EGFR INHIBITORS

PRECLINICAL AND CLINICAL EVALUATIONS OF ABX-EGF, A FULLY HUMAN ANTI-EPIDERMAL GROWTH FACTOR RECEPTOR ANTIBODY

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The epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein, with an extracellular ligand-binding domain and intracellular tyrosine kinase domain. Ligand binding induces EGFR dimerization and autophosphorylation on several tyrosine residues in the intracellular domain, leading to mitogenic signal transduction. EGFR overexpression occurs with a poor prognosis and is often associated with malignant transformation in a variety of epithelial cancers. ABX-EGF is a high-affinity (dissociation constant $K_D = 5 \times 10^{-11}$ M) fully human IgG2 monoclonal antibody against human EGFR. ABX-EGF binds EGFR and blocks receptor binding of EGF and transforming growth factor-$\alpha$, inhibiting EGFR tyrosine phosphorylation and tumor cell activation. ABX-EGF prevents tumor formation and eradicates large, established A431 tumors in xenograft models. Tumor growth inhibition occurs at relatively low doses, without concomitant chemotherapy or radiotherapy. When combined with chemotherapeutic agents, ABX-EGF has resulted in additive antitumor activity. A Phase I clinical trial has demonstrated activity in several tumor types, and the results from a Phase II trial for renal cell cancer also showed modest activity. Therapy was generally well tolerated without statistically significant adverse events. Monoclonal antibody blockade of EGFR represents a new and exciting direction in cancer therapy. © 2004 Elsevier Inc.

INTRODUCTION

The epidermal growth factor receptor (EGFR) is a member of the erbB family of four related cell membrane receptors, including EGFR (Her1 or erbB1), erbB2 (Her2), erbB3 (Her3), and erbB4 (Her4). These are transmembrane glycoprotein receptors, each of which has an extracellular ligand-binding domain. The intracellular domain has tyrosine kinase activity for signal transduction. A number of ligands, including epidermal growth factor (EGF), transforming growth factor-$\alpha$ (TGF-$\alpha$), and amphiregulin, bind to the extracellular domain of EGFR. After ligand binding, the receptor dimerizes with another EGFR monomer (homodimerization) or another member of the erbB family (heterodimerization) (1). This leads to EGFR tyrosine autophosphorylation, followed by phosphorylation of several intracellular molecules, with subsequent signaling of a variety of cellular events (2, 3). The ras-raf-mitogen–activated protein kinase pathway (4) and phosphatidylinositol 3-kinase and the downstream protein kinase Akt that regulates apoptosis, gene expression, and cell proliferation (5–7) are the two major pathways involved in tumorigenesis. EGFR overexpression correlates with a poor prognosis and may be associated with malignant transformation in a variety of epithelial cancers (8, 9). Concomitant with EGFR overexpression, the expression of one of the receptor ligands, usually TGF-$\alpha$, is also typically upregulated (10–12). Blockade of the TGF-$\alpha$/EGFR autocrine pathway is a proposed therapeutic modality (8, 9, 13).

ABX-EGF is a high-affinity (dissociation constant $[K_D] = 5 \times 10^{-11}$ M) fully human IgG2 monoclonal antibody
(mAb) against human EGFR. In this report, we discuss the development of ABX-EGF using XenoMouse® technology and present the preclinical and clinical data.

XENOMOUSE TECHNOLOGY TO GENERATE FULLY HUMAN MONOCLONAL ANTIBODIES

Hybridoma technology, introduced by Kohler and Milstein (14) 25 years ago, suggested the potential of mAbs for human therapy. The first-generation mAbs were murine derived and immunogenic in humans, leading to human anti-mouse antibody responses. To overcome this problem, efforts were made to engineer part-human, part-mouse chimeric mAbs and “humanized” mAbs (Fig. 1). Chimeric mAbs are generated by genetically combining the antigen-binding regions (Fv) of the mouse antibody with human IgG constant domains. The resulting chimeric antibody consists of 34% mouse protein (15). Humanized mAbs are constructed by “implanting” the complementary domain regions of the mouse antibody into the human IgG framework. The humanized antibody still contains 5–10% mouse protein sequences (16). Chimeric and humanized mAbs have improved therapeutic utility and markedly reduced immunogenicity compared with murine mAbs but may still be immunogenic and allergenic in a fraction of patients (17, 18). The goal of establishing a fully human mAb was accomplished by the development of the XenoMouse® mice, in which human immunoglobulin genes were introduced into mice genetically engineered to lack functional mouse immunoglobulin expression (19–23).

ABX-EGF, A FULLY HUMAN MONOCLONAL ANTIBODY TO EGFR

TGF-α and EGF regulate cell proliferation and differentiation through binding to the EGFR. Elevated levels of EGFR expression are found on many malignancies, including most squamous cell carcinomas, adenocarcinomas, and gliomas (10–12). Overexpression may be a consequence of gene amplification, as in the case of many gliomas, or, more commonly, of increased gene transcription (11). It was proposed that EGFR overexpression plays a role in the tumorigenesis of cancer cells (24). Invasiveness and poor differentiation correlate with the high number of EGFRs in some tumors (25). Moreover, several tumors have shown an association of increased EGFR expression with detectable levels of its ligand, TGF-α (26).

Blocking mAbs to the human EGFR have been generated in rodents (8, 9, 13, 27) and characterized in murine xenografts (8, 9, 13). Some mAbs caused tumor growth inhibition and eradication when combined with concomitant chemotherapy (13, 28). The chimeric version of the mouse anti-EGFR mAb (C225) demonstrated improved binding affinity and in vivo antitumor activity at high doses (29), establishing a rationale for antibody therapy targeting EGFR. However, C225 is a mouse-human chimeric antibody and appears to be immunogenic in about 5% of patients (17, 18). We generated a panel of fully human IgG2 mAbs against EGFR using the XenoMouse® technology (Fig. 2) (30, 31). These anti-EGFR mAbs demonstrated high binding affinity to EGFR (Kd = 10^-9 to 10^-10 M) (30). In vitro, ABX-EGF, formerly known as clone E7.6.3 (30), bound EGFR with an affinity of 5 × 10^-11 M, blocked binding of EGF and TGF-α to the receptor, and inhibited EGF-activated EGFR tyrosine phosphorylation and tumor cell activation. This resulted in inhibition of in vitro tumor cell proliferation.

Treatment with ABX-EGF prevented and eradicated A431 tumors in xenografts, without concomitant chemotherapy or radiotherapy (30). The antitumor effect of ABX-EGF was also seen in multiple human solid tumors derived from different tissues and displaying different EGFR expression levels in xenograft models (Fig. 3) (32).

EGFR expression on the cell surface was quantitated using flow cytometric analysis of immunofluorescently stained cells. The EGFR number was determined by analytical flow cytometry in conjunction with fluorescent R-phycocerythrin-bound microbead standards (33). EGFR ex-
pression levels on human breast, epidermal, renal, pancreatic, prostate, ovary, and colon carcinoma cell lines ranged from 0 to 1.6 million copies/cell (Fig. 3). Tumor cells (5 × 10⁶ cells/mouse) from kidney (SK-RC-29), pancreas (BxPC-3, HS766T, and HPAC), prostate (PC3), ovary (IGROVI), or colon (HT-29 and SW707) tissues were inoculated subcutaneously into nude mice. Mice bearing established tumors were treated with 1 mg ABX-EGF twice weekly for 3 weeks. ABX-EGF treatment led to significant growth inhibition of multiple tumor xenografts, including SK-RC-29, BxPC-3, IGROVI, PC3, HS766T, and HT-29 (32). These data supported the concept that EGFR expression may be accompanied by tumor growth dependency on the EGFR pathway and that blockade of the EGFR pathway with EGFR blocking antibodies, such as ABX-EGF, can lead to tumor growth arrest and eradication. These data also indicated that ABX-EGF could inhibit the growth of, not only the tumors that express extremely high EGFR levels, such as A431 and MDA-MB-468, but also other human carcinomas that express lower EGFR levels (Fig. 3). Moreover, ABX-EGF therapy in combination with chemotherapeutic agents resulted in additive antitumor activity in the A431 tumor model (Fig. 4) (34). Recent preclinical and clinical data have suggested that EGFR expression levels alone may not be an adequate predictor of response to EGFR-inhibitory therapy. Therefore, it is critical to understand the mechanism of action of EGFR inhibitors as a cancer therapy.

The mechanism of in vivo ABX-EGF antitumor activity may involve the downregulation of EGFR expression by triggering receptor internalization, induction of apoptosis triggered by blocking EGFR signaling pathways and induction of cell cycle arrest, and inhibition of angiogenesis (30, 32, 34–36). Because ABX-EGF is a human IgG2 antibody that essentially lacks effector functions, complement-dependent and antibody-dependent cell-mediated cytotoxicities likely do not account for the in vivo antitumor activity.

**CLINICAL TRIALS**

A Phase I, multicenter, open-label, multiple-dose, dose-escalating clinical trial was initiated for patients with renal, prostate, pancreatic, non–small-cell lung, colorectal, or gastroesophageal cancer. Patients received four intravenous ABX-EGF infusions once weekly for ≤1 h, from 0.01 to 2.5 mg/kg (37).

The primary objective of this Phase I study was to determine the tolerability of multidose ABX-EGF administration. The primary statistical objective was to determine the maximal tolerated dose of ABX-EGF. The study was designed so that when a single patient within a cohort experienced a dose-limiting toxicity, dosing of a higher dose cohort would not be initiated until 2 additional patients had been enrolled and dosed without exhibiting a dose-limiting toxicity. If one or both of these patients exhibited the same toxicity, the dose level below this dose would be considered the maximal tolerated dose. The secondary objectives of the study were pharmacokinetics of different dose levels of ABX-EGF, the dose–response relationship of different dose levels of ABX-EGF, and assessing the clinical effect of different dose levels of ABX-EGF.

Immunohistochemical analysis was used to determine EGFR overexpression in fresh or archival paraffin-embedded tumor tissues. These tumor tissues had to overexpress EGFR to the level of 2+ or 3+ in ≥10% of evaluated tumor cells. A central laboratory, IMPATH, using an EGFR immunohistochemistry kit developed by Dako Corporation, in collaboration with Abgenix, determined the levels of EGFR expression in patient tumor samples.
Forty-three patients were treated weekly for 4 weeks with ABX-EGF. The patients had renal (n = 10), prostate (n = 13), non–small-cell lung (n = 7), pancreatic (n = 3), gastroesophageal (n = 3), or colorectal (n = 7) cancer. Transient acneiform skin rashes were the predominant toxicity and occurred in a dose-dependent manner. All patients receiving at least one dose of 2.0 or 2.5 mg/kg developed National Cancer Institute Common Toxicity Criteria Grade 1 or worse skin rashes. To date, no infusion-related or serious adverse events have been observed. Human anti-human antibodies (HAHAs) have not been detected. One patient with esophageal cancer had stable disease for 7 months at the 0.1-mg/kg dose; 1 patient with

Fig. 3. EGFR-dependent tumor inhibition by ABX-EGF. Human carcinoma cells (5 × 10⁶) expressing different amounts of EGFR on cell surfaces were inoculated subcutaneously into nude mice (n = 10) at Day 0. Tumor sizes were measured twice weekly for >8 weeks. ABX-EGF (1 mg) (filled triangles) was given intraperitoneally twice weekly for 3 weeks from the first day of tumor measurement. Control mice (open circles) received no treatment. (A) SK-RC-29, renal carcinoma; (B) BxPC-3, pancreatic carcinoma; (C) IGROVI, ovarian carcinoma; (D) PC-3, prostate carcinoma; (E) HS766T, pancreatic carcinoma; (F) HPAC, pancreatic carcinoma; (G) HT-29, colon carcinoma; and (H) SW707, colon carcinoma. ABX-EGF inhibited growth of tumors that expressed >17,000 EGFRs/cell. Data presented as mean tumor size ± SE. Adapted from Yang et al. (32) with permission from Critical Review in Oncology/Hematology 2001, Elsevier Ireland Ltd.
prostate cancer had a minor response lasting for 6 months at the 0.75-mg/kg dose, with three or four lesions regressing 38–76% and prostate-specific antigen levels decreasing by 60%; and 1 patient with colorectal cancer had stable disease for 4 months at the 1.5-mg/kg dose. One patient with colorectal cancer had a partial response at the 2.5 mg/kg dose (Table 1).

Part one of a two-part Phase II monotherapy trial, consisting of eight weekly infusions of ABX-EGF, was performed in patients with renal cell cancer in whom interleukin-2/interferon-α therapy had failed or who were unable to receive it (38). Stable or responding patients were eligible for extended weekly treatment at the assigned dose for eight additional months or until disease progression. In Part 1, 88 patients received at least one dose of ABX-EGF at 1.0 mg/kg (22 patients), 1.5 mg/kg (22 patients), 2.0 mg/kg (23 patients), or 2.5 mg/kg (21 patients). EGFR overexpression was documented in 91% of patients. Only 11% of patients had received no prior biotherapy or chemotherapy. Most patients were heavily pretreated, having received one to two (56%) or three or more (33%) prior regimens. Three patients had a partial response (one each from the 1.0-, 1.5-, and 2.5-mg/kg dose levels) and two had minor responses (one each from the 1.0- and 2.5-mg/kg dose levels). Fifty percent of patients had stable disease at the end of the first 8-week treatment period. Similar to the Phase I study, a transient acneiform skin rash was the primary toxicity, observed in 70%, 91%, 95%, or 100% of patients treated with at least three doses of ABX-EGF at the 1.0-, 1.5-, 2.0-, or 2.5-mg/kg dose levels, respectively. Asthenia, pain, abdominal pain, back pain, constipation, cough, and dyspnea occurred in a dose-independent fashion. Importantly, no statistically significant allergic or infusion-related reactions were observed, and no HAHA responses have been detected. The absence of a HAHA response is consistent with the extremely low intrapatient variability of ABX-EGF exposure. Pharmacokinetics demonstrated that EGFR-mediated clearance of ABX-EGF was saturated at a dose of 2 mg/kg. A 100% skin rash incidence was achieved with increasing doses to 2.5 mg/kg, suggesting full receptor occupancy at this dose level. Serum ABX-EGF concentrations at the dose of 2.5 mg/kg were similar to serum ABX-EGF concentrations that resulted in >90% inhibition of A431 tumor growth in xenograft mice. Acneform rash is considered an indirect marker of clinically relevant EGFR targeting and saturation in vivo. In this respect, recent studies have suggested that the presence and intensity of acneform rash that resulted from treatment with EGFR inhibitors may predict increased survival in patients with different malignancies (39, 40).

**CONCLUSION**

The autocrine-driven EGFR pathway is a rational target for cancer therapy. The blockade of EGFR signaling in cancer cells by ABX-EGF determines not only inhibition of EGFR tyrosine kinase and cell proliferation, but also other effects that are critical for tumor survival, growth, and metastasis. These include antiangiogenesis effects by inhibition of tumor cell production of angiogenic growth factors (vascular endothelial growth factor and interleukin-8), and...
anti-invasive and antimetastatic effects by inhibition of matrix metalloproteinase production (35, 36).

As a potent inhibitor of the EGFR signaling pathway, ABX-EGF has demonstrated in vitro and in vivo antitumor activity and encouraging results in clinical trials. ABX-EGF has been well tolerated and has shown an excellent safety profile with very mild and reversible side effects. As a fully human monoclonal antibody, ABX-EGF has shown no detectable HAHA responses in patients who received varying doses of ABX-EGF. The pharmacokinetic variability between and within patients has been very low, allowing consistent exposure to the drug. The results from Phase I and II trials (Table 1) have demonstrated that ABX-EGF has acceptable tolerability and encouraging clinical activity in patients with a variety of tumor types.

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