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From XenoMouse technology to panitumumab, the first fully human antibody product from transgenic mice

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Therapeutic monoclonal antibodies have shown limited efficacy and safety owing to immunogenicity of mouse sequences in humans. Among the approaches developed to overcome these hurdles were transgenic mice genetically engineered with a 'humanized' humoral immune system. One such transgenic system, the XenoMouse, has succeeded in recapitulating the human antibody response in mice, by introducing nearly the entire human immunoglobulin loci into the germ line of mice with inactivated mouse antibody machinery. XenoMouse strains have been used to generate numerous high-affinity, fully human antibodies to targets in multiple disease indications, many of which are progressing in clinical development. However, validation of the technology has awaited the recent regulatory approval of panitumumab (Vectibix), a fully human antibody directed against epidermal growth factor receptor (EGFR), as treatment for people with advanced colorectal cancer. The successful development of panitumumab represents a milestone for mice engineered with a human humoral immune system and their future applications.

The discovery of mouse monoclonal antibodies (mAbs) in 1975 as high-affinity and high-specificity proteins[1] generated great expectations for their utilization as targeted therapeutics for cancer, inflammation, cardiovascular, autoimmunity and infectious diseases. However, it took more than a quarter century for the therapeutic potential of mAbs to be realized, with the majority of the 21 commercialized mAbs achieving US Food and Drug Administration (FDA) regulatory approval during the last 10 years. Although better selection of targets and disease indications suitable for antibody therapy played a role in this advance, the predominant factor for the surge in successful development of therapeutic mAbs is the technical progress made in generating safer, more stable and efficacious mAbs, primarily by making them more 'human-like'. Until the early 1990s, most product candidates in development were mouse mAbs that elicited production of human anti-mouse antibodies in humans, resulting in rapid clearance, limited efficacy and safety risks, such as immunogenicity and allergic reactions[2]. The limitations of mouse mAbs were especially problematic in the treatment of chronic and recurring human diseases that require repeated antibody administration. These issues became the driving force for the development of numerous approaches to generate partially or fully human mAbs. Application of the hybridoma methodology to generate human mAbs from human B cells was limited by the scarcity of human B cells expressing antibodies of the desired antigen specificity and affinity and by difficulty in achieving immortalization[3]. The replacement of parts of the mouse mAbs with human sequences to generate chimeric[4] or humanized antibodies[5] with reduced immunogenicity requires case-by-case molecular modeling and engineering. Furthermore, these mAbs still retain some mouse sequences. The generation of large human immunoglobulin gene combinatorial libraries has opened an avenue for the cloning of antigen-specific fully human antibodies[6,7]. However, derivation of high-affinity human antibodies by this technology, particularly to human antigens, may require extensive *in vitro* manipulation[8]. The limitations associated with *in vitro* production of partially or fully human therapeutic mAbs redirected attention to the mouse machinery as a simple but robust tool for generation and selection of authentic human mAbs. Mice engineered with the human humoral immune system could harness the natural recombination and affinity maturation processes to generate a large and diverse repertoire of high-affinity antibodies to any target, including human antigens. Furthermore, the well-established mouse hybridoma technology provides an efficient and accessible process to derive and select the desired mAbs.

This rationale was the basis for generating XenoMouse strains, in which the inactivated mouse antibody machinery was 'humanized' with megabase (Mb)-sized human immunoglobulin loci to substantially reproduce the human humoral immune system in mice and to produce a wide diversity of high-affinity human mAbs.

Generation of XenoMouse strains

The strategy for the creation of XenoMouse aimed at recapitulation of the human humoral immune system in mice. It required two major genetic manipulations of the mouse genome: (i) inactivation of mouse antibody–production machinery, and (ii) stable cloning and introduction of human immunoglobulin heavy and light chain loci (**Fig. 1**). Both genetic modifications were carried out in mouse embryonic stem (ES) cells, which provided an effective tool for transmission of defined and selected genetic modifications into the mouse germ line. The mouse heavy and kappa (κ) light chain genes were inactivated in ES cells by gene-targeted deletion of crucial *cis*-acting sequences involved in the process of mouse immunoglobulin gene rearrangement and expression[9]. Deletion of the mouse J_H

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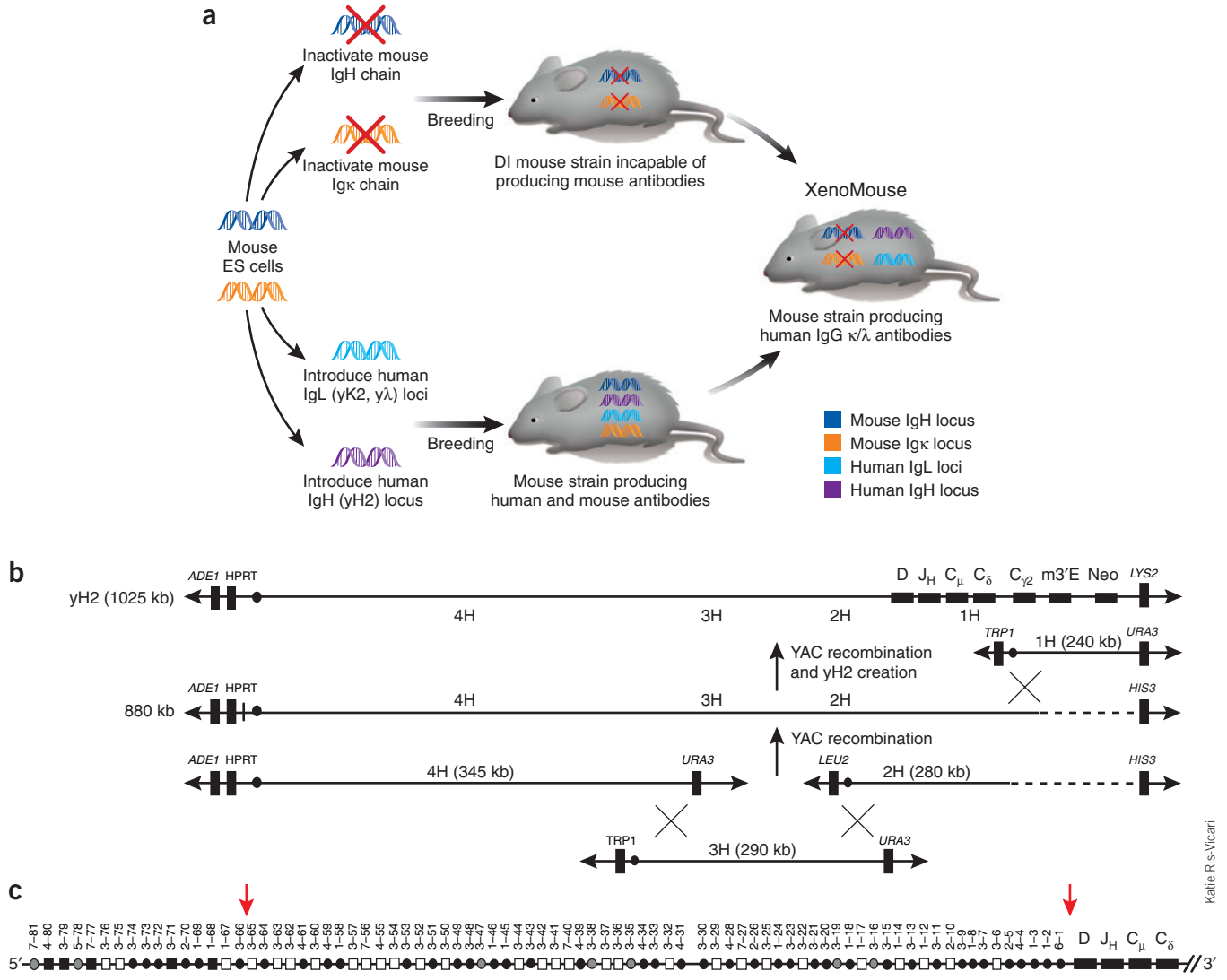

Figure 1 Creation of XenoMouse strains. (**a**) Mouse heavy and κ light chain were inactivated in ES cells by gene-targeting technology. The targeted ES cells were used to generate mice with homozygous deletions, in which mouse antibody production is disrupted. Human heavy, κ and λ light chain loci, present on YACs, were introduced into ES cells and the modified ES cells were used to generate mice containing human immunoglobulin loci and producing fully human antibodies in the presence of mouse antibodies. Breeding of these mice with immunoglobulin-inactivated mice generated XenoMouse strains with four genetic alterations, producing human antibodies in the absence of mouse antibodies. (**b**,**c**) Schematic representation of the reconstruction of human heavy chain locus on yH2 by stepwise homologous recombination in yeast of four cloned YACs (**b**) that span 880 kb out of the 1,000 kb of the human heavy chain variable region on chromosome 14q[16] (**c**). The V_H gene region contained on yH2 is marked by the red arrows. Additional gene segments identified on the locus[83] do not change the extent of the repertoire (80%) contained on the YACs and in XenoMouse strains.

region completely inhibited the heavy chain recombination machinery and thus abolished mouse immunoglobulin production[9]. Deletion of the mouse Cκ region inactivated the mouse Igκ locus. Successive crosses of J_H and Cκ homozygous mice led to a double-inactivated strain in which production of antibodies (**Fig. 1a**) and, therefore, B-cell development, were completely arrested[10,11]. These mice, however, maintained the necessary *trans*-acting factors for antibody rearrangement and expression and therefore provided the proper genetic background for introduction of unrearranged human immunoglobulin loci.

Recapitulation of the human antibody repertoire in mice emphasized the need for cloning and transferring large portions of the human immunoglobulin loci to preserve the large variable gene diversity and proper regulation of antibody maturation and expression. The genes encoding human immunoglobulin heavy and κ light chains each span over 1.5 Mb on chromosomes 14 and 2, respectively, containing hundreds of gene segments that encode and control the expression of the huge diversity of the humoral immune response. In their germline configuration, these loci consist of the variable segments encoding the variable (95 V_H, 76 Vκ genes), diversity (30 D_H genes) and joining (6 J_H and 5 Jκ genes) domains, the segments that encode the constant (C) domains, and the interspersed *cis*-regulatory elements, which control antibody expression, allelic exclusion, class switching and affinity maturation[12–14]. Therefore, reproduction of the human antibody response in mice demanded the ability to clone and reconstruct large portions of the human immunoglobulin heavy and light chain loci in their germline configuration, and their introduction in intact form into ES cells. Cloning of the large human heavy and light chain loci was facilitated by the yeast artificial chromosome (YAC) technology, which permitted the stable isolation and efficient genetic manipulation of Mb-sized DNA fragments[15]. By cloning and recombining DNA fragments in yeast, we reconstructed a 970-kb human heavy chain YAC, encompassing

in germline configuration 66 different consecutive V_H genes (80% of the human V_H gene repertoire), all 30 D_H and 6 J_H genes and the Cμ and Cδ constant regions (**Fig. 1b**,**c**)[16]. The Cδ region was retrofitted with either human γ1, γ2 or γ4 constant region, in conjunction with the mouse 3′ enhancer, to generate three different yH2 YACs, each equipped with a different heavy-chain isotype (IgG_1, IgG_2 or IgG_4). A similar cloning strategy was applied for reconstruction of an 800-kb yK2 YAC containing, in germline configuration, the human κ chain proximal locus, including 32 Vκ genes, 5 Jκ genes, Cκ region, κ deleting element and Igκ intronic and 3′ enhancers[16]. Inasmuch as the κ chain distal locus mainly duplicates the proximal region, and the proximal Vκ genes are the ones most commonly used in human, yK2 captures 80% of the human Vκ chain gene repertoire[17].

The large human yH2 and yK2 YACs were introduced into the mouse genome by the fusion of YAC-containing yeast spheroplasts and ES cells[18]. This methodology yielded a high frequency of ES cells in which the large DNA fragments are integrated in the mouse genome in intact and unrearranged form and are transmitted faithfully through the mouse germ line[18]. ES cells, containing intact yH2 or yK2 YACs, were used to transmit these human immunoglobulin fragments into the mouse germ line with no apparent deletions or rearrangements and to generate yH2- and yK2-bearing transgenic mice expressing human heavy or κ chain protein, respectively[16]. The crossbreeding of these mouse strains yielded a mouse strain expressing fully human antibodies in the presence of mouse antibodies. The crossbreeding of these mice with double-inactivated mice yielded XenoMouse strains that produce fully human antibodies[16] (**Fig. 1a**). Our strategy yielded three different XenoMouse strains, each producing fully human antibodies with only one of the three isotypes (IgG_1κ; IgG_2κ; IgG_4κ), allowing the generation of antigen-specific mAbs with the desired effector function for specific disease indications. Subsequently, the entire human Igλ chain locus on YAC was also introduced into XenoMouse strains to generate three mouse strains, XMG1-KL, XMG2-KL, XMG3-KL, each producing both human IgGκ and IgGλ antibodies (at a 60:40 ratio) with the corresponding isotype[19].

XenoMouse strains produce high-affinity fully human mAbs

Analysis of XenoMouse lymphoid organs and serum demonstrated that the human immunoglobulin YACs were fully compatible with the mouse machinery to restore proper B-cell development and antibody production[10,16]. Characterization of the mouse humoral immune system confirmed the faithful reconstitution of a human antibody response, from a broad primary immune response that utilizes the wide range of variable gene repertoire to a proper secondary response, including class switching and affinity maturation, leading to a large and diverse repertoire of authentic human antibodies[16,20]. Sequence analysis of XenoMouse-derived antibody transcripts revealed a broad and diverse utilization of the different V, D and J genes across the entire length of the YACs that is reminiscent of their utilization in humans[19,20]. The critical role of the large and diverse V gene repertoire in the recapitulation of human humoral response was validated when XenoMouse strains were compared to mice engineered with smaller V gene repertoires, whether contained on YACs[11] or on minigene constructs[21,22]. In addition to more efficient B-cell development and higher levels of circulating antibodies, the utilization at high frequency of V genes, which are rarely used in the overall repertoire, in sequences of mAbs with selected specificity and function, underscores the need for a large V gene array to support generation of antibodies to specific epitopes.

The utilization of the large human repertoire in XenoMouse for generation of a diverse human antibody response was manifested by the production of antigen-specific, high-affinity human antibodies to numerous antigens, including interleukin-8 (IL-8), EGFR and tumor necrosis factor-α[16,23], IL-6, L-selectin, GROα and CD147 (ref. 19), MUC18 (ref. 24), platelet-derived growth factor-D (PDGFR-D)[25], insulin-like growth factor receptor-1 (IGF-1R)[26], cytotoxic T lymphocyte–associated antigen 4 (CTLA-4)[27], CD40 (refs. 28,29), hepatocyte growth factor (HGF)[30], receptor activator of NF-κB (RANK) ligand[31] and prostate stem-cell antigen (PSCA)[32]. XenoMouse animals consistently produced mAbs of high affinity, 10^{-9} to 10^{-11} M, as a result of the broad and diverse utilization of the variable genes and the efficient affinity maturation processes in XenoMouse strains. In addition, the generated antibodies also demonstrated high potency in blocking the *in vitro* and *in vivo* biological effects of their respective antigens on human cells, indicating their potential therapeutic use[23–32]. The pharmacokinetics of mAbs are also influenced by their targets and the related biology, such that a mAb targeting a circulating antigen may have a different pharmacokinetics profile than a mAb targeting a cell surface receptor that can mediate internalization of the receptor-mAb complex. For example, administration of XenoMouse-derived anti-IL-8 mAb to subjects indicated pharmacokinetics similar to that of endogenous IgG_2, which has a half-life of 21 d[33], and a XenoMouse-derived mAb to RANK ligand (AMG 162, denosumab) has shown nonlinear pharmacokinetics and a very long half-life allowing for dosing as infrequently as every 3 or 6 months[31].

Targeting EGFR by therapeutic antibodies

EGFR is one of the most extensively studied growth factor receptors. It is a member of the erbB family of receptor tyrosine kinases, which also includes HER2/neu (erbB2), HER3 (erbB3) and HER4 (erbB4). ErbB receptors are type I membrane glycoproteins that share a very similar structure. Each has an N-terminal extracellular ligand-binding domain, a single transmembrane domain and a C-terminal intracellular tyrosine kinase domain. With the exception of HER2, all erbB receptors bind to one or more peptide ligands. EGFR binds to many ligands, including EGF, transforming growth factor-alpha (TGF-α), amphiregulin, betacellulin, heparin-binding EGF and epiregulin[34]. A recent study has shown that there appear to be two classes of EGFR in human tumor tissues, one with low binding affinity to EGF (K_d = 12.57 nM) and the other one with a much higher affinity (K_d = 0.26 nM)[35]. Early studies indicated that high-affinity receptors may represent the biologically active receptors[36,37]. Ligand binding triggers a conformational change that leads to homodimerization of the same receptor and/or heterodimerization between different receptors of the erbB family. Each form of an erbB receptor dimer has a different affinity for cognate ligands and transduces through different signaling pathways[38]. Receptor dimerization activates the receptor kinase that catalyzes autophosphorylation of C-terminal tyrosine residues, leading to recruitment of adaptor proteins to those phosphorylated tyrosines and subsequent activation of one or more signal transduction pathways. This cascade of signaling pathways mediates a variety of cellular responses, including cell proliferation, differentiation, survival, motility, adhesion and angiogenesis[34] (**Fig. 2a**). Three predominant pathways activated by erbB receptors include the Ras/Raf/mitogen-activated kinase (MAPK), phosphatidylinositol-3-kinase (PI-3K)/Akt and signal transducer and activator of transcription (STAT) pathways (**Fig. 2b**). MAPK regulates cell growth and proliferation, whereas Akt and STAT rather specifically regulate cell survival and apoptosis[39]. Their association with EGFR activation suggests a role for EGFR in modulation of cell growth and survival. EGFR and other erbB family members were initially found to be transmitted as oncogenes by the avian erythroblastosis virus[40]. The concept that EGFR plays a critical role in the development and progression of human solid tumors mainly stems from three pieces of evidence. First, it has been shown that introduction and overexpression of EGFR induced transformed phenotypes in the recipient cells[41]. Upregulation of EGFR expression and aberrant activation of EGFR has been shown in many human epithelial

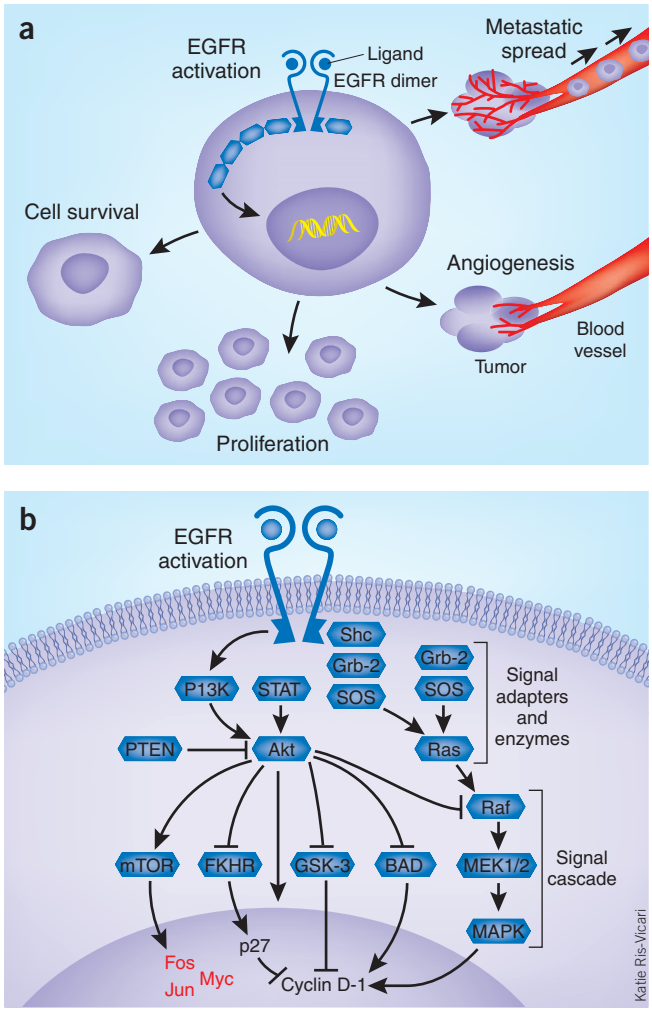

Figure 2 The role of EGFR activation in tumorigenesis (**a**) and its related signal transduction pathways (**b**). Transcription factors are depicted in red.

cancers, including those of the colon, lung, kidney, head and neck, breast, prostate, brain and ovary[42–47]. Importantly, overexpression of EGFR is often associated with poor clinical prognosis[48]. Second, coexpression of EGFR and its ligands TGF-α or EGF was found in the same tumor tissues, indicating that an autocrine regulatory loop may stimulate and control the growth and progression of these tumors[49]. Finally, a number of anti-EGFR agents, including small-molecule inhibitors that block the kinase activity and mouse EGFR-neutralizing mAbs that block ligand binding and EGFR activation, were able to inhibit tumor growth in xenograft mouse models[34,50]. This profile established EGFR as an attractive therapeutic target in oncology and provided the rationale for the development of a therapeutic antibody. The first approved therapeutic anti-EGFR mAb to be developed was cetuximab (IMC-C225; Erbitux), a chimeric $IgG_1\kappa$ derivative of the mouse mAb 225, with a $K_d = 0.39$ nM binding affinity[34]. A fully human antibody was expected to minimize immunogenicity and to improve the safety profile and dosing schedule.

Discovery and preclinical development of panitumumab

Panitumumab (formerly known as E7.6.3 and ABX-EGF) is a fully human $IgG_2\kappa$ mAb that binds specifically to EGFR with very high affinity ($K_d \sim$ 0.05 nM) and not to the other erbB family members[51]. The antibody was raised by immunizing the $IgG_2\kappa$ strain of XenoMouse using human epidermoid cervical carcinoma A431 cells, which overexpress EGFR on their surface (>10^6 receptors per cell). After screening more than 70 specific anti-EGFR mAbs, panitumumab was chosen based on its very high binding affinity and specificity, and its potency to block ligand binding and to inhibit receptor phosphorylation[51]. Panitumumab binds to EGFR with an affinity which is >200-fold higher and >five-fold higher than that of the ligand binding to the low affinity and high affinity receptors, respectively[35]. The high affinity should allow panitumumab to compete more effectively with TGF-α or EGF in binding to EGFR and to saturate EGFR *in vivo* at lower doses relative to mAbs with lower affinity. Because EGFR is widely expressed in many normal epithelial tissues such as skin, liver, kidney and lung, an IgG_2 isotype was chosen to minimize potential toxicity to EGFR-expressing normal tissues from recruitment of antibody-dependent, cell-mediated cytotoxicity and complement-dependent cytotoxicity. The combination of the IgG_2 isotype and the fully human nature of the antibody was also expected to result in a longer half-life and low rate of immunogenicity and infusion-related reactions, which include symptoms such as fever, chills, rigors, difficulty breathing and, in the most severe cases, anaphylaxis.

Panitumumab binds to domain III of EGFR, which is the ligand binding domain. It has been shown that upon binding to the receptor both panitumumab and EGFR are rapidly internalized leading to downregulation of cell surface EGFR[52]. Panitumumab-mediated EGFR downregulation was also detected *in vivo* in human tumor xenografts[53]. In addition to inhibition of phosphorylation of EGFR and MAPK/Akt, panitumumab also causes cell cycle arrest and suppresses production of pro-angiogenic factors such as vascular endothelial growth factor and IL-8 by tumor cells[52], suggesting that panitumumab is capable of stopping tumor growth and progression by directly blocking tumor cell proliferation and indirectly by inhibiting tumor angiogenesis (**Fig. 2**).

As a monotherapy, panitumumab was able to not only prevent and inhibit tumor growth but also completely eradicate large established A431 tumor xenografts[51]. The eradication of A431 tumors is dose dependent and long lasting (for more than 8 months of follow-up). In addition to its ability to inhibit the growth of tumors that overexpress EGFR such as A431 and MDA-MB-468 (>1.0×10^6 receptors per cell), panitumumab also showed anti-tumor activity in tumors that express lower levels of EGFR such as pancreatic tumor BxPC3 and colon tumor HT-29 (63×10^3 and 9×10^3 receptors per cell, respectively). Not all the EGFR-expressing tumors examined were sensitive to panitumumab treatment suggesting that EGFR expression alone is not sufficient to predict tumor response to EGFR-neutralizing mAbs[51,54]. Interestingly, panitumumab is efficacious in treating tumors bearing both wild-type EGFR and the mutant EGFR that renders non-small cell lung tumors resistant to EGFR tyrosine kinase inhibitors such as gefitinib (Iressa). Combination therapy of panitumumab with various standard chemotherapeutic agents has demonstrated additive anti-tumor activity in tumor xenograft models. For instance, monotherapy treatment with either panitumumab or gemcitabine resulted in significant growth inhibition of human pancreatic MiaPaCa-2 (36% and 38% inhibition, respectively) and BxPC-3 (44% and 25% inhibition, respectively) xenograft tumors compared to the control group. Combination therapy with panitumumab plus gemcitabine resulted in enhanced antitumor efficacy in both MiaPaCa-2 and BxPC-3 tumors (58% and 61% inhibition, respectively) compared with either agent alone. The additive anti-tumor activity of combination treatment is accompanied by a >60% reduction in proliferation markers of Ki67 and BrdU staining compared to that of either agent alone[55].

CLINICAL DEVELOPMENT OF PANITUMUMAB

Phase 1 evaluation

The clinical development program for panitumumab began in 1999 with a phase 1 study evaluating the safety and pharmacokinetics of panitumumab in people with previously treated, advanced, EGFR-expressing solid tumors[56]. This phase 1 study enrolled a total of 96 people with advanced solid malignancies including renal cell, prostate, pancreatic, non-small cell lung, colorectal and gastro-esophageal cancer. A range of doses (0.01 – 9 mg/kg) and administration schedules (weekly, every other week and every 3 weeks) were evaluated. In this study, panitumumab was generally well tolerated and no maximum tolerated dose was reached. Skin toxicity (acneiform skin rash) was the most common dose-related adverse event and was chosen as a pharmacodynamic marker of EGFR blockade. Anti-tumor activity was observed in 5 out of the 39 subjects with colorectal cancer for a partial response rate of 13%. Responses were observed for dosings of 2.5 mg/kg weekly, 6 mg/kg every other week and 9 mg/kg every three weeks. These data together with observations made in the development of cetuximab guided the development of panitumumab in colorectal cancer.

Because of frequent and high expression of EGFR in renal cell carcinoma (RCC), a phase 1/2 study was launched in this indication to further characterize panitumumab pharmacokinetics and to evaluate safety and efficacy in this indication. This study evaluated four weekly doses of panitumumab[57]. Across the doses tested (1.0, 1.5, 2.0 and 2.5 mg/kg weekly), panitumumab exhibited nonlinear pharmacokinetics, with longer antibody half-life with increasing dose (**Fig. 3**). This pharmacokinetic profile indicates clearance of the antibody by EGFR expressed in tissues. Upon saturation of this elimination pathway, pharmacokinetics is linear. Overall, the intra- and intersubject variability in panitumumab concentrations was low. The antibody clearance decreased with increasing dose and approached the clearance value for endogenous IgG, suggesting EGFR saturation at weekly doses >2 mg/kg. The 1.5 mg/kg weekly dose was associated with a 90% incidence of skin toxicity, with a 100% incidence observed with the 2.5 mg/kg weekly dose. Based on EGFR saturation, as indicated by the pharmacokinetics and the incidence of skin toxicity, the 2.5 mg/kg was identified as the target weekly dose for future development.

As colorectal cancer was one of the intended indications, dosing schedules of every 2 and every 3 weeks were also explored to potentially match the administration schedule of chemotherapy regimens frequency used in this indication. Pharmacokinetic modeling had indicated that doses of panitumumab at 6 mg/kg every other week and 9 mg/kg every 3 weeks could result in similar exposure as the selected weekly dose. These findings were confirmed in the phase 1 study mentioned above[56]. At these dosing regimens, steady-state panitumumab concentrations were obtained after 6 weeks of treatment, where the mean half-life value during the dosing interval was 8.5 d for 2.5 mg/kg weekly, 7.5 d for 6 mg/kg every other week, and 8.4 d for 9 mg/kg every 3 weeks. A population pharmacokinetic analysis conducted using data from multiple studies and multiple tumor types indicated that age (21–88 years), sex, race (15% non-white), hepatic dysfunction (mild to moderate) and EGFR membrane staining in tumor cells (1 to 3+, staining intensity by immunohistochemistry) had no apparent effect on the pharmacokinetics of panitumumab.

Phase 2 evaluation

The phase 2 evaluation of panitumumab focused largely on people with metastatic colorectal cancer whose disease had progressed during or after one or more prior chemotherapy regimens. These studies investigated efficacy, safety and the potential relationship between panitumumab efficacy and EGFR tumor membrane expression levels as measured by immunohistochemistry using the Dako EGFR PharmDx kit.

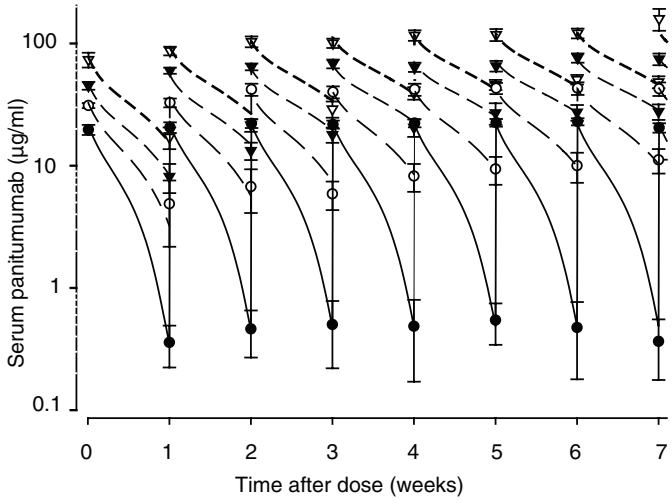

Figure 3 Pharmacokinetics of panitumumab. Mean (± s.e.m.) panitumumab concentration-time data fit to the model. The graph shows the observed panitumumab concentrations for the 1.0-, 1.5-, 2.0- and 2.5-mg/kg weekly doses represented by closed circle, open circle, closed triangle and open triangle, respectively. The different lines represent the model fits for each dose. The horizontal line represents the IC_{90} for saturation of the nonlinear clearance. (From Rowinsky *et al.*, 2004, ref. 57. Reproduced with permission from *J. Clin. Oncol.*)

The first phase 2 single-arm study enrolled 148 people with metastatic colorectal-cancer who had progressive disease during or after a regimen[58] of fluoropyrimidine in combination with irinotecan (Camptosar) or oxaliplatin (Eloxatin) or both. Subjects were stratified into two cohorts according to EGFR-staining intensity and received panitumumab monotherapy 2.5 mg/kg weekly infused over 1 h. The overall response rate was 9%, and the overall stable disease rate was 29%, for an overall disease control rate of 38%. No differences in response rate were observed between subjects with tumors with low and high EGFR-staining intensity. Over 90% of subjects experienced skin-related toxicities, most of which were grade 1 or 2 in severity (grade 1 and 2 identify the severity of an adverse event as mild to moderate out of a 5-grade scale). Only one infusion reaction (grade 2) was reported. No anti-panitumumab antibodies were detected.

Two additional phase 2 studies were initiated to further evaluate panitumumab activity in people with refractory disease with different levels of EGFR expression. These studies, which continue to date, are single-arm phase 2 trials examining panitumumab 6 mg/kg every 2 weeks in 203 subjects with metastatic colorectal cancer with low/negative tumor-membrane EGFR expression (EGFR staining in <10% of tumor cells)[59] and in 185 subjects with high tumor-membrane EGFR expression (staining in ≥10% of tumor cells)[60]. In both studies, eligible subjects had documented disease progression during or following fluoropyrimidine and prespecified doses of irinotecan and oxaliplatin. Interim analyses from these studies (88 and 91 treated subjects, respectively) suggest that panitumumab has similar efficacy in people with tumors that show undetectable (staining in tumor cells <1%), low, or high EGFR expression. For people with EGFR-negative or low-expressing tumors, and those with high-expressing tumors, a response rate of 7% and 8%, respectively, and stable disease rates of 30% and 21%, respectively, were observed. Panitumumab was well tolerated, resulting in low rates of infusion reactions and anti-panitumumab antibody formation.

Another single-arm phase 2 study examined the efficacy and safety of panitumumab administered in combination with irinotecan-based chemotherapy regimens in the first-line setting of metastatic colorectal

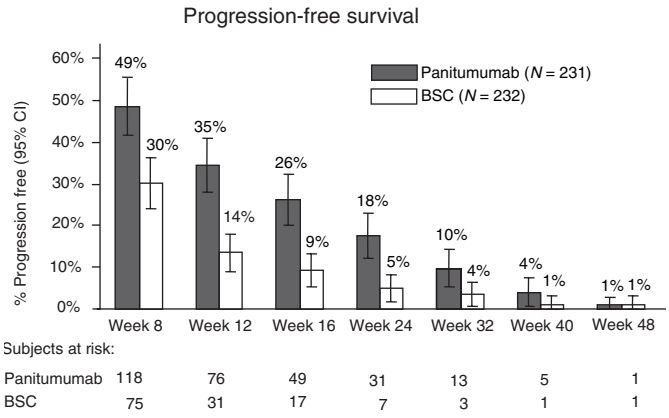

Figure 4 Progression-free survival of panitumumab versus best supportive care at prespecified tumor assessment time points in the phase 3 trial. The primary analysis of progression-free survival for all randomized analysis set was assessed by central review, stratified by Eastern Cooperative Oncology Group (ECOG) status and geographic region. The Kaplan-Meier estimates and 95% confidence intervals over time are presented.

cancer[61]. This study included two cohorts: one evaluated panitumumab in combination with the IFL (bolus irinotecan and 5-fluorouracil) regimen and the other evaluated panitumumab in combination with FOLFIRI (infusional 5-FU, bolus irinotecan). Results indicated that panitumumab plus IFL was associated with a high incidence of grade 3/4 diarrhea (58%). However, panitumumab plus FOLFIRI was well tolerated and had encouraging antitumor activity with a response rate of 33% and disease control rate of 79%. A confirmatory trial is ongoing to investigate the efficacy of panitumumab in combination with FOLFIRI chemotherapy.

The results from these phase 2 studies suggest that panitumumab has activity in colorectal cancer and has a manageable safety profile as monotherapy or in combination with FOLFIRI chemotherapy. Activity does not appear to be dependent on EGFR expression as measured by immunohistochemistry. These results are consistent with those observed with other anti-EGFR antibodies[62–64]. Additional studies with panitumumab are currently examining the predictive value of alternative biomarkers to aid in subject selection.

Pivotal phase 3 study

After evaluation of the phase 2 trials, a pivotal trial was designed to study panitumumab monotherapy in people with previously treated metastatic colorectal cancer. A randomized, phase 3, multicenter, open-label trial comparing panitumumab plus best supportive care (BSC) versus BSC alone in people with metastatic colorectal cancer who had disease progression after fluoropyrimidine-, irinotecan- and oxaliplatin-containing chemotherapy regimens was conducted in Europe, Canada and Australia. EGFR tumor membrane expression in ≥1% of tumor cells by immunohistochemistry was required. Persons were eligible if they had disease progression after prespecified doses of irinotecan and oxaliplatin. These pretreatment and radiographic progression criteria were adjudicated by a blinded independent radiology review committee. The trial allowed for crossover of subjects randomized to the BSC arm to an extension study where they received panitumumab treatment after disease progression had been confirmed by the investigator. The primary endpoint of the pivotal trial was progression-free survival as assessed by blinded independent radiology review; secondary endpoints included response rate and overall survival.

A total of 463 subjects were randomized: 231 subjects to panitumumab administered at a dose of 6 mg/kg every other week plus BSC and 232 subjects to BSC alone[65]. The rate of disease progression or death was reduced by 46% with panitumumab plus BSC compared with BSC alone (hazard ratio, 0.54; 95% confidence interval (CI), 0.44–0.66; $P < 0.0001$). At all pre-specified time points through week 32, the proportion of subjects who were progression free was significantly higher for the panitumumab arm compared with the BSC arm (**Fig. 4**). In subset analyses, progression-free survival favored panitumumab over BSC for all subsets studied, including those based on gender, age, Eastern Cooperative Oncology Group (ECOG) status, primary tumor type, prior number of regimens and EGFR staining categories (**Fig. 5**). Objective responses favored panitumumab over BSC (10% versus 0%, respectively, $P < 0.001$). No differences in overall survival were observed between treatment groups (hazard ratio, 1.00; 95% CI, 0.82–1.22). Overall survival could have been confounded by the fact that about 75% of subjects in the BSC arm crossed over to receive panitumumab at a median time of 7 weeks, as similar activity of panitumumab was observed in the crossover study as in the phase 3 study.

Panitumumab was generally well tolerated, with an adverse event profile similar to that reported in earlier studies. Main toxicities included skin rash, hypomagnesemia and diarrhea. No grade 3 or 4 infusion reactions were observed. As seen with other EGFR inhibitors, response rate, progression-free survival and overall survival were associated with skin toxicity severity[62,63,65,66]. Overall survival favored panitumumab for people who had a worst skin toxicity of grade 2–4 as compared to those with grade 1 (hazard ratio, 0.59; 95% CI, 0.42–0.85) (**Fig. 6**). No one developed *de novo*, persistent anti-panitumumab antibodies. The overall safety profile of panitumumab across multiple studies is summarized below. These results led to the approval of panitumumab by the FDA in September 2006 for the treatment of EGFR-expressing metastatic colorectal cancer with disease progression on or following fluorouracil-, oxaliplatin- or irinotecan-containing chemotherapy regimens.

Safety profile

A pooled safety analysis of 920 persons from ten panitumumab monotherapy studies has shown that panitumumab is generally well tolerated with expected on-target effects related to EGFR inhibition. These toxicities include skin-related events, hypomagnesemia and diarrhea. In panitumumab monotherapy trials, these toxicities were mild to moderate in the majority of subjects and severe in 13% of subjects for skin-related toxicities, 7% for hypomagenesemia and 2% for diarrhea. The nature of the skin toxicity ranged from erythema and rash with macules (flat discolored areas of the skin) and papules (small bumps) to pustular rash, mostly affecting the face, scalp and trunk. Infectious complications including sepsis have been observed, particularly in association with severe skin infections. Other skin-related toxicities included paronychia and fissures, particularly after longer-term exposure. Other safety observations included fatigue, nausea and vomiting. Infusion reactions of any grade were reported in 4% of subjects, and grade 3 reactions were observed in 1% of subjects. Investigators have reported infusion reactions of any grade at a rate of 1% and of grade 3 at a rate of <0.3%. No grade 4 reactions had been reported in this pooled safety analysis. Only one subject discontinued panitumumab because of an infusion reaction (grade 2); all others received additional infusions of panitumumab with premedication without further events. Few (4%) subjects discontinued panitumumab therapy because of a treatment-related event.

Immunogenicity

Fully human antibodies generated from human immunoglobulin transgenic mice[8,16] are expected to have a low risk of immunogenicity and infusion reactions. The development of anti-drug antibodies is a potential concern, as the immune response might neutralize the antibody function, accelerate antibody clearance and elicit hypersensitivity

reactions. However, data from phase 1 and 2 clinical trials of several human mAbs generated from transgenic mice have supported a low risk of immunogenicity with human antibodies[8]. For example, no anti-human antibody responses were observed in subjects with psoriasis, rheumatoid arthritis or chronic obstructive pulmonary disease who received up to 3 months of treatment with ABX-IL8, a XenoMouse-derived anti-IL-8 $IgG_2\kappa$ mAb[33]. In addition, no immune reaction was observed in people treated with the XenoMouse-derived antibody denosumab[31,67,68]. Similar lack of immunogenicity has been observed with other fully human mAbs, including ipilimumab and zanolimumab, generated from another transgenic mouse platform[8].

In both the pivotal trial and in the pooled safety summary of panitumumab, the incidence of antibody formation against panitumumab was low with no discernable effect on pharmacokinetics or the safety profile. The immunogenicity of panitumumab was assessed using two screening immunoassays for the detection of anti-panitumumab antibodies. All antibody-positive samples were also tested for the presence of panitumumab-neutralizing antibodies using a bioassay incorporating detection of EGFR phosphorylation. The first immunoassay used a bridging enzyme-linked immunosorbent assay (ELISA) capable of detecting 10 ng/ml of positive control anti-panitumumab antibody [69]. The ELISA assay incorporated an acid-dissociation step to reduce drug interference. The ELISA detected persistent antibody responses in 2 of 612 (0.3%) subjects, with one person developing neutralizing antibodies. The second immunoassay used a Biacore biosensor immunoassay format capable of detecting 1 μg/ml of positive control antibody while tolerating the presence of equal molar amounts of drug[70]. Although less sensitive and less tolerant to competing drug in the assay, the Biacore assay detected persistent antibody responses in 25 of the 604 (4.1%) subjects. Additionally, the Biacore assay identified eight subjects who developed neutralizing antibodies[70]. The higher rate of immunogenicity observed in the Biacore assay was attributed to its ability to detect low-affinity antibodies not detected in the ELISA. Despite this detection, there was no apparent impact on the pharmacokinetics and safety of panitumumab.

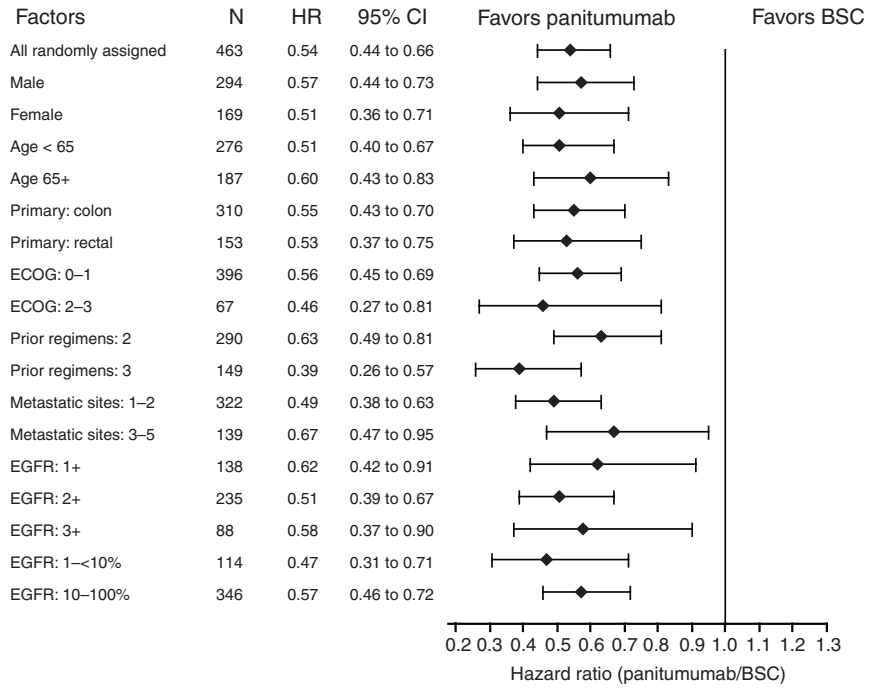

Figure 5 Hazard ratios of progression-free survival in subject subsets in the phase 3 trial. Hazard ratio (HR) for progression-free survival (PFS) and the 95% confidence intervals (CI) for HR are shown and represented by the diamond and horizontal line, respectively. The HR for the randomly assigned analysis was adjusted by ECOG status and geographic region; all other HRs were unadjusted. (From Van Cutsem *et al.*, 2007, ref. 65. Reproduced with permission from *J. Clin. Oncol.*)

Ongoing panitumumab clinical development plan

Multiple studies of panitumumab are currently evaluating efficacy and safety in metastatic colorectal cancer, focusing largely on the combination of panitumumab with chemotherapy and/or other targeted agents in earlier lines of therapy (first-line, second-line and as adjuvant therapy). The PACCE study (Panitumumab Advanced Colorectal Cancer Evaluation) is a phase 3b randomized, open-label clinical trial evaluating oxaliplatin- and irinotecan-based chemotherapy and bevacizumab (Avastin) with and without panitumumab for first-line treatment in people with metastatic colorectal cancer. This trial completed enrollment with 1,053 subjects. A preplanned interim efficacy analysis for the oxaliplatin cohort scheduled after the first 231 events (death or disease progression) was conducted that included 947 randomized subjects, 812 subjects in the oxaliplatin cohort and 135 subjects in the irinotecan cohort. The interim analysis on the oxaliplatin cohort demonstrated an unfavorable risk:benefit profile for panitumumab plus bevacizumab/oxaliplatin–based chemotherapy based on reduced progression-free survival time and additional toxicity[71]. Excess grade 3 and 4 toxicity included skin rash, diarrhea, dehydration, hypokalemia, infections and pulmonary embolism. In addition there was a trend for greater mortality on the panitumumab arm, although interpretation of the significance of this trend was limited by the small number of events and the lack of a prespecified significance boundary to correct for multiplicity testing. No differences in time to treatment failure were observed.

These results indicate a lack of biological synergy between panitumumab and bevacizumab in combination with oxaliplatin-based chemotherapy, at least in the absence of subject selection. Additional toxicity was observed arising from dual-pathway inhibition. Lower dose intensity of standard chemotherapy was also observed in the panitumumab plus bevacizumab/oxaliplatin-chemotherapy arm, which may have contributed to the worsened progression-free survival outcome. Most subjects dropped out of first-line treatment for reasons other than progressive disease or death. In an exploratory, *post hoc* analysis, specific populations with compromised baseline physiology (including elderly and poor performance–status subjects, as well as persons with comorbidities—effects of all other diseases other than the primary disease of interest) had worse outcomes with the five drug combination.

The reasons for these findings continue to be the subject of intense evaluation, with currently ongoing data collection and analyses that include subset analyses based on biomarkers.

Additional studies in colorectal cancer, head and neck cancer, and other indications are currently ongoing. Two additional phase 3 randomized controlled trials are evaluating the role of panitumumab in combination with FOLFOX and FOLFIRI chemotherapy (without bevacizumab) in first- and second-line metastatic colorectal cancer, respectively. These studies are expected to complete accrual in late 2007 and early 2008,

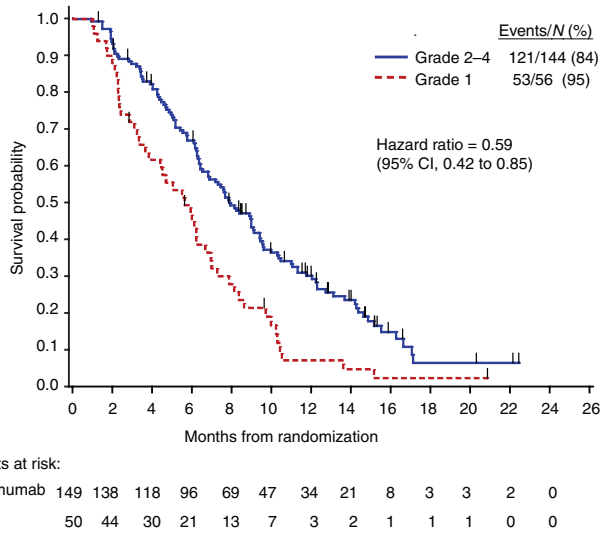

Figure 6 Overall survival by worst severity of skin toxicity in the phase 3 trial. Only panitumumab patients were included; those with no reported skin toxicity or those with progression or death <28 d from randomization were excluded to mitigate lead-time bias. A minimum of 12 months of follow-up is included in this analysis. Hazard ratio for grade 2–4 relative to grade 1 skin toxicity (stratified by ECOG status and geographic region) is shown. The hazard ratio in the primary analysis of overall survival with 12 months of minimum follow-up was 1.00 (95% CI: 0.82–1.22). (From Van Cutsem *et al.*, 2007, ref. 65. Reproduced with permission from *J. Clin. Oncol.*)

respectively. Independent data monitoring committees for these studies have recommended continuation without protocol modification.

EGFR inhibitors have proven activity in squamous cell carcinoma of the head and neck (SCCHN)[72–74]. Studies evaluating the safety and efficacy of panitumumab in combination with radiotherapy or with radiotherapy and chemotherapy in people with locally advanced SCCHN are underway. In addition, combinations of panitumumab and various chemotherapy regimens are being tested in people with metastatic or recurrent SCCHN. Studies in a variety of other malignancies including esophageal and gastric cancer are ongoing or planned for 2007.

Other anti-EGFR mAbs for the treatment of cancer

Additional agents targeting the EGFR are either approved or currently in development for the treatment of solid malignancies. Cetuximab, a chimeric IgG1 antibody that is ~30% murine, was the first therapeutic monoclonal antibody developed against the EGFR. This antibody was approved in 2004 for the treatment of colorectal cancer and recently in 2006 for the treatment of SCCHN and thus validated the use of anti-EGFR antibodies for cancer therapy. Differences in half-life and dosing requirements between cetuximab and panitumumab may reflect biological differences between the two antibodies, such as the fully human nature of panitumumab conferring a longer half-life secondary to slower clearance due to low immunogenicity, and the higher affinity of panitumumab resulting in more avid binding and lower dose requirement for EGFR saturation.

The pivotal trial of cetuximab examined cetuximab plus irinotecan versus cetuximab alone in persons with metastatic colorectal cancer with EGFR-expressing tumors that were refractory to irinotecan[63]. Improved response rates were seen with the addition of cetuximab to irinotecan versus cetuximab alone (22.9% versus 10.8%, respectively; $P = 0.007$). In the irinotecan- and oxaliplatin-refractory subgroup (a similar population as that in the pivotal panitumumab trial), 8.5% of subjects receiving cetuximab alone had a measurable response. The incidence and severity of skin toxicity, but not EGFR expression levels, were associated with response, which is now considered an EGFR inhibitor class effect.

Published evidence indicates a severe infusion reaction rate of 3% with cetuximab and a total infusion reaction rate of ~20%[63,75]. Interestingly, recent institutional reports and results from a panitumumab compassionate-use program have shown successful administration of panitumumab after cetuximab intolerance (that is, severe infusion reactions)[76,77]. The reported cetuximab immunogenicity rate is 5% (Erbitux prescribing information, 2006) based on the use of either double antigen (bridging) radiometric assay or an ELISA. The observed differences of antibody development may be influenced by the format of the assays used, hence comparison of the incidence of antibodies to different therapeutic antibodies is not feasible.

A number of additional mAbs targeting the EGFR are currently in various phases of development in many tumor types. These include matuzumab and nimotuzumab (TheraCIM), both IgG_1 humanized antibodies, and zalutumumab (HuMax-EGFR), a human antibody. Nimotuzumab is approved in Cuba for the treatment of SCCHN in combination with radiotherapy and is currently under investigation in non-small cell lung cancer (NSCLC), pancreatic cancer and gliomas. A pivotal phase 3 trial of zalutumumab has recently been initiated in refractory SCCHN and is currently also under investigation in NSCLC.

Subject selection for treatment with anti-EGFR antibodies

To date, EGFR tumor expression as measured by immunohistochemistry has not proven to be useful for subject selection. Therefore, there is a need to understand molecular characteristics that can prospectively predict clinical benefit to treatment with EGFR antagonists. These characteristics are likely to differ by tumor type. For instance, in the BR21 NSCLC trial, positive fluorescence *in situ* hybridization (FISH) staining for EGFR gene overexpression was found to be a predictive marker for survival associated with erlotinib treatment[78]. In colorectal cancer, Moroni *et al.* performed FISH analysis on tumors of people treated with either panitumumab or cetuximab[79]. They found that 8 out of the 9 responders had an increase in EGFR gene copy number. Conversely only 1 of 21 nonresponsive subjects had increased gene copy number. However, other recent data have not corroborated the association between increased gene copy number and response[75,80].

Recent work has shown that mutations activating the RAS-RAF signaling pathway are predictive and prognostic indicators in persons with metastatic colorectal cancer and are inversely correlated with the response to anti-EGFR antibodies. In persons with metastatic colorectal cancer treated with cetuximab, *KRAS* mutations were found in 13 of 30 subjects (43%) and were significantly associated with the absence of response to cetuximab (*KRAS* mutation in 0% of the 11 responder subjects versus 68.4% of the 19 nonresponding subjects; $P = 0.0003$)[80]. Additionally, overall survival in people with tumors that express wild-type KRAS was significantly higher than in those with tumors that express mutant KRAS ($P = 0.016$; median, 16.3 versus 6.9 months, respectively)[80]. A phase 2 multicenter single-arm study of panitumumab and FOLFIRI as second-line treatment is prospectively investigating the effect of *KRAS* mutation status on efficacy parameters. At the RNA level, gene expression profiles from fresh tissue samples have been shown to predict response to cetuximab treatment[81]. Gene expression signatures identified in pilot studies can be further validated using samples from randomized trials. At the protein level, attempts to identify stratification markers by immunohistochemistry have been largely unsuccessful. Assessing levels of hetero- and homo-dimers of the HER family as a surrogate measurement of pathway activation could potentially predict dependency of tumors on EGFR inhibition[39,82].

Table 1 XenoMouse-derived mAb product candidates in clinical development

mAb	Target	Indication	Company (developer)	Clinical trial stage
Panitumumab (Vectibix)	EGFR	Cancer, solid tumors	Amgen	approved; 2, 3
Denosumab (AMG162)	RANK ligand	Osteoporosis, treatment-induced bone loss, bone metastases, multiple myeloma	Amgen	2, 3
AMG 102	HGF	Cancer, solid tumors	Amgen	1
AMG 655	Trail receptor 2	Cancer, solid tumors	Amgen	1, 2
CP-675,206	CTLA-4	Cancer, solid tumors	Pfizer	3
CP-870, 893	CD40 agonist	Cancer, solid tumors	Pfizer	1
CP-751,871	IGF-IR	Cancer, solid tumors	Pfizer	2
HCD122	CD40 antagonist	Cancer, hematologic tumors	Novartis/ Xoma	1
CRO02	PDGFR	Kidney inflammation	CuraGen	1b
CRO11-vcMMAE	GPNMB	Cancer, melanoma	CuraGen	1
HGS004	CC chemokine receptor 5 (CCR5)	HIV	Human Genome Sciences	2
AGS-PSCA/ MK-4721	PSCA	Cancer, solid tumors	Agensys/Merck	1

Conclusions

Panitumumab represents the first fully human antibody developed from XenoMouse technology to be approved by a regulatory agency. This has been an important milestone in validating XenoMouse strains as well as other human immunoglobulin-producing mouse technologies[8,22] as sources for therapeutic antibodies. The path from initiation of XenoMouse technology development to regulatory approval took ~15 years, including 6 years for mouse strains derivation and mAb development and 6.5 years of clinical development. The drug has a positive risk-benefit profile in advanced, chemotherapy refractory colorectal cancer and has the potential to add to the treatment of this disease in earlier lines of therapy as well as to play a role in the treatment of other malignancies. As expected, given the fully human nature of the antibody, treatment with panitumumab has been associated with low immunogenicity, and more importantly, with low risk of hypersensitivity reactions. When compared to anti-EGFR antibodies that contain mouse sequences, these properties, together with the antibody's longer half-life, confer potentially important safety benefits and dosing flexibility, although a full understanding of the clinical relevance of these differences among antibodies can be gained only in the context of appropriately designed clinical trials comparing both agents. The development of panitumumab validates the XenoMouse platform as a proven technology for generation and selection of a therapeutic antibody with the desired characteristics, including affinity, specificity and isotype. The numerous fully human antibody candidates generated using the XenoMouse platform currently under development (**Table 1**) are expected to yield novel therapeutics for the treatment of a variety of diseases.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology/.

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