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Development of ABX-EGF, a fully human anti-EGF receptor monoclonal antibody, for cancer therapy

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Contents

Abstract

Overexpression of epidermal growth factor receptor (EGFr) has been demonstrated on many human tumors, and the increase in receptor expression levels has been linked with a poor clinical prognosis. Blocking the interaction of EGFr and the growth factors could lead to the arrest of tumor growth and possibly result in tumor cell death. To this end, using XenoMouse™ technology, ABX-EGF, a human IgG2 monoclonal antibody (mAb) specific to human EGFr, has been generated. ABX-EGF binds EGFr with high affinity (5×10^{-11} M), blocks the binding of both EGF and transforming growth factor-α (TGF-α) to various EGFr-expressing human carcinoma cell lines, and inhibits EGF-dependent tumor cell activation, including EGFr tyrosine phosphorylation, increased extracellular acidification rate, and cell proliferation. In vivo ABX-EGF prevents completely the formation of human epidermoid carcinoma A431 xenografts in athymic mice. More importantly, administration of ABX-EGF without concomitant chemotherapy results in complete eradication of established tumors. No tumor recurrence was observed for more than 8 months following the last antibody injection, further indicating complete tumor cell elimination by the antibody. Inhibition of human pancreatic, renal, breast and prostate tumor xenografts which express different levels of EGFr by ABX-EGF was also achieved. Tumor expressing more than 17 000 EGFr molecules per cell showed significant growth inhibition when treated with ABX-EGF. ABX-EGF had no effect on EGFr-negative tumors. The potency of ABX-EGF in eradicating well-established tumors without concomitant chemotherapy indicates its potential as a monotherapeutic agent for treatment of multiple EGFr-expressing human solid tumors, including those where no effective chemotherapy is available. Utilization of mAbs directed to growth factor receptors as cancer therapeutics has been validated recently by the tumor responses obtained from clinical trials with Herceptin, the humanized anti-HER2 antibody, in patients with HER2 overexpressing metastatic breast cancer. Being a fully

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human antibody, ABX-EGF is anticipated to exhibit a long serum half-life and minimal immunogenicity with repeated administration, even in immunocompetent patients. These results demonstrate the potent anti-tumor activity of ABX-EGF and its therapeutic potential for the treatment of multiple human solid tumors that overexpress EGFr.

Keywords: EGFr; Human monoclonal antibody; Cancer therapy; XenoMouse

1. Production of human monoclonal antibody using XenoMouse technology

1.1. Evolution of monoclonal antibody technology

With the development of hybridoma technology by Kohler and Milstein more than 25 years ago, the potential of monoclonal antibodies (mAbs) for human therapy was easily appreciated [1]. However, the promise of the 'magic bullet' was not immediately realized since the first generation of mAbs was derived from mouse and the mouse mAbs were in general immunogenic in humans leading to the generation of human anti-mouse antibody (HAMA) responses, thus limiting efficacy in long term and repeated administration.

To reduce the immunogenicity of the mouse mAbs and to overcome the problem of HAMA, tremendous efforts have been made to engineer part-human, part-mouse 'chimeric' mAbs and mostly human 'humanized' mAbs (Fig. 1). The chimeric mAb is created 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 [2]. The humanized mAbs are constructed by 'implanting' the complementary-determining regions (CDR) of the mouse antibody into the human IgG framework. The humanized antibody still contains approximately 5–10% mouse protein sequences [3]. Although the chimeric and humanized recombinant mAbs have improved the therapeutic utility, they require significant protein engineering efforts and could be still immunogenic [2,3]. Therefore, to fully realize the value of mAb technology, the ultimate goal is to establish a simple and robust system by which fully human mAbs can be readily generated. To this end, transgenic strains of mouse, XenoMouse strains, have been created by introducing human immunoglobulin genes into mice engineered to lack functional mouse immunoglobulin genes.

1.2. XenoMouse technology and fully human monoclonal antibodies

To reconstitute a robust human humoral immune system in mice, the mouse endogenous Ig gene loci were functionally inactivated by gene targeted deletions of crucial *cis*-acting sequences of heavy and light (κ) chains involved in the mouse Ig gene rearrangement and expression, J_H and C_κ, respectively [4,5]. The mouse embryonic stem (ES) cells containing the targeted heavy or κ chain allele were utilized to generate mice homozygous for each of the J_H or C_κ deletions. Crossbreeding of these mice yielded double-inactivated (DI) mice, homozygous for both mutations. In order to preserve the variable gene diversity and to maintain the regulatory elements that control antibody recombination and expression, DNA fragments spanning the majority of the human heavy and light chain loci were cloned and recombined into yeast artificial chromosomes (YACs) in germline configuration. The heavy chain YACs encompassed 66 different consecutive V_H genes (representing about 80% of the human V_H gene repertoire), all 30 D segments, and all six J_H genes. The Cδ region was retro-fitted with the human γ1, γ2 or γ4 constant region.C_κ constant region. The heavy and light chain YACs were introduced into mice via fusion of YAC-containing yeast spheroplasts with mouse ES cells [5–7]. Crossbreeding of these mouse strains resulted in the transgenic mice producing human antibodies in the presence of mouse antibodies. Finally the XenoMouse strains which are homozygous for the inactivated mouse heavy and κ-chain genes

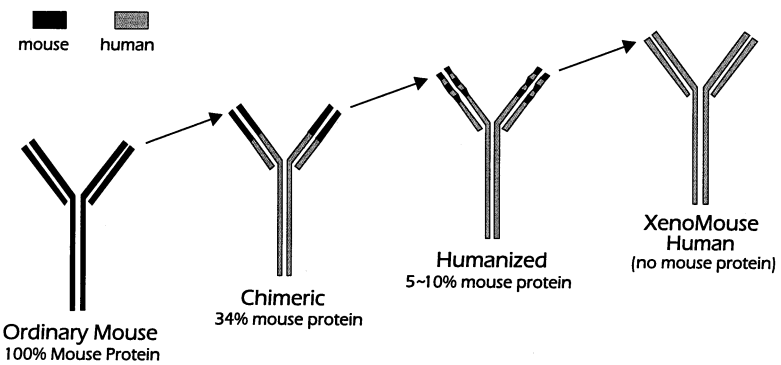

Fig. 1. Evolution of monoclonal antibody technology.

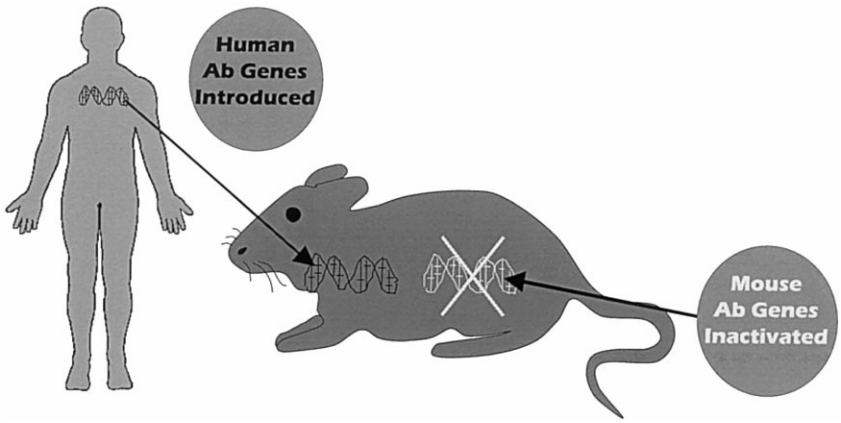

Fig. 2. Creation of XenoMouse technology. XenoMouse strains were created by introducing human Ig genes into the mice, which carry the inactivated mouse Ig gene loci.

and bearing one allele each of the human heavy and the κ chain were created by breeding the human antibody-producing transgenic mice with the DI mouse strain [7] (Fig. 2).

Analysis of XenoMouse lymphoid organs and serum revealed that B cell development and the serum human antibody responses were restored [8]. More importantly, diverse human antibody responses to multiple antigens were demonstrated in the XenoMouse strains. Upon immunization with either soluble antigens such as IL-8 or cell-bound antigens such as EGFr, high affinity fully human mAbs were generated using standard hybridoma technology [7]. XenoMouse technology offers a unique and reliable source for rapid generation and production of antigen-specific and high affinity fully human therapeutic mAbs. Fully human mAbs are anticipated to be nonimmunogenic and thus to allow repeated administration without human anti-human antibody response. This provides a significant advantage in antibody therapy of chronic and recurring human disease, such as cancer. In this article, the properties and the anti-tumor activity of a XenoMouse-derived fully human anti-EGFr antibody, ABX-EGF, are summarized.

2. ABX-EGF, a fully human monoclonal antibody to EGF receptor

2.1. EGFr overexpression on human tumors

Various growth factors and growth factor receptors have been implicated in a wide variety of human cancers. EGF is a mitogenic hormone that regulates the proliferation and differentiation of normal and neoplastic cells in vitro as well as in vivo. Production of TGF-α, an EGF-related polypeptide growth factor, has been associated with cellular transformation. The EGFr, which binds both EGF and TGF-α, is structurally related to the transforming protein encoded by the v-erbB oncogene of avian erythroblastosis virus. This homology between EGFr and an oncogene product suggests that growth factor receptor abnormality may contribute to neoplastic transformation [15].

Elevated levels of EGFr expression characterize a wide range of carcinomas such as epidermal and squamous cell carcinomas, non-small cell lung cancer, and gliomas [9–11]. The overexpression is either the consequence of gene amplification, as in the case of many gliomas, or, more commonly, of increased gene transcription [10]. It was proposed that overexpression of EGFr plays a role in the tumorigenesis of cancer cells [12]. Indeed, invasiveness and poor differentiation were found to correlate with the high number of EGF receptors in some tumors such as bladder [13]. Moreover, several tumors show association of increased EGFr expression with detectable levels of its ligand, TGF-α, and TGF-α can act as a potent mitogen in cells overexpressing EGFr, suggesting the involvement of the EGFr/TGF-α autocrine loop in the development of some human malignancies [14].

Numerous neutralizing mAbs specific to the human EGFr have been generated from rodents [15–19]. Some of these antibodies, such as the murine mAbs 225 and 528, have been extensively characterized and evaluated in mouse xenograft models [15–17]. When administered into mice bearing established human tumor xenografts, 225 and 528 mAbs caused a partial tumor regression and required concomitant chemotherapy for complete eradication of the tumors [16,17]. The chimeric version of the 225 mAb (C225) showed an improved binding affinity and in vivo anti-tumor activity at the high doses [20]. These data established a rationale for antibody therapy targeting EGFr expressing solid tumors. However, being a mouse or mouse-human chimeric antibody and the requirement for chemotherapy can limit the efficacy and utilization of anti-EGFr antibodies for patients who developed HAMA responses and patients with cancers for which chemotherapy is unavailable.

Therefore, the identification of a fully human anti-EGFr mAb that can be used as monotherapy may expand the patient population and allow repeated administrations in immunocompetent patients. A panel of fully human mAbs was generated against EGFr using the XenoMouse technology [21].

2.2. Generation and selection of ABX-EGF

A panel of human IgG2 anti-EGFr mAbs was generated by immunizing the XenoMouse IgG2 strain with cells of the human cervical epidermal carcinoma cell line A431, which expresses more than 10^6 EGFrs per cell. A total of 70 EGFr-specific hybridomas were established from five fusions. Among these, at least 15 are neutralizing antibodies. The anti-EGFr mAbs demonstrated high binding affinity to EGFr (10^{-9}–10^{-11} M) [22]. In vitro, ABX-EGF, formerly known as clone E7.6.3 [21], binds EGFr with an affinity of 5×10^{-11} M, blocks the binding of both EGF and TGF-α to the receptor, and inhibits EGF-activated EGFr tyrosine phosphorylation and tumor cell activation. This results in the inhibition of in vitro tumor cell proliferation. ABX-EGF does not activate the EGFr tyrosine kinase. Upon binding to the receptor, ABX-EGF causes EGFr internalization in tumor cells (unpublished data). The ABX-EGF-producing hybridoma was found to have a relatively high productivity of 12 pg/cell per day in serum-free medium [21].

2.3. EGF receptor-dependent tumor inhibition

Administration of ABX-EGF led to the complete prevention of A431 tumor formation, eradication of established tumors, and inhibition of tumor growth at relatively low doses without concomitant chemotherapy or radiation therapy in xenograft tumor models. The data indicate that ABX-EGF can provide effective therapy to tumors that require the EGFr pathway for their continuous progression and survival. To verify further this concept and to elucidate the mechanism of action, we evaluated the anti-tumor effect of ABX-EGF on multiple human solid tumors that are derived from different tissues and display different expression levels of EGFr in xenograft models.

The EGFr expression level on the cell surface was quantitated using flow cytometric analysis of immunofluorescent stained cells. The number of EGFr was determined by analytical flow cytometry in conjunction with fluorescent R-phycoerythrin (R-PE) bound microbead standards [23]. Cells were incubated in the presence of mouse anti-EGFr antibody 225, washed, and stained with R-PE-conjugated goat-anti-mouse IgG. The resulting cellular fluorescence intensity was extrapolated onto a standard fluorescence calibration curve derived from an R-PE quantitative fluorescence standard kit (Flow Cytometry Standards Corporation, San Juan, PR). The microbead standard consisted of goat-anti-mouse IgG covalently bound to the exterior of polymeric beads. The calibration curve was generated by plotting the given number of equivalent bound R-PE molecules per bead versus the log of its mean fluorescence intensity. As shown in Table 1, the EGFr expression levels on human breast, epidermal, renal, pancreatic, prostate, ovarian and colon carcinoma cell lines ranged from 0 to 1.6 million copies per cell.

To determine whether the EGFr expression level on tumor cells correlates with their responses to ABX-EGF-mediated anti-tumor therapy, the tumor cells

Table 1
Effect of ABX-EGF on human tumor xenografts expressing different levels of EGFr

Tumor	Tissue of origin	EGFr expression[a] (EGFr number/cell)	Inhibition of tumor[b]
MDA-MB-468	Breast	1 600 000	Yes
A431	Epidermal	1 200 000	Yes
SK-RC-29	Renal	77 000	Yes
BxPC-3	Pancreatic	63 000	Yes
PC-3	Prostate	30 000	Yes
IGROVI	Ovarian	30 000	Yes
HS766T	Pancreatic	17 000	Yes
HPAC	Pancreatic	11 000	No
HT29	Colon	9000	No
SW707	Colon	0	No

[a] Cell surface EGFr numbers were quantitated by analytical flow cytometry using fluorescence standard kit.
[b] The inhibition of MDA-MB-468 and A431 tumor xenografts were shown in the previous study [21]. The experimental procedure and data are presented in Fig. 3.

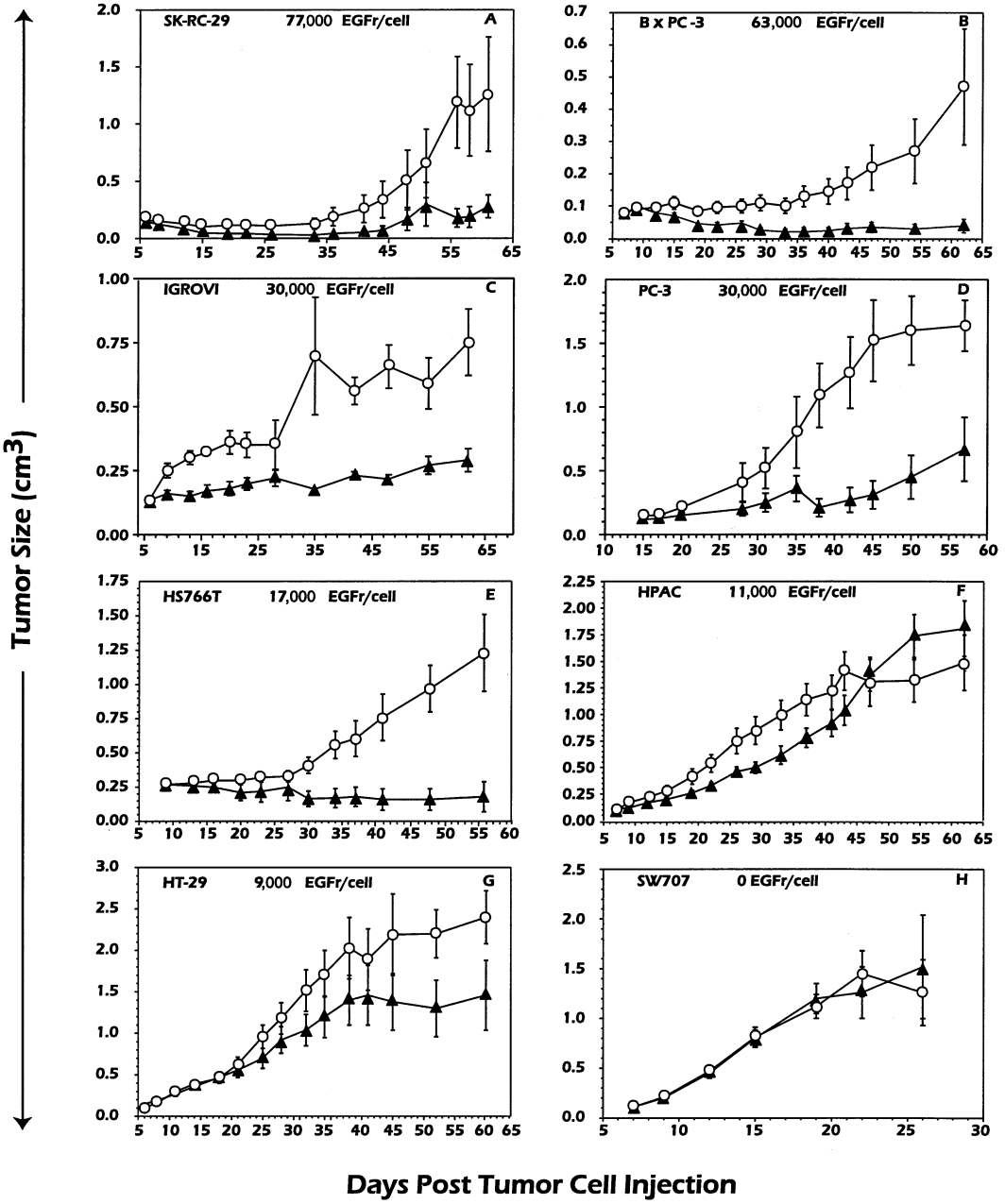

Fig. 3. EGFr-dependent tumor inhibition by ABX-EGF. Human carcinoma cells (5×10^6) expressing different EGFr numbers on the cell surface were inoculated s.c. into nude mice ($n = 10$) at Day 0. Tumor sizes were measured twice a week for more than 8 weeks. ABX-EGF (1 mg) (filled triangles) was given i.p. twice a week for 3 weeks from first day of tumor measurement. Control mice (○) 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 the tumors which express $>17\,000$ EGFrs per cell. The data are presented as the mean tumor size $\pm$ S.E.

derived from kidney (SK-RC-29), pancreas (BxPC-3, HS766T, and HPAC), prostate (PC3), ovary (IGROVI) and colon (HT-29 and SW707) were inoculated subcutaneously (s.c.) into nude mice (5×10^6 cells/mouse). The mice were then treated intraperitoneally with 1mg ABX-EGF twice a week for 3 weeks. Tumor sizes were measured twice a week. As demonstrated in Fig. 3, ABX-EGF treatment led to significant growth inhibition of tumors expressing 17 000 or more EGFrs per cell. In contrast, the growth of tumors expressing 11 000 or fewer EGFrs per cell was unaffected by the ABX-EGF treatment. Importantly, ABX-EGF had no effect at all on the EGFr-negative tumor SW707. These data strongly support the concept that overexpression of EGFr on tumors is accompanied by growth-dependency on the EGFr pathway and that blocking the EGFr pathway with neutralizing anti-EGFr antibodies such as ABX-EGF can lead to tumor growth arrest and

tumor eradication. Furthermore, the data also indicate that ABX-EGF can inhibit not only the tumors that express extremely high level of EGFr such as A431 and MDA-MB-468 but also other human carcinomas which express substantially lower levels of EGFr (Table 1). Considering the fact that normal human skin keratinocytes express approximately 9200 EGFrs per cell, ABX-EGF may not have significant impact on the growth of EGFr-positive normal cells such as keratinocytes [23].

The mechanism behind the potent in vivo anti-tumor activity of ABX-EGF remains elusive but may involve the induction of apoptosis triggered by blocking EGFr signaling pathways, inhibition of angiogenesis, and/or inhibition of differentiation of the tumor cells. Since ABX-EGF is a human IgG2 antibody that essentially lacks effector functions, complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity probably do not account for the in vivo anti-tumor effect. This is further supported by the fact that the E7.5.2 antibody, which is also a human IgG2 anti-EGFr antibody derived from the same XenoMouse IgG2 strain but is nonneutralizing, had no significant inhibitory effect on A431 xenograft tumor growth (Fig. 4).

3. Conclusion

XenoMouse technology has been proven to be a useful tool to develop human therapeutic mAbs for the treatment of variety of diseases, including cancer and inflammatory diseases [21,24]. The XenoMouse-derived fully human anti-EGFr mAb, ABX-EGF, demonstrated potent anti-tumor activity as a monotherapeutic agent in a variety of human carcinoma xenografts that overexpress EGFr. The observation that tumor growth inhibition by ABX-EGF is dependent on EGFr number suggests that the growth of EGFr overexpressing tumors may mainly rely on the EGFr pathway to proliferate and ABX-EGF exerts anti-tumor activity via efficient blockade of this growth pathway. With its significant anti-tumor activity, anticipated minimal antigenicity, and longer serum half-life, ABX-EGF may prove to be a potent therapeutic agent for the treatment of cancer. Based on these data, a phase I clinical trial with ABX-EGF in multiple patient populations with EGFr-expressing solid tumors has been initiated.

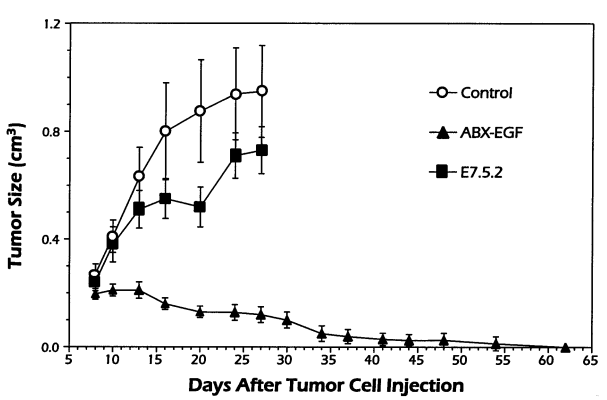

Fig. 4. ABX-EGF mediated anti-tumor effect depends on its neutralizing activity. Human epidermal carcinoma A431 cells (5×10^6) were injected s.c. into nude mice at Day 0 ($n = 10$). Tumor sizes were measured twice a week. ABX-EGF or non-neutralizing human IgG2 anti-EGFr antibody, E7.5.2 (1 mg) were given i.p. twice a week for 3 weeks from the first day of tumor measurement. The anti-tumor effect is dependent upon the neutralizing activity of ABX-EGF. The data are presented as the mean tumor size ± S.E.

Reviewers

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Biography

Xiao-Dong Yang is Senior Scientist and the head of the Preclinical Biology Division at Abgenix, Inc.