Activity of panitumumab alone or with chemotherapy in non-small cell lung carcinoma cell lines expressing mutant epidermal growth factor receptor

Daniel J. Freeman, Tammy Bush, Selam Ogbagabriel, Brian Belmontes, Todd Juan, Cherylene Plewa, Gwyneth Van, Carol Johnson, and Robert Radinsky
Amgen Inc., Thousand Oaks, California

Abstract
Epidermal growth factor receptor (EGFR) kinase domain mutations cause hyperresponsiveness to ligand and hyper-sensitivity to small-molecule tyrosine kinase inhibitors. However, little is known about how these mutations respond to antibodies against EGFR. We investigated the activity of panitumumab, a fully human anti-EGFR monoclonal antibody, in vitro in mutant EGFR-expressing non-small cell lung carcinoma (NSCLC) cells and in vivo with chemotherapy in xenograft models. Mutant EGFR-expressing NSCLC cells (NCI-H1975 [L858R + T790M] and NCI-H1650 [A746-750]) and CHO cells were treated with panitumumab before EGF stimulation to assess the inhibition of EGFR autophosphorylation. Established tumors were treated with panitumumab (25, 100, or 500 µg/mouse twice a week) alone or with docetaxel (10 or 20 mg/kg once a week) or cisplatin (7.5 mg/kg once a week). Antitumor activity and levels of proliferation markers were analyzed. Treatment of mutant EGFR-expressing CHO and NSCLC cells with panitumumab inhibited ligand-dependent autophosphorylation. In NCI-H1975 and NCI-H1650 xenografts, treatment with panitumumab alone or with cisplatin inhibited tumor growth compared with control (P < 0.0003). With panitumumab plus docetaxel, enhanced antitumor activity was seen in both xenografts versus panitumumab alone. Panitumumab treatment alone decreased Ki-67 and phospho-mitogen-activated protein kinase (pMAPK) staining in both xenografts compared with control. Docetaxel enhanced panitumumab activity in NCI-H1650 xenografts (decreased Ki-67 and pMAPK staining by >60%) when compared with either agent alone. Panitumumab inhibits ligand-induced EGFR phosphorylation, tumor growth, and markers of proliferation alone or with docetaxel in NSCLC cell lines that express clinically observed EGFR kinase domain mutations, including the small-molecule tyrosine kinase inhibitor-resistant T790M mutation. [Mol Cancer Ther 2009;8(6):1536–46]

Introduction
The epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase receptor expressed in many different tumor types. The role of EGFR-mediated signaling pathways in the progression of tumor growth and metastases has been well-established (1, 2). EGFR consists of an extracellular ligand-binding domain, a transmembrane domain, and an intracellular kinase domain with tyrosine autophosphorylation activity. Activation of EGFR by EGF or transforming growth factor-α induces receptor heterodimerization or homodimerization and tyrosine kinase phosphorylation of downstream signaling molecules that promote cell proliferation, survival signals, and metastases (2).

Panitumumab is a fully human monoclonal antibody (mAb) that binds to EGFR with high affinity (5 × 10^{-11} mol/L), prevents ligand-induced activation, and arrests tumor cell proliferation (3). In preclinical studies, treatment with panitumumab resulted in inhibition of tumor growth and eradication of tumors in some animal models (3, 4). Because panitumumab is a fully human mAb, it may have greater specificity than EGFR small-molecule tyrosine kinase inhibitors (SM TKI; ref. 5), and fewer immunogenic reactions in patients compared with chimeric or humanized EGFR mAbs (3, 4, 6). In clinical studies, patients with solid tumors, including colorectal, renal cell, and head and neck, and non-small cell lung carcinoma (NSCLC), the use of anti-EGFR agents have shown encouraging efficacy and safety (7–12). More recently, greater efficacy has been observed in patients stratified by tumor biomarkers (13, 14).

Mutations in the intracellular kinase domain of EGFR have been identified in patients with NSCLC but not other tumor types (15–17). Although findings from clinical studies suggest that patients harboring the EGFR mutations may account for the majority of observed clinical responses to small-molecule inhibitors of EGFR (18, 19), the correlation of these mutations with survival benefit is unclear (14, 20–22). Current data suggest that patients...
with EGFR mutations may also have an amplified EGFR gene (22–24), which may be correlated with a survival benefit in patients with or without EGFR mutations. Preclinical data suggest that the EGFR mutations render the receptor hyperresponsive to ligand in both intensity and duration of signal, resulting in increased mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase, and STAT5 signaling. This suggests that patients harboring the EGFR mutations may be more resistant to chemotherapy treatment (25). Preclinical data also suggested that cells expressing the EGFR kinase domain mutations are hypersensitive to inhibition with SM TKIs (17–19, 25) and down-regulated by anti-EGFR antibodies (26, 27).

In addition to the EGFR mutations found in patient samples, several human NSCLC cell lines, including NCI-H1650 and NCI-H1975, have been found to express clinically observed EGFR mutations. Both cell lines have an increased sensitivity to EGF-induced phosphorylation at two COOH-terminal phosphorylation sites, Y992 and Y1068, as well as an increased phosphorylation of AKT and STAT5 (25).

Our objectives were to determine the in vitro activity of panitumumab against clinically observed EGFR mutations and the in vivo activity of panitumumab, either alone or with docetaxel or cisplatin, in NSCLC cell lines expressing mutant EGFR. These chemotherapeutic agents represent the standard of care for patients with advanced NSCLC. We found that panitumumab inhibits ligand-induced EGFR phosphorylation in an isogenic Chinese hamster ovary (CHO) cell system and in NSCLC cell lines expressing both wild-type and clinically observed EGFR kinase domain mutations including the SM TKI-resistant T790M mutation. Panitumumab inhibits ligand-induced EGFR phosphorylation, tumor growth, and markers of proliferation alone or with docetaxel in wild-type and mutant-expressing NSCLC cell lines. Our results may support further evaluation of panitumumab in NSCLC patients, especially if subsets of patients more likely to benefit can be identified based on tumor molecule phenotype.

Materials and Methods

**Cell Lines and Reagents**

Human NSCLC cell lines expressing wild-type EGFR (A549 and SK-MES-PD) or mutant EGFR (NCI-H1650 and NCI-H1975) were purchased from the American Type Culture Collection. Cells were cultured in RPMI 1640 or Ham’s F-12 (Life Technologies) supplemented with 10% fetal bovine serum (HyClone), 2 mmol/L L-glutamine, and 0.1 mmol/L nonessential amino acids (Life Technologies). EGFR-expressing CHO cell lines were cultured in DMEM high-glucose (Life Technologies) supplemented with 10% fetal bovine serum (HyClone), 1× glutathione, 1× hypoxanthine, and 1× thymidine (Life Technologies). EGFR mutations, L858R, T790M, and Δ750-759 [consensus sequence from the EGFR deletion mutations reported by Paez et al. (19)] were introduced into CHO cells. CHO cells were seeded into T-25 flasks; on the following day, all media were replaced with 50% of the medium containing 6 mg/mL polybrene (Sigma-Aldrich) and 50% of the medium containing 0.5 × multiplicity of infection of EGFR lentivirus. Multiplicity of infection was calculated as the number of seeded cells × 0.5 × 0.1 [factor accounted for the difference in expression levels between the neo gene (expressed from IRES EMCV virus) and the EGFR gene (expressed from the CMV promoter)]. Fresh medium containing 500 μg/mL geneticin (Life Technologies) was used for selection for 14 days.

**Investigational Antibody and Chemotherapy**

Panitumumab was provided as a 20 mg/mL stock solution in 50 mmol/L acetate, 100 mmol/L NaCl (pH 5.8; Amgen) in 10 mL sterile vials and diluted in sterile PBS. Human IgG2 control antibody was provided as a 40 mg/mL stock solution (Amgen) and diluted in sterile PBS. Commercially available docetaxel and cisplatin were purchased as a stock concentration of 10 mg/mL for docetaxel in 13% ethanol/water (Aventis Pharmaceuticals) and 1 mg/mL (aqueous injectable; Bedford Labs) for cisplatin. Docetaxel and cisplatin were dosed based on the weight of the mice receiving either treatment before each dosing regimen.

**EGFR Autophosphorylation Assays and Western Blot Analysis**

Cells lines were seeded in 6-well plates at ~85% confluency in the appropriate cell culture medium to determine the ability of panitumumab to inhibit ligand-induced phosphorylation. Cells were serum starved overnight before treatment with panitumumab at 2, 20, 200, or 2,000 nmol/L for 3 h followed by subsequent EGF treatment at 100 ng/mL for 30 min. NCI-H1975 and NCI-H1650 xenografts (~300 mm3) were treated at t = 0 and t = 72 h with 0.5 mg panitumumab via an i.p. injection before a 30 min stimulation with an i.p. injection of 100 μg EGF on day 5 (28). Total cell lysates were prepared, loaded on a 4% to 20% Tris-glycine gel, transferred onto 0.45 μm nitrocellulose membrane, and blocked in 3% bovine serum albumin in TBS (Invitrogen). The membranes were then incubated with an anti-phospho-Y1068 EGFR or an anti-total AKT antibody (Cell Signaling Technology). Expression was detected by enhanced chemiluminescence (Amersham Biosciences) and images were captured with the Versa Doc 5000 imaging system (Bio-Rad). The IC50 was determined using the concentration response data to fit a sigmoid function using nonlinear regression analysis (Prism; Graph Pad). AKT levels were measured as a loading control using an anti-total AKT antibody (Cell Signaling Technology).

**Xenograft Models in Athymic Nude Mice**

All animal studies were conducted under an internal Institutional Animal Care and Use Committee protocol and satisfied all Association for Assessment and Accreditation of Laboratory Animal Care specifications. Athymic nude mice (Harlan-Sprague-Dawley; female, 5-6 weeks old) were housed 5 per cage, supplied with food and water ad libitum, and maintained under aseptic conditions in a ventilated rack system. Human NSCLC cancer cells (5 × 106) were injected s.c. in the right flank area of the mice. After tumors were allowed to establish and grow to an average size of...
Panitumumab in Mutant EGFR-Expressing NSCLC Xenografts

~200 mm³, mice were randomized into experimental groups (n = 10), according to tumor volume, and then treatment began on the following day. Tumors were measured twice a week with a digital caliper using the following equation: \((W^2 \times L) / 2\), where width (W) is defined as the smaller of the two measurements and length (L) is defined as the larger of the two measurements. Body weights were recorded twice a week. Percent inhibition was calculated using the final average tumor volume measurement at the end of the study, \([T / C \times 100]\), where T is the treatment group and C is the control group.

Statistical Analysis

All continuous measurements were expressed as mean ± SE and plotted as a function of time for xenograft data. Statistical significance at the P < 0.05 level of observed differences between growth curves was evaluated by repeated-measures ANOVA followed by Scheffé post hoc testing for nonstatistical differences compared with the control are indicated (P = NS).

Immunohistochemistry

NCI-H1975 and NCI-H1650 xenografts were collected, fixed in buffered zinc formalin, and paraffin embedded. Deparaffinized tissue sections were pretreated with Antigen Retrieval Citra (BioGenex). The sections were blocked with CAS Block (Zymed Laboratories) and incubated with rabbit polyclonal anti-Ki-67 at a dilution of 1:2,000 (Novocastra Laboratories) or anti-phospho-p44/42 MAPK at a dilution of 1:50 (Cell Signaling). The antibody was detected with a biotinylated goat anti-rabbit immunoglobulin (Vector Laboratories) for anti-Ki-67 or with horseradish peroxidase-conjugated goat anti-rabbit (Jackson ImmunoResearch Laboratories) for anti-phospho-MAPK. Slides were quenched with 3% hydrogen peroxide and then incubated with avidin-biotin complex (Vector Laboratories). Reaction sites were visualized with 3,3′-diaminobenzidine (DAKO) and counterstained with hematoxylin.

Tumor sections stained for Ki-67 photographed in three widely spaced images from the peripheral viable regions of the tumor mass using a ×10 objective on a Nikon FXA upright microscope using standard bright-field illumination. The number of Ki-67-positive cells in each field were counted using the Visiomorph Integrator image analysis system (Visiopharm), and the individual field areas were measured. The total positive cells and the total field areas were summed, and the positive cell density was calculated. The results were expressed as the percent of Ki-67-positive cells versus the control treated group.

Tumor sections stained for phospho-MAPK were scored semiquantitatively for the percentage cells with cytoplasmic staining on a scale 0 (negative) to 3. Apoptotic or necrotic cells appeared to differentially take up stain and were excluded. Because of uneven distribution of apparently viable and nonviable cells, it was not possible to perform automated morphometry. Three ×40 high-power fields were examined for each tumor and the percentage of cells at each intensity was scored. A long H score was calculated by combining the components of staining intensity and the percentage of positive cells is defined as \([1 \times \text{(percentage of cells staining at 1)}) + [2 \times \text{(percentage of cells staining at 2)}) + [3 \times \text{(percentage of cells staining at 3)})] = \text{long H score.}\)

The group means were calculated with a n = 4 tumors per group, except in the NCI-H1975 control group where the n was 3 tumors.

Results

Panitumumab Inhibits Ligand-Induced Autophosphorylation in Wild-type and Mutant EGFR-Expressing Cells In vitro

To determine if panitumumab inhibits phosphorylation and activation in vitro, CHO cells were transduced with a multiplicity of infection of 0.5 with lentiviral constructs containing either wild-type or mutant EGFR sequences (L858R, Δ750-759, or T790M). Fluorescence-activated cell sorting analysis revealed that the expression level of cell-surface EGFR in the ligand-inducible CHO cells was similar to that seen in both EGFR-expressing NSCLC cell lines (data not shown). Subconfluent cells were serum-starved, treated with panitumumab or control IgG2 at varying concentrations, before EGF stimulation. Treatment with panitumumab inhibited ligand-induced autophosphorylation of EGFR in CHO cells that overexpressed either wild-type or mutant EGFR in vitro (Fig. 1A).

Because the majority of EGFR mutations have been shown to occur in NSCLC patients, we wanted to determine if panitumumab can inhibit ligand-induced phosphorylation in NSCLC cell lines that express either wild-type or mutant EGFR. Similar to CHO cells, panitumumab treatment inhibited EGF-induced phosphorylation in SK-MES-PD, A549, NCI-H1650, and NCI-H1975 cells (Fig. 1B). However, we noted that there was some basal level of phosphorylation in the mutant EGFR-expressing cell lines (NCI-H1650 and NCI-H1975).

The IC₅₀ for ligand-induced autophosphorylation by panitumumab in CHO cells expressing wild-type or mutant EGFR (L858R, Δ750-759, or T790M) was 0.23, 0.18, 0.17, and 0.23 nmol/L, respectively. In the wild-type (A549 and SK-MES-PD) and mutant (NCI-H1975 and NCI-H1650) expressing NSCLC lines, the IC₅₀ with panitumumab was 0.34, 0.44, 0.92, and 0.25 nmol/L, respectively.

Panitumumab Inhibits Ligand-Induced Autophosphorylation in Mutant EGFR-Expressing NSCLC Xenografts In vivo

To test if panitumumab can inhibit EGFR autophosphorylation in vivo, mice with established NCI-H1650 or NCI-H1975 xenograft tumors of ~300 mm³ were treated at t = 0 and t = 72 h with 500 μg panitumumab or control IgG2. At day 5, based on previous work (28), mice were treated with 100 μg EGF 30 min before tumor excision and analysis. Similar to the in vitro results, panitumumab inhibited ligand-induced EGFR autophosphorylation in NCI-H1650 established tumor xenograft tissues as detected by immunoblotting with anti-phospho-Y1068 EGFR and anti-EGFR antibodies (Fig. 2A). Panitumumab treatment also decreased basal EGFR phosphorylation when compared with
the unstimulated control (Fig. 2A). Additionally, panitumumab treatment of NCI-H1975 tumor xenografts inhibited ligand-induced phosphorylation (Fig. 2B). Interestingly, treatment of NCI-H1975 tumors also reduced total EGFR compared with untreated or control treated tumors (Fig. 2B).

**Panitumumab Treatment Alone Delayed Tumor Growth In vivo in a Dose-Dependent Manner**

To determine if the in vivo inhibition of ligand-induced phosphorylation observed could result in an inhibition of tumor growth, NCI-H1650 and NCI-H1975 NSCLC cells were grown as tumor xenografts. Mice were treated with 0, 25, 100, or 500 μg/mouse panitumumab or 500 μg/mouse IgG2 by i.p. injection twice a week for 3 to 4 weeks. In NCI-H1650 xenografts, panitumumab treatment at 25, 100, and 500 μg significantly delayed tumor growth by 21% (P = 0.3388), 47% (P < 0.0011), and 56% (P < 0.0001), respectively, when compared with the control group (Fig. 3A). Similarly, panitumumab treatment of NCI-H1975 xenografts at the same doses significantly delayed tumor growth by 45%, 54%, and 55% (all treatments; P < 0.0001), respectively, when compared with the control group (Fig. 3B).

**Panitumumab plus Cisplatin Treatment Delayed Tumor Growth In vivo**

Because platinum-based chemotherapies are the standard of care in patients with NSCLC, we assessed whether panitumumab plus cisplatin could enhance antitumor efficacy. In previous experiments, cisplatin treatment alone did not significantly inhibit tumor growth at any doses studied (2.5, 5.0, or 7.5 mg/kg) in either NCI-H1650 or NCI-H1975 xenograft models compared with the control groups (data not shown). Panitumumab or IgG2 control antibody was administered 500 μg twice a week for 3 weeks, with cisplatin treatment of 7.5 mg/kg weekly for 3 weeks the following day. Treatment of panitumumab plus cisplatin significantly delayed tumor growth in NCI-H1650 xenografts (51%, P < 0.0001 versus control and 36%, P = 0.0419 versus cisplatin alone; Fig. 4A). In NCI-H1975 xenografts, tumor growth delay was also observed (63%, P < 0.0001 versus control and 58%, P = 0.009 versus cisplatin alone; Fig. 4B). However, the combination of panitumumab plus cisplatin did not enhance tumor growth delay when compared with panitumumab alone (P > 0.9009 versus panitumumab).

---

**Figure 1.** Panitumumab activity on ligand-induced EGFR phosphorylation in vitro in (A) CHO cells expressing wild-type or mutant EGFR or (B) NSCLC cell lines expressing wild-type or mutant EGFR. Cells were treated with panitumumab (or control IgG2) at varying concentrations for 3 h followed by treatment with 100 ng/mL EGF for 30 min. Total EGFR and EGFR autophosphorylation levels were detected by Western blot analysis using anti-EGFR and anti-phospho-Y1068 EGFR antibodies, respectively. Each experiment was done in duplicate.
Panitumumab plus Docetaxel Treatment Enhanced Tumor Growth Inhibition In vivo

The antitumor activity of panitumumab plus docetaxel was also assessed in both mutant EGFR-expressing NSCLC cell lines. In previous experiments, docetaxel treatment at 20 and 30 mg/kg statistically significantly inhibited tumor growth in established NCI-H1650 xenografts, by 29% (P = 0.0067) and 43% (P < 0.0001), respectively, whereas treatment of NCI-H1975 xenograft with 10, 20, and 30 mg/kg inhibited tumor growth by 53% (P = 0.0215), 73.2% (P < 0.0001), and 70% (P = 0.0005), respectively, compared with IgG control (data not shown). To determine the effect of treatment with panitumumab plus docetaxel versus either agent alone, docetaxel was used at a suboptimal dose in the combination studies. The combination of panitumumab agent alone, docetaxel was used at a suboptimal dose in the combination studies. The combination of panitumumab and docetaxel (20 mg/kg) significantly enhanced tumor growth inhibition in NCI-H1650 xenografts (76%, P < 0.0001 versus control, P = 0.0003 versus docetaxel, and P = 0.0135 versus panitumumab alone; Fig. 5A). Panitumumab (500 μg) plus docetaxel (10 mg/kg) also enhanced tumor growth inhibition in NCI-H1975 (87%, P < 0.0001 versus control, P < 0.0001 versus docetaxel, and P = 0.0672 panitumumab alone; Fig. 5B). These data indicate that panitumumab plus docetaxel treatment significantly enhanced the antitumor activity of panitumumab. Importantly, no negative interaction with this combination treatment was observed as determined by the efficacy and lack of significant weight loss (<10%; data not shown) in the animals.

Panitumumab Treatment Decreased Levels of Proliferation Markers in Tumor Xenografts

Ligand-induced activation of the EGFR can induce cellular proliferation (1). To examine if panitumumab can inhibit markers of cellular proliferation in vivo, mice with established NCI-H1650 or NCI-H1975 tumor xenografts were treated for 18 days with panitumumab or docetaxel alone or the combination of the two agents at the same dose and schedule as the xenograft studies (Fig. 6). Fixed tissue sections from these tumors were measured for changes in Ki-67 or anti-phospho-MAPK by immunohistochemistry.

We show that panitumumab inhibits ligand-induced EGFR autophosphorylation in NSCLC cell lines and in isogenic CHO cells that expresses physiologically relevant levels of mutant or wild-type EGFR, including the SM TKI-resistant T790M mutation. We show that panitumumab inhibits ligand-induced EGFR autophosphorylation in NSCLC cell lines and in isogenic CHO cells that expresses physiologically relevant levels of mutant or wild-type EGFR, including the SM TKI-resistant T790M mutation. We show that panitumumab inhibits ligand-induced EGFR autophosphorylation in NSCLC cell lines and in isogenic CHO cells that expresses physiologically relevant levels of mutant or wild-type EGFR, including the SM TKI-resistant T790M mutation. We show that panitumumab inhibits ligand-induced EGFR autophosphorylation in NSCLC cell lines and in isogenic CHO cells that expresses physiologically relevant levels of mutant or wild-type EGFR, including the SM TKI-resistant T790M mutation. We show that panitumumab inhibits ligand-induced EGFR autophosphorylation in NSCLC cell lines and in isogenic CHO cells that expresses physiologically relevant levels of mutant or wild-type EGFR, including the SM TKI-resistant T790M mutation. We show that panitumumab inhibits ligand-induced EGFR autophosphorylation in NSCLC cell lines and in isogenic CHO cells that expresses physiologically relevant levels of mutant or wild-type EGFR, including the SM TKI-resistant T790M mutation. We show that panitumumab inhibits ligand-induced EGFR autophosphorylation in NSCLC cell lines and in isogenic CHO cells that expresses physiologically relevant levels of mutant or wild-type EGFR, including the SM TKI-resistant T790M mutation. We show that panitumumab inhibits ligand-induced EGFR autophosphorylation in NSCLC cell lines and in isogenic CHO cells that expresses physiologically relevant levels of mutant or wild-type EGFR, including the SM TKI-resistant T790M mutation. We show that panitumumab inhibits ligand-induced EGFR autophosphorylation in NSCLC cell lines and in isogenic CHO cells that expresses physiologically relevant levels of mutant or wild-type EGFR, including the SM TKI-resistant T790M mutation.

Discussion

Understanding the different mechanisms of EGFR tyrosine kinase inhibitors and mAbs may lead to insight into the EGFR pathway and the treatment modality likely to result in clinical benefit. The data here show that panitumumab can inhibit ligand-induced EGFR autophosphorylation, tumor growth, and levels of proliferation markers in mouse models of human NSCLC expressing both wild-type and mutant EGFR, including the SM TKI-resistant T790M mutation.

We show that panitumumab inhibits ligand-induced EGFR autophosphorylation in NSCLC cell lines and in isogenic CHO cells that expresses physiologically relevant levels of mutant or wild-type EGFR. The difference in methodology may explain the differences in the activity of the two EGFR inhibitors. In other studies, EGFR mutants were overexpressed using transient transfections (16–18, 25, 29), which resulted in nonphysiologic levels of the EGFR and therefore ligand-independent phosphorylation of the receptor. Thus, our approach was to transduce CHO cells

![Figure 2](image-url)
with a more physiologically relevant number of EGFR on the cells and to evaluate panitumumab activity in a system that is dependent on EGF for EGFR phosphorylation.

Importantly, panitumumab also inhibited ligand-induced EGFR phosphorylation in cells expressing the T790M mutation. This mutation was identified as an acquired resistance mutation in patients with NSCLC that initially responded to SM TKIs (16–18). Although panitumumab has yet to show clinical utility in patients with NSCLC, its activity against the T790M mutation may suggest a potential role for panitumumab in either patients with \textit{de novo} SM TKI resistance mutations, as seen in the NCI-H1975 NSCLC cell line (17), or in patients who acquire a resistance mutation subsequent to an initial response to a SM TKI (16).

A challenge for antibody-targeted therapies is the requirement to overcome tumor penetration obstacles, such as hydrostatic microvascular pressure and interstitial fluid flow.

Figure 3. Panitumumab treatment of (A) NCI-H1650 and (B) NCI-H1975 mutant EGFR-expressing NSCLC xenografts delayed tumor growth. Mice were treated with panitumumab at 25, 100, or 500 μg/mouse i.p. twice a week once tumors reached 200 mm³. Tumor sizes were measured at prespecified time points. Mean ± SD (n = 10). Statistical analysis was done using StatView 5.0.1 using Scheffé’s ANOVA.

Mol Cancer Ther 2009;8(6). June 2009
pressure (31–33). Although these pressures were not directly measured in our experiments, panitumumab treatment alone effectively inhibited ligand-induced EGFR autophosphorylation and reduced the basal level of phosphorylation in vivo in both NCI-H1650 and NCI-H1975 established xenografts. Of note, there was a measurable level of residual EGFR phosphorylation after treatment with panitumumab presumably because of the high number of receptors that may result in ligand-independent signaling (Fig. 2).

Although treatment of established tumors with panitumumab did not result in a complete inhibition of basal phosphorylation at the day 8 time point, biweekly treatment alone was associated with significant inhibition of tumor growth in both xenograft models. This is significant given that the NCI-H1975 cells harbor a T790M mutation that is completely resistant to EGFR TKIs in preclinical studies and thought to be responsible for clinical resistance to gefitinib and erlotinib (16, 34, 35). This result may be explained by two different hypotheses. First, the “oncogenic addiction” hypothesis asserts that the mutant EGFR-expressing NSCLC cells are addicted to the EGFR signaling pathway such that any perturbation of the proliferation or survival signals results in cell death (36). This hypothesis is potentially supported by data from a large number of patients that harbor EGFR mutations that have shown clinical responses to SM TKIs (19, 22, 25, 29, 37) as well as responses seen in colorectal cancer patients with wild-type EGFR who have responded to antibodies to the receptor (3, 9, 24, 38, 39). A second hypothesis is that long-term panitumumab treatment results in internalization or down-regulation of EGFR expression, which ultimately results in a reduction in EGFR-dependent oncogenic signaling (40, 41).

Although EGFR inhibitors have shown single-agent activity in an unselected patient population to date, in the future they are more likely to be used in a selected patient population and in combination with chemotherapy in earlier lines of treatment (42). Currently, platinum-based chemotherapies are used as the standard of care in patients with NSCLC, so we assessed whether panitumumab with cisplatin could enhance antitumor efficacy over either agent.
alone. Treatment of panitumumab with cisplatin did not result in an increase in antitumor activity over panitumumab alone consistent with other anti-EGFR antibodies in similar preclinical models (43). These data are not completely surprising for several reasons. Although platinum-based therapies have activity in human patient populations, in our studies, the maximum tolerated dose of cisplatin was lower than the efficacious dose. Also, because the NSCLC cell lines have increased signaling through the AKT and STAT survival pathways, they might be more resistant to treatment with chemotherapy agents (25). Of note, although the addition of cisplatin to panitumumab did not have an increased antitumor activity versus panitumumab alone, no negative interaction occurred between the two agents. This observation is important given the results observed with erlotinib, which showed a negative interaction between the SM TKI and chemotherapy in clinical trials for first-line treatment of NSCLC (20, 44–47).

Additionally, we also tested the antitumor activity of panitumumab with docetaxel, another agent approved for first-line treatment of NSCLC. We showed that treatment of panitumumab plus docetaxel has enhanced antitumor activity compared with either agent alone. Results from a first-line therapy trial of erlotinib and chemotherapy in NSCLC suggest that adding a SM TKI EGFR inhibitor before treating with a chemotherapy agent may negate the effects of the chemotherapy (46), but our data showed additive effects with panitumumab and docetaxel, suggesting that the agents are not antagonistic when administered simultaneously.

Activity of EGFR mAbs in combination with platinum-based chemotherapy has been studied in the first-line setting of NSCLC. Clinical benefit has been shown with cetuximab when combined with cisplatin and vinorelbine in a phase III study (FLEX) in NSCLC (48). Although no change in progression-free survival was detected (4.8 months for each arm, cetuximab plus chemotherapy or chemotherapy alone), overall survival benefit was statistically significant in favor of the cetuximab-containing arm versus chemotherapy alone (11.3 versus 10.1 months; \( P = 0.044 \)). Panitumumab has also been evaluated in a randomized phase II first-line NSCLC study in combination with paclitaxel and carboplatin (49). This study failed to show an improvement in time to progression or overall survival when panitumumab was added to chemotherapy in this unselected patient population. Similarly, a phase III study...
comparing carboplatin and paclitaxel with cetuximab versus chemotherapy alone did not show a significant difference in progression-free survival (the primary endpoint of study; ref. 50). Overall, the clinical data suggest that there may be a role for EGFR mAbs in NSCLC but that improved patient selection will be required.

One of the major challenges for targeted therapeutics is to associate treatment with a pharmacodynamic readout of efficacy. Our data indicated that the inhibition of phosphorylation in vivo and the antitumor activity correlated with a reduction in markers of proliferation and downstream kinase signaling, confirming that panitumumab inhibits ligand-induced activation of EGFR, resulting in an inhibition of the MAPK pathway and associated proliferation.

In conclusion, our studies show that panitumumab has antitumor activity both alone and with docetaxel in NSCLC cell lines and xenografts expressing mutant EGFR. These results provide evidence for the potential role of panitumumab in the treatment of NSCLC. Future studies are needed to correlate responsiveness to panitumumab to markers that identify NSCLC populations that would likely respond to therapy.

Disclosure of Potential Conflicts of Interest
All of the authors are employees with ownership interest, Amgen, Inc.

Acknowledgments
We thank Mee Rhan Kim, Ph.D. (Amgen), for assistance with the writing and preparation of this article.

References
4. Yang XD, Jia XC, Corvalan JR, Wang P, Davis CG, Jakobovits A.


Molecular Cancer Therapeutics

Activity of panitumumab alone or with chemotherapy in non-small cell lung carcinoma cell lines expressing mutant epidermal growth factor receptor


Mol Cancer Ther  Published OnlineFirst June 9, 2009.

Updated version  Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-08-0978

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.