Panitumumab a novel drug in cancer treatment

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The epidermal growth factor receptor (EGFR) is a member of the erbB family overexpressed in most of the solid tumors. In cancer cells, the overexpression of EGFR correlates with the development and the progression of tumor. Panitumumab is a fully human monoclonal antibody that blocks the extracellular domain of the EGFR and has not been associated with the formation of any antibodies directed against it. This review summarizes on the preclinical and clinical development of panitumumab in human solid tumors. As bevacizumab and cetuximab have been approved for colorectal cancer because of their improvements in progression-free survival and overall survival when associated with chemotherapy, panitumumab represents an interesting molecule which needs more phase III studies to validate its efficacy.

Key words: colorectal cancer, fully human monoclonal antibody, panitumumab, target therapy

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 with an extracellular ligand-binding domain, a hydrophobic transmembrane domain involved in interactions between receptors within the cell membrane and an intracellular domain. The intracellular domain has a tyrosine kinase activity for signal transduction.

EGFR can be activated through a series of events initiated by binding to its ligands, such as epidermal growth factor (EGF), transforming growth factor-α (TGF-α), amphiregulin, epiregulin, heregulin, neuregulin and betacellulin.

The ligand binding induces a receptor dimerization with another EGFR monomer (homodimerization) or another member of the erbB family (heterodimerization) [1].

The dimerization activates the receptor tyrosine kinase activity and induces EGFR tyrosine autophosphorylation. Activation of EGFR induces a cascade of downstream signaling pathways that mediate a variety of cellular responses, such as cell proliferation, differentiation, survival, motility, adhesion and repair. [2]

Three major pathways are involved in EGFR-mediated signaling. EGFR tyrosine autophosphorylation activates Ras. This causes a multistep phosphorylation event, leading to the activation of mitogen-activating protein kinases, Extracellular signal-regulated protein kinase (ERK1) and ERK2 control, and regulates transcription of molecules that are involved in cell transformation, proliferation and survival [3]. Another important pathway secondary to the EGFR activation is phosphatidylinositol-3-kinase and the downstream protein serine/threonine kinase Akt. Akt transduces intracellular signals linked to cell survival, proliferation and motility [4, 5]. The third pathway is represented by the Jak2/STAT3.

EGF, the mitogenic hormone that activates EGFR, plays an important role in regulating the proliferation and the differentiation of normal and neoplastic cells in vitro as well as in vivo.

Elevated levels of EGFR expression have been found in human tumors including most squamous cell carcinoma, adenocarcinoma and gliomas [6]. Overexpression can derive from a gene amplification, as in case of many gliomas, or from an increased gene transcription [7]. It may be that EGFR overexpression plays a role in the tumorigenesis of cancer cells [8].

The rationale for targeting EGFR as an anticancer therapy is the following.

First, EGFR is overexpressed in many human epithelian carcinomas, including cancer of colorectal, esophagus, prostate, ovarian, head and neck, lung and kidney.

Second, increased EGFR expression seems to be correlated with poor clinical prognosis and outcome [9].

Third, EGFR overexpression is often associated with increased production ligands—in particular TGF-α—by the same tumor cells. This determines an autocrine regulatory loop that stimulates EGFR and amplifies its signaling pathways [10].

Principal strategies for EGFR target therapy include monoclonal antibody (mAbs) and small-molecule tyrosine kinase inhibitors.

mAbs block the receptor extracellular domain inhibiting ligand binding and receptor activation; indeed, the small-molecule tyrosine kinase inhibitors do not permit
autophosphorylation by blocking intracellular domain. Examples of these two categories of EGFR inhibitors are cetuximab and panitumumab as mAbs and gefitinib and erlotinib as small-molecule tyrosine kinase inhibitors.

This review focuses on the preclinical, clinical and future development of panitumumab in human cancer treatment.

**monoclonal antibodies**

The introduction of hybridoma technology by Kohler and Milstein [11] >25 years ago has indicated the potential role of mAbs for human therapy.

The first generation of mAbs was of murine origin. These mAbs have had a limited therapeutic development because of their immunogenicity. Patients who received murine mAbs rapidly developed an immune response to the mouse protein, i.e., the human anti-mouse antibody response (HAMA) [12]. HAMA response can determine important reactions including allergies and anaphylaxis—with repeated administrations—and can reduce mAbs potency and efficacy by enhancing its clearance rate.

To overcome this problem, efforts were made to engineer less immunogen mAbs. These mAbs composed by a minor amount of murine protein are represented by chimeric mAbs, humanized mAbs and fully humanized mAbs.

Chimeric mAbs are generated by genetically combining the antigen-binding regions (Fv) of the mouse antibody with human IgG constant domain. The chimeric antibody still contains 34% of murine proteins [13]. Examples of chimeric antibodies approved for the oncological treatment of human cancer are rituximab (Rituxan, anti-CD20 mAb) and cetuximab (Erbitux, anti-EGFR mAb).

Humanized mAbs are constructed by 'implanting' the murine antibody complementarity-determining regions sequences into the human variable gene framework of the constant region. These mAbs contain 5%–10% mouse protein sequences [14]. Examples of humanized antibodies used in the clinical oncological practice are trastuzumab (the anti-erbB2 humanized mAb) and bevacizumab (Avastin, anti-vascular endothelial growth factor (VEGF) mAb).

Chimeric and humanized mAbs are less immunogenic than murine mAbs and have improved therapeutic utility even if, because of their murine sequences, they might determine allergic reactions in a portion of patients [15, 16].

Therefore, to reduce further on the risk of allergic reactions, fully human mAbs have been developed.

**xenoMouse technology and fully human mAbs**

The first important step to create the XenoMouse animals that produce fully human antibodies is the inactivation of the endogenous mouse antibody genes. This can be achieved by gene-targeting deletions of crucial cis-acting sequences of heavy and light (k) chains involved in the mouse immunoglobulin gene rearrangement and expression, Jh and Ck, respectively [17]. The homozygous mice for each of the Jh or Ck deletion were acquired by mouse embryonic stem cells containing the target heavy or k chain allele. Crossbreeding of these mice yielded double-inactivated mice, homozygous for both mutations. To preserve the variable gene diversity and the presence of regulatory elements that control antibody recombination and expression, DNA fragments containing human heavy- and light-chain loci have been cloned and recombined into yeast artificial chromosomes (YACs) in germline configuration. The heavy-chain YACs encompassed 66 different consecutive Vh genes, all 30 D segments and all 6 Jh genes. The Ck region has been retrofitted with the human γ1, γ2 or γ4 constant region. The heavy- and light-chain YACs were introduced into mice via infusion of YAC-containing yeast spheroplasts with mouse embryonic stem cells. Crossbreeding of these mouse strains resulted in the transgenic mice producing human antibodies in the presence of mouse antibodies. Breeding the human antibody-producing transgenic mice with the double-inactivated mouse strain created the XenoMouse strains which are homozygous for the inactivated mouse heavy- and k-chain genes and bearing one allele each of the human heavy and the k chain.

XenoMouse technology offers a unique reliable source for production of antigen-specific and high-affinity fully human therapeutic mAbs.

Fully human mAbs are nonimmunogenic and thus allow repeated administrations without human anti-human antibody response.

**discovery of panitumumab—a fully human anti-EGFR mAbs**

A panel of human IgG2 anti-EGFR mAbs was generated by immunizing the XenoMouse IgG2 strain with cells of human cervical epidermal carcinoma cell line A431 that expresses >1 million copies of EGFR per cell on the cell surface. The mice were immunized intraperitoneally or subcutaneously with A431 cells in complete Freund’s adjuvant. The standard hybridoma technology was used and the specific antibodies produced were selected through screening by enzyme-linked immunosorbent assay. A total of 70 EGFR-specific antibodies were identified. Thirty-eight antibodies were evaluated for their neutralization activity and 15 out of 38 mAbs blocked binding of EGF to the receptor [18]. The antibody clone E7.6.3, also known as panitumumab or ABX-EGF, was chosen because of its high potency and high affinity to EGFR.

**preclinical development of panitumumab**

Panitumumab is a fully humanized IgG2 mAb acquired by the XenoMouse technology. It does not contain murine protein sequences: clinical data indicate that panitumumab is well tolerated, does not require premedication and is associated with a very low incidence of HAMA. It binds EGFR with high affinity \((K_d = 5 \times 10^{-11} \text{ M})\) and blocks binding of EGF and TGF-α, inhibiting EGF-dependent tumor cell activation and proliferation [19].

Panitumumab causes cell cycle arrest at the G0/G1 interphase \(\text{in vitro}\) and inhibits tumor colony formation. Data indicate that
these fully humanized mAbs exert their antitumor activity by a number of different mechanisms. This could involve the inhibition of proliferation by blocking EGFR signaling pathways, the induction of cycle arrest, the inhibition of angiogenesis and the down-regulation of EGFR expression stimulating the receptor internalization. [20] Besides being a fully humanized IgG2 mAb, other antitumor effects are represented by complement-dependent cytotoxicities and antibody-dependent cell-mediated cytotoxicities.

In vitro it inhibits proliferation of A431 cells and the breast cancer cell line MDA-468. [21]. Panitumumab also blocks autocrine growth stimulation, inhibiting EGF/TGF-α-mediated tumor activation and proliferation. This fully humanized mAbs could also indirectly inhibit tumor angiogenesis because data indicate that treatment of prostate cancer cell line DU145 with panitumumab in vitro resulted in inhibition of VEGF and interleukin-8 production [22].

These mAbs completely prevent the formation of human epidermal carcinoma A431 xenografts in vivo and produce complete eradication of tumors with long-lasting and dose-dependant effects. Complete tumor eradication in xenografts could depend on the fact that panitumumab can penetrate the tumor mass and saturate EGFR expressed in the tumor tissue in a time- and dose-dependent fashion. Panitumumab not only inhibits tumor cell proliferation but also seems to inhibit tumor development and prevent metastasis of human breast cancer MDA-231 cells in severe combined immunodeficient mouse model [23].

Blocking EGFR and using xenografts models, panitumumab has shown to be active against multiple human solid tumors derived from different tissues. These include tumors from breast (MDA-468), pancreas (BxPC-3 and HS766T), prostate (PC-3), kidney (SK-RC-29), ovary (IGROV1) and colon (HT-29). Data indicate that ABX-EGF can 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 tumor expressing lower EGFR levels. More importantly, panitumumab without concomitant chemotherapy completely eradicates well-established A431 EGFR-overexpressing tumors, which do not recur; in comparison, cetuximab has only a limited effect on A431 tumors, and its murine counterpart requires the coadministration of chemotherapeutic drugs to achieve the elimination of the tumors [24, 25].

Panitumumab activity can be increased by the coadministration of chemotherapeutic agents because of additive mechanisms, even if the tumor growth inhibition can be acquired at relatively low doses, without concomitant chemotherapy or radiotherapy.

**clinical applications of panitumumab**

Traditionally, chemotherapy has been the main approach for the treatment of colorectal cancer (CRC); but with the recent advent of targeted therapies, biologic treatments are becoming an area of intense interest. In the past 2 years alone, two molecular targeted antibodies, bevacizumab and cetuximab, have been approved for CRC, targeting VEGF and EGFR, respectively.

The basic combination of 5-fluorouracil (5-FU) and leucovorin (LV) remains the backbone of therapy for metastatic colorectal cancer (mCRC). In recent years, studies combining 5-FU/LV with irinotecan (FOLFIRI [Fluorouracil, leucovorin, irinotecan]) or oxaliplatin (FOLFOX [Fluorouracil, leucovorin, oxaliplatin]) have demonstrated improvements in disease-free and overall survival (OS) [26, 27].

A recent comparison of FOLFOX6 followed by FOLFIRI versus the reverse order as first-line treatment found that both sequences achieved a median survival of ~20 months and were similarly efficacious.

The overall response rate (RR) in the FOLFIRI arm was 56%, with a progression-free survival (PFS) time of 8.3 months, compared with an overall RR of 54% and a PFS of 8 months with FOLFOX6. These results indicate that FOLFIRI and FOLFOX could be considered interchangeable for first-line and second-line treatment of mCRC [28].

In 2004, the approval of bevacizumab, a humanized mAb against VEGF-A, introduced the first biologic therapy for the initial treatment of mCRC. Several studies have shown that bevacizumab improves PFS and OS when combined with chemotherapy as FOLFIRI and FOLFOX [29, 30, 31].

In preclinical studies, treatment with panitumumab resulted in inhibition of EGFR autophosphorylation, intracellular signaling through downstream molecules and tumor growth [32].

Phase I studies evaluated single-agent, multi-dose administration of panitumumab in pretreated patients with advanced EGFR-expressing colorectal, non-small-cell lung, prostate, renal, pancreatic or esophageal/gastroesophageal junction cancer and demonstrated that panitumumab is generally well tolerated and did not require premedication before its administration. The exposure and tolerability profile is comparable between QW, Q2W and Q3W schedules. Panitumumab has single-agent antitumor activity in solid tumor, most notably in subjects with advanced CRC [33].

The first studies of phase II were conducted to evaluate the safety and efficacy of panitumumab monotherapy in patients with mCRC who have failed standard chemotherapy with a fluoropyrimidine (5-FU) with or without LV, and either irinotecan or oxaliplatin or both.

One of the objectives was to evaluate the activity of panitumumab also in patients with lower tumor EGFR expression.

The results confirm safety and response of panitumumab with no significant differences in all efficacy parameters between patients with higher or lower tumor EGFR expression. Response was seen in patients receiving up to four lines of prior therapy.

The four most frequently reported grade 3 or 4 treatment-related adverse events were rash, fatigue, vomiting/nausea and pruritus [34, 35, 36].

These data provide encouraging survival data in patients with mCRC who have failed multiple lines of standard chemotherapy.

In 2006 were presented the first data of a phase III multicenter, randomized controlled trial of panitumumab plus best supportive care (BSC) versus BSC alone in patients with mCRC.

Eligible patients had mCRC with measurable disease with progression during or following fluoropyrimidine, irinotecan
and oxaliplatin treatment, and ≥1% tumor cell membrane staining for EGFR by immunohistochemistry.

A total of 463 patients were randomized 1:1 to receive panitumumab at 6 mg/kg Q2W plus BSC or BSC alone; baseline demographics and clinical characteristics were well balanced between groups (Figure 1). Median follow-up time was 19 weeks and a significant improvement in PFS favoring panitumumab was observed ($P < 0.0001$). Patients receiving panitumumab had a 46% lower relative progression rate than those receiving BSC alone. A higher percentage of patients alive without progression was observed in the panitumumab versus BSC group (49% versus 30%, respectively), 75% of BSC-alone patients entered crossover study.

Panitumumab was well tolerated and, as expected, skin-related toxic effects were the most observed (90%) [37]. It is interesting to observe that skin toxicity seems to correlate to the survival probability (Figure 2).

In 2006 the results of a two-part study of first-line therapy of panitumumab with chemotherapy (irinotecan, 5-FU, LV—IFL (Irinotecan, Fluorouracil, leucovorin) or FOLFIRI—part 1 and part 2, respectively) were also presented that evaluated the safety, the efficacy and the pharmacokinetics in patients with mCRC. Patients enrolled were 19 in part 1 with IFL and 24 in part 2 with FOLFIRI treatment.

The panitumumab was administered at a dose of 2.5 mg/kg QW. The conclusions were the following:

- The incidence of grade 3 or 4 diarrhea was lower with panitumumab plus FOLFIRI compared with panitumumab plus IFL; the incidence of these events appeared to be dependent upon the dose, schedule and delivery of chemotherapy.
- All patients had an integument or eye toxicity; most patients (84% part1 and 83% part 2) had mild or moderate events as the most severe grade.
- Grade 3 or 4 hypomagnesemia was seen with both the IFL (11%) and FOLFIRI (8%) regimens plus panitumumab, which were manageable with magnesium.
- The dose of 2.5 mg/kg QW panitumumab can be administered safely with FOLFIRI for treatment of mCRC.
- The efficacy was as expected for these regimens when used for first-line treatment with mCRC.
- With panitumumab plus FOLFIRI, the objective RR was 33%, disease control rate was 79% and the median PFS was 10.9 months [38].

This study supports ongoing studies (large, randomized phase III trials) with panitumumab also administered Q2W.

The combination of panitumumab and bevacizumab has shown to inhibit two distinct but interacting biologic pathways. Preclinical models have demonstrated synergy with combined blockade of EGFR and VEGF [39].

It is ongoing a large phase III randomized trial of chemotherapy (FOLFOX or FOLFIRI) and bevacizumab with or without panitumumab in the first-line treatment of patients with mCRC. Primary objective is to assess whether the addition of panitumumab to chemotherapy plus bevacizumab improves PFS compared with treatment with chemotherapy and bevacizumab alone. The secondary objectives are to assess whether the addition of panitumumab improves OS, RRs (complete and partial response), time to progression, time to treatment failure, duration of response and/or the rate of stable disease.

Panitumumab is administered at a dose of 6 mg/kg once every 2 weeks on the same day as the chemotherapy and bevacizumab [40].

conclusions

The EGFR overexpression plays an important role in the tumorigenesis of cancer cells. Principal strategies for EGFR

[Diagram: Panitumumab + BSC vs BSC Alone in 3rd line mCRC]

**Randomization stratification**
- ECOG score: 0-1 vs 2
- Geographic region: Western EU vs Central and Eastern EU vs rest of world

**Design of the registered study of Panitumumab in United States**

**Figure 1.** Panitumumab + best supportive care (BSC) versus BSC alone in third-line metastatic colorectal cancer.
Panitumumab demonstrated safety and promising activity in phase I, II and III studies in many human tumors when administered weekly, bi-weekly and every 3 weeks without premedication. It demonstrated safety and promising activity in phase I, II and III studies in many human tumors when administered weekly, bi-weekly and every 3 weeks without premedication. It is active when administered in monotherapy and when associated with chemotherapy, also in heavily pretreated patients. Panitumumab well tolerated and the most common toxicity was represented by skin rash which seems to be dose dependent.

Phase III randomized studies, actually ongoing, are necessary to assess the real efficacy of panitumumab.

Figure 2. Panitumumab + best supportive care (BSC) versus BSC alone in third-line metastatic colorectal cancer.

**Exploratory Analysis of OS by Severity of Skin Toxicity with Panitumumab**

Hazard ratio = 0.61

(95\% CI: 0.40, 0.95)  

*P = 0.0278*

**Patients at risk:**

| Grade 2-4 | 152 | 150 | 138 | 120 | 99 | 78 | 58 | 39 | 29 | 17 | 13 | 9 | 6 | 5 | 1 | 0 | 0 |
| Grade 1   | 57  | 55  | 47  | 34  | 24  | 20  | 12  | 8  | 5  | 4  | 3  | 2  | 0 | 0 | 0 | 0 | 0 |

**References**