antitumor activity from dual blockade. We conducted a randomized, double-blind, placebo-controlled window trial of erlotinib, an EGFR inhibitor; erlotinib plus sulindac, a nonselective COX inhibitor; versus placebo.

Experimental Design: Patients with untreated, operable stage II-IVb HNSCC were randomized 5:5:3 to erlotinib, erlotinib–sulindac, or placebo. Tumor specimens were collected before and after seven to 14 days of treatment. The primary endpoint was change in Ki67 proliferation index. We hypothesized an ordering effect in Ki67 reduction: erlotinib–sulindac > erlotinib > placebo. We evaluated tissue microarrays by immunohistochemistry for pharmacodynamic modulation of EGFR and COX2 signaling intermediates.

Results: From 2005–2009, 47 patients were randomized for the target 39 evaluable patients. Thirty-four tumor pairs were of sufficient quality to assess biomarker modulation. Ki67 was significantly decreased by erlotinib or erlotinib–sulindac (omnibus comparison, two-sided Kruskal–Wallis, \( P = 0.043 \); placebo, \( P = 0.027 \)). There was a significant trend in ordering of Ki67 reduction: erlotinib–sulindac > erlotinib > placebo (two-sided exact Jonckheere–Terpstra, \( P = 0.0185 \)). Low baseline pSrc correlated with greater Ki67 reduction (\( R^2 = 0.312, P = 0.024 \)).

Conclusions: Brief treatment with erlotinib significantly decreased proliferation in HNSCC, with additive effect from sulindac. Efficacy studies of dual EGFR–COX inhibition are justified. pSrc is a potential resistance biomarker for anti-EGFR therapy, and warrants investigation as a molecular target. Clin Cancer Res; 20(12); 3289–98. © 2014 AACR.

Introduction

The EGF receptor (EGFR), a transmembrane receptor tyrosine kinase (RTK) propagating proliferative and anti-apoptotic signals, is amplified and/or overexpressed in approximately 90% of head and neck squamous cell carcinoma (HNSCC; refs. 1, 2). EGFR expression is higher in cancers driven by environmental carcinogens than by human papilloma virus (HPV; ref. 3), and correlates with increased stage, reduced survival, and radioresistance (1, 2, 4). EGFR signaling is also deregulated by HPV oncoproteins independent of EGFR expression level (5). EGFR has been validated as a therapeutic target in phase III trials enrolling both HPV-positive and negative patients (6, 7). Yet absolute benefit is low, and intrinsic resistance to the monoclonal antibody, cetuximab, or small-molecule RTK inhibitors (gefitinib and erlotinib) is implied by low mono-therapeutic response rates (8, 9). Predictive biomarkers for sensitivity to EGFR inhibition represent a major unmet need in HNSCC. Activating mutations in the EGFR kinase...
Translational Relevance

The EGF receptor (EGFR) and COX2 pathways are upregulated in head and neck squamous cell carcinoma (HNSCC). Cross-talk potentiates growth, proliferation, and invasion. Preclinical models indicate synergistic antitumor activity from dual blockade. We conducted a phase 0 trial of erlotinib, an EGFR inhibitor; erlotinib plus sulindac, a nonselective COX inhibitor; versus placebo in operable HNSCC to mechanistically characterize dual targeting. We demonstrated significant down-modulation of the tumor Ki67 proliferation index in both groups treated with erlotinib, and a formal test of trend evidenced potentiation by sulindac. Furthermore, we identified baseline pSrc expression as a candidate biomarker of erlotinib resistance in the clinic. This window trial provides the first in-human mechanistic data to justify efficacy trials of dual EGFR–COX inhibition in HNSCC. pSrc warrants investigation as a resistance biomarker for EGFR inhibition, and is now under investigation as a bona fide molecular target in HNSCC.

domain, associated with clinical response in lung cancer, are rarely identified in HNSCC (10). Furthermore, no alternative biomarker has been found (11, 12).

In HNSCC, an important mechanism of EGFR activation is autocrine secretion of EGFR ligands. G protein-coupled receptors (GPCR) transactivate EGFR by triggering proteolytic shedding of membrane-bound ligands (13, 14) GPCR–EGFR cross-talk may represent a targetable upstream resistance mechanism to EGFR blockade (15–17). COX2, an inflammatory enzyme which synthesizes the GPCR ligand prostaglandin E (PGE)-2, is upregulated in HNSCC and amenable to targeting by nonsteroidal anti-inflammatory drugs (NSAIDs). Available NSAIDs, including the nonselective COX inhibitor sulindac, inhibit HNSCC cell growth in vitro, and suppress formation of preneoplastic lesions in a transgenic HNSCC model (18). Concurrent EGFR–COX targeting demonstrates therapeutic synergism in HNSCC cell line and xenograft models (17, 19, 20).

We conducted a randomized, double-blind, placebo-controlled window trial of erlotinib–sulindac, erlotinib, or placebo in operable HNSCC to mechanistically characterize dual targeting. We demonstrated significant down-modulation of the tumor Ki67 proliferation index to test the hypothesis that dual EGFR–COX targeting would potentiate antitumor effects relative to EGFR inhibition alone. We studied associated pharmacodynamic changes in EGFR–GPCR signaling intermediates, to identify predictive biomarkers for anti-EGFR therapy that may translate to the clinic.

Materials and Methods

Ethics statement

This multicenter trial was approved by the Institutional Review Boards of the University of Pittsburgh (Pittsburgh, PA), Oregon Health Sciences University (Portland, OR), and the Portland Veterans Administration Medical Center (Portland, OR). The trial was nationally registered at clinicaltrials.gov (NCT01515137). All subjects provided written, informed consent.

Eligibility criteria

Eligible subjects met the following key inclusion criteria: histologically confirmed, previously untreated HNSCC (stage II–IVA) of the oral cavity, oropharynx, hypopharynx, or larynx, as defined by the American Joint Committee on Cancer Staging Handbook, 6th edition; planned complete resection of the primary tumor; age ≥ 18 years; Eastern Cooperative Oncology Group performance status 0–1; and adequate hematologic, hepatic, and renal function. Key exclusion criteria included: prior history of HNSCC, hypersensitivity to NSAIDs, interstitial lung disease, and chronic use of NSAIDs or steroids.

Treatment

Subjects were randomized by the University of Pittsburgh Biostatistics Facility to one of the three neoadjuvant treatment groups: erlotinib (150 mg daily), erlotinib (150 mg daily) plus sulindac (150 mg twice daily), or placebo (for erlotinib) in a 5:5:3 ratio. There was no placebo for sulindac. Preoperative therapy was administered in double-blind fashion for 7 to 14 days and discontinued 24 to 36 hours before surgery. Concomitant use of nonstudy NSAIDs or steroids was disallowed. Toxicities were described according to NCI Common Terminology Criteria for Adverse Events, version 3.0. Pre- and posttreatment tumor specimens were obtained at the time of diagnostic evaluation and definitive resection. Specimens were formalin-fixed and paraffin-embedded (FFPE), then shipped to University of Pittsburgh for centralized analyses.

Statistical analysis

The reported window trial was originally nested in a single-arm, phase II study of adjuvant, open-label erlotinib following primary surgery, and (chemo)radiation for locally advanced HNSCC. All subjects entering the parent trial were first randomized to one of the three preoperative treatment groups. After enrollment of 30 patients, the parent trial was discontinued because of nonfeasibility (21). At the time of discontinuation, and before analysis of biospecimens, the nested window trial was redesigned as an independent study. Patient allocation and randomization were unaffected. The primary endpoint was defined as change in the Ki67 proliferation index (ΔKi67) in pre and posttreatment tumor specimens, as validated in neoadjuvant breast cancer trials (22, 23). We hypothesized differential ΔKi67 according to treatment group. With 39 patients randomized 5:5:3, we had 92% power at α = 0.05 for a two-sided Kruskal–Wallis test to detect an omnibus between-group difference of 1 log in ΔKi67. Oversampling was permitted to reach the target of 39 evaluable patients, defined as being randomized, undergoing neoadjuvant treatment, and providing at least one tissue specimen. We
further hypothesized an ordering in Ki67 downmodulation, with erlotinib–sulindac > erlotinib > placebo; an exact, two-sided Jonckheere–Terpstra test formally evaluated for this trend. Pairwise contrasts were evaluated by two-sided Wilcoxon tests. Exact two-sided Kruskal–Wallis tests evaluated for randomization imbalances. For the observed randomization imbalance in baseline Ki67 (Kruskal–Wallis, \(P = 0.022\)), we used analysis of covariance to model the within-group association between baseline and change. Using adjusted values from this model, we verified that the baseline imbalance did not confound analysis of treatment group differences in \(\Delta\text{Ki67}\) (Supplementary Fig. S1). Thus, the presented analysis is based upon observed data.

Secondary endpoints included modulation of 25 candidate protein biomarkers, including 21 empirically selected from preclinical signaling models and four from mass spectrometry (MS) discovery. Before analysis in February 2013, we proposed two biomarker tiers, with specialized alpha spending for priority, hypothesis-driven analytes. For tests of trend, we designated three priority analytes: \(\Delta\text{pSrc}, \Delta\text{pAkt,}\) and \(\Delta\text{pSTAT}\) (17, 24–28). We hypothesized that phosphorylated forms of these key EGFR signaling nodes mediated short-term changes in proliferative signaling. We used the Jonckheere–Terpstra statistic to test the hypothesis that a valid biomarker would recapitulate the ordering of Ki67 downmodulation, that is, erlotinib–sulindac > erlotinib > placebo. For analyses of covariance, we defined four priority baseline proteins that could mechanistically explain resistance to Ki67 modulation: \(\text{pSrc, pAkt, pSTAT,}\) and \(\text{COX2}\). We first estimated flexible parametric regression models to evaluate the association of each baseline protein with \(\Delta\text{Ki67}\) across treatment groups. Significant baseline proteins were then tested for treatment group differences with analysis of covariance. For priority analytes, significance was set at \(\alpha = 0.05\). The remaining analytes were exploratory; multiple testing was corrected for false discovery by the method of Hochberg and Benjamini (29).

**Specimen analysis**

The primary method for analyzing biomarker response was immunohistochemical (IHC) analysis of tissue microarrays (TMA). Using guiding hematoxylin and eosin staining from FFPE blocks, 0.6 mm cores were transferred in triplicate from each pre- and posttreatment block to a blank recipient block. TMAs were assembled and stained after all infection outside the surgical field (2), and bleeding (1). No unusual rate or type of postoperative complication was observed; complications included fistula (2), wound infection (2), free flap necrosis (1), prolonged intubation (2), infection outside the surgical field (2), and bleeding (1). Toxicity

Brief exposure to erlotinib, erlotinib–sulindac, or placebo was well tolerated in the preoperative setting. Clinically significant toxicities attributed to study treatment are summarized in Table 2. Adverse events represented typical class toxicities for EGFR inhibitors, including rash and diarrhea (36). One patient discontinued study treatment for grade 2 anxiety, and one required erlotinib dose reduction for grade 2 mucositis. Median hospitalization for surgery was 9 days. No unusual rate or type of postoperative complication was observed; complications included fistula (2), wound infection (2), free flap necrosis (1), prolonged intubation (2), infection outside the surgical field (2), and bleeding (1).

**Biomarker modulation**

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from 35 of 39 patients (90%). Depending upon the biomarker assayed, 49% to 92% of the 34 paired specimens (median 78%) had measurable protein in both samples.

**Ki67.** Twenty-seven patients (69%) had measurable Ki67 in both samples. The primary, omnibus hypothesis test demonstrated a significant between-group difference in ∆Ki67 (Kruskal–Wallis, two-sided, \( P = 0.04 \)). Box plots depicting treatment group medians and interquartile ranges are presented in Fig. 2A. There was no change in Ki67 attributable to placebo. As compared with placebo, Ki67 was significantly modulated by erlotinib (\( P = 0.04 \)) or erlotinib–sulindac (\( P = 0.03 \)). There was a significant trend in ordering of Ki67 downmodulation: erlotinib–sulindac > erlotinib > placebo (Exact Jonckheere–Terpstra, two-sided, \( P = 0.02 \)), indicating additive antiproliferative effect from sulindac. A waterfall plot depicting per-patient percent change in Ki67 is shown in Fig. 2B.

**Biomarker intermediates.** We hypothesized that pSrc, pAkt, or pSTAT mediated the significant reduction in Ki67 by erlotinib or erlotinib–sulindac. If valid signaling intermediates of reduced proliferation, changes in these proteins would directly correlate with the ordering of ∆Ki67. As shown in Fig 3A and B, none of the three priority analytes, nor 22 exploratory biomarkers, demonstrated a significant ordering effect.

**Resistance biomarkers.** We hypothesized that increased baseline pSrc, pAkt, pSTAT or COX2 might mediate resistance to Ki67 modulation. We first evaluated the association between baseline expression of these candidate resistance biomarkers and ∆Ki67, independent of randomized treatment assignment (Fig. 4A). Higher pretreatment pSrc was associated with a smaller decrease in Ki67 (\( R^2 = 0.3, P = 0.04 \)). To understand whether these findings might represent erlotinib resistance, we plotted pSrc against ∆Ki67 within each treatment group. As displayed in Fig. 4B, resistance to Ki67 modulation was demonstrated only in the active treatment groups (tests that slopes differ from 0: placebo, \( P = 0.8773 \); erlotinib, \( P = 0.0024 \); erlotinib–sulindac, \( P = 0.0150 \)). Collectively, these findings implicate high baseline pSrc expression as a resistance biomarker to erlotinib, irrespective of cotreatment with sulindac. No significant association between baseline expression of 21 exploratory biomarkers and Ki67 modulation was discovered (Supplementary Fig. S2).

**PIK3CA mutations**

Because PIK3CA mutations are associated with chemopreventive benefit from aspirin in patients with colorectal cancer (37), we conducted a posthoc correlation between PIK3CA mutation status and ∆Ki67 in the erlotinib–sulindac arm. Two of 9 patients harbored noncommon exon 9 mutations (p.E522K, c.1564G>A; p.A533V, c.1598C>T AND p.I543I, c.1629C>T), whereas one bore a canonical exon 20 mutation (H1047L, c.3140A>T). There was no relationship between mutation status and ∆Ki67 (data not shown).

**Discussion**

Although EGFR has been validated as the first molecular target in HNSCC, absolute improvement in a clinically reliable endpoint following exposure to an EGFR inhibitor is limited to 10% to 20% of patients, implicating intrinsic resistance despite EGFR overexpression in the vast majority (6, 7, 9). Predictive biomarkers for anti-EGFR therapy in HNSCC represent a major unmet need. The current trial took advantage of a window design to investigate mechanistic signaling hypotheses about response and resistance to short-term erlotinib, with or without sulindac, in HNSCC. The study met its primary endpoint. First, we observed differential downmodulation of the Ki67 proliferation index across treatment groups, attributable to erlotinib or
erlotinib–sulindac as compared with placebo. Second, we confirmed that sulindac potentiates the antiproliferative effect of erlotinib in a formal test of trend, indicating that the forward feedback loop between COX2 and EGFR is a relevant clinical target. Finally, we identified baseline pSrc expression as a potential resistance biomarker to erlotinib.

### Table 1. Subject characteristics by treatment group

<table>
<thead>
<tr>
<th></th>
<th>Placebo (N = 12)</th>
<th>Erlotinib (N = 18)</th>
<th>Erlotinib + Sulindac (N = 16)</th>
<th>Test of equalitya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Median</td>
<td>59</td>
<td>64</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>33–86</td>
<td>44–73</td>
<td>49–68</td>
<td></td>
</tr>
<tr>
<td>Gender, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>3 (25)</td>
<td>4 (22)</td>
<td>3 (19)</td>
<td>0.9924</td>
</tr>
<tr>
<td>Male</td>
<td>9 (75)</td>
<td>14 (78)</td>
<td>13 (81)</td>
<td></td>
</tr>
<tr>
<td>Tumor site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral cavity</td>
<td>8 (67)</td>
<td>9 (50)</td>
<td>9 (56)</td>
<td>0.6606</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>3 (25)</td>
<td>5 (28)</td>
<td>6 (38)</td>
<td></td>
</tr>
<tr>
<td>Hypopharynx</td>
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<td>2 (11)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Larynx</td>
<td>1 (8)</td>
<td>2 (11)</td>
<td>1 (6)</td>
<td></td>
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<tr>
<td>Tumor (T) stageb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1 (9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.2371</td>
</tr>
<tr>
<td>2</td>
<td>5 (42)</td>
<td>3 (18)</td>
<td>3 (20)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3 (28)</td>
<td>4 (24)</td>
<td>5 (33)</td>
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<tr>
<td>4</td>
<td>2 (18)</td>
<td>10 (59)</td>
<td>7 (47)</td>
<td></td>
</tr>
<tr>
<td>Nodal (N) stagec</td>
<td></td>
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<td></td>
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<tr>
<td>0</td>
<td>7 (70)</td>
<td>8 (44)</td>
<td>6 (40)</td>
<td>0.3389</td>
</tr>
<tr>
<td>1</td>
<td>1 (10)</td>
<td>4 (22)</td>
<td>6 (40)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2 (20)</td>
<td>6 (33)</td>
<td>2 (13)</td>
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<tr>
<td>3</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (7)</td>
<td></td>
</tr>
<tr>
<td>p16 statusd</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1 (11)</td>
<td>3 (20)</td>
<td>0 (0)</td>
<td>0.3579</td>
</tr>
<tr>
<td>Negative</td>
<td>10 (89)</td>
<td>12 (80)</td>
<td>11 (100)</td>
<td></td>
</tr>
</tbody>
</table>

aWilcoxon test for age, Fisher exact two-tailed test for others.
bT classification was not available for three subjects.
cN classification was not available for three subjects.
dp16 status was not available for 9 patients. Among 14 oropharynx cases, 11 were p16(−), two were p16(+) and one was unknown.

### Table 2. Toxicities

<table>
<thead>
<tr>
<th>Nonhematologic toxicity</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Total grade ≥ 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergy/immunology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypersensitivity</td>
<td>1 (2%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Constitutional</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>3 (7%)</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td>0</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Dermatologic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash/desquamation</td>
<td>11 (24%)</td>
<td>5 (11%)</td>
<td>2 (4%)</td>
<td>0</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anorexia</td>
<td>0</td>
<td>1 (2%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4 (9%)</td>
<td>1 (2%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mucositis/stomatitis</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>2 (4%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neurologic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>0</td>
<td>1 (2%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Study results were strengthened by incorporation of a placebo control. Lack of significant change in Ki67 in placebo-treated patients raised confidence in its validity as a primary biomarker endpoint. Conversely, recognition of background changes in GPCR–EGFR signaling intermediates in placebo-treated patients avoided overstated mechanistic conclusions.

Ki67 is a nuclear nonhistone protein expressed in proliferating human tissue (38). Although recognized as a marker of poor prognosis in breast cancer, the Ki67 proliferation index is inconsistently prognostic in HNSCC (39, 40). ∆Ki67 is a validated surrogate biomarker in neoadjuvant studies of targeted therapy in breast cancer (22, 23); however, relevance to HNSCC window trials is unknown as Ki67 modulation has not been evaluated directly against clinical outcome (41–43). Here, the nonfeasibility of adjuvant erlotinib in the parent trial precluded our planned correlation of ∆Ki67 and 3-year progression-free survival. An alternate surrogate biomarker, the TUNEL (terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling) apoptotic index, was evaluated in a randomized window trial of lapatinib versus placebo in HNSCC (41). Although apoptosis was increased by lapatinib, the increase was not significant against placebo, underscoring the value of placebo control in short-term biomarker modulation studies. Notably, the Ki67 proliferation index was significantly decreased by lapatinib versus placebo, in line with our results. Collectively, these data suggest that proliferation is a more robust short-term endpoint than apoptosis for assessing response to targeted therapy in HNSCC.

The major secondary objective of this window study was identification of GPCR–EGFR signaling intermediates responsible for pharmacodynamic change in Ki67. However, among 25 protein candidates, none demonstrated an ordering effect consistent with ∆Ki67. Preclinical HNSCC models demonstrate at least additive downregulation of

Figure 2. The primary endpoint, Ki67 proliferation index. A, the primary omnibus hypothesis demonstrated a significant difference in Ki67 modulation among groups (two-sided Kruskal–Wallis, $P = 0.04$). Depicted in this box and whisker plot are the Wilcoxon pairwise contrasts confirming a significant decrease in Ki67 for patients treated with either erlotinib or erlotinib–sulindac versus placebo. Box and whisker plots consist of the median (white line), the interquartile range (box), and the distance to observed values within 1.5 times the interquartile range (whiskers). B, a waterfall plot depicts the intra-patient change in tumoral Ki67 expression, pre- and posttreatment. Bars are color-coded according to treatment arm.
multiple phospho-proteins (e.g., pSrc, pAkt, and pSTAT3) by GPCR-EGFR coinhibition relative to EGFR inhibition alone (17, 20). Our hypothesis test requiring linear ordering of valid intermediates may not reflect in vivo complexity of the GPCR–EGFR signaling network. The candidate biomarker approach was further limited by availability of validated commercial antibodies. Of note, multiple signaling proteins changed significantly in placebo-treated

\[ R^2 = 0.242, P = 0.072 \]
\[ R^2 = 0.109, P = 0.376 \]
\[ R^2 = 0.022, P = 0.812 \]

Figure 3. Biomarker intermediates and \( \Delta \)Ki67. We hypothesized that constituents of the EGFR or COX2 signaling pathways may mediate the observed change in proliferation. The line represents restricted cubic-spline fit for the relationship between \( \Delta \)analyte and \( \Delta \)Ki67. No significant correlation was identified between \( \Delta \)Ki67 and the priority analytes, \( \Delta \)pSTAT3, \( \Delta \)pSrc, or \( \Delta \)pAkt (A) or 21 other candidate biomarkers (B).
The paucity of predictive biomarkers for EGFR targeting in HNSCC impairs selection of patients who will benefit, and redirection of those who will not. This study identified high baseline pSrc expression as a potential resistance biomarker for erlotinib, an observation consistent with mechanistic preclinical data. Src family kinases are activated in response to EGFR signaling (13, 45). GPCRs also activate pSrc upstream of EGFR, recruiting pSrc to the complex mediating EGFR transactivation (17). Finally, pSrc drives ligand-independent activation of cMet, a major resistance mechanism to erlotinib in HNSCC models (46, 47). Because sulindac reduces PGE2-GPCR activation, and as a consequence pSrc (17), we hypothesized that the association between pSrc and resistance to Ki67 modulation would be muted in the sulindac group. This was not the case, suggesting that baseline pSrc is not driven dominantly by PGE2, but the convergent influence of multiple GPCR ligands and accessory RTKs. Contrary to our hypothesis, no other GPCR–EGFR signaling protein was associated with ΔKi67, including the PI3K/Akt and STAT3 resistance nodes described in preclinical models (17, 20). Although we recently reported that PI3KCA mutations are associated with erlotinib resistance in HNSCC cell lines (48), this has not been observed in HNSCC clinical cohorts. Here, PI3KCA mutation status in the erlotinib–sulindac arm did not correlate with ΔKi67; this post hoc finding warrants cautious interpretation due to small sample size and the potential interaction between genomic activation of PI3KCA and NSAIDs (37).

This study has several important limitations. First, the CONSORT diagram (Fig. 1) highlights the challenges in executing window studies. Although 35 of 39 patients had sufficient quality tissue for analysis of one biomarker, only 27 (69%) pairs were analyzable for the primary endpoint. Second, a sulindac-alone arm was not incorporated. This decision was based upon two factors: (i) EGFR is an established therapeutic target in HNSCC, whereas NSAIDs alone have not proven effective; and (ii) our biomarker hypothesis emphasized the potentiation of EGFR inhibition by sulindac, in the setting of GPCR-EGFR transactivation. Third, a potential criticism is use of Ki67 IHC as the primary endpoint, given the observed heterogeneity of tumor protein expression and unknown reliability of many IHC antibodies. Although Ki67 is subject to variability from preanalytic processing and interobserver scoring, the MIB1 antibody has been well validated, is tolerant of a range of fixation times, demonstrates durable antigenicity, and is the subject of international consensus standards followed here (30). Finally, the absence of a clinical endpoint limits interpretation of our mechanistic findings. Correlation of ΔKi67 to radiologic response or disease outcome will be critical to validating Ki67 modulation as a short-term biomarker endpoint in HNSCC window trials.

Despite acknowledged limitations, we affirmed that erlotinib significantly reduced proliferation in operable HNSCC after short-term exposure, an effect potentiated by sulindac. Efficacy studies evaluating dual EGFR–COX2 targeting are justified, particularly in light of a phase I clinical trial of gefitinib and celecoxib, demonstrating a 22% response rate.
in recurrent/metastatic HNSCC (49). Such a strategy may be more fruitful in patients with low tumoral pSrc expression, based upon our identification of baseline pSrc as a candidate resistance biomarker for erlotinib. Given pSrc’s central role in activating compensatory pathways, pSrc also warrants investigations as a bona fide cotarget in HNSCC. Although the Src family kinase inhibitor, dasatinib, demonstrated limited single-agent activity in HNSCC (50), dual EGFR–Src inhibition remains of interest. Two translational studies are ongoing in HNSCC, including the combination of erlotinib–dasatinib in the window setting and cetuximab–dasatinib in the recurrent/metastatic setting (NCIT00779389 and NCIT01488318).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions


Study supervision: S.M. Thomas, J.R. Grandis

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