Review

Targeting the Epidermal Growth Factor Receptor in Non-Small Cell Lung Cancer

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Abstract

Fifteen % or fewer of patients with non-small cell lung cancer (NSCLC) survive 5 years. The current standard of care for patients with locally advanced or metastatic NSCLC is systemic chemotherapy with a two-drug combination regimen that includes a platinum agent. Although systemic chemotherapy reduces the rate of death attributable to lung cancer, disease progression is inevitable and dose-limiting toxicities restrict their use. New molecularly targeted therapies aim to inhibit specific pathways and key molecules implicated in tumor growth and progression while sparing normal cells. Several therapies, which target signal transduction pathways involved in angiogenesis, metastasis, and apoptosis, are in clinical development to treat lung cancer. Among these targeted therapies are the oral, small-molecule epidermal growth factor receptor-tyrosine kinase (EGFR-TK) inhibitors gefitinib and erlotinib. Both therapies have been validated preclinically as new treatment approaches for NSCLC and have shown single-agent activity against advanced, chemorefractory NSCLC in clinical trials. This article focuses on the biology of the EGFR-TK signal transduction pathway, its role in the proliferation of solid tumors, and the rationale for the clinical development of EGFR-TK inhibitors. We also review clinical trials with EGFR-TK inhibitors in NSCLC and future directions of investigation with these targeted agents.

Introduction

The TK[3] activity of the EGFR has received considerable attention as a target for cancer therapy (1, 2). In recent clinical trials, selective and orally active EGFR-TK inhibitors gefitinib [IRESSA (ZD1839); AstraZeneca] and erlotinib [Tarceva (OSI-774); OSI and Genentech] produced objective tumor responses and symptom improvement in some patients with NSCLC who had previously received chemotherapy (3–5). This was the first class of oral targeted therapies to produce such responses in advanced NSCLC. Although chemotherapy can result in life-threatening toxicities, the EGFR-TK inhibitors have far better safety profiles in patients with advanced NSCLC.

Lung cancer is the leading cause of cancer death in both men and women in the United States and throughout the world (6, 7). The 5-year survival rate for lung cancer patients remains very poor with 15% or less surviving 5 years (6). Nonetheless, this is improved compared with the 5% 5-year survival rate in the United States in the 1960s and the 5% rate still seen in many parts of the world. The major reasons for the poor survival rate for lung cancer are the lack of effective screening and early diagnosis procedures, the propensity for early metastasis, and the inability of systemic therapies to cure patients with widely metastatic disease. This is not to conclude that there have been no advances in lung cancer therapy. Systemic chemotherapy produces a 26–32% reduction in the hazard rate of death for patients with advanced stage III/IV NSCLC that includes adenocarcinomas, squamous carcinomas, and large cell carcinomas (8–10). Chemotherapy also reduces lung cancer-related symptoms and improves quality of life in patients with advanced NSCLC (8–11).

The current standard of care for patients with locally advanced or metastatic NSCLC is systemic chemotherapy with a two-drug combination regimen that includes a platinum agent (8). Such two-drug combinations, developed in the 1990s, were shown to be more effective than the best supportive care or treatment with a single chemotherapy agent. These two-drug combinations were also shown to be as effective as, but less toxic than, combinations of three or more chemotherapy drugs. The efficacy is similar for several of these two-drug combinations. Trials of the SWOG and the ECOG compared five different two-drug combinations in previously untreated patients with advanced NSCLC and found that they had similar efficacy in terms of tumor response rates and overall survival (Table 1; Refs. 12 and 13). Other large randomized trials from the United States and Europe have also shown the equivalence of a number of two-drug combination regimens, and superiority compared with single-agent chemotherapy regardless of whether the single agent is an older agent such as cisplatin or a newer agent such as paclitaxel, docetaxel, or gemcitabine (14–18).

The development of NSCLC disease progression on chem-

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[3] The abbreviations used are: TK, tyrosine kinase; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; SWOG, protein kinase; mAb, monoclonal antibody; IDEAL, IRESSA dose

Table 1 Overview of efficacy results from large comparative trials of first-line chemotherapy regimens for advanced NSCLC[a]

Trial end point	SWOG 9509	ECOG 1594
Response, %	25–28	17–22
Median survival, mo	8.1–8.6	7.4–8.1
Time to tumor progression, mo	4	3.1–4.2
1-year survival, %	36–38	31–36

[a] See Refs. 12, 13.

otherapy is inevitable, because these regimens do not result in cure. Until recently, there were no Food and Drug Administration approved agents for use in the second-line setting. Docetaxel was approved on the basis of randomized trials in patients whose disease had progressed on platinum-based chemotherapy (19, 20). The objective response rates of docetaxel were only 5–10% associated with a modest survival improvement. No agent produced tumor response in more than 5% of patients in the third-line treatment setting.

The cytotoxic mechanism of action of chemotherapy agents imposes inherent limitations on their use. These agents nonspecifically kill normal proliferating cells and, as a result, are frequently associated with dose-limiting toxicities. Many of these effects, such as nausea, vomiting, and hair loss, are troubling to the patient but are not life threatening. Perhaps the most troubling effect is fatigue. Other frequent toxicities may be disabling even if not life threatening. Among these would be the neuropathy associated with paclitaxel and the severe fluid retention or effusion associated with docetaxel. All of the cytotoxic chemotherapy agents produce hematological toxicities that are often life threatening and occasionally fatal. The careful observation of sequential blood counts and the i.v. infusions of chemotherapeutic agents and their supportive agents are expensive and inconvenient for the patient. Such toxicities often result in treatment adjustments such as dose reduction, delayed administration or, in some cases, discontinuation. Furthermore, with increasing rounds of chemotherapy, there is an increased risk that tumors will develop multidrug resistance, thus limiting future therapeutic options.

Toxicities associated with chemotherapy may interfere with the ability of some patients with advanced NSCLC to receive the standard two-drug combination chemotherapy regimens. Such patients include the elderly (≥70 years of age), patients with poor performance status, and patients with comorbidities. Several studies in elderly patients show that less than one-third receive therapy although it may prolong survival (21). In addition, patients with a poor performance status of 2 experienced a high rate of serious adverse events in the ECOG 1594 study of combination chemotherapy regimens (13). The study design was subsequently amended to include only patients with an ECOG performance status of 0 or 1, because patients with poorer performance status are, in general, more likely to experience adverse events.

There is a need for new therapies with novel mechanisms of action that are well tolerated, effective, and convenient. The molecularly targeted agents that are in clinical development aim at inhibiting specific pathways and key molecules in tumor agents that were recently approved by the Food and Drug Administration include trastuzumab, a mAb targeting the HER2/*neu* receptor protein in breast cancer; imatinib [Gleevec (STI571); Novartis], a small molecule receptor TK inhibitor targeting Bcr/abl in chronic myelogenous leukemia and c-Kit in gastrointestinal stromal tumors, (22, 23); and gefitinib, an orally active EGFR-TK inhibitor used as monotherapy in the treatment of patients with advanced or metastatic NSCLC who have failed to respond to platinum-based and docetaxel chemotherapies. Depending on the specific molecule targeted and the mechanism of inhibition, these agents may offer novel clinical benefits compared with outcomes with cytotoxic chemotherapy, or at least the minimum comparable benefits with reduced general toxicity and improved convenience.

A variety of new approaches to treat lung cancer that target signal transduction pathways involved in angiogenesis, metastasis, and apoptosis (24–26) are in clinical development. These agents inhibit a wide variety of tumor-associated molecules including matrix metalloproteinase, farnesyltransferase, and a number of protein kinases. The various therapeutic approaches to inhibiting these molecules include mAbs, small-molecule inhibitors, antisense oligonucleotides, biological response modifiers, and vaccines (24–26). Among these various approaches are small-molecule inhibitors of tumor cell TKs. Gefitinib and imatinib have been validated clinically as new treatment approaches for malignancies (3–5, 23). Furthermore, the EGFR-TK inhibitors gefitinib and erlotinib have shown single-agent activity against advanced, chemorefractory NSCLC in clinical trials described below (3–5, 27).

EGFR-TK: A Molecular Target in NSCLC

EGFR-TK Biology and Signaling in Solid Tumors. The EGFR is a cell surface receptor encoded by the *HER1* (HER type 1) or ErbB1 gene (1). EGFR belongs to a family of receptor TKs that includes HER2/*neu* (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). EGF and TGF-α are the two predominant ligands for EGFR (28, 29). The binding of these ligands to the extracellular domain of EGFR results in dimerization of the receptor monomer with another EGFR molecule or another member of the HER family (Fig. 1). Dimerization produces structural changes in the intracellular portion of the receptor that activate the TK domain. The enzymatic activity of EGFR-TK transfers phosphate moieties from ATP to specific tyrosine residues in the cytoplasmic tail of the EGFR protein. These phosphotyrosine residues then act as docking sites for various downstream effectors (Fig. 2). Some of these effectors are adapter molecules, such as growth factor receptor-bound protein 2 (Grb2) and Src homology collagen protein (Shc), which serve as platforms to assemble the downstream signaling elements necessary for activating cellular proliferation (30). Other molecules are enzymes that are activated on EGFR-TK-dependent phosphorylation, including son of sevenless (SOS), PI3K, and Grb 2-associated binder-1 (Gab-1). Multiple major signal transduction pathways are initiated by EGFR autophosphorylation, including the Ras-MAPK signaling cascade, Src, and the signal transducers and activators of transcription (STAT) pathways,

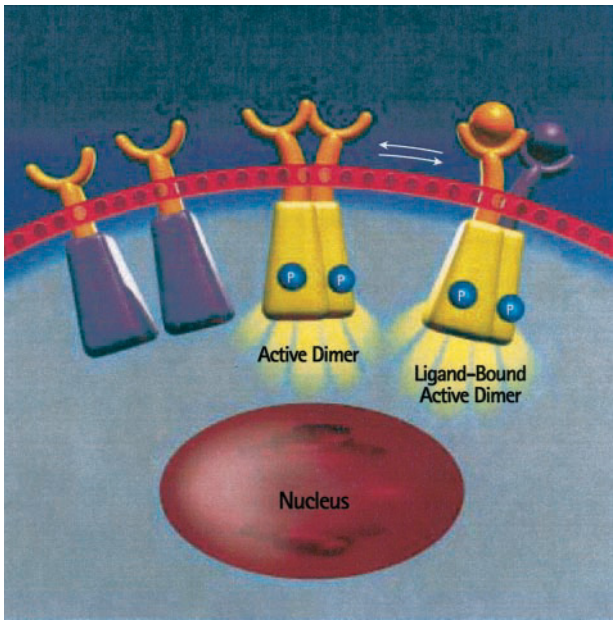

Fig. 1 Homodimers and heterodimers within the human EGFR (HER)/ErbB family, and subsequent phosphorylation.

scription and promote diverse cell responses. The particular dimer combinations that form at the cell surface after ligand binding determine which signaling molecules will be recruited to the surface (29–33).

Ligand binding is the most extensively studied mechanism of EGFR-TK activation, but a variety of other cellular mechanisms are now known to influence EGFR-TK activity in tumor cells. For example, some mutations in the *EGFR* gene result in expression of EGFR proteins with constitutively activated TK activity, the most well-known being the EGFRvIII mutation. Defective inactivation mechanisms (*e.g.,* phosphatases, and receptor endocytosis and degradation) may also result in sustained signaling. Heterologous receptors and signal transduction pathways, including interactions or dimerization with other ErbB receptor types, have been shown to cross-activate EGFR-TK.

Cellular proliferation as a result of EGFR-TK activation may occur via several signal transduction pathways; however, proliferation signals are strongly mediated by the MAPK pathway. After recruitment of adapter molecules on the activated EGFR complex, stepwise activation of Ras, Raf, MAP/Erk kinase (MEK1), and extracellular regulated kinase (Erk) proteins leads to increased activity of transcription factors, such as Elk1 and c-*fos*, key molecules that prime the cell for proliferation and activate cell cycle progression (29). Activated EGFR has been shown to induce the expression of cyclin D, which is crucial in cell cycle progression and is commonly increased in solid tumors.

Activated EGFR-TK also influences the malignant progression of solid tumors. TGF-α and EGF induce angiogenesis by up-regulating the expression of vascular endothelial growth factor (VEGF) in tumor cells. Increased microvessel EGFR-TK (34). In addition, EGFR-TK interacts with components of the integrin pathway involved in cell–cell adhesion, which is crucial for tumor cell invasion of adjacent tissues (29, 35, 36). EGFR-TK also promotes invasiveness through the up-regulation or activation of matrix metalloproteinases and stimulates tumor cell motility that further contributes to metastasis (37–39).

EGFR-TK activation indirectly inhibits apoptosis in tumor cells, promoting tumor cell survival and resistance to cytotoxic therapies. This activity is mediated by PI3K, which activates Akt, an important signaling molecule in antiapoptotic pathways involving the transcription factor nuclear factor κB. Akt also regulates activity of the Ras-MAPK pathway, which is important for cellular proliferation (29). Interaction with signals from heterologous pathways, including those activated by stress inducers, neurotransmitters, hormones, and lymphokines, adds additional complexity to the EGFR-TK signaling network (31). These pathways involve G-protein-coupled receptors, which can transactivate EGFR. Cross-talk between EGFR and other receptors allows for EGFR-TK signals to activate other pathways.

Increased expression of EGFR and its signaling pathways has been associated with a high percentage of tumors in the lung, breast, head and neck, colon, prostate, esophagus, and cervix (1, 2). These elevated levels of EGFR may be the result of transcriptional or posttranscriptional alterations or genomic mutation (34). Differences in the methodologies used and in the criteria for determining EGFR expression levels make it difficult to compare study results (2, 40). Various methods of meas-

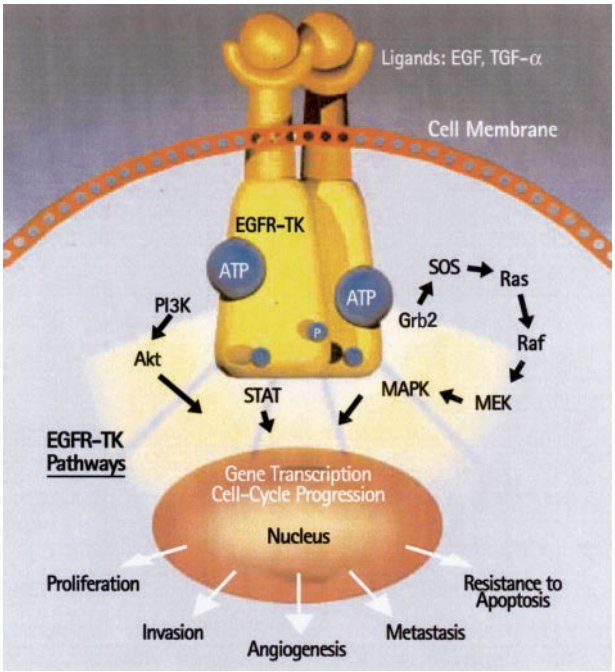

Fig. 2 Key signal transduction pathways of activated EGFR-TK and the various pathways affected. *Grb2,* growth factor receptor-bound protein 2; *SOS,* son of sevenless; *STAT,* signal transducer(s) and activator(s) of transcription; MEK, MAP/Erk (extracellular regulated ki-

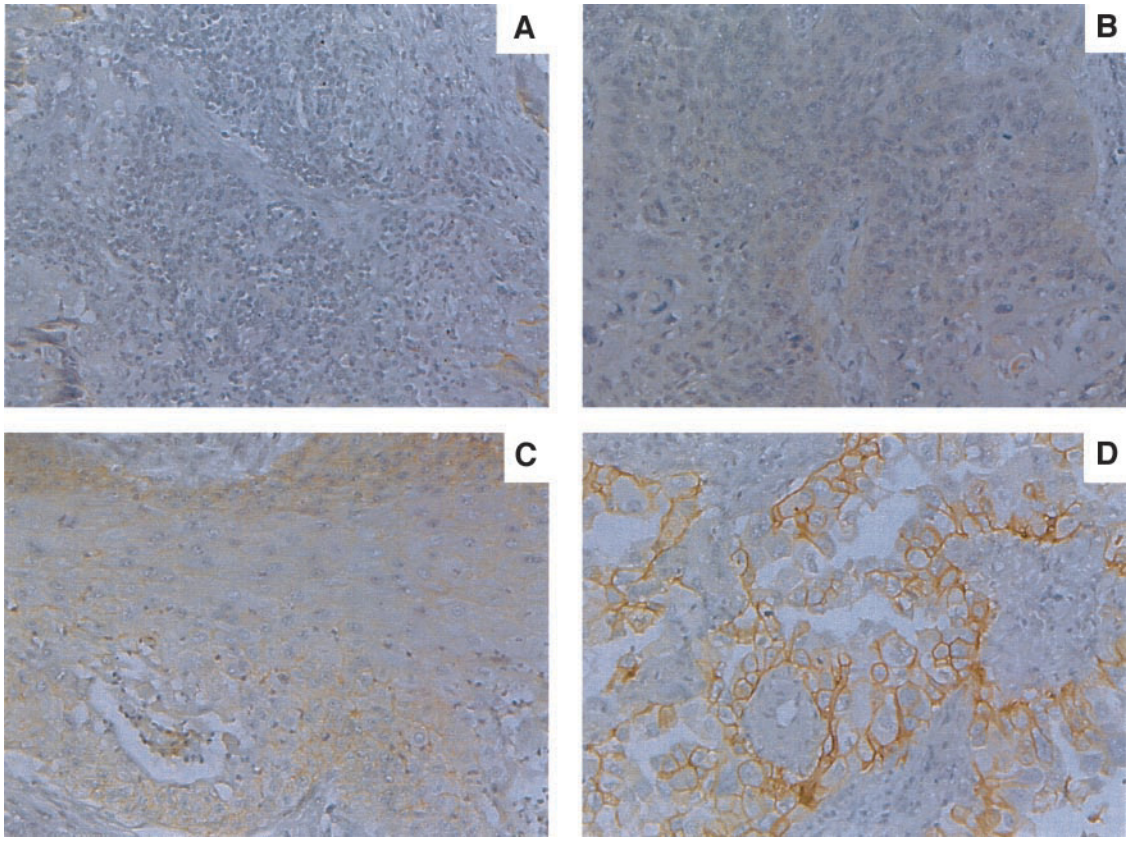

Fig. 3 Expression patterns of EGFR in NSCLC. Scoring on immunohistochemistry scale, staining intensities: 0, none (*A*); 1+, mild (*B*); 2+, moderate (*C*); 3+, strong (*D*). All of the panels are stained with an anti-EGFR antibody.

uring EGFR levels in tumor tissues include immunohistochemistry (Fig. 3), immunoassays, and assessment of RNA levels. Although some studies have shown a correlation between high expression of EGFR and decreased survival times, most studies of NSCLC patients have failed to show that EGFR expression is independently prognostic of survival (2, 34). The prognostic value of EGFR expression is increased when analyzed in conjunction with its dimeric partners, such as HER2/*neu*/ErbB2, or with ligands, such as TGF-α or EGF (41–43). High levels of EGFR in tumors result in an increase in EGFR ligand-binding sites and higher levels of the TK enzyme, as well as an increase in initiation sites for signal transduction inside the tumor cell. These findings indicate that there is an important role for aberrant EGFR signaling in the development and progression of various human tumors. In addition, they provide a strong rationale for EGFR-TK as a target molecule for the development of new cancer therapies.

EGFR-TK Inhibitors. Different approaches to inhibiting EGFR have resulted in a number of EGFR-targeted agents in clinical development including small-molecule EGFR-TK inhibitors, mAbs, vaccines, immunotoxins, and recombinant ligand–toxin fusion proteins (1, 44).

Small-molecule EGFR-TK inhibitors act by blocking the ATP binding site of the EGFR-TK enzyme inside tumor cells inhibitors have the potential to inhibit all mechanisms of EGFR-TK activation, including constitutively activating mutations and receptor cross-talk. EGFR-TK inhibitors were designed to selectively inhibit EGFR-TK relative to other kinase enzymes present in normal tissues (28). Gefitinib erlotinib and CI-1033 (Pfizer) are among the EGFR-TK inhibitors in clinical development (Table 2; Refs. 1 and 45–48). Both gefitinib and erlotinib selectively and reversibly inhibit EGFR-TK, whereas CI-1033 is an irreversible pan-ErbB family inhibitor. The small-molecule EGFR-TK inhibitors also inhibit signals induced by EGFR heterodimerization with other members of the ErbB family. Compared with anti-EGFR mAbs such as cetuximab [Erbitux (C225); ImClone], EGFR-TK inhibitors offer the advantages of oral bioavailability and once-daily treatment.

The targeted agent cetuximab, [Erbitux (C225); ImClone], is a chimeric mAb directed against the extracellular, ligand-binding domain of EGFR that competes with ligand for receptor binding (1, 49, 50). Cetuximab was not studied as a single agent in NSCLC but is currently being evaluated in combination with carboplatin/paclitaxel and cisplatin/gemcitabine in untreated patients with stage IV NSCLC, and with docetaxel in patients with chemotherapy-refractory tumors. ABX-EGF (Abgenix) is another anti-EGFR mAb in Phase I clinical trials. mAbs can also

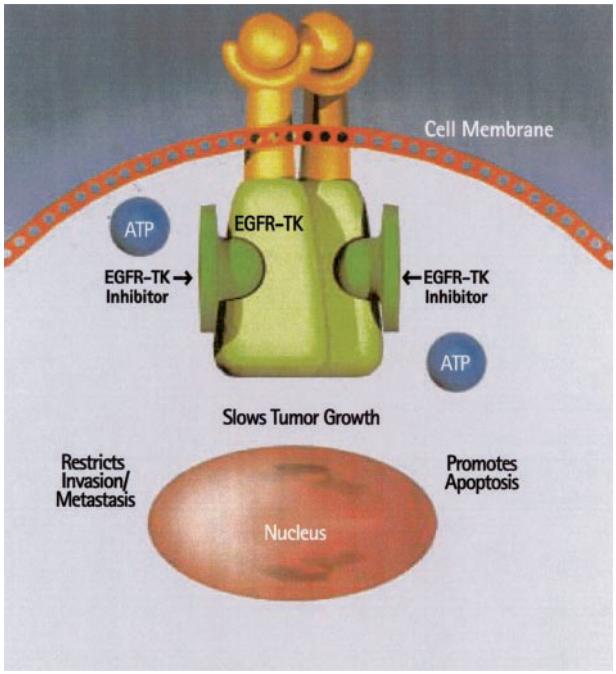

Fig. 4 The mechanism of action of EGFR-TK inhibitors in blocking signal transduction through EGFR-TK.

radioactive particles, which may be used as delivery devices. The binding of the mAbs to the extracellular domain of EGFR triggers endocytosis of the receptor-immunotoxin complex to the cytoplasm, in which the various toxins act to inhibit protein synthesis and induce apoptosis (51). In another approach for targeting toxins to EGFR-expressing tumor cells, chimeric molecules are created by fusing portions of the genes for ligands (EGF, TGF-α) with a toxin gene. One example of such a toxin-fusion protein, DAB389-EGF, is in Phase II clinical trials for NSCLC (24).

Both EGFR-TK inhibitors and anti-EGFR antibodies are effective in preclinical models for inhibiting the growth of a variety of human tumor cell lines, including lung, colorectal, breast, and prostate, suggesting their potential for broad applicability for solid tumor types (1). Preclinical studies also showed that EGFR inhibition results in synergy with chemotherapy agents or radiation therapy in cell lines that are sensitive to EGFR inhibitors (52). For example, in cell viability assays, gefitinib treatment was synergistic with the cytotoxic chemotherapy agents, vinorelbine and paclitaxel, and had additive effects with cisplatin (52). Similarly, gefitinib in combination with radiation has shown growth-inhibitory effects ranging from synergistic to additive in gefitinib-sensitive cell lines (52). Lung tumor xenografts have also been inhibited by gefitinib alone or in combination with chemotherapy agents (53). Gefitinib, erlotinib, and cetuximab have all been shown to potentiate the antitumor effects of most cytotoxic agents, including platinum-based chemotherapy agents in preclinical models with cell lines sensitive to EGFR inhibition. Gefitinib also showed activity against NSCLC xenografts in combination with taxanes, doxo-

Pharmacodynamic studies indicate that EGFR-TK inhibitors and anti-EGFR antibodies block cell cycle progression in the G_1 phase by up-regulating $p27^{Kip1}$, a cell cycle inhibitor, and down-regulating c-*fos*, a transcriptional activator that is prominent in EGFR-mediated signaling (45, 52–55). Elevated levels of $p27^{Kip1}$ block cell cycle progression in the G_1 phase of growth. This sustains the hypophosphorylated state of the retinoblastoma (RB) gene product, which is necessary to keep cells from progressing in the cell cycle (37, 56).

The inhibition of tumor growth seen with EGFR-TK inhibition is also accompanied by decreases in vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and TGF-α, all potent inducers of tumor angiogenesis (57). Thus, inhibitors of EGFR/EGFR-TK may also inhibit tumor growth by interfering with angiogenesis (58, 59). These observations suggest that by inhibiting EGFR-TK, gefitinib and erlotinib treatment alters expression levels of key molecules in tumor cells that are important for stimulating proliferation, cell cycle progression, tumor angiogenesis, metastasis, and inhibition of apoptosis.

Clinical Trials of EGFR-TK Inhibitors in NSCLC

Phase I Trials. Several anti-EGFR agents have been tested alone or in combination with other agents in Phase I trials that included patients with NSCLC. Phase I trials of gefitinib followed two escalating dose schedules: (*a*) once-daily gefitinib given continuously for 28 days; or (*b*) intermittent gefitinib, with 14 days on and 14 days off treatment (60–62). In the intermittent-dosing trials, doses ranged from 50 to 925 mg/day. In the continuous-dosing trials, doses ranged from 150 to 1000 mg/day. Tumor EGFR status

Table 2 EGFR-targeted agents in clinical development[a]

Agent	Mechanism of action *in vitro* IC_{50} HER1 (KB[b,c]; CG[d]) HER2 (KB[c]; CG[d])	Clinical status
ZD1839 gefitinib)	EGFR-TK inhibitor HER1 (23–79 nM; ≤80 nM) HER2 (3.7–10 μM; N/A)	Phase III (Approved)
OSI-774 (erlotinib)	EGFR-TK inhibitor HER1 (2–20 nM; ≤100 nM) HER2 (0.2 μM; <3 μM)	Phase III
CI-1033 (none)	EGFR-TK/HER2 inhibitor HER1 (1.7 nM; 7.4 nM) HER2 (5 nM; N/A)	Phase I
C225 (cetuximab)	Anti-EGFR antibody	Phase II/III
ABX-EGF (none)	Anti-EGFR antibody	Phase I/II
EGF-P64K (none)	Vaccine	Phase II/III
DAB389-EGF (none)	Immunotoxin	Phase II

[a] See Refs. 45–48.
[b] KB, kinase/binding inhibition; CG, cell growth inhibition; N/A, not available.
[c] KB varies based on the source of purified HER1/2.

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