Minireview

Clinical Trial Design and End Points for Epidermal Growth Factor Receptor-targeted Therapies: Implications for Drug Development and Practice

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Introduction

There is an increased perception that current chemotherapeutic drugs have almost reached a ceiling as systemic anticancer therapies. In great part because of their unpredictable side effects, the development of these cytotoxic drugs followed certain patterns and principles that may not necessarily apply to modern molecule-targeted antineoplastic strategies. In the following commentary, some of these differences will be highlighted. Using the EGFR1 network as a model of a therapeutic target in cancer, key aspects that apply to the clinical development of EGFR-directed drugs will be discussed. These issues may well apply to many other molecule-targeted strategies in preclinical and clinical development.

Different Targeted Therapies Can Inhibit the Same Molecular Target

The EGFR provides an example in which understanding first the functional role of different receptor domains in its pathophysiology led to several potentially complementary rational approaches designed to eliminate EGFR function. One anti-receptor strategy has been the development of humanized mAbs against the receptor’s EC domain (1). These compete for the binding of receptor ligands and can induce EGFR dimerization and down-regulation from the tumor cell surface (2, 3). In addition, it has been proposed that some antibodies can recruit Fc-receptor-expressing immune effector cells that, in turn, lead to antibody-dependent cell-mediated cytotoxicity (ADCC; Refs. 4–7).

A second class of EGFR inhibitors are small molecules that compete with ATP for binding to the ATP site in the EGFR tyrosine kinase domain and, therefore, like EGFR antibodies, abrogate the receptor’s catalytic activity, receptor autophosphorylation, and its engagement with signal transducers (8, 9). Some of these small molecules can induce inactive EGFR homodimers and EGFR/HER2 heterodimers (10, 11) and, thus, impair EGFR-mediated transactivation of the potent HER2 tyrosine kinase, an EGFR-homologous coreceptor that enhances the positive effects of the EGFR on tumor progression (12). In principle, these small molecules should be able to block ligand-independent EGFR kinase activity as well as the catalytic activity of EGFR mutants lacking EC epitopes that are required for the binding of EGFR antibodies. Because of the >80% homology in the kinase domain between EGFR and HER2 (13), some ATP-competitive small molecules can block the catalytic activity of both receptors (reviewed in Ref. 14), a property not shared by antibodies targeted to the less conserved EC domain of the EGFR family of receptors.

Despite these mechanistic differences, both EGFR antibodies and low-molecular-weight ATP-competitive inhibitors of the EGFR kinase induce similar toxicity in patients treated with these drugs (15–18), suggesting that they are equally capable of disabling the EGFR function. Single-agent clinical activity of small-molecule EGFR kinase inhibitors (see heading Clinical End Points with Anti-EGFR Therapies) and EGFR antibodies (19, 20) has been reported recently. The preclinical data with these agents indicate that EGFR function can be blocked by receptor antibodies and ATP-competitive kinase inhibitors via molecular mechanisms that do not completely overlap, suggesting the possibility of therapeutic synergy when used in combination. Two reports using tumor cells in culture have already shown synergistic activity of the EGFR antibody C225 in combination with the small molecule PD 153035 (21, 22). Finally, the existence of nonoverlapping mechanisms of action would also suggest similar, but not identical, single-agent clinical activity of antibodies and small molecules.

Patient Selection and Timing of EGFR-targeted Therapies

Ideally, a molecular therapeutic target is one causally involved in tumor progression that can be identified in tumor diagnostic tissue. Several human cancers including cancers of the upper aerodigestive tract (non-small-cell lung, head and neck, esophagus, and gastric), colon, pancreas, breast, ovary, bladder, and kidney and gliomas display EGFR RNA and/or protein overexpression. This occurs with or without gene amplification of the EGFR locus and is often associated with overexpression of receptor ligands such as TGFα or amphiregulin (23–26). Studies evaluating EGFR expression in human

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3 The abbreviations used are: EGF, epidermal growth factor; EGFR, EGF receptor; EC, extracellular; TGF, transforming growth factor; mAb, monoclonal antibody; NSCLC, non-small cell lung cancer; QOL, quality of life; FACT-L, Functional Assessment of Cancer Therapy-Lung (scale); OBD, optimal biological dose; ER, estrogen receptor; IHC, immunohistochemistry; VEGF, vascular endothelial growth factor.
tumor tissues have used a plethora of methods and, in general, have defined overexpression rather loosely without an adequate quantitation of receptor levels. A clinical trial with Indium-labeled EGFR mouse mAb 225 revealed selective localization of the EGFR antibody in 11 of 11 squamous cancers of the lung that had not been prescreened for EGFR levels (27). The results from this study suggest that (a) differential expression of EGFR in tumor versus nontumor host tissues can provide an exploitable therapeutic window in cancers with high frequency of EGFR expression; and (b) the determination of tumor EGFR levels a priori may not always be necessary to predict targeting of the tumor. More recently, studies with the humanized EGFR antibody C225 and the EGFR tyrosine kinase inhibitor ZD1839 (“Iressa”) have demonstrated responses in human tumors and cell lines with EGFR levels ranging from very low to very high (28–32). One implication from these data are that EGFR content does not reflect the level of receptor utilization. This is illustrated by the EGFR-positive colon cancers shown in Fig. 1. Only the tumor coexpressing the EGFR ligand TGFα exhibits EGFR phosphorylation and evidence of high proliferation. It is also possible that low-EGFR-expressing but still inhibitor-sensitive cells may not score as positive with available immunohistochemical method. These data support the critical need for assays that will determine either a threshold level of total and/or activated (phosphorylated) tumor EGFR or other molecular marker(s) predictive of benefit from an EGFR-targeted therapy. Until such predictive assay(s) is/are available, the exclusion of patients with EGFR-“negative” cancers, as defined by current laboratory methods, from enrollment into clinical trials of EGFR inhibitors is difficult to justify.

The EGFR has been reported to be overexpressed in hyperplastic and preneoplastic epithelial lesions (33, 34). Similarly, the EGFR homologous HER2 receptor has at least the same rate of gene amplification in ductal carcinoma in situ (DCIS) of the breast as in metastatic breast carcinomas (35), which suggests that, like for EGFR, HER2 overexpression occurs much earlier than the time of onset of invasive cancer and metastatic disease. The role of inhibitors of these receptor tyrosine kinases in preventing the progression of preneoplastic and/or preinvasive lesions is not known. Preclinical studies in transgenic mice overexpressing the EGFR ligand TGFα and neu (rat/mouse homologue of HER2) in the mammary gland indicate that EGFR kinase inhibitors are more effective in preventing mammary hyperplasias than in inhibiting established carcinomas (36). Pharmacological inhibition of the EGFR with EKI-785 in APCMin mice markedly reduces intestinal polyp number but not polyp size (37), implying that EGFR function is required for the initiation of intestinal tumors but that it might be dispensable for polyp expansion and progression. Nonetheless, the overall tolerability of EGFR and HER2 inhibitors provides an opportunity for testing them against preneoplastic lesions and/or in subjects at high risk, an approach not defensible with cytotoxic chemotherapy. Interestingly, treatment with ZD1839 results in a significant inhibition of proliferation and an increase in the apoptotic index in human xenografts consisting of ductal carcinoma in situ and adjacent normal breast epithelium established in athymic nude mice (38).

Clinical End Points with Anti-EGFR Therapies

A critical aspect in the development of anti-EGFR therapies (and other targeted agents) is the choice of appropriate clinical end points that will allow investigators to establish whether the activity of anti-EGFR therapies warrants their introduction into clinical practice. Taking into account that these agents are considerably less toxic than conventional anticancer agents and are perceived as predominantly cytostatic, investigators have proposed softer end points, such as time to disease progression or clinical benefit, including stabilization of disease as clinical endpoints. The implication is that objective tumor

![Fig. 1](image-url) EGFR levels do not correlate with evidence of EGFR activation. Colon carcinomas expressing EGFR by IHC. Tumor in B but not in A coexpresses the EGFR ligand TGFα. Phospho-EGFR (pEGFR) and tumor cell proliferation as determined by Ki67 IHC are both high in tumor B but low in tumor A.
response would not be any longer the “gold standard” end point. The frequent and prolonged disease stabilization induced by antiestrogens in patients with breast cancer provides a powerful argument in support for this line of thought. However, there is considerable evidence to suggest that an approach based exclusively on nonclassical end points has not been validated. First, the concept that these agents in preclinical models are cytostatic may not be accurate: Even though anti-EGFR compounds are mainly cytostatic against tumor cells in culture, they can exhibit a marked apoptotic effect (32, 39) as well as eradicate human xenografts from nude mice under conditions of high tumor burden when chemotherapy is not active (40). Second, all of the approved (targeted) agents for the treatment of cancer have some type of single-agent activity as defined by their capacity to induce either partial and/or complete objective tumor response. This is true for trastuzumab, a humanized IgG1 directed against HER2, which showed single-agent activity in previously treated and untreated patients with metastatic breast cancer (41–43). Single-agent activity in advanced NSCLC has been reported with the small-molecule tyrosine kinase inhibitors ZD1839 and OSI-774 (44–46). Similarly, the EGFR antibodies ABX-EGF and C225 have shown clinical activity in renal cell cancer and colorectal cancer, respectively (19, 20).

Although classical responses may provide evidence of the clinical activity of targeted agents and, as a consequence, be used as a signal to proceed with their clinical development, it is unclear whether objective tumor response per se is an indication of true clinical benefit. In the search for more clinically meaningful and stringent end points, an improvement in survival is increasingly becoming the standard by which these agents are being evaluated as first-line therapies. This has been exemplified by the studies with trastuzumab in patients with HER2-overexpressing breast cancers. These studies documented a 25% improvement in survival in patients that received concurrent trastuzumab plus chemotherapy versus chemotherapy alone (47). Placebo-controlled large Phase III trials of chemotherapy ± the EGFR tyrosine kinase inhibitor ZD1839 in patients with advanced NSCLC were recently completed. In these trials, the combination failed to improve overall and progression-free survival over chemotherapy alone (48, 49). Similarly, the EGFR antibody C225 failed to improve the efficacy of cisplatin-based chemotherapy over chemotherapy alone in patients with metastatic head and neck squamous cancers of the head and neck (50). It is possible, however, that in patients selected by an appropriate predictive assay (to be developed) of response to EGFR inhibitors, these trials might have had a positive outcome in favor of the combination. The development of such predictive assay(s) is currently the focus of very active research.

Additional secondary end points include disease stabilization, progression-free survival, and improvement in disease-related symptoms. Disease-related symptoms may become an important end point in tumor types such as NSCLC that are frequently associated with an impaired QOL. For example, in a large Phase II randomized trial with the EGFR kinase inhibitor ZD1839 in patients with NSCLC, the FACT-L was completed monthly by patients to assess QOL (44). QOL improvement rate as measured by FACT-L was 23.9 and 21.9% in patients treated with ZD1839 at 250 and 500 mg/day, respectively. Overall, there was a high degree of concordance between objective tumor response and symptom improvement. Among patients who responded to therapy, 52% showed improved QOL by FACT-L, with an impressive median time-to-symptom improvement of less than 8 days. These results require confirmation in placebo-controlled Phase III trials comparing single-agent EGFR inhibitors with best supportive care in patients with advanced NSCLC.

Clinical Trial Design and Target Validation

Several issues need to be taken into account when designing trials with anti-EGFR therapies: (a) these agents are less toxic and better tolerated than conventional chemotherapy; (b) their OBD may not match their maximally tolerated dose and, in many cases, it has not been established; (c) the tumor types that will derive the most benefit from these agents are unknown; (d) the EGFR expression level and/or other molecular determinants predictive of a therapeutic benefit are unknown as well; and, finally, (e) preclinical models suggest that they are supra-additive when added to conventional chemotherapy or hormonal agents and, in some cases, may reverse acquired resistance to these drugs.

The favorable safety profile of anti-EGFR agents has allowed for an accelerated clinical development strategy (Table 1). Anti-EGFR agents have been tested initially in healthy volunteers, thus shortening early phases of clinical development. In the case of ZD1839, initial studies in healthy volunteers provided information on important pharmacokinetic parameters such as bioavailability, peak plasma concentrations, and terminal half-life, in addition to preliminary safety data (51).

A central issue in clinical trials with anti-EGFR is to define the OBD and optimal schedule at which complete and sustained receptor saturation and/or inhibition are achieved. This premise is based on preclinical studies that suggested that complete receptor occupancy was required for maximal inhibition of function (52, 53). This approach is radically different from dose-finding approaches for conventional nontargeted chemotherapeutic agents, for which dose selection has been based on determining dose-limiting toxicities. The OBD may be chosen by extrapolating from preclinical models (i.e., establishing a parallelism between doses resulting in steady-state concentrations in plasma that are equivalent to those required to inhibit tumor cell growth ex vivo). This approach has been proposed with both anti-HER2 (41) and anti-EGFR therapies (15). However, possible differences between tumor and nontumor tissues in EGFR function and turnover, EGFR and/or receptor ligand levels, intracellular ATP concentrations, drug-protein binding in situ, and so forth, could lead to choosing a suboptimal dose and/or schedule. This has been exemplified in studies with the humanized antibody C225. In the initial Phase I studies, a difference in dose was found between the OBD projected from preclinical mouse models and the higher C225 dose required to achieve saturation of drug clearance in humans, a finding probably related to the fact that the mAb binds to human but not to mouse EGFR (15).

Additional factors include a wide variation in interpatient pharmacokinetic parameters that has been observed with both mAbs and small-molecule tyrosine kinase inhibitors (18). Therefore, the best way to identify the right dose for these
compounds might be by analyzing appropriate pharmacodynamic end points. A recent report suggests that this approach might have been fruitful. Pharmacodynamic studies with ZD1839 suggested that doses of $\pm$150 mg/day resulted in maximal inhibition of EGFR kinase activity in normal tissues (54). In part because of these data, two well-tolerated doses (250 and 500 mg/day) were chosen for Phase II and Phase III studies. Interestingly, the response rate in advanced NSCLC was identical at both dose levels of ZD1839 (44), suggesting the possibility that, as predicted by the pharmacodynamic studies, both doses might have been equivalent in binding to the ATP site of the EGFR and inhibiting the EGFR tyrosine kinase.

The patients within different cancer types that, based on tumor EGFR levels or other molecular profiles, are best suited for enrollment into trials with EGFR inhibitors are unknown. One possible exception is breast cancer in which a strong correlation between ER-negative and EGFR-positive tumors has been reported (55, 56). One potential exploratory trial design to address this issue of patient selection consists of the administration of EGFR inhibitors to treatment-naive, newly diagnosed patients with operable cancers for 10–14 days from the time of definitive surgery. A flow diagram illustrating such trial design in a breast cancer model is shown in Fig. 2. This approach has been used effectively with antiestrogens. These studies have shown that as little as 14 days of therapy with tamoxifen results in marked reduction of breast cancer proliferation as measured by Ki67 IHC in tumor sections. Treatment-induced inhibition of proliferation was limited to ER-positive tumors (57–59), suggesting the possibility that this approach could potentially identify a molecular signature or marker of response to therapy. Preclinical studies in transgenic mice suggested that 5 days of treatment with an EGFR tyrosine kinase inhibitor are adequate to inhibit the proliferation of EGFR-dependent mammary tumors (36). Overall, these studies suggest that the detection of a signal of a cellular response in situ to a molecule-targeted therapy may not require prolonged drug administration for effective blockade of its target. Therefore, by measuring total and activated EGFR levels in pre- and posttherapy sections and correlating these with evidence of an antiproliferative or an apoptotic effect, it would be possible to determine a threshold level of receptors that predicts for an antitumor response. Because all patients will be subjected to an operation as part of their standard of care, in situ cellular “response” data should be available in as many as 100% of subjects enrolled. Data from this type of exploratory trial can generate a tumor profile that can be used for the identification of patients and their enrollment into future trials with EGFR inhibitors, similar to the selection of ER-positive or HER2-amplified patients into trials of antiestrogens and trastuzumab, respectively.

Additional information could be derived from trials with this design. For example, a consistent loss or reduction in the levels of activated EGFR by IHC without indication of an antitumor effect would suggest a lack of efficacy of EGFR inhibitors as single agents in the cancer type focused by the trial. In addition, evidence of an antitumor effect without reduction or loss of activated receptors by IHC would suggest a lack of efficacy of EGFR inhibitors as single agents. Preclinical and clinical studies have shown that tumor sections subjected to an operation as part of their standard of care, in situ cellular “response” data should be available in as many as 100% of subjects enrolled. Data from this type of exploratory trial can generate a tumor profile that can be used for the identification of patients and their enrollment into future trials with EGFR inhibitors, similar to the selection of ER-positive or HER2-amplified patients into trials of antiestrogens and trastuzumab, respectively.

One area of great interest, once dose and safety issues are resolved, is how to translate to human trials the synergy of these agents with chemotherapy or radiation observed in preclinical
cancer models. The Phase III study of trastuzumab given concurrently with chemotherapy versus chemotherapy alone had overall survival as the primary end point and may be envisioned as the model type of study to perform (47). Unfortunately, this type of study design is not very different from the ones used with conventional agents and requires the inclusion of a large number of patients. Finally, an innovative study design has been based on the possible resensitization of resistant tumors to chemotherapy on therapeutic inhibition of the EGFR. In the case of C225, an initial observation that the EGFR antibody could revert resistance to chemotherapy (60, 61) led to studies in CPT-11-resistant and cisplatin-resistant patients with colorectal and head and neck cancers, respectively. In both of these studies, using patients as their own controls, the addition of C225 resensitized tumors to the previously ineffective chemotherapy regimen (30, 62). In fairness, however, an effect of the antibody alone was not appropriately excluded by these studies.

**Pharmacodynamics**

As mentioned above, the definition of the OBD of a targeted therapy should be based on pharmacokinetic end points or, preferably, by demonstrating the desired biochemical effect on the target molecule. An example of selection of an OBD based on pharmacokinetic end points is the selection of the dose and schedule for the EGFR antibody C225 (15). Recent studies suggest that the administration of the selected dose of C225 results in EGFR inhibition in patients’ skin (63). Pharmacodynamic studies have been incorporated earlier in the clinical development of low-molecular-weight EGFR tyrosine kinase inhibitors. In the initial Phase I studies of ZD1839, skin biopsies were performed sequentially prior to and after 4 weeks of therapy (54). The skin was selected as the target tissue because of its easy access and the established role of the EGFR in renewal of the dermis (64, 65). In normal adult human skin, the EGFR is strongly expressed in keratinocytes and in cells of eccrine and sebaceous glands. ZD1839-induced changes in the phosphorylation of EGFR, mitogen-activated protein kinase (MAPK), and STAT-3, as well as in the levels of the Cdk inhibitor 27Kip1, the proliferation marker Ki67, and skin maturation markers. All of these effects were seen at doses of ≥150 mg/day, well below the maximal tolerated dose of 700 mg/day. Similar data have been obtained in skin biopsies of patients participating in clinical trials with other ATP-competitive inhibitors of the EGFR kinase such as OSI-774 (66), PKI-166 (67), and CI-1033 (68). These findings support the use of doses below the maximal tolerated dose for subsequent clinical studies with these compounds. On the basis of these data, a Phase II study in NSCLC randomized patients to receive ZD1839 at either 250 or 500 mg/day. The overall objective response rate was 18.7%, and there was no difference between the two dose levels for any of the efficacy endpoints (44). On the other hand, fewer patients receiving the 250 mg/day experienced severe (grade 3 or 4) adverse reactions, required interruption of therapy, or withdrew from study than those receiving 500 mg/day ZD1839. Whether pharmacodynamic changes in the skin correlate with the inhibition of the EGFR at tumor sites and whether qualitative changes in these biochemical markers at either site predict for tumor sensitivity will require further investigation.

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![Fig. 2](image-url)

**Fig. 2** Exploratory trial design for molecular target validation and identification of predictive markers of response to EGFR inhibitors. Patients with operable breast cancer will be treated with the OBD of an EGFR inhibitor for a period of ≤2 weeks. Ki67, terminal deoxynucleotidyltransferase-mediated nick end labeling (TUNEL), EGFR, phospho-EGFR (P-EGFR), and ER will be determined in both a diagnostic core biopsy and surgical specimen to assess (a) whether there is a reduction of tumor cell proliferation and/or increase in apoptosis; and (b) whether drug-induced inhibition of EGFR signaling occurs. Evidence of antitumor action in situ (as defined by the inhibition of Ki67 or increase in TUNEL) versus none, can be used initially to determine a level of total, and/or a change in, P-EGFRs that predict for an antitumor effect. Loss of P-EGFR without in situ evidence of response would strongly suggest individual patient resistance. By comparing end points in pre- and posttherapy specimens in this treatment-naive, in vivo model, a predictive assay for response to EGFR inhibitors can be developed. This same assay can then be used to select patients for entry into larger randomized trials that will test the EGFR inhibitor versus placebo, after the standard of care (Std. of care) has been completed (bottom row).
Therapeutic Targeting of the EGFR Network in Human Cancer

Integration with Conventional Anticancer Therapies

Aberant EGFR signaling up-regulates several survival pathways (12, 13) that can protect tumor cells from the cytotoxic effects of conventional cancer therapies. Therefore, EGFR inhibitors have been combined with ionizing radiation and most standard anticancer drugs and tested against human tumor xenografts. Overall, these studies show a supra-additive antitumor effect of the combinations over either chemotherapy or radiation alone with no increased host toxicity (29, 61, 69). These observations plus the absence of myelosuppression in Phase I studies of EGFR inhibitors led to the design of a number of pilot trials of chemotherapy in combination with anti-EGFR therapies. Preliminary data from these trials suggest that some of these combinations are safe and do not limit chemotherapy action. In patients with advanced solid tumors, a combination of ZD1839 (250 or 500 mg/day) and full-dose gemcitabine and cisplatin, given on a 21-day cycle, results in partial responses in patients with NSCLC without any dose-limiting toxicities (48). There are no significant pharmacokinetic interactions, and the toxicity profile was predictable. Similarly, the combination of ZD1839 (250 or 500 mg/day) with full doses of carboplatin and paclitaxel is well tolerated with no alterations in the pharmacokinetic profile of either chemotherapeutic agent (49). Albeit limited, the available information suggests, thus far, that there is no need for the cumbersome dose escalation schemes frequently used with chemotherapy-containing combinations.

Several preclinical and fewer clinical studies do not suggest that inhibition of the EGFR sensitizes tumors to a type of chemotherapy preferentially over others. It is possible, however, that as EGFR inhibitors are randomly combined with standard drugs, unanticipated toxicity might be encountered with some combinations. One relevant example with a therapy targeted against HER2 is the enhanced cardiotoxicity observed with the combination of trastuzumab and doxorubicin (47). Hence, considering the role of the EGFR in renewal of the intestinal mucosa and pending elucidation of the pathogenesis of the gastrointestinal toxicity seen with some EGFR inhibitors, combinations of these drugs with cytotoxic agents associated with similar gastrointestinal toxicity should be viewed with caution. One alternative that obviates the concomitant use of EGFR inhibitors with chemo/radiotherapy would be administering them as single agents after primary therapy is completed at a time of lower tumor burden. Such strategy in a placebo-controlled design in patients with a high likelihood of early recurrence (i.e., stage IIIB NSCLC and T4 head and neck cancers post-chemo/radiotherapy, lymph node-positive colon and bladder cancer post-adjuvant chemotherapy, and so forth; Fig. 3), using time-to-first-recurrence as an end point, should provide a signal of single-agent clinical activity worthy of exploration in earlier phases of the disease and/or in the adjuvant setting.

Overexpression of the EGFR and its ligand TGFα as well as HER2 have been associated with antiestrogen resistance in breast cancer (reviewed in Refs. 73, 74). Both EGFR and HER2 inhibitors have been shown to enhance the antitumor effect of antiestrogens or reverse antiestrogen resistance in erbB receptor-overexpressing, hormone receptor-positive breast cancer models (75–77). MCF-7 human breast cancer cells selected for resistance to the pure antiestrogen ICI182,780 (fulvestrant) exhibit increased dependence on EGFR signaling for proliferation and survival because they are extremely sensitive to the EGFR inhibitor ZD1839 (78). In a recent study, MCF-7 cells, selected for resistance to the antiestrogen tamoxifen, exhibited markedly elevated levels of EGFR and HER2 compared with tamoxifen-sensitive parental cells. Tamoxifen resistance did not occur if the selection was done in the presence of ZD1839 (79). Taken together, these data suggest that interruption of the EGFR/HER2 signaling network may increase the antitumor effect of hormonal therapies in breast cancer by abrogating an important mechanism of de novo or acquired antiestrogen resistance. This hypothesis is currently being investigated in clinical trials using aromatase inhibitors or pure antiestrogens, each with EGFR/HER2 tyrosine kinase inhibitors.

Integration with Other Molecule-targeted Therapies

An area of clinical research with surprisingly less activity is the combination of EGFR inhibitors with other molecule-targeted therapies. In this endeavor, the basic science has provided important leads. For example, it is well established that overexpression of HER2 (or its rat/mouse homologue neu) can potentiate EGFR signaling (80) and contribute to EGFR-mediated transformation and tumor progression (81). Cancers that co-overexpress both EGFR and HER2 fare worse than those that overexpress either receptor (82–84). In some experimental sys-
tems, the inactivation of HER2 is required to block EGFR-mediated transformation (85, 86). Overexpression of HER2 counteracts the ability of EGFR kinase inhibitors to block EGFR activity (87). Conversely, high levels of activated EGFR abrogate the efficacy of trastuzumab against HER2-amplified gastric cancer cells (88), and this resistance is reversed by the EGFR inhibitor PKI-166 (89). In addition, the EGFR antibody C225 synergizes with HER2 antibodies against HER2-overexpressing ovarian cancer cells (90). Finally, ZD1839 inhibits HER2 phosphorylation per se (31, 32, 91) and potentiates the antitumor effect of trastuzumab against breast cancer xenografts (32). Taken together, these results lead to the hypothesis that overexpression of HER2 is a preferential mechanism of de novo or acquired resistance to EGFR inhibitors and that combinations of EGFR and HER2 inhibitors will be synergistic against EGFR-positive tumors with high levels of HER2. This hypothesis is currently being tested by Phase II studies of trastuzumab in combination with either ZD1839 or OSI-774.

Several studies have shown that both EGFR antibodies and small-molecule kinase inhibitors reduce VEGF and factor VIII levels (by IHC) and microvessel density in tumors that regress on EGFR blockade (92, 93). Interestingly, A431 tumor cells with acquired resistance to C225 exhibit increased expression and secretion of VEGF. Forced expression of VEGF in sensitive A431 cells renders them resistant to EGFR antibodies in vivo (94). These data imply that (a) subversion of EGFR-dependent tumor neo-angiogenesis is central for the antitumor effect of EGFR inhibitors; and (b) enhanced angiogenesis can endow tumors with resistance to EGFR blockade. In addition, these results provide a strong rationale for combinations of anti-EGFR agents with angiogenesis inhibitors.

It is anticipated that elucidation of the preferential molecular mechanisms of escape from anti-EGFR and anti-HER2 therapies will define new rational targets against which, drugs are either available or to be developed. Drugs against these targets can be combined with EGFR and HER2 inhibitors to prevent de novo or acquired resistance in advanced tumors and can enhance therapeutic efficacy. For example, overexpression of the insulin-like growth factor I (IGF-I) receptor has been recently reported to abrogate the antitumor effect of EGFR tyrosine kinase inhibitors and trastuzumab against human cancer cells (95, 96). In these studies, simultaneous blockade of IGF-I receptor signaling restored tumor cell sensitivity to EGFR and HER2 inhibitors, providing a rationale for combined anti-angiogenesis and anti-EGFR therapies. Similarly, loss of the phosphatase and tensin homolog PTEN in human tumor cell lines leads to phosphatidylinositol 3-kinase and Akt hyperactivity and results in relative resistance to EGFR inhibitors (97). In this report, resistance to ZD1839 was reversed by reconstitution of PTEN and inhibition of Akt. Taken together, data like these imply that combinations of antia signaling agents against multiple molecular targets are worth pursuing. If cancer selective and well tolerated, combinations like those suggested above will become a robust alternative to cytotoxic chemotherapy.

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