Therapies Directed Against Epidermal Growth Factor Receptor in Aerodigestive Carcinomas

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Cancer that originates from the aerodigestive epithelium, including carcinomas of the head and neck, lung, and esophagus (referred to as aerodigestive carcinomas [ADCs]), are the leading causes of cancer-related mortality worldwide, accounting for about 2 million deaths annually. Their pathogenesis is causally linked with exposure to carcinogens, mainly tobacco and alcohol, and they are usually diagnosed at advanced stages, in which available treatments have limited curative potential. Even small advances made in the management of ADC might affect millions of lives. The cornerstone of the current era of therapeutics is the rational development of anticancer agents that has emerged from the in-depth understanding of cancer biology. Seminal features of cancer that relate to cell growth, replication potential, signaling pathways, cell-to-cell interactions, angiogenesis, and other factors are being elucidated and successfully exploited for therapeutic purposes, thus enabling accelerated development of novel compounds.

Epithelial carcinogenesis is the phenotypic outcome of serial genetic and epigenetic deviations resulting in the deregulation of cellular homeostasis. Accumulated evidence suggests that membrane receptor signaling pathways may participate in the acquisition of malignant phenotype. Epidermal growth factor receptor (EGFR) is a type I tyrosine kinase (TK) membrane receptor that regulates key cellular functions in epithelial malignancies. Multiple studies that used various methods have demonstrated that EGFR is commonly overexpressed in ADCs, typically more in squamous cell carcinoma than in adenocarcinomas. EGFR is commonly overexpressed in ADCs, typically more in squamous cell carcinoma than in adenocarcinomas.

Context Malignancies arising from the aerodigestive epithelium, including lung, head and neck, and esophageal carcinomas, are the leading causes of cancer-related mortality worldwide. Given the biological importance of epidermal growth factor receptor (EGFR) in cancer development and progression, EGFR inhibitors have emerged as promising novel therapies.

Objectives To summarize the current status of EGFR inhibitors in aerodigestive carcinomas (ADCs), highlight ongoing research designed to optimize their therapeutic effectiveness, and consider the future role of these agents.

Evidence Acquisition Systematic MEDLINE search of English-language literature (1966-April 2007) performed using the terms EGFR, EGFR inhibitors, monoclonal antibodies, tyrosine kinase inhibitors, lung cancer, head and neck cancer, esophageal cancer, and EGFR predictive factors. Quality assessment of selected studies included clinical pertinence, with an emphasis on controlled study design, publication in peer-reviewed journals, adequate number of enrolled patients, objectivity of measurements, and techniques used to minimize bias.

Evidence Synthesis The role of EGFR in ADC pathogenesis has been extensively studied, and multiple EGFR inhibition strategies are under evaluation. Erlotinib, an EGFR tyrosine kinase inhibitor used as a single agent, and cetuximab, an anti-EGFR monoclonal antibody used in combination with radiation, have conferred survival benefit in 1 trial of patients with advanced non–small cell lung cancer (median survival, 6.7 vs 4.7 months; hazard ratio, 0.70; 95% confidence interval, 0.58-0.87; P < .001) and in 1 trial of patients with locally advanced head and neck squamous cell carcinoma (median survival, 49 vs 29.3 months; hazard ratio, 0.74; 95% confidence interval, 0.57-0.97; P = .03), respectively. However, other trials have not shown these degrees of improvement. EGFR inhibitors toxicities include rash, diarrhea, and hypomagnesemia. Somatic mutations and other molecular tumoral characteristics offer opportunities for treatment individualization and optimal patient selection for anti-EGFR therapy.

Conclusions EGFR is a promising therapeutic target in ADC. Further translational research is needed to optimize ways of inhibiting EGFR using single-agent or combination regimens and to identify patients who benefit the most from these therapies.

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Clinical Review Section Editor: Michael S. Lauer, MD. We encourage authors to submit papers for consideration as a Clinical Review. Please contact Michael S. Lauer, MD, at michael.lauer@jama-archives.org.
nomas than in adenocarcinomas; EGFR protein expression is detected in about 50% or more of non–small cell lung cancers (NSCLCs),4,7 more than 90% of head and neck squamous cell carcinomas (HNSCCs),8 and 30% to 70% of esophageal carcinomas.9-11 High levels of EGFR protein expression have been correlated with worse patient survival in NSCLC, although the association has not been robust and results not consistent between studies.12-18 HNSCC,8,19 and esophageal carcinoma.10,11,20 Moreover, a high EGFR gene copy number, which varies with EGFR protein expression, has also been reported as a poor prognostic marker in ADC.5,21 Therefore, EGFR represents a rational target for ADC therapeutics.

Although blockade of EGFR function can be accomplished by several methods (Table 1), 2 approaches have been most extensively studied in the laboratory and clinic: monoclonal antibodies (mAbs) directed against the extracellular receptor domain, and small-molecule TK inhibitors of the EGFR intracellular domain (EGFR-TKIs). Agents of both categories have been incorporated into the standard management of ADC. However, their clinical application has generated a number of challenges.22 Foremost, the identification of molecular predictors of outcome that may potentially optimize patient selection and therapeutic efficacy remains the subject of intense ongoing basic and clinical investigation. Here, we will summarize the current status of clinical testing with EGFR inhibitors in ADC, highlight ongoing research regarding molecular predictive factors, and consider the future perspectives of these agents.

Table 1. Treatment Strategies Against Epidermal Growth Factor Receptor (EGFR)

<table>
<thead>
<tr>
<th>Strategy/Mode of Action</th>
<th>Anti-EGFR Strategy Parameter</th>
<th>Representative Agents</th>
<th>Route</th>
<th>Specificity</th>
<th>Adverse Effect Profile</th>
</tr>
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<tbody>
<tr>
<td>Tyrosine kinase inhibitors</td>
<td></td>
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<tr>
<td>EGFR phosphorylation inhibition</td>
<td></td>
<td>Gefitinib (R)</td>
<td>Oral</td>
<td>Moderate</td>
<td>Cutaneous toxicity, diarrhea</td>
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<tr>
<td></td>
<td></td>
<td>Erlotinib (R)</td>
<td></td>
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<td></td>
<td></td>
<td>EKB-569 (IRR)</td>
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<tr>
<td>EGFR/ERBB2 phosphorylation inhibition</td>
<td></td>
<td>Lapatinib (R)</td>
<td>Oral</td>
<td>Moderate</td>
<td>Cutaneous toxicity, diarrhea</td>
</tr>
<tr>
<td>EGFR/VEGFR phosphorylation inhibition</td>
<td></td>
<td>Vandetanib (R)</td>
<td>Oral</td>
<td>Moderate</td>
<td>Cutaneous toxicity, diarrhea, QT prolongation</td>
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<tr>
<td>Monoclonal antibodies</td>
<td></td>
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<tr>
<td>EGFR dimerization and activation blockade, immune-mediated action</td>
<td></td>
<td>Cetuximab</td>
<td>Intravenous</td>
<td>High</td>
<td>Cutaneous toxicity, infusion reactions, hypomagnesemia</td>
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<tr>
<td></td>
<td></td>
<td>Panitumumab</td>
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<td></td>
<td></td>
<td>Matuzumab</td>
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<tr>
<td>EGFR/ERBB2 heterodimerization blockade, immune-mediated action</td>
<td></td>
<td>Pertuzumab</td>
<td>Intravenous</td>
<td>High</td>
<td>Cutaneous toxicity, diarrhea</td>
</tr>
<tr>
<td>Antisense oligodeoxynucleotides</td>
<td>Decrease in mRNA expression/EGFR protein production</td>
<td>NA</td>
<td>Intravenous or intratumoral</td>
<td>High</td>
<td>Under study</td>
</tr>
<tr>
<td>Small interfering RNA</td>
<td>Decrease in mRNA expression/EGFR protein production</td>
<td>NA</td>
<td>Intravenous or intratumoral</td>
<td>High</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Abbreviations: IRR, irreversible; mRNA, messenger RNA; NA, not applicable; R, reversible; VEGF, vascular endothelial growth factor.
as epidermal growth factor or transforming growth factor α causes the EGFR to dimerize with itself or with another member of the ERBB family. EGFR dimerization causes activation of the receptor-linked TK, recruitment of signaling complexes, and phosphorylation (activation) of multiple downstream cascades.

**Figure.** Epidermal Growth Factor Receptor Activation, Processing, and Signaling

EGFR activation initiates activation of downstream signaling pathways.

When EGFR or other ligands bind to EGFR, the receptor dimerizes and activates TK.

Ligand-induced endocytosis limits EGFR activity. Activated surface receptors are internalized in endosomes and either undergo degradation through ubiquitination or are recycled to the cell surface.

ATP indicates adenosine triphosphate; Cbl, a ubiquitin E3 ligase; IGF, insulin-like growth factor; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; P, phosphate; PDGF, platelet-derived growth factor; PI3K, phosphatidylinositol-3-kinase; PKC, protein kinase C; PLC-γ, phospholipase Cγ; STAT, signal transducers and activators of transcription; TK, tyrosine kinase; and Ub, ubiquitin.
The EGFR-activated networks include the RAS/mitogen-activated protein kinase, 23 phosphatidylinositol-3-kinase/AKT, 24 Janus kinase/signal transducers and activators of transcription (JAK/STAT), 27 and phospholipase-Cy/ protein kinase C pathways 28 (Figure). These cascades are potent regulators of a variety of intracellular and intercellular processes such as proliferation, apoptosis, invasion, angiogenesis, and metastasis. EGFR activation also can be achieved through cross-talk with other receptors, such as G-protein-coupled receptors, 29 platelet-derived growth factor receptor, 30 insulin-like growth factor receptor 1, 31 and hormone receptors 32 (Figure).

An important regulatory component of the cell surface EGFR consists of a 2-step endocytosis process. 33 First, a rapid ligand-dependent internalization removes activated receptors from the cell membrane and enclaves them in endosomes. Second, internalized receptors either are targeted to lysosomes, where they undergo degradation, or are recycled back to the plasma membrane. EGFR sorting for lysosomal degradation requires receptor autophosphorylation, which then recruits Cbl—a ubiquitin E3 ligase responsible for EGFR ubiquitination 44 (Figure). Ligand-induced receptor endocytosis generally down-regulates EGFR signaling, even though there is some evidence that the internalized receptors may retain their capability of coupling effector proteins and activating signaling cascades. 33

The pathological manifestations of EGFR in ADC are achieved by at least 4 major mechanisms: (1) overexpression of EGFR ligands and establishment of autocrine/paracrine loops, (2) mutational activation of EGFR, (3) amplification of EGFR, and (4) transactivation by other receptor and nonreceptor TKs. 23,33 Two main categories of EGFR mutations have been identified: deletion mutations of the extracellular domain and somatic mutations in the TK activity of intracellular domain. Several classes of EGFR ectodomain mutations (vl-vVII) have been discovered, although EGFRvIII is the one most commonly found in ADC. It was recently shown that EGFRvIII in HNSCC leads to constitutive ligand-independent receptor activation, enhanced downstream effects, and resistance to wild-type EGFR targeting. 36 Activating mutations of EGFR-TK domain are more commonly found in NSCLCs than other ADCs. 37-40 Deletions in exon 19 and nucleotide substitutions in exon 21 are the most common EGFR mutations in NSCLCs. These mutations affect the adenosine triphosphate binding pocket of the EGFR-TK domain and endow the receptor with opposed TK activity. Tumors harboring EGFR mutations respond better to EGFR-TKIs, suggesting that they are dependent on mutant EGFR signaling. However, it should be noted that although EGFR-driven molecular pathway aberrations are important, they cannot be considered the dominant sole genetic alteration in the molecular pathogenesis of ADC. 41

EGFR signaling is important for a variety of normal tissues, including those of the skin, gut, and kidney, which explains the development of certain toxicities from EGFR targeting, such as rash, diarrhea, and hypomagnesemia. For example, aceneform rash and other skin toxicities are encountered in the majority of patients who receive EGFR inhibitors. 42 Moreover, EGFR blockade may interfere with magnesium transport, possibly due to the strong expression of EGFR in the ascending limb of the loop of Henle, where most of the filtered magnesium is reabsorbed, which could explain the high frequency of hypomagnesemia during cetuximab treatment. 43,44

**Strategies of EGFR Inhibition**

Various strategies have been developed to disrupt the EGFR signal transduction pathway (Table 1). Among them, anti-EGFR mAbs and EGFR-TKIs have undergone the most extensive investigation. Putative mechanisms of EGFR mAb–based anticaner activity can be classified into 2 categories. First, they prevent ligands from binding to the EGFR extracellular domain, inhibit subsequently receptor dimerization/activation, and finally induce receptor degradation. 45 The second potential mechanism of EGFR mAb therapy is indirect action mediated by the immune system. There are unique immune-effector mechanisms that have been found to be triggered by therapeutic mAbs, such as antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, and complement-dependent cell-mediated cytotoxicity. 46-47 An alternative mode of targeting EGFR is by conjugating anti-EGFR mAbs with toxins to selectively attack tumor cells overexpressing EGFR. 48

Receptor and nonreceptor TKs are critical mediators in EGFR signaling pathways, and many are deregulated during ADC tumorigenesis. Small-molecule inhibitors that target these TKs are directly acting in tumor cells rather than mediating immune responses. Most small-molecule TK inhibitors are adenose triphosphate mimetics, thus competing (reversibly or irreversibly) with adenose triphosphate binding within the catalytic kinase cleft. Unlike mAbs, they cross the plasma membrane and interact with the cytoplasmic domain of cell-surface receptors or modulate intracellular signaling molecules. Tyrosine kinase inhibitors have a variable selectivity for TKs, and some are dual-selective or multisitelective (Table 1). EGFR-TKIs are generally thought to be less specific than mAbs, which is potentially advantageous for antitumor activity but may be associated with increased toxicities due to the inhibition of several signaling pathways that potentially interfere with normal cell functions. A number of newer-generation TK inhibitors show a broad-spectrum activity against various receptor TKs (eg, EGFR, vascular endothelial growth factor [VEGF] receptor), nonreceptor TKs (eg, SRC kinase), and/or downstream molecules (eg, RAS/mitogen-activated protein kinase). 49

The development of new EGFR inhibitors has also been attempted using high-throughput screening approaches, rational drug design, or a combination of both. High-throughput screening of compounds is a way of iden-

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The EGFR-TKIs gefitinib and erlotinib achieved responses of 10% to 20%, respectively, in phase 2 clinical trials in patients with previously treated, advanced NSCLC.55-57 Based on these observations, 2 randomized, double-blind, placebo-controlled phase 3 trials were initiated to evaluate the overall survival with EGFR-TKIs compared with best supportive care alone in patients with advanced NSCLC who had received first- or second-line chemotherapy (TABLE 2). In the first study, patients were randomized to receive either erlotinib (150 mg orally, once daily) or placebo. Overall survival was 6.7 months for patients in the erlotinib group compared with 4.7 months in patients in the control group (P < .001).58 Analysis of quality of life showed that patients in the erlotinib group had longer time to deterioration of tumor-related symptoms.68

In the second trial, gefitinib at a daily dose of 250 mg did not improve overall survival compared with placebo in a similar cohort of patients with NSCLC, even though subset analysis showed a survival benefit in Asians and nonsmokers.61 The reasons for the discrepancy of the results of these 2 similarly designed clinical trials are unclear. The possibility that gefitinib was given at a suboptimal biological dose has been suggested as a plausible explanation and is also supported by preclinical observations.69 Whether gefitinib dose escalation beyond 500 mg would improve its clinical efficacy remains to be demonstrated in clinical studies.70 Gefitinib also produced negative results as adjuvant monotherapy after combined-modality therapy with chemotherapy and radiotherapy in a phase 3 trial in patients with stage III NSCLC64 (Table 2). Although a phase 3 study of adjuvant gefitinib in patients with surgically resected NSCLC was prematurely terminated, the use of erlotinib as adjuvant or maintenance therapy is currently under investigation (TABLE 3).

Four phase 3 trials evaluated an EGFR-TKI, erlotinib or gefitinib, in combination with standard first-line chemotherapy in patients with advanced NSCLC59,60,62,63 (Table 2). No advantage could be elicited with the addition of an EGFR-TKI to chemotherapy in survival or any other efficacy end point in these trials, in spite of preclinical work on human tumor xenografts supporting such combinations.53 It has been postulated that EGFR-TKIs and chemotherapy would be more efficacious when administered sequentially, not simultaneously, and this concept is undergoing clinical evaluation61 (Table 3). However, it is likely that the lack of advantage found in the phase 3 trials evaluating EGFR inhibitors in combination with chemotherapy was due to the lack of patient selection, stratification based on molecular tumor characteristics, or both. For example, the design of these studies did not take into consideration the presence of EGFR-activating mutations, EGFR gene copy number, or both, which recently emerged as predictors of EGFR-TKI efficacy. Thus, small benefits may have been obscured due to the molecular heterogeneity of the study population.

The anti-EGFR antibodies, mainly cetuximab, also have been studied in NSCLC as single agents and in combination with chemotherapy. In 1 study in 66 patients with previously treated, advanced NSCLC, cetuximab monotherapy yielded a low response rate (4.5%) but a promising median overall survival of 8.9 months.72 A number of ongoing phase 3 randomized trials are evaluating the addition of cetuximab to chemotherapy in the first- and second-line treatment of advanced NSCLC (Table 3).

Head and Neck Cancer. EGFR is important in the pathogenesis of HNSCC, and its overexpression has been reported in more than 80% of cases.23 Moreover, EGFR up-regulation also has been documented in the normal-appearing epithelium adjacent to malignant tissue, thus supporting the “field cancerization” concept, which refers to the multifocal development of premalignant and malignant lesions within the entire carcinogen-exposed epithelium.73 Therefore, inhibition of EGFR...
signaling represents a rational new strategy in HNSCC therapeutics.

Several TK inhibitors with preclinical activity against EGFR have been tested in HNSCC. Many single-group phase 2 trials using gefitinib or erlotinib have shown modest single-agent activity (responses of 1%-11%) in patients with recurrent or metastatic HNSCCs, whereas lapatinib, a novel dual EGFR and ERBB2 inhibitor, was not active in the same setting. Recently, promising preliminary results of clinical trials evaluating the combination of gefitinib or erlotinib with chemotherapeutic agents or other biological agents (eg, bevacizumab) and radiotherapy have been reported. Moreover, 2 phase 3 studies have evaluated gefitinib in recurrent or metastatic HNSCC. The first is an international multicenter trial that compared gefitinib (at either a 250-mg or a 500-mg daily dose) with methotrexate and that recently reported results showing that gefitinib is not superior to methotrexate in this setting (Table 2); the second is an ongoing study that compares docetaxel with or without gefitinib as first- or second-line treatment (Table 3).

Cetuximab was the first novel agent to obtain regulatory approval in the United States for the treatment of patients with HNSCC. This was based on positive survival data from a phase 3 randomized clinical trial that compared radiation and cetuximab with radiation alone in patients with locally advanced HNSCC. (Table 2). The improvement in locoregional control and survival achieved with the addition of cetuximab to radiation was comparable to that achieved with platinum-based regimens. However, no randomized trial has yet compared

### Table 2. Completed Phase 3 Clinical Trials Using Anti-EGFR Agents in Aerodigestive Carcinoma

<table>
<thead>
<tr>
<th>Anti-EGFR Strategy</th>
<th>Source</th>
<th>Patient Population</th>
<th>Treatment (No. of Patients)</th>
<th>Primary End Point</th>
<th>Result</th>
<th>Other End Point Results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR-TKIs</td>
<td><strong>Erlokin</strong></td>
<td>Shepherd et al.2005</td>
<td>Previously treated, advanced</td>
<td>Erlotinib (488) vs placebo (243)</td>
<td>Overall survival</td>
<td>6.7 vs 4.7 mo (HR, 0.70; 95% CI, 0.55-0.87; P &lt; .001)</td>
<td>PFS, 2.2 vs 1.8 mo (HR, 0.61; 95% CI, 0.51-0.74; P &lt; .001)</td>
</tr>
<tr>
<td></td>
<td><strong>Herbst et al.2005</strong></td>
<td>First-line, advanced</td>
<td>Carboplatin/paclitaxel plus erlotinib (539) or placebo (540)</td>
<td>Overall survival</td>
<td>10.6 vs 10.5 mo (HR, 0.99; 95% CI, 0.86-1.16; P = .96)</td>
<td>TTP, 5.1 vs 4.9 mo (HR, 0.70; 95% CI, 0.58-0.87; P &lt; .001)</td>
<td>No benefit from the addition of an EGFR-TKI (erlotinib) to chemotherapy in advanced disease</td>
</tr>
<tr>
<td></td>
<td><strong>Catzemeier et al.2007</strong></td>
<td>First-line, advanced</td>
<td>Cisplatin/gemcitabine (580) plus erlotinib (583) or placebo (579)</td>
<td>Overall survival</td>
<td>43 vs 44.1 wk (HR, 1.00; 95% CI, 0.90-1.23; P = .49)</td>
<td>TTP, 23.7 vs 24.6 wk (HR, 0.70; 95% CI, 0.58-0.87; P &lt; .001)</td>
<td>No benefit from the addition of an EGFR-TKI (erlotinib) to chemotherapy in advanced disease</td>
</tr>
<tr>
<td></td>
<td><strong>Gatema et al.2005</strong></td>
<td>Previously treated, advanced</td>
<td>Gefitinib 250 mg (112) vs placebo (112)</td>
<td>Overall survival</td>
<td>5.6 vs 5.1 mo (HR, 0.99; 95% CI, 0.77-1.02; P = .09)</td>
<td>TTP, 3 vs 2.6 mo (HR, 0.70; 95% CI, 0.55-0.87; P &lt; .001)</td>
<td>No statistically significant survival benefit from an EGFR-TKI (gefitinib) over placebo in patients with advanced NSCLC and the subset of patients with adenocarcinomas</td>
</tr>
<tr>
<td></td>
<td><strong>Herbst et al.2004</strong></td>
<td>First-line, advanced</td>
<td>Carboplatin/paclitaxel plus gefitinib 250 mg (345) or gefitinib 500 mg (347) or placebo (345)</td>
<td>Overall survival</td>
<td>9.8 vs 8.7 vs 9.9 mo (P = .64)</td>
<td>PFS, 5.3 vs 4.6 vs 5 mo (HR, 0.58; 95% CI, 0.47-0.70; P &lt; .001)</td>
<td>No benefit from the addition of an EGFR-TKI (gefitinib) to chemotherapy in advanced disease. Two doses of gefitinib evaluated.</td>
</tr>
<tr>
<td></td>
<td><strong>Giaccone et al.2004</strong></td>
<td>First-line, advanced</td>
<td>Cisplatin/gemcitabine plus gefitinib 250 mg (365) or gefitinib 500 mg (365) or placebo (365)</td>
<td>Overall survival</td>
<td>9.9 vs 9.9 vs 9.9 mo (P = .46)</td>
<td>PFS, 5.8 vs 5.5 vs 5.5 mo (HR, 0.70; 95% CI, 0.51-0.92; P &lt; .001)</td>
<td>No benefit from the addition of an EGFR-TKI (gefitinib) to chemotherapy in advanced disease. Two doses of gefitinib evaluated.</td>
</tr>
<tr>
<td></td>
<td><strong>Kelly et al.2005</strong></td>
<td>Inoperable, stage III</td>
<td>Gefitinib 250 mg or 500 mg (124) or placebo (131) following chemotherapy and radiotherapy</td>
<td>Overall survival</td>
<td>19 (both gefitinib dose levels combined) vs 29 mo (HR, 0.55; P = .09)</td>
<td>PFS, 11 vs 10 mo (HR, 0.55; P = .04)</td>
<td>Prematurely closed; the possibility of a detrimental effect of gefitinib was suggested</td>
</tr>
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EGFR-TKIs have been extensively studied in the treatment of recurrent or metastatic HNSCC. The Eastern Cooperative Oncology Group conducted a randomized study that compared cisplatin with or without cetuximab for the first-line treatment of recurrent or metastatic HNSCC. Although this study had a small sample size of 117 analyzable patients, it showed a significant improvement in response rates and a trend toward improved median progression-free survival with the addition of cetuximab (Table 2). Cetuximab has also shown efficacy in patients with platinum-refractory HNSCC, either as a single agent, which formed the basis for regulatory approval in the United States for this indication as well, or in combination with platinum. A recently completed phase 3 trial investigated the combination of a platinum agent (cisplatin or carboplatin) and 5-fluorouracil with or without cetuximab in recurrent or metastatic HNSCC (Table 3). Esophageal Carcinoma. Squamous cell carcinoma of the esophagus remains a common disease; however, the incidence of adenocarcinoma of the esophagus is gradually increasing. These 2 entities may be distinct in their etiology, pathogenesis, and prognosis. Several studies have reported EGFR protein expression of 30% to 70% in esophageal carcinomas, which is more common in squamous cell carcinomas than in adenocarcinomas and has been correlated with poor patient prognosis and inferior response to conventional treatment. EGFR amplification has been documented in approximately 15% of esophageal carcinomas, whereas EGFR mutations are rarely seen (0%-11%). Phase 2 studies using EGFR-TKIs as monotherapy in the first- and second-line setting in unsselected patients with advanced esophageal cancer showed modest activity, while the combination of TK inhibitors or mAbs with chemotherapy, radiotherapy, or both is currently under evaluation.

### Table 2. Completed Phase 3 Clinical Trials Using Anti-EGFR Agents in Aerodigestive Carcinoma (cont)

<table>
<thead>
<tr>
<th>Anti-EGFR Strategy</th>
<th>Source</th>
<th>Patient Population</th>
<th>Treatment (No. of Patients)</th>
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<th>Other End Point Results</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EGFR-TKIs</strong></td>
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<tr>
<td><strong>Gefitinib</strong></td>
<td>Stewart et al, 2007</td>
<td>Recurrent or metastatic</td>
<td>Gefitinib 250 mg (158) or 500 mg (167) vs methotrexate (161)</td>
<td>Overall survival</td>
<td>5.6 vs 6 vs 6.7 mo (HR, 1.22; 95% CI, 0.95-1.57; P = .12 and HR, 1.12; 95% CI, 0.87-1.43; P = .39)</td>
<td>RR, 2.7% vs 7.8% vs 3.9% (P = .57; P = .16)</td>
<td>No survival benefit with EGFR-TKI (gefitinib) monotherapy over standard methotrexate</td>
</tr>
<tr>
<td><strong>EGFR-MoAbs</strong></td>
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<tr>
<td><strong>Cetuximab</strong></td>
<td>Burtness et al, 2005</td>
<td>Recurrent or metastatic</td>
<td>Cisplatin (100mg/m²) plus cetuximab (57) or placebo (60)</td>
<td>Progression-free survival</td>
<td>4.2 vs 2.7 mo (HR, 0.78; 95% CI, 0.54-1.12; P = .07)</td>
<td>Overall survival, 9.2 vs 8 mo (P = .21) (RR, 26% vs 10%; P = .03)</td>
<td>Placebo-controlled study; insufficient sample size</td>
</tr>
<tr>
<td><strong>Bonomi et al, 2006</strong></td>
<td>Locally advanced</td>
<td>RT plus cetuximab (211) vs RT alone (213)</td>
<td></td>
<td>Locoregional control</td>
<td>24.4 vs 14.9 mo (HR, 0.68; 95% CI, 0.52-0.89; P = .009)</td>
<td>Overall survival, 49 vs 29.3 mo (HR, 0.74; 95% CI, 0.57-0.97; P = .03); PFS, 17.1 vs 12.4 mo (HR, 0.70; 95% CI, 0.54-0.90; P = .006)</td>
<td>First randomized study showing survival benefit with an EGFR targeting agent in locally advanced HNSCC</td>
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</table>

**Abbreviations:** CI, confidence interval; EGFR, epidermal growth factor receptor; EGFR-TKI, small-molecule tyrosine kinase inhibitor of the EGFR intracellular domain; HNSCC, head and neck squamous cell carcinoma; HR, hazard ratio; MoAb, monoclonal antibody, NS, not significant; NSCLC, non–small cell lung cancer; RR, response rate; RT, radiotherapy; TTF, time to treatment failure; TTP: time to disease progression.

aMedian values reported.

bAll P values are 2-sided.
NSCLC have identified a number of characteristics that predict increased benefit with EGFR-TKIs, such as no prior smoking history, female sex, adenocarcinoma or bronchioloalveolar carcinoma (BAC) histology, and Asian ethnicity. The molecular basis of these associations is not always apparent. Also, the distinction between prognos-

Table 3. Selected Ongoing Phase 3 Clinical Trials Using Anti-EGFR Agents in Aerodigestive Carcinoma

<table>
<thead>
<tr>
<th>Anti-EGFR Strategy</th>
<th>ClinicalTrials.gov Identifier</th>
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<th>Treatment (No. of patients)</th>
<th>Primary End Point</th>
<th>Question Asked</th>
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<td><strong>Non–Small Cell Lung Cancer</strong></td>
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<tr>
<td>EGFR-TKIs</td>
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<tr>
<td>Gefitinib</td>
<td>NCT00455936</td>
<td>First-line, advanced, never smokers</td>
<td>Gefitinib vs cisplatin/ gemcitabine (314)</td>
<td>Overall survival</td>
<td>Is first-line gefitinib better than chemotherapy in never smokers?</td>
</tr>
<tr>
<td></td>
<td>NCT00091156</td>
<td>First-line, advanced disease</td>
<td>Gefitinib vs placebo following first-line chemotherapy (380)</td>
<td>Overall survival</td>
<td>Can adjuvant gefitinib improve survival for patients with advanced-stage NSCLC who have not progressed after first-line chemotherapy?</td>
</tr>
<tr>
<td></td>
<td>NCT00076388 (Accrual completed)</td>
<td>Previously treated, advanced</td>
<td>Gefitinib vs docetaxel (1440)</td>
<td>Overall survival</td>
<td>Is gefitinib as efficacious as docetaxel in the second- or third-line treatment of NSCLC?</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>NCT00153803</td>
<td>Inoperable, stage III</td>
<td>Erlotinib vs placebo following concurrent docetaxel, carboplatin, and thoracic radiotherapy (380)</td>
<td>Progression-free survival</td>
<td>Can maintenance erlotinib improve progression-free survival in patients with inoperable, stage III NSCLC?</td>
</tr>
<tr>
<td></td>
<td>NCT00440414</td>
<td>Previously treated, advanced</td>
<td>Erlotinib vs pemetrexed (450)</td>
<td>Overall survival</td>
<td>Is erlotinib or pemetrexed preferable as second-line therapy of advanced NSCLC?</td>
</tr>
<tr>
<td></td>
<td>NCT00349219</td>
<td>First-line, advanced</td>
<td>Erlotinib followed by cisplatin/gemcitabine vs cisplatin/ gemcitabine followed by erlotinib (900)</td>
<td>Overall survival</td>
<td>Which is the best sequence of erlotinib and chemotherapy in advanced NSCLC?</td>
</tr>
<tr>
<td></td>
<td>NCT00446225</td>
<td>First-line, advanced, positive for EGFR-TK mutations</td>
<td>Erlotinib vs chemotherapy (146)</td>
<td>Progression-free survival</td>
<td>Is erlotinib better than chemotherapy in NSCLC with EGFR-TK mutations?</td>
</tr>
<tr>
<td></td>
<td>NCT00373425</td>
<td>Resected, stage IB-IIIA, EGFR-positive</td>
<td>Erlotinib vs placebo</td>
<td>Overall survival</td>
<td>Is adjuvant erlotinib beneficial in patients with resected NSCLC?</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>NCT00095199</td>
<td>Previously treated, advanced</td>
<td>Docetaxel or pemetrexed with/without cetuximab (800)</td>
<td>Progression-free survival</td>
<td>Is the addition of cetuximab to second- or third-line chemotherapy beneficial?</td>
</tr>
<tr>
<td></td>
<td>NCT00122460 (Accrual completed)</td>
<td>First-line, advanced, EGFR-positive</td>
<td>Docetaxel plus cetuximab (890)</td>
<td>Overall survival</td>
<td>Does the addition of cetuximab in the first-line treatment of advanced NSCLC improve survival?</td>
</tr>
<tr>
<td></td>
<td>NCT00112294 (Accrual completed)</td>
<td>First-line, advanced</td>
<td>Docetaxel plus cetuximab (250 mg or placebo (330))</td>
<td>Overall survival</td>
<td>Does the addition of gefitinib to docetaxel improve survival in recurrent or metastatic HNSCC?</td>
</tr>
<tr>
<td>EGFR-MoAbs</td>
<td>NCT000265941</td>
<td>Locally advanced</td>
<td>RT plus cisplatin with/without cetuximab (720)</td>
<td>Disease-free survival</td>
<td>Does the addition of cetuximab to standard chemoradiotherapy improve disease-free survival in locally advanced HNSCC?</td>
</tr>
</tbody>
</table>

Abbreviations: EGFR, epidermal growth factor receptor; EGFR-TKI, small-molecule tyrosine kinase inhibitor of the EGFR intracellular domain; HNSCC, head and neck squamous cell carcinoma; NSCLC, non–small cell lung cancer; RT, radiotherapy; TK, tyrosine kinase.
tic and predictor factors can be potentially made only in randomized trials that included a treatment group without an EGFR inhibitor. The higher probability of response to EGFR-TKIs in never smokers with NSCLC has been a robust and particular intriguing observation that may be partially related to the higher frequency of EGFR mutations in this group. Moreover, patients with pure BAC or with adenocarcinoma having BAC features appear to derive substantial benefit from EGFR-TKIs, as demonstrated in phase 2 trials, though confirmatory data have not yet been available.

It also has been consistently shown that the onset and severity of skin rash due to EGFR-TKIs and mAbs correlate with patient outcome across many tumor types, including HNSCC and NSCLC. The skin toxicities associated with EGFR inhibitors are attributable to the prominent role of EGFR in skin physiology. An interesting hypothesis is that patients with certain polymorphisms in intron 1 of the EGFR gene may have a higher likelihood of a favorable tumor response as well as of developing skin toxicities with EGFR inhibitor treatment.

Molecular Predictive Markers. EGFR Mutations. Activating somatic mutations in the EGFR-TK domain were first described in NSCLC. It is noteworthy that these mutations are present in a fraction of patients with NSCLC that varies with race or ethnicity (approximately 10% in unselected populations in the United States but markedly higher in East Asians), smoking history, sex, and histology. Similar EGFR-TK mutations have been detected but appear to be less frequent in HNSCCs (1%-7%) and esophageal carcinomas (0%-11%). The presence of activating EGFR mutations appears to be a prognostic factor associated with prolonged survival in patients with advanced NSCLCs treated with chemotherapy with or without an EGFR-TKI. Moreover, in multiple studies of NSCLCs but not in a placebo-controlled randomized trial of advanced NSCLC treated with erlotinib, EGFR mutations were predictive of survival in patients treated with EGFR-TKIs. A secondary point mutation in the EGFR-TK domain, T790M, that is associated with acquired resistance has also been reported.

Although EGFR mutations are potentially important predictors of outcome, they can only partially account for the documented clinical benefit from EGFR-TKIs in patients with NSCLC, while in other ADCs, EGFR mutations appear to be of limited significance. Multiple clinical trials are evaluating EGFR-TKIs as upfront treatment in chemotherapy-naive patients with advanced NSCLC and EGFR-activating mutations. Both gefitinib and erlotinib have so far produced dramatic antitumor activity, with objective response rates of 75% to 82% in this setting. A potential limitation for the prospective evaluation of EGFR mutations is the difficulty in obtaining sufficient tumor tissue for analysis in patients with inoperable NSCLC. Detection of the mutations in malignant pleural effusions is under study.

EGFR Gene Copy Number and Polymorphisms. The EGFR gene copy number has emerged as an important predictor of the efficacy of EGFR inhibitors in NSCLC. High EGFR gene copy number, amplification, or high polysomy, as evaluated by fluorescent in-situ hybridization, has been documented in 31% to 45% of cases of NSCLC and associated with increased responsiveness and longer survival after treatment with EGFR-TKIs. The potential role of EGFR amplification as a prognostic factor is also being studied in HNSCC and squamous cell cancer of the esophagus, whereas data on its use as a predictor of outcome in studies with EGFR inhibitors in these cancers are awaited. The significance of genomic gain for other ERBB family members (eg, ERBB2, ERBB3) in patients treated with EGFR inhibitors is also under investigation.

The regulation of EGFR expression is complex and involves multiple regulatory steps. Interindividual or ethnic polymorphisms in the EGFR gene also can alter EGFR expression, activity, or both. Such variations also might account for the observed diverse anti-EGFR treatment efficacy and should be further investigated. Recently, a pharmacokinetic model was developed to assess the influence of cytochrome P450 3A activity on EGFR-TKI metabolism, antitumor activity, and toxicity.

EGFR Protein Expression and Other Molecular Markers. High EGFR protein expression, as determined by immunohistochemistry, is observed in the vast majority of ADCs, with the highest expression seen in HNSCC. However, EGFR protein expression has not been found to consistently correlate with the antitumor activity of EGFR inhibitors. For example, mixed results have been obtained in studies that evaluated EGFR protein expression and EGFR-TKI activity in advanced NSCLC. The discordant results regarding the predictive value of EGFR protein expression may be partially attributed to variability in population characteristics and methodology. Nevertheless, it is suggested by analysis of phase 3 trials that patients with NSCLC negative for EGFR protein expression derive no survival benefit from EGFR-TKIs.

Perturbations in the RAS/mitogen-activated protein kinase, phosphatidylinositol-3-kinase/AKT, and JAK/STAT pathways, which are the major downstream effectors of EGFR signaling, are being evaluated as potential predictors of outcome. For example, it was recently shown in vitro that AKT phosphorylation without EGFR ligand stimulation has an important role in sensitivity to EGFR-TKI, probably due to ERBB3-mediated activation. NSCLC arising in smokers appears to have a different spectrum of molecular abnormalities than NSCLC in nonsmokers, suggesting differences that emerge early in carcinogenesis and may possibly relate to prognosis. K-RAS mutations strongly correlate with smoking history and have been associated with poor prognosis. Activating mutations in K-RAS...
and EGFR may be mutually exclusive, since they almost never occur in the same tumor.\(^{120}\) It appears that tumor growth can depend on activation of either pathway but not both. Accumulating preclinical and clinical evidence suggests that patients with NSCLC and K-RAS mutations may represent a distinct patient population with intrinsic resistance to anti-EGFR treatment approaches.\(^{92,130,131}\)

Although data on predictors of efficacy are rapidly accumulating, at present there is no single biomarker that can be recommended for patient selection for anti-EGFR therapies. It is possible that an algorithm that incorporates a panel of biomarkers, including EGFR mutations, EGFR gene copy number, or EGFR protein levels may provide a useful clinical tool.

**Future Perspectives**

The introduction of EGFR inhibitors was one of the first steps in the development of successful molecularly targeted therapies in ADC. Certainly, optimizing strategies and learning from failures in the developmental process of these agents is critical. The molecular events implicated in intrinsic or acquired resistance to anti-EGFR therapy are yet to be fully unveiled. One of the potential strategies for improving antitumor effects is combined treatment with distinct anti-EGFR agents that can maximize EGFR signaling inhibition.\(^{132}\) Thus, the combination of EGFR-TKIs and mAbs is of interest and has entered clinical testing.\(^{133}\) However, anti-EGFR treatment alone may not be sufficient. Aerodigestive carcinomas usually acquire aberrations in more than 1 signaling pathway during carcinogenesis, and EGFR is only one of many targets in a complex system. Thus, EGFR targeting may rather become a component of a dual-targeting or multitargeting strategy that addresses the complexity of signaling pathways and avoids the emergence of resistance. For example, EGFR cross-talk networks with the VEGF/VEGF-receptor pathway are well established. Preclinical and clinical resistance to EGFR inhibitors has been attributed to increased VEGF levels.\(^{134}\) Therefore, the combination of EGFR inhibitors and antiangiogenesis agents has surfaced as an appealing novel strategy. Dual inhibitors of EGFR and VEGF receptor, such as vanetubin, also have been generated.\(^{135}\)

Molecular profiling of ADCs has considerable potential to improve the results of anti-EGFR therapies by identifying tumors that are susceptible to this approach. Conducting clinical trials in patient populations enriched with certain molecular features is more likely to yield the desired outcomes. Moreover, tumor sampling before and after treatment may elucidate the mechanisms of action and resistance to novel agents.

The biological effect of EGFR inhibitors also has been assessed in biopsy samples of the skin, an easily accessible surrogate tissue; however, results from skin and tumor biopsies may not be concordant.\(^{136}\) Validation of appropriate pharmacodynamic end points that could guide dosing and response assessment remains of paramount importance for the development of anti-EGFR strategies. Furthermore, because tumor stabilization, not only tumor shrinkage, is relevant to the antitumor efficacy of EGFR inhibitors, study end points in early clinical trials that include time to progression are desirable. Finally, it might be important to consider the most appropriate treatment sequence of EGFR inhibitors and chemotherapy. It has been suggested that better results are likely to be obtained with intermittent dosing of EGFR-targeted agents when combined with chemotherapeutic agents.\(^{137}\) Clinical trials with EGFR-TKIs in advanced NSCLC are ongoing to test these hypotheses.

Therapies against the EGFR are becoming an integral component of the antitumor armamentarium and currently are being evaluated in a variety of clinical settings, including combinations with chemotherapy and radiotherapy for potentially curable disease. At the same time, newer EGFR inhibitors and multitargeted approaches are being explored in ADCs and other malignancies. A deeper understanding of the biology of ADCs should enable further optimization of anti-EGFR and other targeted therapies.

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