Minireview

Clinical Trial Design and End Points for Epidermal Growth Factor Receptor-targeted Therapies: Implications for Drug Development and Practice[1]

Carlos L. Arteaga[2] and Jose Baselga

Departments of Medicine and Cancer Biology and Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-6307 [C. .L. A.]; Medical Oncology Service, Vall d'Hebron University Hospital [J. B.], and Universidad Autónoma de Barcelona, Barcelona, Spain [J. B.]

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 EGFR[3] 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 antireceptor 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 EGF 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

Received 1/3/03; revised 3/18/03; accepted 3/26/03.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by the Spanish Health Ministry "Fondo de Investigación Sanitaria" (Grant 99/0020-01, to J. B.), National Cancer Institute Grant R01 CA80195 (to C. L. A.), and Vanderbilt-Ingram Cancer Center Support Grant CA68485.

[2] To whom requests for reprints should be addressed, at Division of Oncology, Vanderbilt University, 2220 Pierce Avenue, 777 PRB, Nashville, TN 37232-6307. Phone: (615) 936-3524; Fax: (615) 936-1790; E-mail: carlos.arteaga@vanderbilt.edu.

[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.

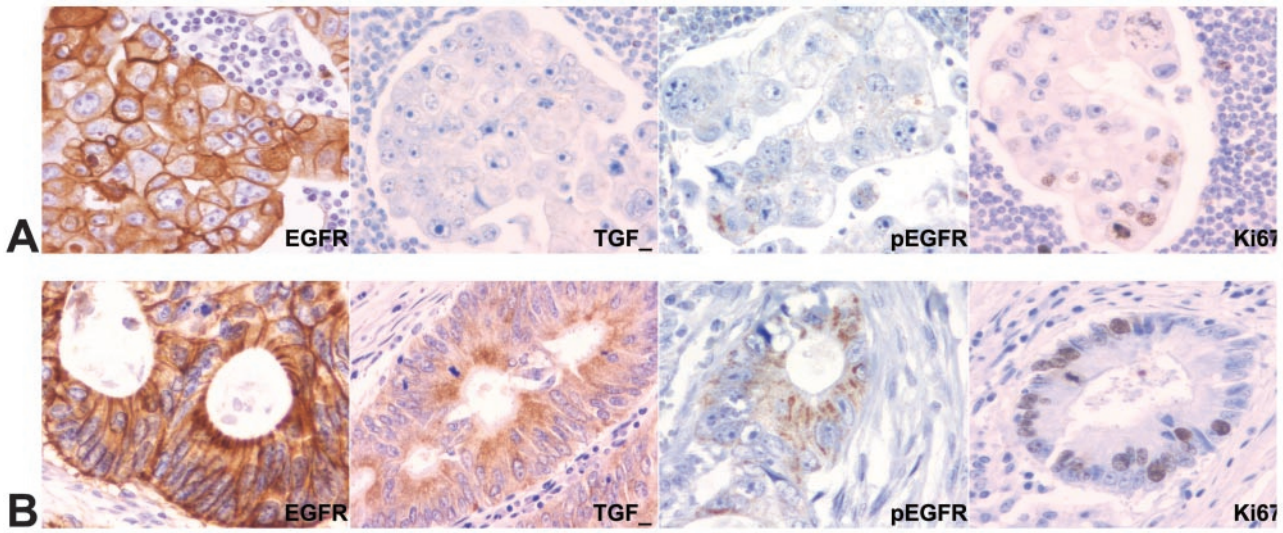

Fig. 1 EGFR levels do not correlate with evidence of EGFR activation. Colon carcinomas expressing EGFR by IHC. Tumor in *B* but not in *A* coexpresses the EGFR ligand TGFα. Phospho-EGFR (*pEGFR*) and tumor cell proliferation as determined by Ki67 IHC are both high in tumor *B* but low in tumor *A*.

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 APC^{Min} 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

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

Table 1 Current state of clinical development of EGFR inhibitors

Agent	Class	Development stage[a]	Clinical activity
ZD1839	Quinazoline EGFR-specific	Phase II: NSCLC (44, 45); Prostate (98); Colon (99); Head and neck (100) Phase III: NSCLC (48, 49)	NSCLC (single agent); Head and neck
OSI-774	Quinazoline EGFR-specific	Phase II: NSCLC (46); Colon (101); Head and neck (102); Ovarian (103)	NSCLC; Head and neck; Ovarian
CI-1033	Quinazoline EGFR/HER2	Phase I (68)	
EKB-569	Quinolone EGFR/HER2	Phase I (104)	
GW-2016	Quinazoline EGFR/HER2	Phase I	
IMC-225	Humanized IgG1	Phase II: Head and Neck (62, 105); Colon (20, 30); NSCLC (106); Pancreas (107). Phase III: Head and neck (50); Colon	Head and neck; Colon
MDX-447	Bispecific mAb EGFR/FcRγ1[b]	Phase I (108)	
h-R3		Phase II	
EMD72000	Humanized IgG1	Phase I (109, 110)	Colon
ABX-EGF	Human IgG2	Phase II (19)	
mAb 806	EGFR VIII mAb	Preclinical (111)	Renal cell

[a] Reference citations are in parentheses.
[b] FcR, Fc receptor.

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 ≥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 an initial diagnostic core biopsy to 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 the possibility of nonspecific mechanisms of action worthy of investigation. Any excess tissue from the initial core biopsies and the surgical specimens could be used for additional biochemical surrogate markers of drug action as well as the discovery of novel RNAs and/or proteins and their changes as a function of response or lack of response to the EGFR inhibitor.

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

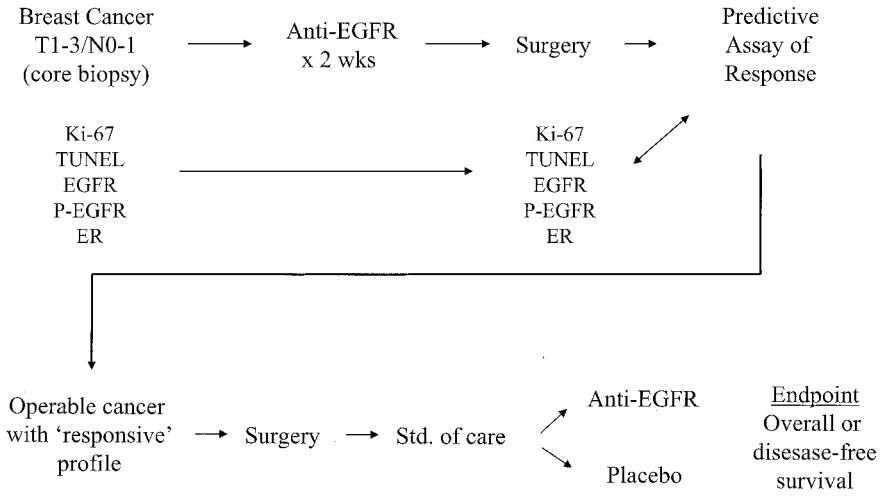

Fig. 2 Exploratory trial design for molecular target validation and identification of predictive markers of response to EGFR inhibitors. Patients with operable breast cancer will be treated with the OBD of an EGFR inhibitor for a period of ≤2 weeks. Ki67, terminal deoxynucleotidyl-transferase-mediated 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-naïve, *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*).

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 27^{Kip1}, 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.

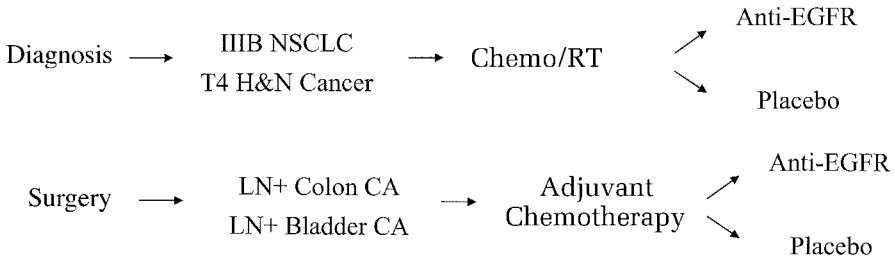

Fig. 3 Trial designs in advanced cancers to generate signals of clinical activity. Patients with the indicated tumor types and stages can be randomized to an EGFR inhibitor *versus* placebo trial after completion of their approved standard treatment. Tumor measurements of EGFR levels will not be required for study entry but will be analyzed retrospectively in all of the subjects enrolled to ensure that EGFR expression is balanced between both arms of the study. Clinical activity can be assessed in "EGFR-positive" and "EGFR-negative" tumors as measured by current methods. The length of time of treatment with the EGFR inhibitor is unknown. *RT,* radiation therapy; *T_4 H&N Cancer,* T_4 head and neck cancer; *Chemo,* chemotherapy; *LN,* lymph node; *CA,* cancer.

Integration with Conventional Anticancer Therapies

Aberrant 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, 69–72). 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 T_4 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 antireceptor 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 antisignaling 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.

References

1. Mendelsohn, J., and Baselga, J. The EGF receptor family as targets for cancer therapy. Oncogene, *19:* 6550–6565, 2000.

2. Fan, Z., Masui, H., Altas, I., and Mendelsohn, J. Blockade of epidermal growth factor receptor function by bivalent and monovalent fragments of 225 anti-epidermal growth factor receptor monoclonal antibodies. Cancer Res., *53:* 4322–4328, 1993.

3. Fan, Z., Lu, Y., Wu, X., and Mendelsohn, J. Antibody-induced epidermal growth factor receptor dimerization mediates inhibition of autocrine proliferation of A431 squamous carcinoma cells. J. Biol. Chem., *269:* 27595–27602, 1994.

4. Rodeck, U., Herlyn, M., Herlyn, D., Molthoff, C., Atkinson, B., Varello, M., Steplewski, Z., and Koprowski, H. Tumor growth modulation by a monoclonal antibody to the epidermal growth factor receptor: immunologically mediated and effector cell-independent effects. Cancer Res., *47:* 3692–3696, 1987.

5. Modjtahedi, H., Eccles, S., Sandle, J., Box, G., Titley, J., and Dean, C. Differentiation or immune destruction: two pathways for therapy of squamous cell carcinomas with antibodies to the epidermal growth factor receptor. Cancer Res., *54:* 1695–1701, 1994.

6. Bier, H., Hoffmann, T., Haas, I., and van Lierop, A. Anti-(epidermal growth factor) receptor monoclonal antibodies for the induction of antibody-dependent cell-mediated cytotoxicity against squamous cell carcinoma lines of the head and neck. Cancer Immunol. Immunother., *46:* 167–173, 1998.

7. Clynes, R. A., Towers, T. L., Presta, L. G., and Ravetch, J. V. Inhibitory Fc receptors modulate *in vivo* cytotoxicity against tumor targets. Nat. Med., *6:* 443–446, 2000.

8. Toledo, L. M., Lydon, N. B., and Elbaum, D. The structure-based design of ATP-site directed protein kinase inhibitors. Curr. Med. Chem., *6:* 775–805, 1999.

9. Al-Abeidi, F., and Lam, K. S. Development of inhibitors for protein tyrosine kinases. Oncogene, *19:* 5690–5701, 2000.

10. Arteaga, C. L., Ramsey, T. T., Shawver, L. K., and Guyer, C. A. Unliganded epidermal growth factor receptor dimerization induced by direct interaction of quinazolines with the ATP binding site. J. Biol. Chem., *272:* 23247–23254, 1997.

11. Lichtner, R. B., Menrad, A., Sommer, A., Klar, U., and Schneider, M. R. Signaling-inactive epidermal growth factor receptor/ligand complexes in intact carcinoma cells by quinazoline tyrosine kinase inhibitors. Cancer Res., *61:* 5790–5795, 2001.

12. Yarden, Y., and Sliwkowski, M. X. Untangling the ErbB signalling network. Nat. Rev. Mol. Cell. Biol., *2:* 127–137, 2001.

13. Olayioye, M. A., Neve, R. M., Lane, H. A., and Hynes, N. E. The ErbB signaling network: receptor heterodimerization in development and cancer. EMBO J., *19:* 3159–3167, 2000.

14. Fry, D. W. Site-directed irreversible inhibitors of the erbB family of receptor tyrosine kinases as novel chemotherapeutic agents for cancer. Anticancer Drug Des., *15:* 3–16, 2000.

15. Baselga, J., Pfister, D., Cooper, M. R., Cohen, R., Burtness, B., Bos, M., D'Andrea, G., Seidman, A., Norton, L., Gunnett, K., Falcey, J., Anderson, V., Waksal, H., and Mendelsohn, J. Phase I studies of anti-epidermal growth factor receptor chimeric antibody C225 alone and in combination with cisplatin. J. Clin. Oncol., *18:* 904–914, 2000.

16. Hidalgo, M., Siu, L. L., Nemunaitis, J., Rizzo, J., Hammond, L. A., Takimoto, C., Eckhardt, S. G., Tolcher, A., Britten, C. D., Denis, L., Ferrante, K., Von Hoff, D. D., Silberman, S., and Rowinsky, E. K. Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. J. Clin. Oncol., *19:* 3267–3279, 2001.

17. Ranson, M., Hammond, L. A., Ferry, D., Kris, M., Tullo, A., Murray, P. I., Miller, V., Averbuch, S., Ochs, J., Morris, C., Feyereislova, A., Swaisland, H., and Rowinsky, E. K. ZD1839, a selective oral epidermal growth factor receptor-tyrosine kinase inhibitor, is well tolerated and active in patients with solid, malignant tumors: results of a Phase I trial. J. Clin. Oncol., *20:* 2240–2250, 2002.

18. Baselga, J., Rischin, D., Ranson, M., Calvert, H., Raymond, E., Kieback, D. G., Kaye, S. B., Gianni, L., Harris, A., Bjork, T., Averbuch, S. D., Feyereislova, A., Swaisland, H., Rojo, F., and Albanell, J. Phase I safety, pharmacokinetic, and pharmacodynamic trial of ZD1839, a selective oral epidermal growth factor receptor tyrosine kinase inhibitor,

in patients with five selected solid tumor types. J. Clin. Oncol., *20:* 4292–4302, 2002.

19. Schwartz, G., Dutcher, J. P., Vogelzang, N. J., Gollob, J., Thompson, J., Bukowski, R. M., Figlin, R. A., Lohner, M., Roskos, L., Hwang, C. C., Foon, K., Schwab, G., and Rowinsky, E. Phase 2 clinical trial evaluating the safety and effectiveness of ABX-EGF in renal cell cancer (RCC). Proc. Am. Soc. Clin. Oncol., *21:* 24, 2002.

20. Saltz, L., Meropol, N. J., Loehrer, P. J., Waksal, H., Needle, M. N., and Mayer, R. J. Single agent IMC-C225 (Erbitux) has activity in CPT-11-refractory colorectal cancer (CRC) that expresses the epidermal growth factor receptor (EGFR). Proc. Am. Soc. Clin. Oncol., *21:* 127, 2002.

21. Martinez-Lacaci, I., Kannan, S., De Santis, M., Bianco, C., Kim, N., Wallace-Jones, B., Ebert, A. D., Wechselberger, C., and Salomon, D. S. RAS transformation causes sustained activation of epidermal growth factor receptor and elevation of mitogen-activated protein kinase in human mammary epithelial cells. Int. J. Cancer, *88:* 44–52, 2000.

22. Bos, M., Mendelsohn, J., Kim, Y. M., Albanell, J., Fry, D. W., and Baselga, J. PD153035, a tyrosine kinase inhibitor, prevents epidermal growth factor receptor activation and inhibits growth of cancer cells in a receptor number-dependent manner. Clin. Cancer Res., *3:* 2099–2106, 1997.

23. Salomon, D. S., Brandt, R., Ciardiello, F., and Normanno, N. Epidermal growth factor-related peptides and their receptors in human malignancies. Crit. Rev. Oncol. Hematol., *19:* 183–232, 1995.

24. Rusch, V., Baselga, J., Cordon-Cardo, C., Orazem, J., Zaman, M., Hoda, S., McIntosh, J., Kurie, J., and Dmitrovsky, E. Differential expression of the epidermal growth factor receptor and its ligands in primary non-small cell lung cancers and adjacent benign lung. Cancer Res., *53:* 2379–2385, 1993.

25. Grandis, J. R., Melhem, M. F., Gooding, W. E., Day, R., Holst, V. A., Wagener, M. M., Drenning, S. D., and Tweardy, D. J. Levels of TGF-alpha and EGFR protein in head and neck squamous cell carcinoma and patient survival. J. Natl. Cancer Inst. (Bethesda), *90:* 824–832, 1998.

26. Fontanini, G., De Laurentiis, M., Vignati, S., Chine, S., Lucchi, M., Silvestri, V., Mussi, A., De Placido, S., Tortora, G., Bianco, A. R., Gullick, W., Angeletti, C. A., Bevilacqua, G., and Ciardiello, F. Evaluation of epidermal growth factor-related growth factors and receptors and of neoangiogenesis in completely resected stage I-IIIA non-small-cell lung cancer: amphiregulin and microvessel count are independent prognostic indicators of survival. Clin. Cancer Res., *4:* 241–249, 1998.

27. Divgi, C. R., Welt, S., Kris, M., Real, F. X., Yeh, S. D., Gralla, R., Merchant, B., Schweighart, S., Unger, M., Larson, S. M., *et al.* Phase I and imaging trial of indium 111-labeled anti-epidermal growth factor receptor monoclonal antibody 225 in patients with squamous cell lung carcinoma. J. Natl. Cancer Inst. (Bethesda), *83:* 97–104, 1991.

28. Sirotnak, F. M., Zakowski, M. F., Miller, V. A., Scher, H. I., and Kris, M. G. Efficacy of cytotoxic agents against human tumor xenografts is markedly enhanced by coadministration of ZD1839 (Iressa), an inhibitor of EGFR tyrosine kinase. Clin. Cancer Res., *6:* 4885–4892, 2000.

29. Ciardiello, F., Caputo, R., Bianco, R., Damiano, V., Pomatico, G., De Placido, S., Bianco, A. R., and Tortora, G. Antitumor effect and potentiation of cytotoxic drugs activity in human cancer cells by ZD-1839 (Iressa), an epidermal growth factor receptor-selective tyrosine kinase inhibitor. Clin. Cancer Res., *6:* 2053–2063, 2000.

30. Saltz, L., Rubin, M., Hochster, H., Tchekmeydian, N. S., Waksal, H., Needle, M., and LoBuglio. A. Cetuximab (IMC-225) plus irinotecan (CPT-11) is active in CPT-11-refractory colorectal cancer that expresses epidermal growth factor receptors. Proc. Am. Soc. Clin. Oncol., *20:* 3, 2001.

31. Moasser, M. M., Basso, A., Averbuch, S. D., and Rosen, N. The tyrosine kinase inhibitor ZD1839 ("Iressa") inhibits HER2-driven signaling and suppresses the growth of HER2-overexpressing tumor cells. Cancer Res., *61:* 7184–7188, 2001.

32. Moulder, S. L., Yakes, F. M., Muthuswamy, S. K., Bianco, R., Simpson, J. F., and Arteaga, C. L. Epidermal growth factor receptor (HER1) tyrosine kinase inhibitor (Iressa) inhibits HER2/neu (erbB2)-overexpressing breast cancer cells *in vitro* and *in vivo*. Cancer Res., *61:* 8887–8895, 2001.

33. Rusch, V., Klimstra, D., Linkov, I., and Dmitrovsky, E. Aberrant expression of p53 or the epidermal growth factor receptor is frequent in early bronchial neoplasia and coexpression precedes squamous cell carcinoma development. Cancer Res., *55:* 1365–1372, 1995.

34. Kurie, J. M., Shin, H. J., Lee, J. S., Morice, R. C., Ro, J. Y., Lippman, S. M., Hittelman, W. N., Yu, R., Lee, J. J., and Hong, W. K. Increased epidermal growth factor receptor expression in metaplastic bronchial epithelium. Clin. Cancer Res., *2:* 1787–1793, 1996.

35. van de Vijver, M. J., Peterse, J. L., Mooi, W. J., Wisman, P., Lomans, J., Dalesio, O., and Nusse, R. Neu-protein overexpression in breast cancer. Association with comedo-type ductal carcinoma *in situ* and limited prognostic value in stage II breast cancer. N. Engl. J. Med., *319:* 1239–1245, 1988.

36. Lenferink, A. E., Simpson, J. F., Shawver, L. K., Coffey, R. J., Forbes, J. T., and Arteaga, C. L. Blockade of the epidermal growth factor receptor tyrosine kinase suppresses tumorigenesis in MMTV/Neu + MMTV/TGF-α bigenic mice. Proc. Natl. Acad. Sci. USA, *97:* 9609–9614, 2000.

37. Roberts, R. B., Min, L., Washington, M. K., Olsen, S. J., Settle, S. H., Coffey, R. J., and Threadgill, D. W. Importance of epidermal growth factor receptor signaling in establishment of adenomas and maintenance of carcinomas during intestinal tumorigenesis. Proc. Natl. Acad. Sci. USA, *99:* 1521–1526, 2002.

38. Chan, K. C., Knox, W. F., Gee, J. M., Morris, J., Nicholson, R. I., Potten, C. S., and Bundred, N. J. Effect of epidermal growth factor receptor tyrosine kinase inhibition on epithelial proliferation in normal and premalignant breast. Cancer Res., *62:* 122–128, 2002.

39. Wu, X., Fan, Z., Masui, H., Rosen, N., and Mendelsohn, J. Apoptosis induced by an anti-epidermal growth factor receptor monoclonal antibody in a human colorectal carcinoma cell line and its delay by insulin. J Clin. Investig., *95:* 1897–1905, 1995.

40. Wakeling, A. E., Guy, S. P., Woodburn, J. R., Ashton, S. E., Curry, B. J., Barker, A. J., and Gibson, K. H. ZD1839 (Iressa): an orally active inhibitor of epidermal growth factor signaling with potential for cancer therapy. Cancer Res., *62:* 5749–5754, 2002.

41. Baselga, J., Tripathy, D., Mendelsohn, J., Baughman, S., Benz, C. C., Dantis, L., Sklarin, N. T., Seidman, A. D., Hudis, C. A., Moore, J., Rosen, P. P., Twaddell, T., Henderson, I. C., and Norton, L. Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. J. Clin. Oncol., *14:* 737–744, 1996.

42. Cobleigh, M. A., Vogel, C. L., Tripathy, D., Robert, N. J., Scholl, S., Fehrenbacher, L., Wolter, J. M., Paton, V., Shak, S., Lieberman, G., and Slamon, D. J. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J. Clin. Oncol., *17:* 2639–2648, 1999.

43. Vogel, C. L., Cobleigh, M. A., Tripathy, D., Gutheil, J. C., Harris, L. N., Fehrenbacher, L., Slamon, D. J., Murphy, M., Novotny, W. F., Burchmore, M., Shak, S., Stewart, S. J., and Press, M. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J. Clin. Oncol., *20:* 719–726, 2002.

44. Fukuoka, M., Yano, S., Giaccone, G., Tamura, T., Nakagawa, K., Douillard, J-Y., Nishiwaki, Y., Vansteenkiste, J., Kudoh, S., Rischin, D., Eek, R., Horai, T., Noda, K., Takata, I., Smit, E., Averbuch, S., MacLeod, A., Feyereislova, A., Dong, R-P., and Baselga, J. A multi-institutional randomized Phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (the IDEAL 1 trial). J. Clin. Oncol., *21:* in press, 2003.

45. Kris, M. G., *et al.* A Phase II trial of ZD1839 ('Iressa') in advanced non-small-cell lung cancer (NSCLC) patients who have failed platinum- and docetaxel-based regimens. J. Clin. Oncol., *21:* in press, 2003.

46. Perez-Soler, R., Chachoua, A., Huberman, M., Karp, D., Rigas, J., Hammond, L., Rowinsky, E., Preston, G., Ferrante, K. J., Allen, L. F.,

Nadler, P. I., and Bonomi, P. A Phase II trial of the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor OSI-774. after platinum-based chemotherapy, in patients (pts) with advanced, EGFR-expressing, non-small cell lung cancer (NSCLC). Proc. Am. Soc. Clin. Oncol., *20:* 310a, 2001.

47. Slamon, D. J., Leyland-Jones, B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A., Fleming, T., Eiermann, W., Wolter, J., Pegram, M., Baselga, J., and Norton, L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N. Engl. J. Med., *344:* 783–792, 2001.

48. Giaccone, g., Johnson, D. H., Manegold, C., Scagliotti, G. V., Rosell, R., Wolf, M., Rennie, P., Ochs, J., Averbuch, S., and Fandi, A. A. Phase III clinical trial of ZD1839 ('Iressa') in combination with gemcitabine and cisplatin in chemotherapy-naive patients with advances non-small-cell lung cancer (INTACT 1). Ann. Oncol., *13* (Suppl. 5)*:* 2, 2002.

49. Johnson, D. H., Herbst, R., Giaconne, G., Schiller, J., Natale, R. B., Miller, V., Wolf, M., Helton, A., Averbuch, S., and Grous, J. Z. D.1839 ("Iressa") in combination with paclitaxel and carboplatin in chemotherapy-naive patients with advanced non-small-cell lung cancer (NSCLC): results from a Phase III clinical trial (INTACT 2). Ann. Oncol., *13* (Suppl. 5)*:* 127, 2002.

50. Burtness, B., Li, Y., Flood, W., Mattar, B. I., and Forastiere, A. A. Phase III trial comparing cisplatin (C) + placebo to C + anti-epidermal growth factor receptor (EGF-R) antibody C225 in patients (pts) with metastatic/recurrent head and neck cancer (HNC). Proc. Am. Soc. Clin. Oncol., *21:* 226, 2002.

51. Kelly, H. C., Laight, A., Morris, C. Q., Woodburn, J. R., and Richmond, G. H. P. Phase I data of ZD1839—an oral epidermal growth factor receptor tyrosine kinase inhibitor. Ann. Oncol., *2:* 109, 1998.

52. Sato, J. D., Kawamoto, T., Le, A. D., Mendelsohn, J., Polikoff, J., and Sato, G. H. Biological effects *in vitro* of monoclonal antibodies to human epidermal growth factor receptors. Mol. Biol. Med., *1:* 511–529, 1983.

53. Kawamoto, T., Mendelsohn, J., Le, A., Sato, G. H., Lazar, C. S., and Gill, G. N. Relation of epidermal growth factor receptor concentration to growth of human epidermoid carcinoma A431 cells. J. Biol. Chem., *259:* 7761–7766, 1984.

54. Albanell, J., Rojo, F., Averbuch, S., Feyereislova, A., Mascaro, J. M., Herbst, R., LoRusso, P., Rischin, D., Sauleda, S., Gee, J., Nicholson, R. I., and Baselga, J. Pharmacodynamic studies of the epidermal growth factor receptor inhibitor ZD1839 in skin from cancer patients: histopathologic and molecular consequences of receptor inhibition. J. Clin. Oncol., *20:* 110–124, 2002.

55. Klijn, J. G., Berns, P. M., Schmitz, P. I., and Foekens, J. A. The clinical significance of epidermal growth factor receptor (EGF-R) in human breast cancer: a review on 5232 patients. Endocr. Rev., *13:* 3–17, 1992.

56. Sharma, A. K., Horgan, K., Douglas-Jones, A., McClelland, R., Gee, J., and Nicholson, R. Dual immunocytochemical analysis of oestrogen and epidermal growth factor receptors in human breast cancer. Br. J. Cancer, *69:* 1032–1037, 1994.

57. Ellis, P. A., Saccani-Jotti, G., Clarke, R., Johnston, S. R., Anderson, E., Howell, A., A'Hern, R., Salter, J., Detre, S., Nicholson, R., Robertson, J., Smith, I. E., and Dowsett, M. Induction of apoptosis by tamoxifen and ICI 182780 in primary breast cancer. Int. J. Cancer, *72:* 608–613, 1997.

58. Chang, J., Powles, T. J., Allred, D. C., Ashley, S. E., Makris, A., Gregory, R. K., Osborne, C. K., and Dowsett, M. Prediction of clinical outcome from primary tamoxifen by expression of biologic markers in breast cancer patients. Clin. Cancer Res., *6:* 616–621, 2000.

59. Robertson, J. F., Nicholson, R. I., Bundred, N. J., Anderson, E., Rayter, Z., Dowsett, M., Fox, J. N., Gee, J. M., Webster, A., Wakeling, A. E., Morris, C., and Dixon, M. Comparison of the short-term biological effects of 7α-[9-(4,4,5,5,5-pentafluoropentylsulfinyl)-nonyl]estra-1,3,5,(10)-triene-3,17β-diol (Faslodex) *versus* tamoxifen in postmenopausal women with primary breast cancer. Cancer Res., *61:* 6739–6746, 2001.

60. Rubin, M., Shin, D., Pasmantier, M., Falcey, J. W., Paulter, V. J., Fetzer, K. M., Waksal, H. W., Mendelsohn, J., and Hong, W. K. Monoclonal antibody (MoAb) IMC-225, an anti-epidermal growth factor receptor (EGFR) for patients with EGFR-positive tumors refractory to or in relapse from previous therapeutic regimens. Proc. Am. Soc. Clin. Oncol., *19:* 474a, 2000.

61. Prewett, M. C., Hooper, A. T., Bassi, R., Ellis, L. M., Waksal, H. W., and Hicklin, D. J. Enhanced antitumor activity of anti-epidermal growth factor receptor monoclonal antibody IMC-C225 in combination with irinotecan (CPT-11) against human colorectal tumor xenografts. Clin. Cancer Res., *8:* 994–1003, 2002.

62. Baselga, J. M., Trigo, J., Bourhis, J., Tortochaux, J., Cortes-Funes, H., Hitt, R., Gascon, P., Muesser, M., Harstrick, A., and Eckardt, A. Cetuximab (C225) plus cisplatin/carboplatin is active in patients with recurrent/metastatic squamous cell carcinoma of the head and neck (SCCHN) progressing on a same dose and schedule platinum-based regimen. Proc. Am. Soc. Clin. Oncol., *21:* 226, 2002.

63. Albanell, J., Codony-Servat, J., Rojo, F., Del Campo, J. M., Sauleda, S., Anido, J., Raspall, G., Giralt, J., Rosello, J., Nicholson, R. I., Mendelsohn, J., and Baselga, J. Activated extracellular signal-regulated kinases: association with epidermal growth factor receptor/transforming growth factor α expression in head and neck squamous carcinoma and inhibition by anti-epidermal growth factor receptor treatments. Cancer Res., *61:* 6500–6510, 2001.

64. Stoll, S. W., Benedict, M., Mitra, R., Hiniker, A., Elder, J. T., and Nunez, G. EGF receptor signaling inhibits keratinocyte apoptosis: evidence for mediation by Bcl-XL. Oncogene, *16:* 1493–1499, 1998.

65. Jost, M., Kari, C., and Rodeck, U. The EGF receptor—an essential regulator of multiple epidermal functions. Eur. J. Dermatol., *10:* 505–510, 2000.

66. Hidalgo, M., Malik, S., Rowinsky, E., Miller, A., Duffey, D., de'Grafenreid, L., Siu, L., Simmons, C., Kreisberg, J., and Brattain, M. Inhibition of the epidermal growth factor receptor (EGFR) by OSI-774, a specific EGFR inhibitor in malignant and normal tissues of cancer patients. Proc. Am. Soc. Clin. Oncol. *20:* 71a, 2001.

67. Hoekstra, R., Dumez, H., Eskens, F., Sizer, K., Cohen, P., Ravera, C., Verweij, J., and Van Oosterom, A. A Phase I and pharmacological study of intermittent dosing of PKI166, a novel epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, administered orally to patients with advanced cancer. Clin. Cancer Res., *7* (Suppl.)*:* 3771s, 2001.

68. Zinner, R. G., Nemunaitis, J. J., Donato, N. J., Shin, H. C., Myers, J. N., Zhang, P. S., Zentgraft, R. E., Lee, J. J., Khuri, F. R., Glisson, B. S., *et al.* A Phase I clinical and biomarker study of the novel pan-erbB tyrosine kinase inhibitor, CI-1033, in patients with solid tumors. Clin. Cancer Res., *7* (Suppl.)*:* 3767s, 2001.

69. Aboud-Pirak, E., Hurwitz, E., Pirak, M. E., Bellot, F., Schlessinger, J., and Sela, M. Efficacy of antibodies to epidermal growth factor receptor against KB carcinoma *in vitro* and in nude mice. J. Natl. Cancer Inst. (Bethesda), *80:* 1605–1611, 1988.

70. Fan, Z., Baselga, J., Masui, H., and Mendelsohn, J. Antitumor effect of anti-epidermal growth factor receptor monoclonal antibodies plus *cis*-diamminedichloroplatinum on well-established A431 cell xenografts. Cancer Res., *53:* 4637–4642, 1993.

71. Baselga, J., Norton, L., Masui, H., Pandiella, A., Coplan, K., Miller, W. H., Jr., and Mendelsohn, J. Antitumor effects of doxorubicin in combination with anti-epidermal growth factor receptor monoclonal antibodies. J. Natl. Cancer Inst. (Bethesda), *85:* 1327–1333, 1993.

72. Huang, S. M., and Harari, P. M. Modulation of radiation response after epidermal growth factor receptor blockade in squamous cell carcinomas: inhibition of damage repair, cell cycle kinetics, and tumor angiogenesis. Clin. Cancer Res., *6:* 2166–2174, 2000.

73. Simak, A., and Coombes, C. Endocrine-responsive breast cancer and strategies for combating resistance. Nat. Rev. Cancer, *22:* 101–112, 2002.

74. Kurokawa, H., and Arteaga, C. L. Inhibition of erbB receptor (HER) tyrosine kinases as a strategy to abrogate antiestrogen resistance in human breast cancer. Clin. Cancer Res., *7* (Suppl.)*:* 4436s–4442s, 2001.

75. Kurokawa, H., Lenferink, A. E., Simpson, J. F., Pisacane, P. I., Sliwkowski, M. X., Forbes, J. T., and Arteaga, C. L. Inhibition of HER2/neu (erbB-2) and mitogen-activated protein kinases enhances tamoxifen action against HER2-overexpressing, tamoxifen-resistant breast cancer cells. Cancer Res., *60:* 5887–5894, 2000.

76. Witters, L. M., Kumar, R., Chinchilli, V. M., and Lipton, A. Enhanced anti-proliferative activity of the combination of tamoxifen plus HER-2-neu antibody. Breast Cancer Res. Treat., *42:* 1–5, 1997.

77. Kunisue, H., Kurebayashi, J., Otsuki, T., Tang, C. K., Kurosumi, M., Yamamoto, S., Tanaka, K., Doihara, H., Shimizu, N., and Sonoo, H. Anti-HER2 antibody enhances the growth inhibitory effect of anti-oestrogen on breast cancer cells expressing both oestrogen receptors and HER2. Br. J. Cancer, *82:* 46–51, 2000.

78. McClelland, R. A., Barrow, D., Madden, T. A., Dutkowski, C. M., Pamment, J., Knowlden, J. M., Gee, J. M., and Nicholson, R. I. Enhanced epidermal growth factor receptor signaling in MCF7 breast cancer cells after long-term culture in the presence of the pure antiestrogen ICI 182, 780 (Faslodex). Endocrinology, *142:* 2776–2788, 2001.

79. Nicholson, R. I., Hutcheson, I. R., Harper, M. E., Knowlden, J. M., Barrow, D., McClelland, R. A., Jones, H. E., Wakeling, A. E., and Gee, J. M. Modulation of epidermal growth factor receptor in endocrine-resistant, oestrogen receptor-positive breast cancer. Endocr.-Relat. Cancer, *8:* 175–182, 2001.

80. Karunagaran, D., Tzahar, E., Beerli, R. R., Chen, X., Graus-Porta, D., Ratzkin, B. J., Seger, R., Hynes, N. E., and Yarden, Y. ErbB-2 is a common auxiliary subunit of NDF and EGF receptors: implications for breast cancer. EMBO J., *15:* 254–264, 1996.

81. Muller, W. J., Arteaga, C. L., Muthuswamy, S. K., Siegel, P. M., Webster, M. A., Cardiff, R. D., Meise, K. S., Li, F., Halter, S. A., and Coffey, R. J. Synergistic interaction of the Neu proto-oncogene product and transforming growth factor α in the mammary epithelium of transgenic mice. Mol. Cell. Biol., *16:* 5726–5736, 1996.

82. Tateishi, M., Ishida, T., Kohdono, S., Hamatake, M., Fukuyama, Y., and Sugimachi, K. Prognostic influence of the co-expression of epidermal growth factor receptor and c-erbB-2 protein in human lung adenocarcinoma. Surg. Oncol., *3:* 109–113, 1994.

83. Xia, W., Lau, Y. K., Zhang, H. Z., Xiao, F. Y., Johnston, D. A., Liu, A. R., Li, L., Katz, R. L., and Hung, M. C. Combination of EGFR, HER-2/neu, and HER-3 is a stronger predictor for the outcome of oral squamous cell carcinoma than any individual family members. Clin. Cancer Res., *5:* 4164–4174, 1999.

84. Nicholson, R. I., McClelland, R. A., Finlay, P., Eaton, C. L., Gullick, W. J., Dixon, A. R., Robertson, J. F., Ellis, I. O., and Blamey, R. W. Relationship between EGF-R, c-erbB-2 protein expression and Ki67 immunostaining in breast cancer and hormone sensitivity. Eur. J. Cancer, *7:* 1018–1023, 1993.

85. Qian, X., Dougall, W. C., Hellman, M. E., and Greene, M. I. Kinase-deficient neu proteins suppress epidermal growth factor receptor function and abolish cell transformation. Oncogene, *9:* 1507–1514, 1994.

86. Graus-Porta, D., Beerli, R. R., and Hynes, N. E. Single-chain antibody-mediated intracellular retention of ErbB-2 impairs Neu differentiation factor and epidermal growth factor signaling. Mol. Cell. Biol., *15:* 1182–1191, 1995.

87. Christensen, J. G., Schreck, R. E., Chan, E., Wang, X., Yang, C., Liu, L., Cui, J., Sun, L., Wei, J., Cherrington, J. M., and Mendel, D. B. High levels of HER-2 expression alter the ability of epidermal growth factor receptor (EGFR) family tyrosine kinase inhibitors to inhibit EGFR phosphorylation *in vivo*. Clin. Cancer Res., *7:* 4230–4238, 2001.

88. Lane, H. A., Beuvink, I., Motoyama, A. B., Daly, J. M., Neve, R. M., and Hynes, N. E. ErbB2 potentiates breast tumor proliferation through modulation of p27(Kip1)-Cdk2 complex formation: receptor overexpression does not determine growth dependency. Mol. Cell. Biol., *20:* 3210–3223, 2000.

89. Motoyama, A. B., Hynes, N. E., and Lane, H. A. The efficacy of ErbB receptor-targeted anticancer therapeutics is influenced by the availability of epidermal growth factor-related peptides. Cancer Res., *62:* 3151–3158, 2002.

90. Ye, D., Mendelsohn, J., and Fan, Z. Augmentation of a humanized anti-HER2 mAb 4D5 induced growth inhibition by a human-mouse chimeric anti-EGF receptor mAb C225. Oncogene, *18:* 731–738, 1999.

91. Anderson, N. G., Ahmad, T., Chan, K., Dobson, R., and Bundred, N. J. ZD1839 (Iressa), a novel epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, potently inhibits the growth of EGFR- positive cancer cell lines with or without erbB2 overexpression. Int. J. Cancer, *94:* 774–782, 2001.

92. Petit, A. M., Rak, J., Hung, M. C., Rockwell, P., Goldstein, N., Fendly, B., and Kerbel, R. S. Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor production by tumor cells *in vitro* and *in vivo*: angiogenic implications for signal transduction therapy of solid tumors. Am. J. Pathol., *151:* 1523–1530, 1997.

93. Ciardiello, F., Caputo, R., Bianco, R., Damiano, V., Fontanini, G., Cuccato, S., De Placido, S., Bianco, A. R., and Tortora, G. Inhibition of growth factor production and angiogenesis in human cancer cells by ZD1839 (Iressa), a selective epidermal growth factor receptor tyrosine kinase inhibitor. Clin. Cancer Res., *7:* 1459–1465, 2001.

94. Viloria-Petit, A., Crombet, T., Jothy, S., Hicklin, D., Bohlen, P., Schlaeppi, J. M., Rak, J., and Kerbel, R. S. Acquired resistance to the antitumor effect of epidermal growth factor receptor-blocking antibodies *in vivo*: a role for altered tumor angiogenesis. Cancer Res., *61:* 5090–5101, 2001.

95. Chakravarti, A., Loeffler, J. S., and Dyson, N. J. Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling. Cancer Res., *62:* 200–207, 2002.

96. Lu, Y., Zi, X., Zhao, Y., Mascarenhas, D., and Pollak, M. Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). J. Natl. Cancer Inst. (Bethesda), *93:* 1852–1857, 2001.

97. Bianco, R., Shin, I., Ritter, C. A., Yakes, F. M., Basso, A., Rosen, N., Tsurutani, J., Dennis, P. A., Mills, G. B., and Arteaga, C. L. Loss of PTEN/MMAC1/TEP in EGF receptor-expressing tumor cells counteracts the antitumor action of EGFR tyrosine kinase inhibitors. Oncogene, *22:* in press, 2003.

98. Moore, M., Winquist, E., Pollak, M., Chi, K., Berry, S., Ernst, S., Douglas, L., Baetz, T., Fisher, B., and Seymour, L. A randomised Phase II study of two doses of ZD1839 in patients (pts) with hormone refractory prostate cancer (HRPC): a NCI Canada Clinical Trials Group Study. Ann. Oncol., *13* (Suppl. 5)*:* 90, 2002.

99. Seymour, L., Goss, G., Stewart, D., Hirte, H., Miller, W., Major, P, Batist, G., Matthews, S., Lorimer, I., and Douglass, L. A translational research study of ZD1839 at a dose of 750 mg in patients with pretreated advanced or metastatic colorectal cancer: NCIC CTG IND. 122. Ann. Oncol., *2002:* 73, 2002.

100. Cohen, E. E., Hidalgo, M., Erlichman, C., Rowinsky, E. K., Koepp-Norris, J., Jensen, K., Boni, J., Korth-Bradley, J., Quinn, S., and Zacharchuk, C. Phase II study of ZD1839 (Iressa) in recurrent or metastatic squamous cell carcinoma of the head and neck (SCCHN). Proc. Am. Soc. Clin. Oncol., *21:* 225a, 2002.

101. Townsley, C., Tabernero, J., Rojo, F., Jimenez, E., Montaner, I., Santome, L., Guix, M., Rosen, O., Kovar, A., Viaplana, I., and Baselga, J. Phase II study of OSI-774 in patients with metastatic colorectal cancer. Eur. J. Cancer, *38* (Suppl. 7)*:* 69, 2002.

102. Senzer, N. N., Soulieres, D., Siu, L., Agarwala, S., Vokes, E., Hidalgo, M., Silberman, S., Allen, L., Ferrante, K., Fisher, D., Marsolais, C., and Nadler, P. Phase 2 evaluation of OSI-774, a potent oral antagonist of the EGFR-TK in patients with advanced squamous cell carcinoma of the head and neck. Proc. Am. Soc. Clin. Oncol., *20:* 2a, 2001.

103. Finkler, N., Gordon, A., Crozier, M., Edwards, R., Figueroa, J., Garcia, A., Hainsworth, J., Irwin, D., Silberman, S., Allen, L., Ferrante, K., Fisher, D., and Nadler, P. Phase 2 evaluation of OSI-774, a potent oral antagonist of the EGFR-TK in patients with advanced ovarian carcinoma. Proc. Am. Soc. Clin. Oncol., *20:* 208a, 2001.

104. Hidalgo, M., Wong, W. K., Arquette, M., Nabell, L., Needle, M. N., Waksal, H. W., and Herbst, R. S. Phase 1 trial of EKB-569, an irreversible inhibitor of the epidermal growth factor receptor (EGFR), in patients with advanced solid tumors. Proc. Am. Soc. Clin. Oncol. *21:* 17a, 2002.

105. Hong, W. K., Kim, E. S., Mauer, A. M., Fossella, F. V., Jamison, T. A., Kies, M. S., Pisters, K. M., Glisson, B. S., Blumenschein, G. R., Zinner, R. G., Tran, H. T., Windt, P., Liu, D., Khuri, F., Dicke, K., Needle, M., Vokes, E. E., Hong, W. K., and Herbst, R. S. Efficacy and safety of the anti-epidermal growth factor antibody IMC-C225 in combination with cisplatin in patients with recurrent squamous cell carcinoma of the head and neck refractory to cisplatin containing chemotherapy. Proc. Am. Soc. Clin. Oncol., *20:* 224a, 2001.

106. Kim, E. S., Mauer, A. M., Fossella, F. V., Jamison, T. A., Kies, M. S., Pisters, K. M., Glisson, B. S., Blumenschein, G. R., Zinner, R. G., Tran, H. T., Windt, P., Liu, D., Khuri, F., Dicke, K., Needle, M., Vokes, E. E., Hong, W. K., and Herbst, R. S. A Phase II study of Erbitux (IMC-C225), an epidermal growth factor receptor (EGFR) blocking antibody, in combination with docetaxel in chemotherapy refractory/resistant patients with advanced non-small cell lung cancer (NSCLC). Proc. Am. Soc. Clin. Oncol., *21:* 293a, 2002.

107. Abbruzzese, J. L., Rosenberg, A., Xiong, Q., LoBuglio, A., Schmidt, W., Wolff, R., Needle, M., and Waksal, H. Phase II study of anti-epidermal growth factor receptor (EGFr) antibody cetuximab (IMC-C225) in combination with gemcitabine in patients with advanced pancreatic cancer. Proc. Am. Soc. Clin. Oncol., *20:* 518, 2001.

108. Curnow, R. T. Clinical experience with CD64-directed immunotherapy. An overview. Cancer Immunol. Immunother., *45:* 210–215, 1997.

109. Bier, H., Hoffmann, T., Hauser, U., Wink, M., Ochler, M., Kovar, A., Muser, M., and Knecht, R. Clinical trial with escalating doses of the antiepidermal growth factor receptor humanized monoclonal antibody EMD 72 000 in patients with advanced squamous cell carcinoma of the larynx and hypopharynx. Cancer Chemother. Pharmacol., *47:* 519–524, 2001.

110. Townsley, C., Major, P., Siu, L. L., Dancey, J., Vincent, M., Pond, G., and MacLean, M. A Phase I pharmacokinetic (PK) and serial tumor and skin pharmacodynamic (PD) study of weekly, every 2 weeks or every 3 weeks 1-hour (h) infusion EMD72000, an humanized monoclonal anti-epidermal growth factor receptor (EGFR) antibody, in patients (p) with advanced tumors known to overexpress the EGFR. Eur. J. Cancer, *38* (Suppl. 7)*:* 69, 2002.

111. Luwor, R. B., Johns, T. G., Murone, C., Huang, H. J., Cavenee, W. K., Ritter, G., Old, L. J., Burgess, A. W., and Scott, A. M. Monoclonal antibody 806 inhibits the growth of tumor xenografts expressing either the de2–7 or amplified epidermal growth factor receptor (EGFR) but not wild-type EGFR. Cancer Res., *61:* 5355–5361, 2001.