Epidermal Growth Factor Receptor Inhibitors: A Moving Target?

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It has been over 20 years since the first agents targeting the epidermal growth factor receptor (EGFR) pathway were developed by Mendelsohn and Baselga (1). In vitro studies demonstrating that antibodies inhibiting the pathway could retard cell growth, and markedly so in some cell lines, led ultimately to clinical trials testing EGFR inhibition as a therapeutic strategy. As the EGFR pathway was dissected, it was recognized that EGFR was a member of a receptor tyrosine kinase family comprising four members that dimerize or heterodimerize with activation (2). Diversity in dimerization and differences in ligand binding likely result in a spectrum of biological phenotypes. HER2/ErbB2, the second member of the family to be identified, engenders an aggressive subtype of breast cancer but is inhibited by the antibody Herceptin, generating major responses in breast cancer. A decade after the introduction of antibodies inhibiting EGFR, small-molecule inhibitors were identified that impaired EGFR tyrosine kinase activity (3). Several inhibitors have now been brought to clinical trials, leading to the approval and marketing of two of them—gefitinib and erlotinib—in lung cancer. Much has been written recently about these agents and it will not be the task of this editorial to provide a detailed review.

Previous studies, including one by the authors of this editorial, have noted that the level of expression of EGFR cannot predict the sensitivity of cells to inhibitors (4). Bishop et al.’s examination of the National Cancer Institute Drug Screen database revealed that the 60 cell lines could be grouped based on sensitivity to EGFR inhibition. Consistent with the notion that a threshold level of EGFR is required for sensitivity to agents targeting the EGFR pathway, cells with low levels of EGFR expression were found to be insensitive to quinazoline inhibitors. Moreover, cells with high levels of EGFR could be divided into two groups composed of cells sensitive or insensitive to EGFR inhibition. Yet, inhibition of EGFR and mitogen-activated protein kinase phosphorylations could be shown in both groups following exposure to AG1478, an antecedent to gefitinib and erlotinib. These and the observations of others indicated that there were differences in the EGFR pathway among cell lines and an independence from mitogen-mediated signaling was inferred as an explanation for insensitivity to EGFR inhibition. Clinical trials have confirmed these in vitro findings, demonstrating that inhibition of both EGFR and mitogen-activated protein kinase phosphorylations in tumors can occur in the absence of disease response (5–8).

Just a few years ago, it was widely expected that EGFR inhibitors would be effective in a wide range of solid tumors with high levels of EGFR including breast, ovarian, lung, renal, head and neck, pancreatic, and colorectal malignancies (9). Thus, it was disappointing to find that response rates in phase II trials in these diseases were low, as in lung cancer, or nonexistent, as in renal, colon, ovarian, or breast cancer (5, 10–15). Why were our expectations so underserved? Our original paradigm—that EGFR expression could be equated with dependence on the pathway and could predict eventual drug sensitivity—was incorrect.

Gefitinib first, and then erlotinib, was licensed for lung cancer based on results in patients with locally advanced or metastatic non–small cell lung cancer after failure of front-line chemotherapy. For erlotinib, approval was based on a study of 731 patients in which the response rate was 8.9% with a survival advantage of only 2 months (16). Despite the initial approval of gefitinib based on a 9% to 19% response rate in non–small cell lung cancer, a survival advantage could not be shown in later randomized trials and the Food and Drug Administration has since limited the scope of its approval (17). Neither agent showed a survival benefit in combination with chemotherapy in randomized trials. The low response rates underscore the critical question of how to predict which subset of patients will benefit from the targeted therapy.

How then can differential responsiveness be explained? Herceptin offered the first paradigm for successful inhibition of an EGFR family member: successful inhibition required significant overexpression. Immunohistochemical analysis of ErbB2 expression showed that a 2+ or 3+ level of expression, predominantly due to amplification of the encoding gene, was required for a meaningful response (18). This observation led to the development of diagnostic tests for HER2 expression and amplification and to careful selection of patients for therapy (19).

The applicability of the HER2 paradigm to EGFR is uncertain. Until recently, EGFR gene amplification had been principally described in glioma as a cause of overexpression. About 50% of human glioblastomas display amplification of EGFR and about 40% of these have amplified EGFRvIII, a variant receptor with ligand-independent kinase activity due to deletion of the extracellular ligand-binding domain. The EGFRvIII receptor has been shown to be resistant to gefitinib and erlotinib (20, 21). In a recently reported phase I trial of erlotinib in glioma, eight responses were noted in 41 patients (22). Ten of 39 tumors were noted to have EGFR amplification; four responses were noted in the group with amplification and four in the group without amplification. Two of the amplified, nonresponder tumors expressed mutant EGFRvIII. Evaluating phospho-Akt as a downstream marker, no tumor positive for phospho-Akt responded to erlotinib. Thus, gene amplification alone is not sufficient to provoke a response to EGFR inhibitors.
Before the clinical trials with gefitinib, overexpression of EGFR was thought to be the principal mechanism of pathway activation in non–small cell lung cancer and solid tumors except for gliomas. This fueled speculation that response to inhibitors would correlate with expression. However, mutations in the EGFR tyrosine kinase domain were then discovered among a subset of lung cancers in patients with tumor responses following gefitinib or erlotinib (23–26). These mutations result in a higher level of receptor phosphorylation and activation. In the initial reports, a high concordance was found between the presence of a mutant EGFR and response to therapy, with mutations reported in 25 of 31 (81%) patients with an objective tumor response (23). By comparison, no mutations were detected in 29 patients whose tumors did not respond to therapy. This observation created an evolving paradigm that EGFR inhibitors might be effective only in cells dependent on EGFR signaling, such as would occur in cells with an activating EGFR mutation. Three subsequent studies have confirmed and extended these findings (26–28). Further, in the studies by Taron et al. from the Spanish Lung Cancer Group and by Mitsudomi et al., in which a high response rate was documented in patients with tumors harboring mutations, a convincing increase in median survival following gefitinib was also reported (26, 28). Whereas the presence of EGFR mutations in non–small cell lung cancer is well documented, similar EGFR mutations have not been systematically observed in other tumor types, potentially limiting this paradigm to non–small cell lung cancer (29).

The discovery of these mutations led to a paradigm shift for lung cancer that did not resolve the question of whether uncomplicated high-level or even low-level expression of EGFR can confer sensitivity to EGFR inhibitors. Two studies that conflict with the mutation paradigm suggest that in non–small cell lung cancer, survival following a tyrosine kinase inhibitor is enhanced in patients whose tumors contain a high copy number of EGFR (27, 30). What mechanisms might regulate EGFR expression and be important in other tumors? One example is an intron 1 polymorphism in the EGFR gene—the number of CA single sequence repeats (31, 32). This polymorphism has been correlated with EGFR expression levels and has been suggested as a mediator of responsiveness to EGFR inhibition. Without gene amplification, head and neck cancer cells with a lower number of CA dinucleotides had higher levels of EGFR and enhanced responsiveness to erlotinib (33). Although these results suggest that EGFR expression alone could create an EGFR-dependent environment that would be susceptible to inhibition, the failure of EGFR inhibitors in colon and renal cell carcinoma argues against such a simple explanation. These results do, however, suggest that mutation or gene amplification is not required to activate the EGFR pathway.

Two trials examining the activity of gefitinib in breast cancer showed disappointing results without evidence of objective responses (8, 15). In one of the studies, the effect of gefitinib on the EGFR pathway was examined in both skin and tumor biopsies (8). Inhibition of both EGFR and mitogen-activated protein kinase phosphorylations was observed in normal and malignant cells. However, Ki67 staining, a marker of proliferation, was reduced in skin but not in tumors. Similarly, a clinical trial evaluating erlotinib in breast cancer found only 1 of the 15 tumors examined to be EGFR positive; in this tumor, both EGFR and mitogen-activated protein kinase phosphorylations were reduced after erlotinib therapy. Skin samples from all 15 patients showed inhibition of phospho–mitogen-activated protein kinase and Ki67 (5). None of the tumors responded to erlotinib. Both studies concluded that the absence of tumor responses was due to a lack of EGFR dependence in the tumors rather than a failure of receptor inhibition.

Writing in the current issue of *Clinical Cancer Research*, Van Schaeybroeck and colleagues offer a surrogate marker for successful inhibition of the EGFR pathway in colon cancer cell lines. The identification of a surrogate marker for sensitivity provides insight into the occasional responses—those tumors capable of activating the pathway are those likely to be sensitive to its inhibition. Van Schaeybroeck and colleagues show that the sensitivity of colon cancer cells to the EGFR inhibitor gefitinib can be discriminated by the higher basal level of EGFR autophosphorylation and by the induction of phosphorylation that results from exposure to the chemotherapeutic agent oxaliplatin. These results, if confirmed clinically, provide both a potential surrogate for successful therapy with an EGFR inhibitor and a potential insight into another mechanism whereby the EGFR pathway plays a critical role in oncology. Beyond mutation or constitutive activation, there might be tumors in which cellular stress such as DNA damage results in activation and phosphorylation of the EGFR pathway. This activation of the pathway could result in proliferation or enhanced survival so that disruption of the pathway by an EGFR inhibitor could conceivably enhance drug sensitivity.

Thus, we can now hypothesize rules for successful EGFR inhibitor therapy. The gene can be amplified, mutated, or overexpressed, but it must be critical to the oncogenic phenotype. The overriding paradigm seems to be that the pathway must be involved in maintenance of the malignant phenotype or in cell survival to achieve a successful antineoplastic effect. Considering the alacrity with which paradigms have been amended in recent years, this one will undoubtedly be amended as well and eventually we will get it right. As a scientific community, we must deliver on the promise of targeted therapy. Inherent in the often-used (and abused) term “targeted therapy” is the implication that those who will benefit from such therapy can be identified prospectively. We cannot develop targeted therapy and then fail to meet the challenge of finding the subset of patients who are likely to benefit. A 2-month survival advantage in a population derived from a therapy administered to every patient with lung cancer cannot be considered to be molecularly targeted therapy. Really, how different is the latter from vincristine, a therapy that *specifically targets tubulin* and has been available to us for nearly 50 years? Let us agree that by definition, a molecularly targeted therapy will be individualized therapy. Developing a clinically relevant assay to detect mutations will be a first step. Developing a surrogate assay that will detect tumors with activation of the pathway will be a second step. Then we will see the day when agents such as erlotinib and gefitinib will be prescribed only for patients who will benefit and the 85% who will not benefit will be spared the cost, toxicity, and disappointment of an ineffective therapy.
References

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