CENTER FOR DRUG EVALUATION AND RESEARCH AND CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

APPLICATION NUMBER:

125147/0

PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

STN BLA NUMBER:	**125147**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**12/15/2005**
PRODUCT:	**recombinant, fully human monoclonal antibody (panitumumab), directed against the human epidermal growth factor receptor**
INTENDED CLINICAL POPULATION:	
SPONSOR:	**AMGEN, INC.**
DOCUMENTS REVIEWED:	**electronic submission (eCTD format)**
REVIEW DIVISION:	**Division of Biologic Oncology Drug Products (HFD-107)**
PHARM/TOX REVIEWER:	**Anne M. Pilaro, Ph.D.**
PHARM/TOX SUPERVISOR:	**M. David Green, Ph.D.**
DIVISION DIRECTOR:	**Patricia Keegan, M.D.**
PROJECT MANAGER:	**Monica Hughes, M.S.**

Date of review submission to Division File System (DFS):

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Based upon the nonclinical data contained in the original submission, the pharmacology and toxicology discipline review of Biologics Licensing Application STN BLA#125147 of panitumumab is complete, and should allow for approval pending completion of the clinical and chemistry sections. Observed panitumumab toxicities are extensions of its pharmacologic activity, were reflected in the clinical studies, and may be monitored and treated appropriately in the clinical setting.

B. Recommendation for nonclinical studies

No additional nonclinical studies of panitumumab are recommended at the present time.

C. Recommendations on labeling

Modifications to the PRECAUTIONS section of the label, including revision of the language regarding potential impairment of fertility by panitumumab, and to the Pregnancy subsections are included for communication to the sponsor. Additionally, a subsection to the WARNINGS section of the labeling, regarding the severe dermatologic toxicities and deaths in monkeys treated with panitumumab, is included for communication to the sponsor. Copies of the proposed, revised language for these sections are included as Appendix I to this review.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Panitumumab (ABX-EGF, AMG 954; VECTIBIX™) was evaluated for pharmacologic activity in human tumor cell lines *in vitro* and in human tumor xenografts in nude mice, and for toxicity and pharmacokinetics in nude mice and cynomolgus monkeys. Tissue binding studies demonstrated that ABX-EGF bound with moderate to strong intensity to surface epidermal growth factor receptor (EGFr) in samples of both human and cynomolgus monkey skin, tonsil, breast, and prostate, and in urothelium of the ureter and urinary bladder, and uterine endometrium and cervical squamous epithelium in monkeys. Treatment of tumor-bearing nude mice with panitumumab alone or in combination with several different biologic or chemotherapy regimens resulted in delayed tumor growth in human colon, epidermoid, breast, or pancreatic cancers. Where effective, combination therapy with panitumumab and selected chemotherapy or biologic anti-tumor treatments resulted in approximately additive, but not synergistic effects. Pharmacokinetic profiles of panitumumab in cynomolgus monkeys following initial, i/v injections of 7.5, 15, 30, or 60 mg/kg doses showed linear, dose-related increases in C_{max} and AUC_{0-t}, dose-related decreases in clearance with a concomitant increase in apparent elimination half-life, and steady state volumes of distribution approximately equal to the plasma space. Steady state, as evidenced by peak and trough serum ABX-EGF levels was achieved in repeat dose studies following approximately 5 to 6 doses of panitumumab. With repeated administration for 4 to 26 weeks, the dose-related decreases in clearance and increases elimination half-life were slightly higher than following the initial dose; however, the C_{max} and AUC_{0-last} were only slightly

(< 2-fold) increased over the initial, observed values. Therefore, the toxicokinetic evaluations confirmed that exposure to ABX-EGF was continuous over the duration of these studies with little accumulation of drug. Although group mean values for C_{max} and $AUC_{0\text{-}last}$ were frequently not different for the same dose levels of ABX-EGF over the study durations, anti-panitumumab antibodies developed in several monkeys in all repeat-dose studies, resulting in decreased ABX-EGF exposure in these individual animals, and in some cases, reversal of some of the panitumumab-related toxicities. Severe dermatologic and gastrointestinal toxicities were noted at all dose levels in cynomolgus monkeys treated weekly with 7.5, 15, 30, or 60 mg/kg panitumumab for 4, 13, or 26 weeks. These doses correspond to approximately 1.25 to 10-fold greater than the proposed human dose of 6 mg/kg ABX-EGF administered every two weeks, and approximately 3 to 24-fold higher than the proposed 2.5 mg/kg/week panitumumab dose, when adjusted for body weight. Observed toxicities included decreases in body weight and food consumption, decreases in serum calcium, phosphate, and magnesium, and dose-dependent clinical signs consisting of soft or watery stool, alopecia, skin rash, erythema, flaking and/or dryness, suppurative dermatitis, erosions, sloughing, and ulcerations, and in several studies, early mortalities secondary to the severity of the skin lesions. These changes occurred with increased frequency and severity as both the dose and duration of ABX-EGF increased, and only partially reversible following discontinuation of panitumumab treatment. Panitumumab treatment inhibited ovarian function in non-pregnant female monkeys, and was abortifacient, although not teratogenic when administered to pregnant animals from GD20 through GD48, throughout organogenesis.

B. Pharmacologic activity

VECTIBIX™ binding to the EGFr competitively inhibits the binding of its normal ligands including EGF and transforming growth factor-alpha, which are implicated in tumor growth, and stimulates receptor internalization, leading to a reduction of EGFr expression on the cell surface. This antagonist action inhibits phosphorylation and activation of EGFr-associated kinases, resulting in inhibition of cell growth, and decreased vascular endothelial growth factor, interleukin-8, and other growth factor production. The epidermal growth factor receptor (EGFr) is constitutively expressed in many normal epithelial tissues, including the skin follicle, placenta, and mammary gland. Over-expression of EGFr is also detected in many human cancers including those of the colon and rectum. *In vitro* assays and *in vivo* animal studies have shown that VECTIBIX™, alone or in combination with irinotecan, but not fluorouracil, oxaliplatin, or cis-platinum chemotherapy inhibits the growth and survival of several human tumor cells that over-express the EGFr. No anti-tumor effects of panitumumab were observed in immune deficient mouse models bearing human tumor xenografts with levels of EGFr expression below 10,000 receptors per cell, suggesting that a threshold level of EGFr expression is required for tumor response to VECTIBIX™ to occur.

C. Nonclinical safety issues relevant to clinical use

The dermatologic toxicities following panitumumab treatment were observed both in cynomolgus monkeys following repeat administration, and in clinical trials of ABX-EGF in patients with metastatic, colorectal cancer. In the non-human primate models, dermatologic toxicities included severe erythema, skin flaking, scaling and sloughing, pustule formation, infections, and erosions or ulcerations. Early mortalities were observed in several studies secondary to the severe skin lesions, and the incidence and timing were related to the dose of ABX-EGF. These doses correspond to approximately 1.25 to 10-fold greater than the proposed human dose of 6 mg/kg ABX-EGF administered every two weeks, and approximately 3 to 24-fold higher than the

proposed 2.5 mg/kg/week panitumumab dose, when adjusted for body weight. The dermatologic toxicities observed in these studies are consistent with the pharmacodynamic effects of ABX-EGF in inhibiting critical intracellular pathways involved with the activation and function of EGFr expressed on skin cells, and subsequent inhibition of epidermal cell growth and maturation.

Skin lesions, including acneform rash, pruritis, dry skin, exfoliation, skin fissures, and paronychia were also observed clinically in approximately 90% of 789 metastatic colorectal cancer patients treated with panitumumab. Dermatologic toxicities in these patients were generally Grade 2 in severity, with approximately 12% of subjects (95/789) reporting Grade 3 or higher skin changes. Although not observed in the nonclinical toxicity studies, Grade 1/2 stomatitis and oral mucositis were also reported in approximately 7% of these patients. Development of severe dermatologic toxicity occasionally resulted in infectious complications including sepsis and in rare occasions, death, but most frequently led to either panitumumab dose interruption or dose modification. The clinical skin toxicities have been adequately described in the WARNINGS and ADVERSE REACTIONS sections of the proposed packaged insert; however, additional modifications to these sections to include the findings in cynomolgus monkeys will be requested by the reviewer.

Panitumumab treatment of non-pregnant, female cynomolgus monkeys inhibited ovarian function, resulting in dose-related irregularities in menstrual cycling (prolonged menstrual cycles and/or amenorrhea), decreased pregnancy rates, and decreases in serum 17β-estradiol and progesterone levels, at doses corresponding to approximately 1.25 to 5-fold higher than the proposed, clinical doses when adjusted for body weight. Although no teratogenic effects were observed, panitumumab was abortifacient at all dose levels tested in pregnant female cynomolgus monkeys, following weekly injection from GD20 through GD48 (approximately 1.25 to 6-fold greater than the highest proposed human dose). While these findings may not be relevant to the indicated, clinical population (metastatic colorectal cancer), they have been included in the PRECAUTIONS section of the panitumumab label. However, additional language regarding both the fertility and developmental effects of panitumumab treatment is included in Appendix 1 of this review, for communication to the sponsor.

Hypomagnesemia, hypocalcemia, and hypophosphatemia were observed in several of the nonclinical, repeat-dose toxicity studies of ABX-EGF in cynomolgus monkeys, and have also been reported in clinical trials of panitumumab. These toxicities may be secondary to the moderate to severe diarrhea and dehydration observed in both the non-human primate studies, and in metastatic colorectal patients treated with panitumumab, alone or in combination with irinotecan and 5-fluorouracil chemotherapy. The electrolyte disturbances and diarrhea have been adequately described in the — section of the proposed package insert for VECTBIX™, and recommendations for periodic monitoring of patients for hypomagnesemia and hypocalcemia during and for 8 weeks following completion of panitumumab are included in the label under Laboratory Monitoring, in the PRECAUTIONS section.

Clinical toxicities not predicted by the animal studies included infusion reactions in < 2% of panitumumab treated, colorectal cancer patients, occurring within 24 hours of the first dose. No patients had life-threatening or fatal, ABX-EGF associated infusion reactions, and the events were reported as severe in only 5/789 (<1%) patients. Most of the potential infusion reactions were mild in intensity, resolved without treatment, were isolated occurrences and did not require alteration or interruption of panitumumab dosing. The infusion reactions have been identified in the — ection of the package insert, and require no further action from the pharmacology and toxicology review staff.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

STN BLA number: 125147/000
Review number: 001
Sequence number/date/type of submission: 000/12-16-05/original licensing application
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: AMGEN, INC.
One Amgen Center Drive
Thousand Oaks, CA 91320-1799 USA

Manufacturer for drug substance: ABGENIX, INC.
6701 Kaiser Drive
Fremont, CA 94555 USA

Reviewer name: Anne M. Pilaro, Ph.D.
Division name: Division of Biologic Oncology Products, Office of Oncology Drug Products

HFD #: 107
Review completion date: June 15, 2006

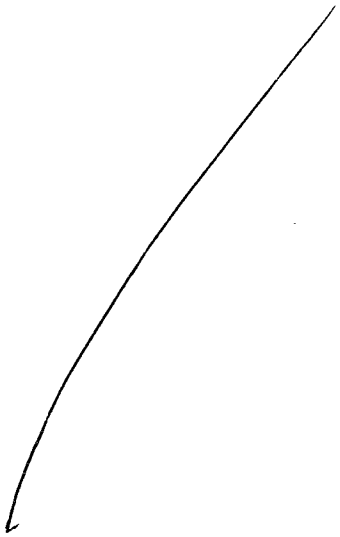
Drug:

Trade name: VECTIBIX™
Generic name: panitumumab
Code name: ABX-EGF, AMG 954
Chemical name: not applicable
CAS registry number: not available
Molecular formula/molecular weight:

Structure: Panitumumab is a fully human, recombinant IgG2 monoclonal antibody, with an approximate molecular weight of 147 kDa. It is

The structure of panitumumab is abstracted from the Chemistry, Manufacturing, and Controls section of the BLA submission (Figure 1, Section 3.2.S.1.2.1), and is included, below as Figure 1.

Figure 1. Schematic Structure of Panitumumab (Predominant Isoform)



Relevant INDs/NDAs/DMFs: BB IND #8382

Drug class: monoclonal antibody; epidermal growth factor inhibitor

Intended clinical population: (from the proposed label for panitumumab, as submitted by the sponsor): "VECTIBIX™ is indicated for the treatment of metastatic carcinoma of the colon or rectum after failure of oxaliplatin- and/or irinotecan-containing chemotherapy regimens."

Clinical formulation: sterile, preservative-free, clear, colorless liquid containing —ng/ml panitumumab, — sodium acetate, — NaCl, and Water for Injection, USP, —

Route of administration: intravenous infusion (over 60 minutes)

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Pharmacology Studies:

Primary Pharmacodynamics:

1. Study #R2003094. Test of expression of EGFr on pancreatic and lung tumor cell lines to be used in xenograft experiments.
2. Study #R2003110. Modulation of *in vitro* growth inhibitory activity of selected anticancer agents by ABX-EGF, a fully-human monoclonal antibody against the EGF receptor.
3. Study #R2003112. *In vitro* growth inhibitory activity and *in vitro* antitumor activity of ABX-EGF, a fully human monoclonal antibody against the EGF receptor, in the human A431 vulvar carcinoma model.
4. Study #R2003113. Evaluation of ABX-EGF, a fully human mAb against the EGF receptor, in athymic mice bearing human A431 vulvar and SKMES lung carcinoma xenograft derived from tumor cell suspensions or tumor fragments.
5. Study #R2003197. Inhibition of EGF binding to A431 cells by ABX-EGF.
6. Study #R2003198. Inhibition of EGFr tyrosine-phosphorylation by ABX-EGF.
7. Study #R2003199. Inhibition of tumor cell activation by ABX-EGF.
8. Study #R2003200. Inhibition of A431 cell growth *in vitro* by ABX-EGF.
9. Study #R2003201. Evaluation of ABX-EGF in athymic nude mice bearing A431 xenografts.
10. Study #R2003202. Eradication of established human epidermoid tumor in nude mice by ABX-EGF.
11. Study #R2003203. Complete eradication of larger tumors by ABX-EGF.
12. Study #R2003204. Effect of ABX-EGF or the 225 antibody on A431 tumor growth in nude mice.
13. Study #R2003205. Effect of ABX-EGF on the growth of multiple human tumors derived from different tissues and expressing different levels of EGFr.
14. Study #R2003206. ABX-EGF prolonged survival of SCID mice bearing MDA231 human breast cancer.
15. Study #R2003207. EGFR expression in normal human tissues and human tumors. A specificity analysis performed by — for Abgenix, Inc.
16. Study #R2003211. Inhibition of IL-8 production by ABX-EGF in human renal cell carcinoma (RCC) cells.
17. Study #R2003212. Inhibition of VEGF production by ABX-EGF in human RCC cells.
18. Study #R2003225. Evaluation of differential effects of tyrosine phosphorylation in EGFr by mass spectrometry.
19. Study #R2003280. Effects of ABX-EGF antibody in established SK-MES PD, a human squamous cell carcinoma, in CD1 nude mice.
20. Study #R2003281. Effects of ABX-EGF antibody in established MiaPaCa-2, a human pancreatic carcinoma, in female Harlan athymic nude mice.
21. Study #R2003283. Evaluation of ABX-EGF antibody in established NCI-H1299, a human non-small cell lung carcinoma, in Harlan athymic nude mice.
22. Study #R2003325. The effect of ABX-EGF on Colo 205 tumor xenografts.
23. Study #R2003327. The effect of AMG 954 on HT29 subcutaneous tumor growth.
24. Study #R2003330. Effects of ABX-EGF on activation of human vascular smooth muscle cells *in vitro*.
25. Study #R2003331. Evaluation of downstream signaling molecules in A431 after ABX-EGF treatment.
26. Study #R2003332. Evaluation of downstream signaling molecules in HCT-116 after ABX-EGF treatment.

27. Study #R2003366. Evaluation of ABX-EGF in nude mice bearing A431 xenografts.
28. Study #R2003371. Inhibition of human renal carcinoma cells growth *in vitro* and *in vivo* tumor xenograft models by ABX-EGF.
29. Study #R2003373. Inhibition of IL-8 and VEGF production in human prostate tumor cells.
30. Study #R2003473. Evaluation of hybridoma derived ABX-EGF in A431 tumor xenograft.
31. Study #R2003520. Effects of ABX-EGF antibody against established MDA-MB-468, human breast carcinoma model in athymic nude mice.
32. Study #R2003537. Effects of combination ABX-EGF and AMG 706 in HT29 human colon carcinoma xenograft model in nude mice.
33. Study #R2003538. Effects of combination ABX-EGF and AMG 706 in A431 human epidermoid carcinoma xenograft model in nude mice.
34. Study #R2003558. Evaluation of combination therapy of ABX-EGF with 5-FU in HT-29 tumor xenograft model.
35. Study #R2003558. Evaluation of combination therapy of ABX-EGF with CPT-11 in HT-29 tumor xenograft model.
36. Study #R2003560. Evaluation of combination therapy of ABX-EGF with oxaliplatin in HT-29 tumor xenograft model.
37. Study #R2003561. Evaluation of combination therapy of ABX-EGF with anti-hVEGF antibody in HT-29 tumor xenograft model.
38. Study #R2003576. Immunohistological evaluation of ABX-EGF localization and penetration in tumors from nude mice with established NCI-H1299 xenografts.
39. Study #R2004035. *In vitro* analysis of ABX-EGF in combination with chemotherapeutic drugs.
40. Study #R2004090. Measurement of EGFr internalization with [redacted] imaging technology.
41. Study #R2004135. Effects of ABX-EGF against established BxPC-3 and Capan I, human pancreatic carcinomas, in nude mice.
42. Study #R2004283. Effects of ABX-EGF and AMG 612153 against established MDA-MB-468, human breast carcinoma model.
43. Study #R2004292. Efficacy of panitumumab in pancreatic (BXPC3 and CAPAN I), epidermoid (A431), breast (MCF7 and MDA MB 468) and colon (HT29) cancer cells and tumors and the analysis of the signal transduction pathways in panitumumab responder and non-responder tumors.
44. Study #R2004446. Evaluation of combination therapy of panitumumab with rapamycin in HT-29 tumor xenograft model.
45. Study #R2004496. Immunohistological evaluation of ABX-EGF administration and penetration in tumors from nude mice with established A-431 xenografts.
46. Study #R2004497. Immunohistological evaluation of ABX-EGF localization and penetration in HT-29 xenograft tumors.
47. Study #R2004498. Immunohistological evaluation of ABX-EGF penetration, cell proliferation, and signaling cascade in MDA-MB-468 human breast carcinoma xenograft tumors from nude mice treated with ABX-EGF.
48. Study #R2004500. Immunohistological evaluation of ABX-EGF penetration, cell proliferation, and signaling cascade in HT-29 xenograft tumors treated with mono and combination therapy of ABX-EGF, oxaliplatin, and CPT-11.
49. Study #R2004503. Immunohistological evaluation of cell proliferation, kinase signaling, and ABX-EGF penetration in tumors from nude mice with established A-431 xenografts.

50. Study #R2004656. EGFR:HER2 ratio in panitumumab responder and non-responder xenograft tumor cell lines.
51. Study #R2004657. Pattern of gene expression can prospectively predict panitumumab (ABX-EGF) monotherapy responsiveness in xenograft models.
52. Study #R2004660. Dose response of panitumumab in EGFr mutant NSCLC NCI-H1650 xenograft.
53. Study #R2005421. ABX-EGF induced internalization of EGF receptor expressed on A431 and HeLa cells.
54. Study #R2005497. Epitope mapping of AMG954, an anti-human epidermal growth factor receptor (EGFR) antibody.
55. Study #R2005530. ABX-EGF induced EGFR internalization.
56. Study #R2005539. Efficacy of panitumumab in the inhibition of EGFR phosphorylation upon stimulation with different EGFR ligands.
57. Study #R2005548. Evaluation of EGFR levels in xenograft tissue using the EGFR pharmDx kit.
58. Study #R2005552. Affinity measurement of ABX-EGF produced by hybridoma.
59. Study #R2005581. Inhibition of ^{125}I-ABX-EGF binding to cell surface expressed human EGFr by unlabeled ABX-EGF.
60. Study #R2005582. Affinity measurement of ABX-EGF derived from CHO cells.

Secondary Pharmacodynamics:

1. Study #R2003090. Test of Expression of EGFr on pancreatic and lung xenografts.
2. Study #R2003091. Evaluation of ABX-EGF monotherapies and combinations with Gemzar against MiaPaCa and Panc-1 human pancreatic carcinoma xenografts in athymic nude mice.
3. Study #2003092. Evaluation of ABX-EGF monotherapies and combinations with Taxotere® or cisplatin against H1299 and SKMES human non-small cell lung carcinoma xenografts in athymic nude mice.
4. Study #R2003093. Test of potency of ABX-EGF used in *in vivo* studies with pancreatic and lung tumors.
5. Study #R2003104. Evaluation of downstream signaling molecules in A431 and SK-MES after ABX-EGF treatment *in vivo*.
6. Study #R2003170. Effects of ABX-EGF and taxotere against established SK-MES PD_1, a human squamous cell carcinoma in CD1 nude mice.
7. Study #R2003208. Effects of ABX-EGF and cisplatin combination therapy on A431 tumor xenografts.
8. Study #R2003209. Effects of ABX-EGF and doxorubicin combination therapy on prostate DU145 tumor xenografts.
9. Study #R2003210. Effects of ABX-EGF and docetaxel combination therapy on NSCLC A549 tumor xenografts.
10. Study #R2003213. Inhibition of EGFr phosphorylation by ABX-EGF in human RCC cells.
11. Study #R2003258. Effects of ABX-EGF and cisplatin against established SK-MES, a human squamous cell carcinoma in CD1 nude mice.
12. Study #R2003277. Effects of ABX-EGF and cisplatin against established NCI-H1299 in CD1 nude mice.
13. Study #2003278. Effects of ABX-EGF and taxotere against established NCI-H1299 tumors in CD1 nude mice.
14. Study #R2003370. Effect of ABX-EGF and taxotere combination therapy on NSCLC A549 tumor xenografts.

15. Study #R2003479. Effects of ABX-EGF on established MCF-7tb human breast cancer model in female nude mice.
16. Study #2003518. Effects of ABX-EGF combined with anti-VEGF antibody against established MDA-MB-468 human breast carcinoma model in athymic nude mice.
17. Study #R2003527. Effects of ABX-EGF against established MDA-MB-231, a human breast carcinoma in female nude mice.
18. Study #R2003547. Effects of ABX-EGF in combination with anti-VEGF mAb against established SK-MES PD, a human squamous cell carcinoma in CD1 nude mice.
19. Study #R2003548. Effects of ABX-EGF in combination with anti-VEGF mAb against established NCI-H1299, a human squamous cell carcinoma in CD1 nude mice.
20. Study #R2003550. Effects of ABX-EGF against established U87 MG vIII, a human glioblastoma tumor model in female nude mice.
21. Study #R2004013. Evaluation of panitumumab (ABX-EGF) alone and in combination with anti-IGF-1R mAb, MAB 391 in nude mice bearing Calu-6 xenografts.
22. Study #R2004082. Effects of ABX-EGF in combination with Herceptin against established NCI-H1299, a human squamous cell carcinoma in HSD athymic nude mice.
23. Study #R2004083. Effects of ABX-EGF in combination with Herceptin against established SK-MES PD, a human squamous cell carcinoma in CD1 nude mice.
24. Study #R2004084. Effects of ABX-EGF in combination with rapamycin against established NCI-H1299, a human squamous cell carcinoma in HSD athymic nude mice.
25. Study #R2004086. Effects of ABX-EGF on established ZR75-1 human breast cancer model in athymic female nude mice.
26. Study #R2004087. Effects of ABX-EGF combined with rapamycin against established MDA-MB-468 in athymic nude mice, human breast carcinoma model.
27. Study #R2004154. Effects of ABX-EGF against established NCI-H82, a human small cell lung carcinoma in female athymic nude mice.
28. Study #R2004155. Effects of ABX-EGF against established NCI-H460, a human large cell lung carcinoma in female athymic nude mice.
29. Study #R2004156. Effects of ABX-EGF against established A549, a human lung carcinoma in female athymic nude mice.
30. Study #R2004279. Effects of ABX-EGF and Herceptin against established BT-474 mammary carcinoma in female nude mice.
31. Study #R2004280. Effects of ABX-EGF in combination with Herceptin against established A549, a human lung carcinoma in female HSD athymic nude mice.
32. Study #R2004281. Effects of ABX-EGF in combination with low-dose rapamycin against established NCI-H1299, a human non-small cell carcinoma in HSD athymic nude mice.
33. Study #R2004282. The effects of ABX-EGF in combination with rapamycin in SK-MES-PD human lung cancer tumor bearing athymic nude mice.
34. Study #2004287. Effects of ABX-EGF in combination with low-dose rapamycin against established NCI-H1299, a human non-small cell carcinoma in HSD athymic nude mice.
35. Study #R2004440. Efficacy of EGFR inhibitors in EGFR-vIII mutation in U87 cells.
36. Study #R2004499. Immunohistochemical evaluation of ABX-EGF penetration, cell proliferation, and signaling cascade in MCF-7tb human breast carcinoma xenograft tumors from nude mice treated with ABX-EGF.
37. Study #R2004501. Immunohistochemical evaluation of cell proliferation, kinase signaling, and ABX-EGF penetration in tumors from nude mice with established BxPC-3 and Capan-1 human pancreatic carcinoma xenografts.
38. Study #R2004637. Administration of panitumumab against established NCI-H1975 NSCLC in female athymic nude mice.

39. Study #R2004720. Efficacy of EGFR inhibitors in kinase domain EGFR mutations in transfected and transduced CHO cells and NCI-H1975, NCI-H1650, SK-MES-PD, and A549 cells and tumors.
40. Study #R2005181. The effects of panitumumab and taxotere combination in NCI-H1975, EGFR mutant NSCLC, xenograft.
41. Study #R2005182. The effects of panitumumab and taxotere combination in NCI-H1650, EGFR mutant NSCLC, xenograft.
42. Study #R2005183. The effects of panitumumab and cisplatin combination in NCI-H1975, EGFR mutant NSCLC, xenograft.
43. Study #R2005184. The effects of panitumumab and taxotere combination in NCI-H1975, EGFR mutant NSCLC, xenograft.
44. Study #R2005428. The effects of panitumumab and taxotere combination in NCI-H1975, EGFR mutant NSCLC, xenograft.
45. Study #R2005549. The effect of panitumumab in the U118 xenograft tumor model.

Safety Pharmacology:

1. Study #104119 (Abgenix Study #ABX-P0307, — Study #04-6567). Cardiovascular, respiratory, and central nervous system assessment of ABX-EGF (panitumumab) administered as a single intravenous dose to conscious cynomolgus monkeys.

Pharmacokinetics Studies:

Absorption:

1. Study #104275 (Abgenix Study #ABX-P0306, — Study # — .243.14). A pharmacokinetic study of ABX-EGF in male nude mice following a single intraperitoneal injection.

Distribution:

1. Study #103619 (Abgenix Study #ABX-T0311, — Study #6271-611). Quantitative whole-body autoradiography of cynomolgus monkeys after a single intravenous administration of ^{125}I-ABX-EGF.
2. Study #103620 (Abgenix Study #ABX-T0312, — Study #6271-612). Absorption, distribution and excretion of ^{125}I-ABX-EGF after a single intravenous administration to cynomolgus monkeys.
3. Study #104274 (— Study #7153-105). Distribution (quantitative whole-body autoradiography) and excretion of radioactivity in monkeys following administration of a single intravenous dose of ABX-EGF and [^{125}I]ABX-EGF.

Excretion:

1. Study #103619 (Abgenix Study #ABX-T0311, — Study #6271-611). Quantitative whole-body autoradiography of cynomolgus monkeys after a single intravenous administration of ^{125}I-ABX-EGF.
2. Study #103620 (Abgenix Study #ABX-T0312, — Study #6271-612). Absorption, distribution and excretion of ^{125}I-ABX-EGF after a single intravenous administration to cynomolgus monkeys.
3. Study #104274 , — Study #7153-105). Distribution (quantitative whole-body autoradiography) and excretion of radioactivity in monkeys following administration of a single intravenous dose of ABX-EGF and [^{125}I]ABX-EGF.

Other Pharmacokinetics Studies:

1. Study #102876 (Abgenix Study #ABX-P0304; — Study # — .026.39). Comparison of the pharmacokinetics of ABX-EGF derived from hybridoma and Chinese hamster ovary (CHO) expression systems in cynomolgus monkeys after intravenous administration.
2. Study #104273 (— Study #054.0501). Comparison of the pharmacokinetics of hybridoma-origin versus CHO-origin ABX-EGF in mice following intravenous injection.

Toxicology Studies:

Single-Dose Toxicology: No single-dose toxicology studies are included in the BLA submission.

Repeat-Dose Toxicology:

1. Study #054-0401 (Abgenix Study #ABX-T0305, — Study #00-3691). A four-week mechanistic toxicity study of ABX-EGF administered once per week by intravenous injection to cynomolgus monkeys followed by a two-month recovery period.
2. Study #BQAW-100. (Abgenix Study #ABX-EGF-99-001). Toxicity, tissue binding, and pharmacokinetics of ABX-EGF following single and multiple intravenous bolus dose administration in cynomolgus monkeys.
3. Study #BQAW-102. Toxicity and toxicokinetics of ABX-EGF following multiple intravenous dose administration (via bolus and infusion) in cynomolgus monkeys.
4. Study # BQAW-103. Toxicity and toxicokinetics of ABX-EGF following intravenous bolus administration in cynomolgus monkeys for three months with a six-week recovery.
5. Study #103419 (Abgenix Study #ABX-T0308, — Study # — 243.15). A six-month multiple dose toxicity study of ABX-EGF administered intravenously to cynomolgus monkeys followed by a two-month recovery period.

Reproductive Toxicology:

Fertility and Embryonic Development:

1. Study #103409 (Abgenix Study #ABX-T0309, — Study # — .026.56). An assessment of the effects of ABXEGF on female fertility and early embryonic development to implantation when administered by weekly intravenous injection to cynomolgus monkeys.

Embryo-fetal Development:

1. Study #103410 (Abgenix Study #ABX-T0310, — Study # — 026.57). An assessment of the effects of ABX-EGF on embryo-fetal development when administered weekly by intravenous injection to pregnant cynomolgus monkeys.

Other Toxicology Studies:

1. Study #102906 (Abgenix Study #ABX-T0307, 02-3032). A 4-week comparison study of two forms of ABX-EGF administered by intravenous injection once per week to cynomolgus monkeys with a 4-week recovery period.
2. Study #102920 (Abgenix Study #ABX-P0305, — Study #1473-31). Cross-reactivity of ABX-EGF (CHO) and ABX-EGF (hybridoma) with human and cynomolgus monkey tissue *ex vivo* and ABX-EGF (CHO) with rat, mouse, and rabbit tissue *ex vivo*.
3. Study #103917 (Abgenix Study #ABX-T0311, — Study #03-3060). A 3-month intravenous toxicity study of ABX-EGF in cynomolgus monkeys with a 6-week recovery period.

4. Study #2005_IT_027_GE. Cloning and sequence homology analysis of cynomolgus EGF receptor.
5. Study #ABG02. EGFr expression in normal cynomolgus monkey tissues.
6. Study #ABG09. Binding of ABX-EGF to normal cynomolgus monkey tissues.

Studies not reviewed within this submission:
Pharmacokinetics Studies:
Analytical Methods and Validation Reports:

1. Study #ABX-V-EGF-02-R. Validation report for electro chemiluminescence assay (ECS) for the determination of ABX-EGF in normal monkey serum.
2. Study #ABX-EGF-Q03-R. Comparison of ECL PLK assay results in cynomolgus monkey serum using — and — analyzers.
3. Study #ABX-V-EGF-04-R. Validation report for ELISA for the detection of monkey antibody against ABX-EGF in monkey serum.
4. Study #ABX-V-EGF-05-R. Report for evaluation of ABX-EGF concentration in cynomolgus monkey serum obtained by ELISA and ECL assay systems.

Additionally, numerous publications from the open literature, describing the pharmacologic effects of ABX-EGF treatment in human tumor xenograft models, the role of EGFr in cancer growth and metastasis, and in developmental biology of the embryo and fetus were included in the pharmacology and toxicology section of the original BLA submission. A listing of these citations is included below, as Appendix 2. Unless specifically noted in the study evaluations below, data from these published studies were not included in this review.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

VECTIBIX™ (panitumumab, ABX-EGF) binds to the epidermal growth factor receptor (EGFr), which is constitutively expressed on the surface of normal human epithelial cells including skin and hair follicle, placenta, gastrointestinal mucosa, corneal epithelium, salivary, and mammary glands. Over-expression of EGFr is also detected in many human cancers, including those of the colon and rectum.

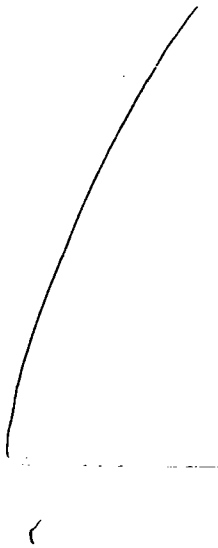
Panitumumab acts as a competitive antagonist by blocking the binding of the normal ligands epidermal growth factor (EGF), and transforming growth factor-α (TGFα) to the EGFr. These two growth factors are implicated in tumor growth, and the overall anti-tumor effects of ABX-EGF are related to its inhibition of ligand-induced EGFr function. These cellular effects of panitumumab-mediated inhibition of EGFr function include blockade of ligand-induced phosphorylation and activation of EGFr-associated kinases, with subsequent inhibition of cellular signal transduction, initiation of cell cycle arrest resulting in inhibition of tumor cell proliferation, induction of apoptosis, and decreased secretion of other growth factors (*e.g.*, vascular endothelial growth factor [VEGF], interleukin-8) that are associated with tumor growth and angiogenesis.

In vitro studies with various human tumor cell lines, as well as *in vivo* tumor xenograft studies in immunodeficient mice bearing human HT-29 colon carcinoma tumors have shown that VECTIBIX™ administered either as monotherapy, or in combination with irinotecan, and to a

lesser extent oxaliplatin, 5-fluorouracil, or rapamycin can inhibit tumor growth, and prolong survival in panitumumab-treated animals. No anti-tumor effects of panitumumab were observed when human tumor cell lines lacking EGFr expression were used to establish the *in vivo* xenografts.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: *(from the sponsor's proposed labeling, CLINICAL PHARMACOLOGY section)*:



Comment: A list of the preclinical studies conducted in support of the pharmacologic activity of VECTIBIX™ is provided, above ("Studies reviewed within this submission; Pharmacology Studies"). A total of 60 primary and 45 secondary pharmacology studies evaluating the tissue distribution and EGFr receptor binding, inactivation of EGFr-associated tyrosine kinases, direct cytotoxic or growth inhibition of human EGFr expressing tumor cells, and the *in vivo* anti-tumor effects of panitumumab alone or in combination with selected chemotherapy regimens were reviewed for this BLA submission. Although all studies were reviewed for this BLA submission, only those primary pharmacology studies which address the mechanism of ABX-EGF binding and interaction with EGFr, or the *in vitro* and *in vivo* cytotoxicity of panitumumab against human colon tumor cell lines, or the A431 vulvar epidermoid cell lines received written review. The data from all of these studies will be summarized and reported below, with the exception of three studies demonstrating the effects of panitumumab treatment in human tumor xenograft models expressing different levels of EGFr, or the tissue distribution of ABX-EGF binding to a panel of human tissues (Studies #R2003094, #R2003205; and #R2003207, respectively), which are reviewed in detail, below. The data from Studies #R2003094 and #R2003205 were used by the sponsor as the basis for not including patients with EGFr-negative colorectal tumors in the pivotal clinical studies.

In vitro binding of panitumumab to EGFr

The pharmacologic activity of VECTIBIX™ was evaluated in a series of *in vitro* EGFr cell surface expression, binding, receptor kinase activation, and cytotoxicity assays, and *in vivo* in established human tumor xenografts in athymic nude mice treated with either panitumumab alone, or in combination with 5-fluorouracil, oxaliplatin, irinotecan, rapamycin, taxotere or docetaxel, or cis-platinum chemotherapy, or anti-VEGF monoclonal antibody treatment.

The affinity of ABX-EGF for human EGFr was measured *in vitro* by plasmon resonance spectroscopy using the BIAcore 2000 instrument, with soluble, recombinant extracellular domain of EGFr immobilized on the BIAcore sensor surface. The initial study (Study #2005552) was conducted using ABX-EGF produced in a cloned, hybridoma cell line, while the later study (Study #2005582) used panitumumab produced by the commercial process, in Chinese hamster ovary (CHO) cells. In Study #2005582, the affinity of the commercial lot of CHO-derived ABX-EGF was also compared to that of a reference lot ($ABX\text{-}EGF_{std}$). In both of these studies, panitumumab was demonstrated to bind EGFr with high affinity (approximately 6 – 60 pM). A summary of these data are presented in Table 1, below.

Table 1. Affinity of Panitumumab for Human EGFr Using the BIAcore *In Vitro* Assay

Study #2005552	**k_a ($M^{-1}s^{-1}$)**	**k_d (s^{-1})**	**K_D (M)**
ABX-EGF (hybridoma)	1.97×10^{6}	1.13×10^{-4}	5.7×10^{-11}
Study #2005582			
ABX-EGF (— CHO)	6.8×10^{5}	3.9×10^{-6}	5.7×10^{-12}
$ABX\text{-}EGF_{std}$ (reference)	6.5×10^{5}	2.3×10^{-6}	3.5×10^{-12}

Comment: The reported affinity for the hybridoma-derived panitumumab appears to be approximately 10-fold less than that of the CHO-derived material. Study #R2005552, which evaluated the affinity of hybridoma-derived, ABX-EGF was conducted in 1996, while Study #R2005582, with the CHO-derived material produced at commercial scale was conducted in October of 2005. This difference in apparent affinity of panitumumab for EGFr will be considered when evaluating the results of the toxicology studies with the material produced by the two different manufacturing processes (please see Section 2.6.6.8, "Other Toxicity Studies," below).

Comment: The final report for Study #R2005552 describes the binding affinity of the native ligand EGF for the EGFr as 3×10^{-9} M, or approximately 60-fold less than that of panitumumab. However, the data in the literature article cited in the final study report show that the K_D for native EGF to EGFr is 7×10^{-9}M, 5×10^{-9}M, or 3×10^{-9}M on A431 human epidermoid carcinoma cells, and the 29R2 and 4 EGFr variant clones of A431 human tumor cells, respectively.[1] It is not clear from the information cited in the final report whether the stated affinity constant of EGF for the EGFr was measured using the same conditions as the present study *i.e.* the BIAcore assay, or whether it was measured in the cellular assay, and which cell line was described.

Similar binding affinity of CHO-derived panitumumab for cell surface EGFr was observed using ^{125}I-labeled ABX-EGF, and intact SK-MES human lung carcinoma cells, with an average, apparent K_D value from 4 experiments of 44.4 ± 8.3 pM (mean ± S.E.; Study #R2005581), and an average EGFr receptor number of approximately 76,200 sites/cell. Panitumumab also

[1] Gill, G.N., T. Kawamoto, C. Cochet, A. Le, J.D. Sato, H. Masui, C. McLeod, and J. Mendelsohn. 1994. Monoclonal anti-epidermal growth factor receptor antibodies which are inhibitors of epidermal growth factor binding and antagonists of epidermal growth factor-stimulated tyrosine protein kinase activity. J. Biol. Chem., **259**:7755-7760.

competitively inhibited binding of native EGF to the EGFr on intact human A431 tumor cells, with a calculated IC_{50} value of 3.1 nM (Study #R2003197).

Comment: Although receptor occupancy was not specifically addressed in this study, additional *in vitro* evaluation of ABX-EGF cell surface EGFr binding by flow cytometry in A549 human lung carcinoma, and HeLa human cervical carcinoma cells demonstrated that receptor saturation was achieved at approximately 4 µg/ml concentrations of ABX-EGF (Study #R2005421).

Study title: Test of expression of EGFr on pancreatic and lung tumor cell lines to be used in xenograft experiments.

Key findings: Four cultured human non-small lung carcinoma and four human pancreatic carcinoma tumor cell lines were evaluated for EGFR expression by flow cytometry, using ABX-EGF or Ab225 anti-human EGFR monoclonal antibodies. All tested tumor cell lines expressed detectable EGFR, with levels ranging from approximately 39,000 to 227,000 receptors per cell.
Study #: R2003094

Methods: Cultured human non-small cell lung and pancreatic cell lines were evaluated for ABX-EGF binding to the cell surface, and the number of cellular EGF receptors quantified using flow cytometry. The non-small cell lung cancer lines tested were H2126, SK-MES-1, H1299, and MV522, and BxPc3, Panc-1, MiaPaCa, and CaPan-1 were the pancreatic cancer lines tested. Following culture to approximately 75% confluence, 5 x 10^5 cells were incubated on ice in the presence of 5 mg/sample of either ABX-EGF, Ab225 (murine anti-human EGFR monoclonal antibody; used as a positive control), or isotype-matched murine IgG1 or PK16.3.1 human IgG2a as controls. After staining with the primary antibody, cells were then incubated on ice with either phycoerythrin (PE)-conjugated, goat-anti-human or fluorescein isothiocyanate (FITC)-conjugated, goat-anti-mouse antibodies, washed, and EGFr expression levels were analyzed by flow cytometry using a —— instrument. The number of EGF receptors per cell type was determined by analytical flow cytometry, using calibrated microbeads as the standard. A calibration curve was generated by plotting the given number of equivalent bound fluorescence molecules per bead, versus the log of its fluorescence intensity.

Results: All eight human tumor lines tested showed positive expression of EGFR by flow cytometry. However, the levels of EGFR expression per cell were variable between the two different tissue types, with expression being higher on pancreatic tumor cells than on lung cancer cells. Additionally, the levels of EGFR per cell varied approximately 4-fold between tumor cell lines of the same tissue origin. These data are presented in Table 2, which was abstracted in part from the sponsor's final study report, below.

Table 2. Quantitation of cell-surface EGFR expression on human lung and pancreatic tumor cell lines.

Tumor Cell Line	Tissue Origin	Number of EGFR per Cell
H2126	lung (non-small cell)	129,297
SK-MES-1	lung (squamous cell)	98,539
H1299	lung (non-small cell)	41,486
MV522	lung (cell origin unknown)	38,714
BxPC3	pancreas (adenocarcinoma)	225,666
Panc-1	pancreas (ductal epithelial Ca)	226,758
MiaPaCa	pancreas (cell origin unknown)	129,486
CaPan-1	pancreas (adenocarcinoma)	69,496

Comment: The final study report does not indicate which antibody (ABX-EGF or M225) was used to generate the quantitative data on receptor number for each of the different cell types. It is feasible that the two antibodies may bind EGFR with different affinities, and may not accurately reflect the actual receptor number present on the tumor cell lines.

Study conclusion: Eight human tumor cell lines, four each originating from pancreatic and lung carcinomas were found to express relatively high levels of EGFR by flow cytometry following staining with ABX-EGF or Ab225 antibodies directed against the human receptor. These data suggest that these cell lines will be useful for *in vivo* efficacy testing of ABX-EGF as an anti-tumor agent, in additional non-clinical studies.

In vitro cross-reactivity of ABX-EGF with a panel of cryopreserved, human tissues

A panel of normal human tissue sections and human tumor samples was evaluated to determine the distribution of binding of ABX-EGF to EGFr present on target human organs and tissues (Study #R2003207). This study is reviewed in detail, below.

Study title: EGFr expression in normal human tissues and human tumors.

Key findings: Panitumumab binding to cell surface EGFr was demonstrated in normal human tissue samples of epithelial origin, including bladder, breast, esophagus, kidney, placenta, prostate, skin, tonsil, ureter, and cervix.

Study #: R2003207

Methods: The purpose of this study was to evaluate the binding of ABX-EGF to a selected panel of human tissues and human renal and prostate tumor samples. Five micron sections of histologically normal, cryopreserved human tissues or formalin-fixed, tumor biopsy specimens were prepared, and labeled with 20 µg/ml of either biotinylated ABX-EGF (lot #098-068-01) or isotype-matched, biotinylated human IgG2 as a negative control (clone PK 16.3.1, lot #004-186-2). Previous studies had demonstrated that using human skin as a positive tissue control, this concentration of ABX-EGF gave the optimal staining of cell surface EGFr with minimal cytoplasmic or non-specific, background staining. For the formalin-fixed tumor samples, 5.8

μg/ml of a biotinylated murine anti-EGFr antibody , was used for the primary antibody labeling, and a murine IgG1 monoclonal antibody , was used as the isotype-matched control. Frozen and formalin-fixed, paraffin embedded sections of human skin and heart were used as the positive and negative tissue controls, respectively, for EGFr staining with the respective antibody preparations. Following incubation of the sections with primary antibody, samples were then incubated with peroxidase-conjugated streptavidin, and positive reactivity (deposition of a colored reaction product) was detected by visualization under light microscopy, after reduction of 3, 3'-diaminobenzidine tetrahydrochloride as the capture reagent.

Results: Cross-reactivity of panitumumab with EGFr was detected only in a limited number of normal human epithelial tissues. Intensity of ABX-EGF staining was variable between the different tissues. Human skin, which was used as the positive control for panitumumab binding, also displayed variability in staining intensity. The strongest staining was noted in the placenta, skin, prostate, and tonsil, with additional specific, although weaker staining noted in the urinary bladder and ureter epithelia, breast, esophagus, and cervix. No ABX-EGF staining was observed in the eye, stomach, or large or small intestines. No staining of EGFr was observed when the irrelevant, biotinylated human IgG2 or murine IgG1 antibodies were used as the negative control. The results of this assay are presented in Table 3, below.

Table 3: Cross-Reactivity of Monoclonal Antibody ABX-EGF with Normal Human Tissues			
Normal Human Tissue	Incidence (# Positive / #Tested)	% of Cells Stained Positive	Staining Intensity and Location[a]
Skin (positive control)	2/2	60-80	1^+ - 3^+ (M)
Heart (negative control)	0/2	0	(-)
Adrenal	0/3	0	(-)
Bladder	1/3	30	1^+ (C, M)
Blood Cells	0/3	0	(-)
Bone Marrow	0/3	0	(-)
Brain – cerebellum	0/3	0	(-)
Brain – cerebrum	0/3	0	(-)
Breast	2/3	10 20	1^+ (C, M) 1^+ (C)
Esophagus	2/3	20 80	1^+ (C, M) 1^+ (C, M)
Eye	0/3[b]	0	(-)
Fallopian tube	0/3	0	(-)
Heart	0/3	0	(-)
Kidney	1/3[c]	50	1^+ (C, M)
Large intestine	0/3	0	(-)
Liver	2/3	80	1^+ (C)
Lung	0/3	0	(-)
Lymph node	0/3	0	(-)
Ovary	0/3[d]	0	(-)
Pancreas	0/3	0	(-)
Parathyroid	0/3	0	(-)
Pituitary	0/3	0	(-)
Placenta	3/3[e]	50 (2/3) 80 (1/3)	1^+ (C, M) 1^+ (C, M)
Prostate	3/3[f]	50 80 80	1^+ (C, M) 1^+ (C, M) 2^+ (C, M)
Skeletal muscle	0/3[g]	0	(-)
Skin	3/3[h]	20 50 80	3^+ (C,M) 1^+ (C,M) 1^+ (M)
Small intestine	0/3	0	(-)
Spinal cord	0/3	0	(-)
Spleen	0/3	0	(-)
Stomach	0/2	0	(-)
Testis	0/2[d]	0	(-)
Thymus	0/3[d]	0	(-)
Thyroid	0/3	0	(-)
Tonsil	3/3	80	1^+ (1/3; C, M) 2^+ (2/3; C, M)
Ureter	2/3	80	1^+ (C, M)[i]
Uterus	1/3	20	1^+ (C, M)[j]

[a] intensity

[b] 1^+ staining on vitreal membrane

[c] weak, specific staining of distal tubules

[d] non-specific staining of stroma in 1/3 ovary, 1/2 testis, and 2/3 thymus samples

[e] weak, specific staining of cytotrophoblastic cells

[f] weak to moderate, specific staining of glandular epithelial cells

[g] non-specific staining of fibroblasts in 1/3 samples

[h] weak to strong, specific staining of squamous epithelium

[i] epithelium staining in 2/3 samples; no epithelium seen in 1/3

[j] moderate, specific staining of the cervix only

The cross-reactivity of panitumumab was also evaluated against several human renal carcinoma and prostate tumor samples. Weak to strong, although specific membrane-associated ABX-EGF staining was noted in 2/5 renal carcinoma, and 1/5 prostate tumor samples. The results of this assay are presented in Table 4, below.

Table 4. Study #R2003207. Cross-Reactivity of Monoclonal Antibody ABX-EGF with Human Tumor Samples

Normal Human Tissue	Incidence (# Positive / #Tested)	% of Cells Stained Positive	Staining Intensity and Location[a]
Skin (positive control)	2/2	50	2^+ (C, M)
Heart (negative control)	0/2	0	(-)
Tumor Sample			
Renal carcinoma	2/5	20-80	1^+ - 3^+ (C, M)
Prostate carcinoma	1/5	20	1^+ (C, M)

Comment: The tumor samples used in this assay were from formalin-fixed, paraffin-embedded specimens. It is not known whether the membrane-associated ABX-EGF binding would have been more intense, or present in more samples or on a greater number of tumor cells if fresh frozen samples had been evaluated.

Study conclusion: Panitumumab binding to EGFr on cell membranes with weak to strong cross-reactivity was observed in glandular, ductular, and/or squamous epithelial cells of human breast, skin, prostate, kidney, tonsil, cervix, and bladder/ureter. Weak to moderate staining was also observed in 2/5 renal, and 1/5 prostate carcinoma specimens. These findings are consistent with the known localization of EGFr in human tissues, and the anticipated localization in tumor cells.

Inhibition of intracellular EGFr-associated receptor phosphorylation and internalization

A series of *in vitro* and *in vivo* experiments was conducted to evaluate the effects of panitumumab treatment on EGFR-mediated intracellular signaling, receptor internalization, phosphorylation of downstream signaling molecules, and cellular proliferation using A431 human epidermoid vulva cancer cells, HCT116 or HeLa human cervical cancer cells. Incubation of A431 cells with ABX-EGF resulted in a dose-related inhibition of cellular proliferation, with an IC_{50} value of 0.08 nM (Study #R2003200), and dose-related inhibition of EGF-mediated, intracellular receptor tyrosine kinase activity and extracellular acidification with concentrations of ABX-EGF ranging from 10 to 1000 nM (Studies #R2003198 and #R2003199, respectively). Mechanistic studies using mass spectrometery to measure panitumumab's effects on receptor kinase signaling at the molecular level revealed that treatment of A431 cells with 10 – 1000 ng/ml ABC-EGF resulted in site-specific inhibition of tyrosine phosphorylation at residues Y1045, Y1086, Y1148, and Y1173, while residues Y992, T669 and Y1068 were still phosphorylated at significant levels even in the

presence of the highest panitumumab concentrations (Study #2003225). *In vitro* pantiumumab treatment of human A549 lung carcinoma cells also inhibited the ligand-induced, autophosphorylation of residue p1068 of the EGFr, in response to exogenously added TGF-α, EGF, amphiregulin, or epiregulin (Study #R2005539). Inhibition of receptor phosphorylation after *in vivo* treatment with panitumumab was confirmed by *ex vivo* immunohistochemical staining for specific, pERK, pAKT, and Ki-67 intracellular phosphokinases in tumor tissue sections from panitumumab-treated A431 (Study #R2003331), but not from ABX-EGF non-responsive HCT116, tumor-bearing mice (Study #2003332).

Incubation of cultured human HeLa, A549, or A431 EGFr-expressing tumor cells with 2-200, 0.5-8, or 2-25 μg/ml ABX-EGF, respectively resulted in dose-related increases in EGF receptor internalization, as measured by flow cytometery (Studies #R2005421 and #R2005530). Internalization of EGFr by these cell types was detectable as early as 5 minutes after addition of panitumumab, reached a maximum at approximately 60 min of incubation, and could be blocked by either exposing the cells to ABX-EGF at 4°C, or by the addition of 0.1% sodium azide (data not shown).

Other potential mechanisms of panitumumab inhibition of tumor cell growth were evaluated in a series of *in vitro* studies examining the effects of ABX-EGF on EGF-induced production of different tumor growth factors (*e.g.* VEGF), or inflammatory mediators (*i.e.* interleukin-8) by cultured human Caki-1 and Caki-2 renal carcinoma cells (Studies #R2003212 and #R2003211, respectively) and DU145 human prostate tumor cells (Study #R2003373). In all three studies, treatment of the different tumor cell lines with 10 – 50 μg/ml ABX-EGF could significantly inhibit either constitutive or EGF-induced growth factor production. Other potential mechanisms by which panitumumab mediates its antitumor activity *in vivo* may include induction of antibody-dependent, cellular cytotoxicity through interaction of ABX-EGF bound tumor cells to Fc receptor present on activated macrophages or NK cells, alterations in tumor cell invasion, migration, and metastasis, decreases in tumor angiogenesis, and induction of programmed cell death or apoptosis.

In vitro cytotoxicity of panitumumab

The ability of ABX-EGF to mediate inhibition of EGFr-positive tumor cell growth was measured in a series of in vitro experiments using the highly positive, A431 human epidermoid cancer cell line, as well as the SK-MES-1, H1299, and MV522 human lung carcinomas, and the Panc-1 and MiaPaCa human pancreatic carcinoma cell lines, (Studies #R2003110, #R2003112, #R2003200, and #R20004035). In all studies, tumor cells were incubated with increasing concentrations of ABX-EGF, or with an isotype-matched, human IgG2 control antibody and growth inhibition was measured by either MTT reduction or crystal violet uptake, colorimetric assays.

In Study #R2003110, no effects of ABX-EGF antibody treatment at concentrations ranging from 0.03 nM to 3300 nM were observed in *in vitro* cellular cytotoxicity assays using the EGFR-positive, human H1299 and MV522 lung carcinoma, and Panc-1 and MiaPaCa pancreatic tumor cell lines. Growth inhibition in the human SK-MES, EGFR-positive human lung tumor cell line was related to the concentration of ABX-EGF in the culture medium, but was incomplete with approximately 20% of the tumor cells surviving under the conditions of the assay. No reproducible, additive or synergistic cytotoxicity was observed when SK-MES or H1299 tumor cells were incubated in the presence of increasing concentrations of cisplatin or taxotere and ABX-EGF. Confirmatory studies were then performed using the A431 human vulvar squamous cell carcinoma line, obtained from two different sources and cultured under different conditions.

In three separate experiments, incomplete inhibition of A431 tumor cell growth *in vitro* was also shown following culture of the cells in the presence of 0.03 nM to 3300 nM ABX-EGF, with approximately 30-40% growth inhibition demonstrated even at the highest dose levels (data not shown).

Comment: Study #R2003094 (reviewed, above) demonstrated relatively high numbers of EGF receptors on the surface of all five of the tested human lung and pancreatic tumor cell lines. However, the present study fails to demonstrate any *in vitro* anti-proliferative effects of ABX-EGF on four of the five tested cell lines including Panc-1, which had the highest level of EGFR expression in the previous study. Additionally, the anti-proliferative effect of ABX-EGF was much weaker than anticipated in the SK-MES line, which demonstrated high levels of receptor binding (approximately 98,000 EGF receptors/cell) in Study #R2003094. Taken together, these data would appear to refute the hypothesis that the cytotoxic effects of ABX-EGF are related to the expression of EGFR on the target tumor cells.

Comment: In the above-described experiments, a concentration-related inhibition of Panc-1 tumor cell growth is observed in the cultures exposed to increasing levels of the human IgG negative control, with approximately 90% growth inhibition observed at 300 nM concentration, and an apparent IC_{50} value of 50 nM. There is no explanation provided in the final study report for this result, although the report does note that this effect was observed.

In Study #R2003112, addition of 0.03 to 300 nM ABX-EGF to cultured A431 epithelial carcinoma cells resulted in a concentration-dependent inhibition of tumor growth, as detected by either the MTT (Figure 2, panels A and B) or crystal violet (Figure 2, panels C and D) cytotoxicity assays. However, the growth inhibitory effect of the antibody was less when A431 tumor cells were cultured in medium supplemented with 0.5% fetal bovine serum (Figure 2, panels A and C), as compared to A431 cells were cultured in serum-free medium and treated with ABX-EGF (Figure 2, panels B and D). Using the MTT assay, the IC_{50} for ABX-EGF in the presence of supplemental fetal bovine serum was 3.93 nM with an approximate 43% maximal inhibition of tumor proliferation, as compared to an IC_{50} value of 0.82 nM, with a maximal growth inhibition of 60% in serum-free medium. An IC_{50} value could not be determined for A431 cells cultured with ABX-EGF in 0.5% fetal bovine serum using the crystal violet assay; however, this same assay resulted in an IC_{50} for ABX-EGF in A431 cells cultured in serum-free medium was 0.96 nM, with a maximal growth inhibition of 77%.

Comment: Figure 2 below, was abstracted *in toto* from Figure 1 in the sponsor's final study report

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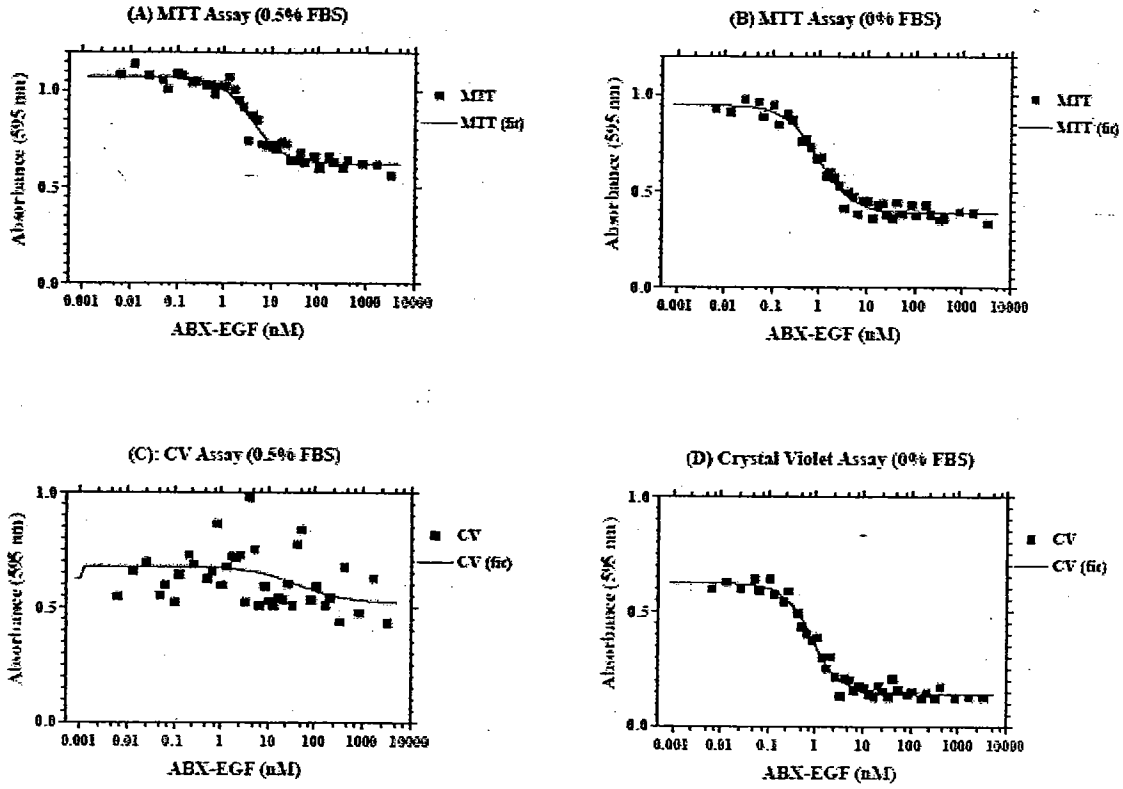

Figure 2. Comparison of ABX-EGF cytotoxicity as measured by MTT (panels A and B) or crystal violet (Panels C and D) staining, in the presence of 0.5% or 0% fetal bovine serum.

Comment: The final study report states that the more than 3-fold increase in IC_{50} value for ABX-EGF observed when A431 tumor cells are cultured in the presence of supplemental fetal bovine serum may result from competition of low levels of growth factors present in the serum with ABX-EGF for binding to the EGFR, or from alterations in the affinity of the EGFR on A431 cells for the ABX-EGF binding. However, the previous study conducted at this same contractor found very similar IC_{50} values for ABX-EGF inhibition of A431 tumor cell growth, with values of 2.54 nM and 3.82 nM in the presence of either 1% or 10% fetal bovine serum, respectively (Study #R2003110, above). These previous findings, coupled with the IC_{50} values obtained in the present study suggest a lack of a dose-relationship for any potential competing growth factors present in the serum. Additionally, saturating concentrations of ABX-EGF were present in the culture medium at the highest concentrations tested in both studies (100 – 3300 nM). At these levels of antibody tested, and given the relatively high number of EGFR present on A431 cells, ABX-EGF would be expected to out-compete any growth factors present at such at low serum concentrations. Taken together, these data do not provide a satisfactory explanation for the *in vitro* loss of ABX-EGF growth inhibitory activity in the presence of supplemental fetal bovine serum, and suggest that the hypothesis of competition of growth factors in serum with ABX-EGF for binding to the EGFR may not be correct.

In vivo treatment of A431 tumor-bearing nude mice with ABX-EGF twice weekly for 3 weeks resulted in dose-related delays in tumor growth and decreased tumor volume, as well as significant increases in survival. The survival data are presented as a Kaplan-Meier plot in Figure

3, below (abstracted from Figure 3 in the sponsor's final study report). All mice treated with the vehicle (saline) control group, and 9/10 mice each in the human IgG2k and 20 µg/mouse/dose ABX-EGF groups developed large tumors (≥ 2g), and were euthanized by Study Day (SD) 26-32. One mouse in the human IgG2k control group underwent spontaneous regression of its tumor beginning SD 33, and was a near complete responder by study termination on SD 61. Tumor growth in one mouse in the 20 µg/dose was remarkably delayed, and tumor size remained stable at approximately 20% of the control tumor volumes beginning on SD 28, continuing until SD 61 (data not shown).

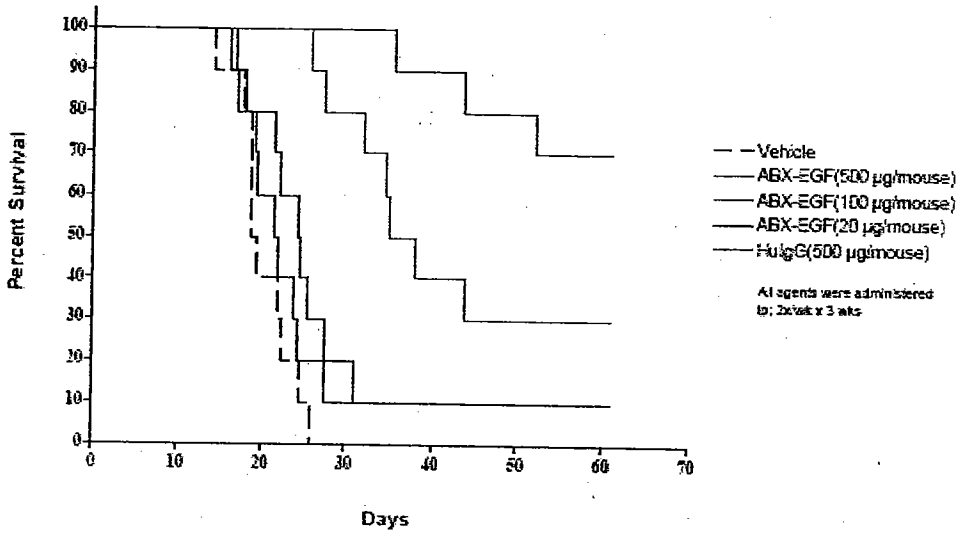

Figure 3. Kaplan-Meier survival curve for A431 tumor-bearing mice, treated with ABX-EGF or controls. Established tumors in mice (n = 10/group) were treated as described, and measurements obtained twice weekly until SD 61. Any animals with tumor burden ≥ 2 g were euthanized, and the day of death recorded.

Tumor growth of A431 tumors was significantly delayed in mice treated with either 100 or 500 µg/dose ABX-EGF, as compared to the vehicle or IgG control groups ($p < 0.0001$, *t*-test; data not shown). Median survival times were also significantly prolonged compared to the control groups, with 3/10 and 7/10 mice still alive at SD 61 in the groups treated with 100 or 500 µg/dose ABX-EGF, respectively (Table 5, below). Complete, or near complete regression of tumor was observed in 2/10 mice, and partial tumor regression (> 25% - < 75% of control tumor size) was observed in 3/10 mice in the 500 µg ABX-EGF /injection group at SD 61. Partial regression of established A431 tumor was also observed in all 3 surviving mice treated with 100 µg ABX-EGF/dose. Median survival times, and mean tumor sizes at SD 61 for each dose group are presented in Table 5, below.

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Table 5. Tumor Responses in A431 Human Squamous Epidermoid Tumor-Bearing Nude Mice following treatment with ABX-EGF or controls			
Treatment Group	**Surviving mice at SD 61**	**Median Survival Time (days), ± SD**	**Mean Tumor Size at SD 61 (mg), ± SD**
Vehicle control	0/10	20.2 ± 1.1	≥ 2 g[a]
Human IgG2k control	1/10	21.3 ± 1.2	18 (n = 1)
20 mg/dose ABX-EGF	1/10	23.2 ± 1.6	256 (n = 1)
100 mg/dose ABX-EGF	3/10	33.8 ± 2.4	860 ± 102
500 mg/dose ABX-EGF	7/10	43.8 ± 4.8[b]	512 ± 205

[a] all mice were euthanized with tumor burden ≥ 2 g by SD 26

[b] $p < 0.0001$ as compared to vehicle, human IgG2κ control groups, log-rank test

Comment: The inhibition of *in vivo* tumor growth of A431 tumors following treatment with ABX-EGF does not achieve the 95-100% regression rate of A431 tumors after ABX-EGF treatment observed in Studies #R2003202 and #R2003203, nor that which has been previously reported in the published literature for this xenograft model.[2] One possible explanation is that the tumors in the present study were allowed to establish until a mean tumor volume of 75 mg was reached before treatment was begun, while it appears that A431 tumor burdens in the study by Yang *et al.*[2], and in the two previous studies were smaller when ABX-EGF treatment was initiated.

Study conclusion: The data in the present study confirm the biologic activity of ABX-EGF in both *in vitro* and *in vivo* inhibition of A431 tumor growth; however, the same levels of anti-tumor effect as observed in previously published studies were not achieved[2]. ABX-EGF treatment of cultured A431 epidermoid tumor cells could inhibit growth in a dose-related fashion in both *in vitro* cytotoxicity assays, and in A431 tumor xenograft models in nude mice. The IC_{50} value for ABX-EGF in the *in vitro* cytotoxicity assay was approximately 3-fold higher when cells were treated in the presence of 0.5% fetal bovine serum in the culture medium, as compared to serum-free conditions. *In vivo* treatment of established A431 tumor bearing mice resulted in delayed tumor growth, decreased tumor burden, and prolonged survival; however, the 95-100% complete regression rates previously described for the treatment regimen employed were not achieved in the present study.

In vivo anti-tumor activity of panitumumab correlates with EGF receptor number on tumor cells

Study title: Effect of ABX-EGF on the growth of multiple human tumors derived from different tissues and expressing different levels of EGFr.

Key findings: Anti-tumor activity of panitumumab in murine human tumor xenograft models correlated with the number of EGFr expressed per cell, with an apparent threshold of 17,000 EGFr/cell required for effect.

[2] Yang, X. D., X.C. Jia, J. R.F. Corvalan, P. Wang, C.G. Davis, and A. Jakobovits. 1999. Eradication of established tumors by a fully human monoclonal antibody to the epidermal growth factor receptor without concomitant chemotherapy. *Cancer Res.*, **59**:1236-1243.

Study #: R2003205

Methods: This study evaluated the anti-tumor effects of panitumumab treatment of BALB/c athymic, *nu/nu* mice bearing established human tumor xenografts from different tissue origins and expressing different levels of EGFr. The human tumor cell lines tested were A431 (vulvar epidermoid carcinoma), MDA-MB-468 (breast carcinoma), SK-RC-29 renal cell carcinoma, HPAC, Hs766T, and BxPC2 human pancreatic carcinomas, PC3 prostate carcinoma, IGROV-1 ovarian carcinoma, and HT29 and SW707 human colon carcinomas. All cell lines were evaluated for ABX-EGF binding and cell surface EGFr number quantitated by flow cytometry. Mice were injected s/c with various numbers of the human tumor cells and the xenograft was allowed to establish to measurable size at which time treatment was initiated. Ten mice per group were treated twice weekly by i/p injection with 1 mg/mouse/dose of ABX-EGF for 3 weeks, or with 2 mg/mouse once weekly for 2 weeks (MDA-MB-468). Control mice received either no treatment, or injections with PBS (MDA-MB-468). As an additional control group, ten A431 tumor-bearing mice were injected with 2 mg/dose E7.5.2 monoclonal antibody which binds to cell surface EGFr, but does not inhibit either ligand binding or downstream cellular activation cascades. Tumor volumes were measured twice weekly until study termination, and the results reported graphically in the final study report as tumor volume vs. time on study (data not shown).

Results: ABX-EGF binding to cell surface EGFr, as detected by flow cytometry is depicted in Table 6 below, which was abstracted from the sponsor's final study report. The anti-tumor effects of panitumumab appeared to correlate with the expression of EGFr, with near complete eradication of tumor observed for mice bearing xenografts of MDA-MB-468, A431, SK-RC-29, BxPC3, and HS766t tumors, and > 50% reductions in tumor burden as compared to control animals at study termination for mice bearing xenografts of IGROV-1 or PC3 tumor cells. A slight, although less than 30% inhibition of HT29 tumor growth was also observed following panitumumab treatment., when compared to the control group. Treatment of A431 tumor-bearing mice with the non-inhibitory, anti-EGFr antibody E7.5.2 had no effect on tumor growth or survival of animals, as compared to mice in the control group (data not shown).

Table 6. Study #R2003205. Expression of EGFr and Anti-Tumor Effect of ABX-EGF on Human Tumor Xenografts in Athymic, Nude Mice

Tumor Cell Line	Tissue of Origin	EGFr Expression (number/cell)	Inhibition of Tumor Growth
MDA-MB-468	Breast	1,800,000	Yes
A431	Epidermal	1,200,000	Yes
SK-RC-29	Renal	77,000	Yes
BxPC3	Pancreatic	63,000	Yes
PC3	Prostate	31,000	Yes
IGROV-1	Ovarian	30000	Yes
HS766T	Pancreatic	17,000	Yes
HPAC	Pancreatic	11,000	No
HT29	Colon	9,000	Yes
SW707	Colon	1057	No

Comment: The raw data for this study were not provided by the sponsor in the final study report; therefore, it was not possible to calculate the percent inhibition of tumor growth.

Comment: Cell surface expression of both EGFr and HER2/*neu* in ABX-EGF responsive and non-responsive human tumor cell lines were also evaluated in Study #R2004656. However, in that specific study, EGF receptor numbers/cell was not identified; rather, the mean fluorescent intensity (MFI) of cell surface binding was measured by flow cytometry, and the data were presented as the ratio of EGFr:Her2 MFIs. In Study #R2004656, the MFI ratios of EGFr:Her2 in the MDA-MB-468 breast, A431 epidermoid, HT-29 colon, PC-3 prostate, and NCI-H1650 and NCI-H1975 (EGFr mutant NSCLC) carcinoma cell lines were 17.6, 10.4, 3.5, 2.1, 3.4, and 1.5 respectively. The ratios of MFI for EGFR:HER2 in the non-responsive cell lines U87 microglioma, H1299 and SK-MES-PD lung carcinomas, and Colo205 colon carcinoma were 1.5, 1.0, 0.76 and 0.2 respectively, demonstrating a lower level of EGFr expression on tumor cells that do not respond to panitumumab *in vivo*.

Study conclusion: Under the conditions of Study #R2003205, the anti-tumor effects of panitumimab were correlated with the number of EGF receptors on the tumor cell surface. There appeared to be a threshold, above which tumors expressing more than 17,000 EGFr per cell responded to ABX-EGF treatment, while tumor cell lines with less than 17,000 EGFr per cell have variable response to panitumumab.

Study title: Patterns of gene expression can prospectively predict panitumumab (ABX-EGF) monotherapy in xenograft models.
Key findings: Using microarray techniques, 11 genes that are differentially expressed in a group of ten different, panitumumab responsive or unresponsive xenograft models were identified. This training set was then used to prospectively determine responsiveness in a dataset from 19 different, ABX-treated human tumor xenograft models, resulting in an 85% probability of predicting response of the selected xenograft models to panitumumab treatment.
Study #: R2004657
Methods: Responsiveness to panitumumab was determined from human tumor xenograft models in 23 previous experiments. In these studies, tumor-bearing mice were treated twice weekly by i/p injection with 20, 100, 200, and 500 µg/mouse/dose ABX-EGF for 2 to 5 weeks, and tumor measurements were obtained twice weekly. Response was determined as a 40% reduction of tumor volume in the treated groups, as compared to mice injected with placebo or no control article. Microarray data using the Affymetrix gene chip were generated, and used to determine a set of genes that could potentially identify responding tumors. Supervised ANOVA, univariate and multivariate analysis were performed to determine transcripts that predict responsiveness to panitumumab.

Comment: The study numbers from which the ABX-EGF responding or non-responding tumors were identified were not provided in the final report for this study.

Results: Analysis of previously conducted, anti-tumor ABX-EGF efficacy studies revealed that treatment of immunodeficient, tumor-bearing mice with established (approximately 300 mm^3 at start of dosing) human A431 vulvar epidermoid, PC3 and DU145 prostate, BxPC-3 and MiaPaCa pancreatic, MDA-468 breast, and HT-29 and DLD-1 colon carcinomas with panitumumab resulted in effective anti-tumor responses, by the criteria outlined above. By contrast, xenografted H460, H1299, Calu-6, and SK-MES-PD lung tumors, BT474, MCF-7, ZR75-1, and MDA 231 breast carcinomas, U87 microglioma, and CaPan-1 pancreatic and Colo 205 and

HCT115 colon carcinoma models were unresponsive to panitumumab treatment at the above doses and schedule. Cluster analysis of the gene expression profiles by microarray techniques revealed 2156 genes that were differentially expressed in responding, vs. non-responding xenograft models (FDR corrected p-value $\leq$ 0.05, two-way ANOVA). However, there were no genes identified that specifically correlated with the anti-tumor response to panitumumab ($p \leq$ 0.05, modified Student's *t* test with Bonferoni correction). Supervised univariate and multivariate analysis of the microarray data identified a list of 11 gene candidates that could that could prospectively determine responsiveness in 8/9 tumor xenograft models tested; additional, "leave one out" analysis of the complete data set from 19 different tumor models resulted in an 85% probability of predicting response of the selected xenograft models to panitumumab treatment.

Study Conclusion: Using a supervised analysis, a selected gene list was generated from microarray data that may prospectively predict panitumumab response in xenograft models.

Study title: Evaluation of EGFr levels in xenograft tissue using the EGFr pharmDx kit.
Key findings: The level of EGFr staining detected on *ex vivo* tumor xenograft samples with the commercial, EGF2 pharmDx kit was determined to correlate with the responsiveness of the xenograft models to ABX-EGF treatment observed in Study #R2004657 (reviewed, above).

Study #:R2005548
Methods: Paraffin-embedded tumor samples isolated from xenografts of both ABX-EGF responsive and unresponsive tumors from untreated animals were stained with the commercial, murine anti-EGFr immunohistochemical detection kit that was used in the clinical studies. Human HT-29 colon tumor cells were used as the positive control for EGFr expression. Five micron sections were stained with the mouse antibody followed by peroxidase conjugated, goat anti-mouse antibody, and the colored reaction product was developed by incubation in hydrogen peroxide and diaminobenzidine. Following counterstaining with hematoxylin, slides were evaluated microscopically for EGFr pharmDx staining. Results were graded on a 0 to 4^+ scale, with 0 negative (0), low positive (1^+), moderate positive (2^+), high positive (3^+), or strong positive (4^+) based on the amount of chromagen staining observed in the tumor tissue. Equivocal staining (-/+) was reported when an apparent majority of the tumor tissue stained negative; however, multifocal groups of strongly positive tumor cells were present in the section.

Results: The HT-29 tumor positive control sample included in the pharmDx kit was reported by the manufacturer to stain 2^+ in this assay, and also stained 2^+ for EGFr with the HT-29 sample isolated from the xenograft tumor. EGFr cell surface labeling by the pharmDx kit was variable across the other tumor types; however, there was no correlation with the level of EGFr staining using this assay with that observed in the previous, flow cytometry studies of tumor cell surface staining with ABX-EGF (Studies #R2003094 and #, above), nor with the in vivo responsiveness to ABX-EGF treatment. The data for EGFr cell surface labeling by the pharmDx kit for the different tumor xenograft models are presented in Table 7, below.

Comment: The strongest, cell surface EGFr staining with the commercial, EGFr pharmDx kit was observed in A431 human vulvar epidermoid carcinoma cells (4^+), which was the only tumor xenograft model which consistently responded to ABX-EGF treatment in the *in vivo* mouse pharmacology studies (above).

Table 7. Study #R2005548. Cell Surface EGFr Expression on Panitumumab Responsive and on-Responsive Tumor Cell Lines Used in Xenograft Efficacy Models					
ABX-EGF Responding Tumor Xenografts			ABX-EGF Non-responding Tumor Xenografts		
Tumor Line	Tissue Origin	EGFr staining	Tumor Line	Tissue Origin	EGFr staining
A431	Epidermoid	4+	BT474	Breast	0
MDA-468	Breast	3+	MCF-7	Breast	1+
BxPC-3	Pancreas	3+	MDA-231	Breast	2+
MiaPaCa	Pancreas	3+	ZR75-1	Breast	negative
DU-145	Prostate	3+	Colo 205	Colon	2+
PC-3	Prostate	3+	HCT116	Colon	2+
HT-29	Colon	2+	Calu-6	Lung	3+
DLD-1	Colon	n.r.[a]	H460	Lung	1+
			H1299	Lung	1+
			NCI-H82	Lung	n.r.
			SK-MES-PD	Lung	1+
			U87	Glioblastoma	3+
			CaPan-1	Pancreas	1+

Comment: Additional tumor samples, including LnCaP prostate, Panc-1 pancreatic, and Colo 320 colon carcinomas, as well as HL60, Daudi, Raji, and Namalwa leukemia or lymphoma lines were also evaluated in this assay. While several of these cell lines stained positively for EGFr using the commercial pharmDx assay (*e.g.*, Panc-1 and AsPC1 were both 3^+), their responsiveness to panitumumab was not tested.

Comment: Based on the data presented in Table 7 above, there does not appear to be any correlation between the level of EGFr expression detected on the cell surface by the EGFr pharmDx kit, and the apparent responsiveness of the tumor to panitumumab treatment in xenograft models in nude mice. For example, both HT-29 and Colo 205 were found to express EGFr at a 2^+ level in this study, but Colo 205 was not responsive to ABX-EGF treatment in vivo (Study #R2003325), while the HT-29 tumor did respond (Study #R2003327). Additionally, the U87 glioblastoma tumor line did not respond to panitumumab treatment in two separate studies (Studies #2003550 and #R2004440), but was 3^+ for cell surface EGFr expression in this assay.

Study conclusion: Immunohistochemical staining of tumor xenograft sections from untreated, nude mice using the commercial EGFr pharmDx kit showed cell surface EGFr staining from 0 to 4^+ in the different tumor models. The positive control HT-29 colon tumor cells when stained with this kit were reported by the manufacturer to give a 2^+ reading, while HT-29 from tumor xenografts gave a 2^+ EGFr cell surface labeling as well. Analysis by the sponsor of tumor xenograft staining with EGFr pharmDx kit (single factor ANOVA) determined that there was a statistically significant ($p = 0.004$) difference in EGFr between tumor models that responded, and non-responders to ABX-EGF.

In vivo treatment of human colon tumor-bearing xenograft models with ABX-EGF, either alone or in combination with chemotherapy

The antitumor activity of ABX-EGF against human Colo205, or HT29 established, colon tumors was evaluated in CD1 *nu/nu* athymic nude mice, either alone (Studies #R2003325 and #R2003327, respectively), or in combination with 5-flurouracil (5-FU; Study #R2003558), irinotecan (CPT-11; Study #R2003358), oxaliplatin (Study #R2003560), or monoclonal antibodies directed against either the human insulin-like growth factor receptor (Study #R2003537) or human VEGF (Study #R2003561). In all studies, tumor was implanted s/c and

allowed to establish for approximately 10 to 14 d prior to initiating twice weekly treatment with 20, 200, 500, or 1000 µg/injection ABX-EGF, i/p twice weekly for 3 to 5 weeks. Tumor measurements and body weights were obtained twice weekly. Mice were euthanized either at a defined study endpoint, or when tumor burden reached or exceeded a calculated volume of 1.2 gm. In some studies, mice were euthanized at study termination and tumors were harvested, and weighed.

Panitumumab monotherapy of HT-29 tumor-bearing mice resulted in a dose-related, 18-44% inhibition of tumor growth as compared to mice treated with either PBS or the irrelevant, human IgG2 as controls (Study #R2003327). By contrast, the same dose and schedule of panitumumab treatment of Colo 205 tumor-bearing nude mice did not inhibit tumor growth as compared to either the vehicle, or the IgG2 control groups (data not shown). Immunohistochemical evaluation of panitumumab localization and penetration in HT-29 tumor xenografts showed that positive, ABX-EGF related staining could be detected at low levels in sections of xenograft tumors from mice treated with 200, 500, or 1000 µg/injection twice weekly for 4 weeks (Study #R2004497), indicating that the antibody was capable of localizing to tumor and inhibiting its growth. All combination chemotherapy studies were therefore conducted using the HT-29 cell line as the only colon tumor xenograft model, along with a wide range of other tumor cell lines from different tissue types (*e.g.*, non-small cell lung cancer, pancreatic cancer; reviewed under Secondary Pharmacodynamics Studies, below).

Panitumumab in combination with standard chemotherapy and targeted anticancer agents has also been evaluated in a wide range of tumor cell lines and xenograft model systems. Additive antitumor effects were demonstrated in some but not many tumor xenograft models, as compared with the treatment effects of either agent alone. Treatment of HT-29 tumor-bearing nude mice with 500 µg/injection panitumumab twice weekly, or either 25, or 50 mg/kg/week 5-FU for 5 weeks as monotherapy significantly inhibited HT-29 tumor growth (Study #R2003558; data not shown). However, combination therapy with panitumumab and 5-FU at the above doses and schedule had no additional effect inhibiting HT-29 tumor growth over either agent alone.

Similar results were obtained when HT-29 tumor xenograft-bearing mice were treated with 500 µg/injection of panitumumab twice weekly for 5 or 6 weeks, either alone or in combination with 5, 10, or 20 mg/kg/week oxaliplatin (Study #R2003560). Treatment with oxaliplatin alone resulted in a statistically significant inhibition of tumor growth only at the 20 mg/kg/dose level ($p \leq 0.0001$ as compared to vehicle or IgG control; ANOVA with Dunnett's test). However, this dose was associated with severe toxicity in the treated mice, including anemia and body weight losses > 10%, and resulting in euthanasia of several animals prior to study completion. By contrast, panitumumab monotherapy in this model resulted in significant inhibition of tumor growth ($p=0.0023$, ANOVA), as did combination treatment with ABX-EGF and 10 mg/kg/dose oxaliplatin ($p=0.0012$). However, there was no additional inhibition of tumor growth in the combination therapy group, as compared to either monotherapy with ABX-EGF or oxaliplatin at these dose levels alone. The results of a representative replicate from this experiment, as abstracted from the sponsor's final study report are presented in Figure 4, below.

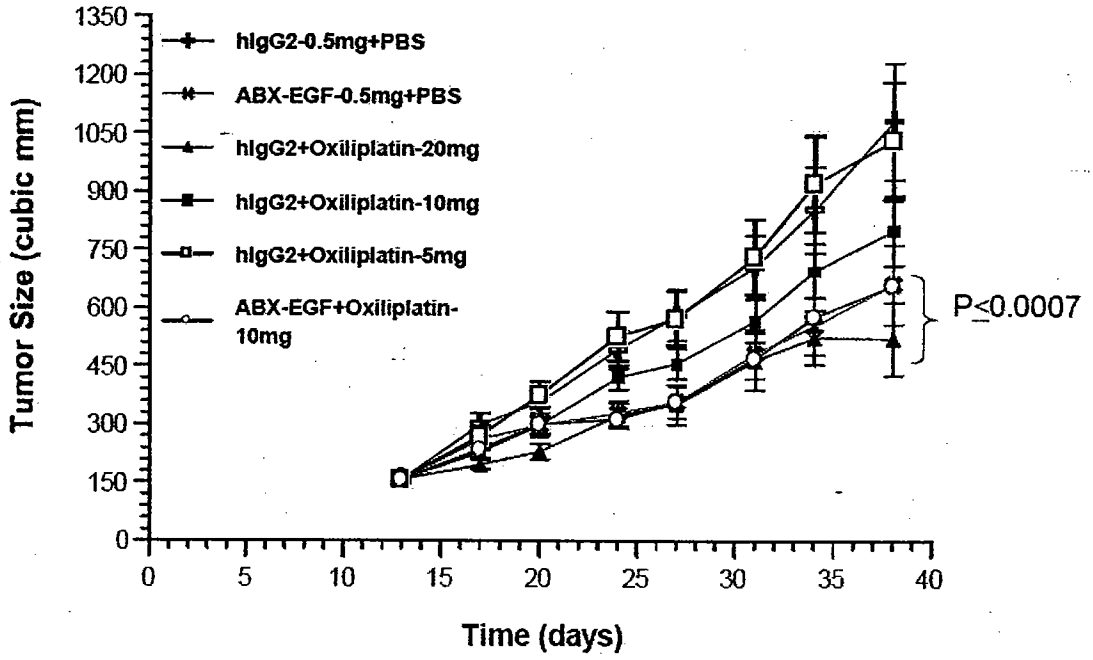

Figure 4. Effects of ABX-EGF alone or in combination with oxaliplatin in athymic, nude mice, bearing HT29 human colon tumor xenografts. Note that statistical significance is as compared to the irrelevant, IgG2 control group (ANOVA with Dunnett's test).

An additional experiment was conducted using the same dose and schedule of panitumumab, administered alone or in combination with once weekly injection of 100 mg/kg CPT-11 in HT-29 tumor xenografts in nude mice Study #R2003559). In this study, the combination of ABX-EGF and irinotecan significantly inhibited HT-29 colon carcinoma xenograft growth as compared with the control group, and to a greater extent than either agent alone. However, this regimen resulted in toxicity that was related to the chemotherapy agent; mice treated with CPT-11 at this dose level, either alone or in combination with panitumumab lost greater than 10% of their body weight during irinotecan administration, which they recovered after the cessation of treatment. No changes in body weight were observed in any of the other treatment groups. These results of the combination treatment on tumor growth are represented in Figure 5 below, which was duplicated from the sponsor's final study report.

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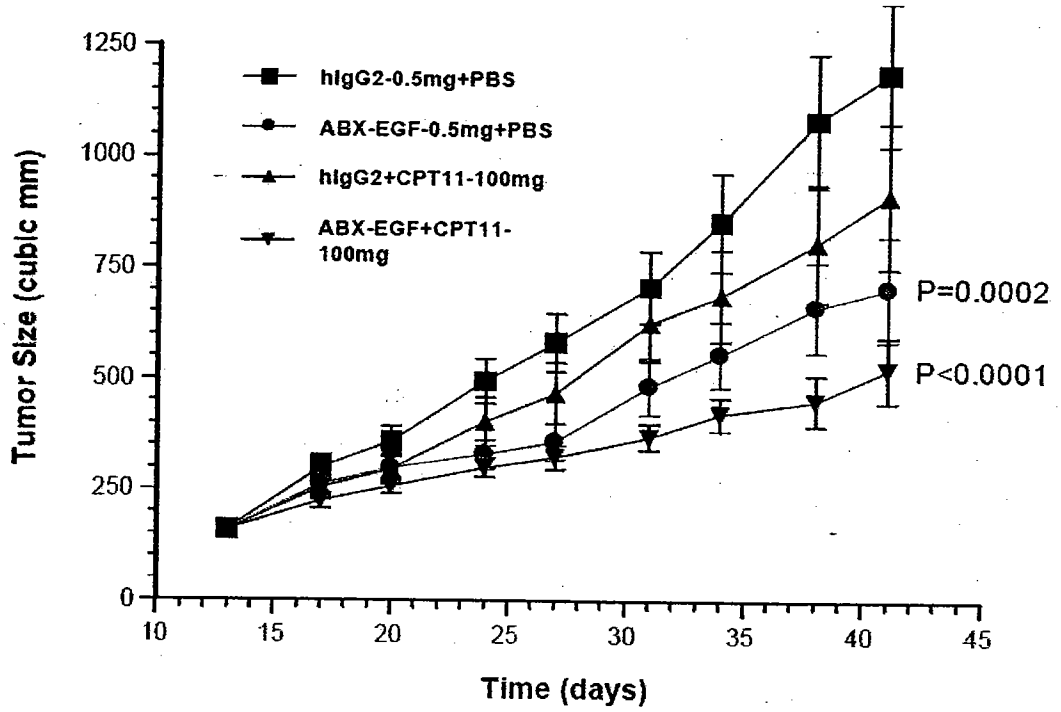

Figure 5. Effects of ABX-EGF alone or in combination with irinotecan (CPT-11) in athymic, nude mice, bearing HT29 human colon tumor xenografts. Note that statistical significance is as compared to the irrelevant, IgG2 control group (ANOVA with Dunnett's test).

In summary, the pharmacologic activity of panitumumab has demonstrated that it binds specifically with EGFr expressed on the surface of human tumor cells. Treatment of EGFr expressing tumor cells *in vitro* with ABX-EGF leads to loss of EGFr function via inhibition of receptor autophosphorylation, enhanced internalization and down-modulation of cell surface EGFr, inhibition of EGFr-associated tyrosine kinase phosphorylation and associated signal transduction, and direct suppression of tumor cell growth. *In vivo*, panitumumab treatment, either alone or in combination with irinotecan but not 5-FU or oxaliplatin chemotherapy could significantly delay tumor growth in human colon (HT-29) xenograft tumor models, in an approximately additive fashion. There was an apparent, threshold for the expression of cell surface EGF receptor required for the anti-tumor activity of panitumumab to be effective in these models, with cells expressing less than 17,000 EGF receptors/cell showing variable responses to ABX-EGF therapy. Taken together, these proof-of-concept data provide the rationale for the use of panitumumab as monotherapy for

2.6.2.3 Secondary pharmacodynamics

Comment: A series of forty-five additional *in vitro* and *in vivo* pharmacology studies were submitted to the original BLA, under the e-CTD heading Secondary Pharmacodynamics. For the most part these studies were additional, *in vitro* and *in vivo* assays evaluating the pharmacologic activity of panitumumab, either alone or in combination with various chemotherapy or biological therapy agents in tumor cell lines other than those from colon or rectal carcinomas. Because the results observed in these additional pharmacology studies were not markedly different from those

seen with ABX-EGF in the colorectal tumor models, they will only be briefly summarized in this section of the review.

In vivo activity of ABX-EGF, alone or in combination with gemcitabine chemotherapy against human pancreatic tumor cell lines

A single study (Study #R2003091) was conducted to evaluate the anti-tumor activity of ABX-EGF alone or in combination with gemcitabine in athymic nude mice bearing established, human MiaPaCa or Panc-1 xenografts. Mice were treated with 20, 100, or 500 µg/injection ABX-EGF twice weekly for three weeks, with or without concomitant gemcitabine (160 mg/kg/dose, q3d) therapy. In MiaPaCa tumor-bearing mice, survival time for the saline control group was 13.8 days, and was 14.6 days for mice treated with an irrelevant, human IgG2 control antibody. ABX-EGF monotherapy at any dose level gave survival times of 15.6 to 15.8 days, which were not significantly different from control. Gemcitabine, either alone or in combination with the human IgG2 control antibody gave survival times of 19.1 and 20.2 days, and TGD values of 5.3 and 5.6 days, respectively. These values represented a statistically significant improval in survival time as compared to the saline injected mice. However, in Panc-1 tumor bearing mice, control survival times were 22.7 to 25.1 days, and no delay in tumor growth or improvement in survival times was observed for either ABX-EGF or gemcitabine monotherapy. There was no further increase in survival times in either MiaPaCa or Panc-1 tumor bearing mice treated with the combination of gemcitabine, and any dose level of panitumumab.

In vivo activity of ABX-EGF, alone or in combination with chemotherapy against human lung tumor cell lines

A series of studies was conducted to evaluate the effects of panitumumab alone or in combination with a number of different chemotherapy agents used in the treatment of lung cancer, in athymic nude mice bearing lung tumors from different sources. A summary of these studies, including the origin of the tumor type and the chemotherapy agent employed is presented in Table 8, below.

Table 8. Summary of all In Vivo Studies of Anti-Tumor Activity of ABX-EGF in Combination or as Monotherapy in Human EGFr-Expressing and Mutant, Lung Tumor Xenograft Models

Human Tumor Line	Monotherapy Study #	Combination Chemo- or Biologic Therapy Given with ABX-EGF				
		CDDP	Taxotere	Rapamycin	Anti-VEGF	Herceptin
A549 (NSCLC)	R2004156		R2003210 R2003370		R2004280	
SK-MES (lung squamous cell)		R2003092 R2003258	R2003092 R2003170	R2004282	R2003547	R2004083
H1299		R2003092 R2003277	R2003092 R2003278	R2004084 R2004281 R2004287	R2003548	R2004082
NCI-H82 (small cell)	R2004154					
NCI-H460 (large cell)	R2004155					
NCI-H1975 (EGFr-mutant NSCLC)		R2005183	R2005181 R2005184 R2005428			
NCI-H1650 (EGFr-mutant NSCLC)			R2005182			

In general, ABX-EGF administered as a single agent delayed tumor growth, and prolonged either the survival of treated, A549, SK-MES, or H1299 tumor-bearing mice, or increased the time for the tumor to reach a defined, maximal allowable tumor burden. Combinations of cis-platinum (CDDP) and panitumumab in both H1299 and SK-MES xenografts (Study #R20030192), or taxotere and ABX-EGF in A549 (Studies #R2003210 and #R2003370), SK-MES (Study #R2003092), and H1299 tumor-bearing mice (Study #R2003092) showed additive anti-tumor activity of panitumumab with either chemotherapeutic, with complete eradication of the tumor seen in the combination therapy arms. Delay in tumor growth was also observed in A549 human NSCLC tumor-bearing mice treated with either 0.5 mg (Study #R2003210) or 1 mg/injection (Study A#R2003370) ABX-EGF monotherapy, which was additive when combined with 4 doses of taxotere at 20 mg/kg. Similar results were observed when the chemotherapeutic agent rapamycin (Studies #R2004282, #R2004084, #R2004281, and #2004287) was used in combination with panitumumab (data not shown). Representative results from Study #R2003210 are included in Figure 6, below which was copied directly from the sponsor's final study report.

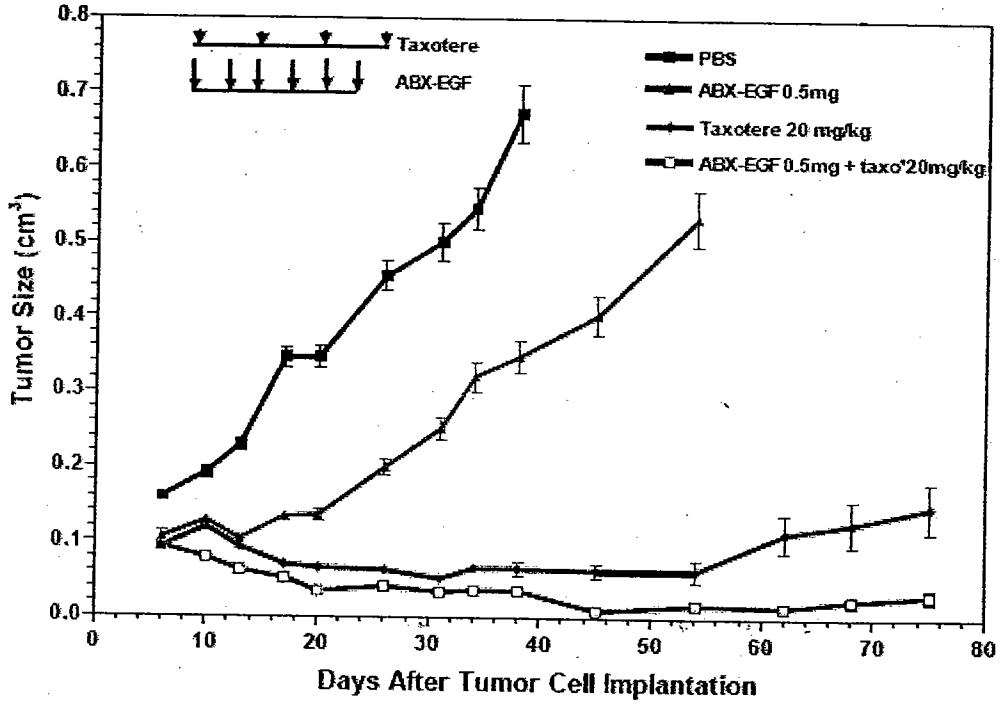

Figure 6. Effects of panitumumab and taxotere combination therapy on the growth of established, A549 human non-small cell lung carcinoma tumors in nude mice

There were no anti-tumor effects of panitumumab observed in either the NCI-H82 small cell, or the NCI-H460 large cell lung carcinoma xenograft models, following i/p injection of established tumor bearing mice with 20, 200, or 500 µg/mouse/injection twice weekly for two weeks (Studies #R2004154 and #R2004155, respectively; data not shown). Neither twice weekly x 4 weeks of either 200 µg or 500 µg/dose ABX-EGF monotherapy, or in combination with 300 µg/dose of a monoclonal antibody directed against the insulin-like growth factor, had any anti-tumor effect against Calu-6 human lung carcinoma xenografts in athymic nude mice (Study #R2004103).

Comment: While study #R2003092 showed dramatic anti-tumor activity of both monotherapy with ABX-EGF and the combination of ABX-EGF with cis-platinum or taxotere against

xenografts of human SK-MES or H1299 lung tumors, repeat of these studies (#R2003170 or #R2003278, respectively) failed to demonstrate any anti-tumor effects of either panitumumab alone or in combination with the chemotherapeutic agent. Similarly, in Studies #R2004082 and #R2004083, there was no activity of ABX-EGF either alone or in combination with the biologic therapeutic agents Herceptin® (anti-Her2/*neu* monoclonal antibody) or an anti-VEGF monoclonal antibody in SK-MES (Study #R2003547) or H1299 ((Study #R2003548) tumor xenografts. A separate study was conducted to evaluate the potency of the ABX-EGF used in Study #R2003092 and Study #2003091, above (Study #R2003093), as well as independent review of the Certificates of Analysis where available for the lots of ABX-EGF used in these studies did not demonstrate any differences in the *in vitro* potency assays (*i.e.*, phosphorylation of EGFr-tyrosine kinases and inhibition of EGF binding), as compared to the reference standard lots of ABX-EGF.

Anti-tumor effects of panitumumab, either as monotherapy or in combination with doxorubicin or cis-platinum could also significantly inhibit the growth of established U118 glioblastoma (Study #R2005549), DU145 human prostate carcinoma (Study #R2003209), or A431 vulvar epidermoid carcinoma xenografts in athymic, nude mice (Study #R2003208). Although the data are not shown in this review, all three cell lines were found to respond to panitumumab monotherapy.

Comment: Although A431 cells have previously been demonstrated to express high levels of EGF3 (Study #R2003094, above), the number of EGF receptors expressed on the DU145 and U118 cell lines is unknown. There was no information provided in the final reports for these three studies regarding the level of EGFr expression on the surface of these tumor cell lines. However, *in vitro* staining of DU145tumor samples with the EGFr pharmDx commercial kit used for the clinical studies (Study #R2004548) showed positive staining (3^+ intensity) for EGFr expression.

In vivo activity of ABX-EGF, alone or in combination with chemotherapy against human breast tumor cell lines

A second series of studies evaluated the anti-tumor activity of ABX-EGF monotherapy, or in combination with a number of different chemotherapy agents used in the treatment of athymic nude mice bearing human breast carcinoma xenografts. A summary of these studies, including the chemo- or biologic agent employed is presented in Table 9, below.

Table 9. *In Vivo* Studies of Anti-Tumor Activity of ABX-EGF Alone or in Combination with MAb Therapies in Human Breast Tumor Xenograft Models

Human Tumor Line	Combination Therapy Given with ABX-EGF		
	Monotherapy	Anti-VEGF MAb	Herceptin
MCF-7tb	R2003479		
MDA-MB-468		R2003518	
MDA-MB-231	R2003527		
ZR75-1	R2004086		
BT-474			R2004279

In general, ABX-EGF administered as a single agent had no effects on the growth of MCF-7tb, MDA-MB-231, ZR75-1, or BT-474 human breast carcinoma xenografts, at doses as high as 500 µg given i/p twice weekly for 2 to 3 weeks to mice with established tumor (studies as listed, above). The one exception was in Study #R2004086, in which ZR75-1 tumor-bearing mice injected with 200 µg ABX-EGF/dose twice weekly for 3 weeks did show statistically significant inhibition of tumor growth (mean tumor volume ± SE at Study Day 42, 773 ± 120 mm^3 as compared to 1324 ± 202 mm^3 in IgG2 control group; $p = 0.025$, ANOVA with Scheffe's post-hoc test).

Comment: The anti-tumor effects of panitumumab against ZR75-1 tumor xenografts were not observed at either the lower (50 μg/dose) or higher (500 μg/dose) tested in this study; therefore, the biologic relevance of this finding is unknown. Cell surface EGFr staining was negative for the ZR75-1 breast carcinoma sample in Study #R2005548 (above).

By contrast, panitumumab treatment of MDA-MB-468 tumor-bearing mice resulted in statistically significant, dose-related delay in tumor growth and improvement in survival, when given either alone or in combination with either 30 μg or 200 μg/dose of an anti-VEGF monoclonal antibody. However, there was no additive effect of the anti-VEGF monoclonal antibody to the effects of panitumumab, and treatment of mice in this study with 200 μg/dose of the anti-VEGF antibody alone did not significantly inhibit tumor growth. The results from Study #R2003518 are included in Figure 7 below, which was reproduced directly from the sponsor's final study report.

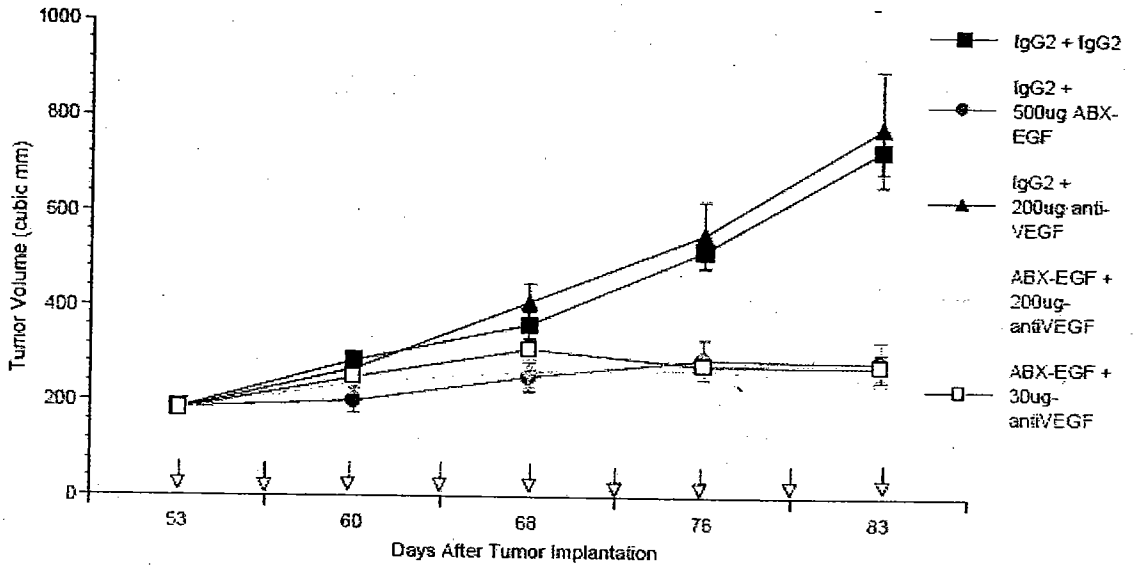

Figure 7. Effects of panitumumab and anti-VEGF monoclonal antibody combination therapy on the growth of established, MDA-MB-468 human breast carcinoma tumors in nude mice

An additional study using sub-therapeutic (20 μg/injection) doses of ABX-EGF in MDA-MB-468 tumor bearing mice resulted in no inhibition of tumor growth, with tumor volumes from SD62 to SD97 that were similar to the control group (Study #R2004087; data not shown). Rapamycin treatment alone (1, 5, or 10 mg/kg/day, 5 d/week x 4 weeks) did result in a dose-related, statistically significant decrease in tumor burdens as compared to the control group; however, there was no additive effect when low dose ABX-EGF was co-administered with the different doses of rapamycin in this study.

Comment: Data provided in the BLA submission in Study Report #R2003518 cites the number of EGF receptors present on MDA-MB-468 tumor as approximately 1.8 x 10^6/cell. It is likely that the 20 μg/dose ABX-EGF used in this study was too low to result in sufficient receptor occupancy/competitive inhibition to overcome activation of EGFr on established tumor by locally acting, endogenous and/or autocrine tumor growth factors, *e.g.* TGF-α or EGF.

In vivo activity of ABX-EGF, alone or in combination with chemotherapy against EGFr-mutant, human lung tumor cell lines

The anti-tumor effects of panitumumab were evaluated alone or in combination with taxotere or cisplatinum in two human, mutant EGFr tumor xenograft models, NCI-H1975 and NCI-H1650. The non-small cell lung carcinoma cell line NCI-H1975 has an amino acid stubstitution in the kinase domain of the EGFr, while the NCI-H1650 human non-small cell carcinoma cell line has an in-frame deletion in the kinase domain of EGFr. Both of these mutations result in increased EGF-induced phosphorylation of the tyrosine residues Y992 and Y1068, as well as increased phosphorylation of several downstream kinases including Akt and STAT5. The overall effect is to enhance tumor growth in response to either exogenous or autocrine EGF and related growth factors.

Treatment of NCI-H1650 tumor-bearing nude mice with 25, 100, or 500 μg/dose twice weekly x 3 weeks resulted in inhibition of tumor growth by 41.3, 67.0, and 50.5%, respectively as compared to the IgG2 antibody control group. These effects were highly significant ($p \leq 0.0036$, ANOVA with post-hoc Scheffe's test). In this same study, taxotere (20 mg/kg/dose) resulted in significant tumor growth inhibition by 48.6%, while the combination of 500 μg panitumumab and taxotere resulted in a 76.6% decrease in tumor burden as compared to the control group. While the anti-tumor effects of the combination of taxotere and ABX-EGF were less than additive, the inhibition of tumor growth was still highly statistically significant as compared to the IgG2 control, but not either monotherapy group ($p \leq 0.0001$ for both groups vs. control, ANOVA with Scheffe's test).

Panitumumab treatment of established, NCI-H1975 tumor bearing nude mice resulted in dose-related inhibition of tumor cell growth following injection of 25, 100, or 500 μg/dose given twice weekly for 3 weeks (Studies #R2004637, #R2005181, #R2005184, and #R2005428). In these four studies, the anti-tumor effects of ABX-EGF were highly statistically significant ($p \leq 0.01$, ANOVA with Scheffe's test) at all dose levels tested. Taxotere monotherapy was also effective in inhibiting NCI-H1975 tumor growth (Studies # R2005181, #R2005184, and #R2005428), with mean decreases in tumor volume of 42.6 – 64.9% from the IgG2 control tumors across the three studies ($p \leq 0.0126$, ANOVA with Scheffe's). Although less than additive effects were seen, combination treatment with taxotere and 100 μg/dose (Studies #R2005181 and #R2005428), or 500 μg/dose panitumumab (#R2004184) resulted in highly significant inhibition of tumor growth (70.7 – 87.0%) as compared to the IgG2-treated control groups ($p \leq 0.001$, ANOVA). Similar effects were observed with panitumumab alone or in combination with cis-platinum treatment in NCI-H1975 tumor xenograft models (Study #R2005183). Mice treated twice weekly for 3 weeks with 500 μg/dose ABX-EGF, i/p showed a 58% reduction in tumor burden as compared to animals treated with 500 μg/dose of the irrelevant, IgG2 control antibody ($p \leq 0.0001$, ANOVA with Scheffe's test). Administration of 7.5mg/kg/dose of CDDP showed no significant inhibition of tumor growth. However, combined treatment of NCI-H1975 tumor-bearing mice with cis-platinum and ABX-EGF resulted in a 62.8% decrease in tumor volume, which was statistically significant ($p \leq 0.0001$, ANOVA with Scheffe's test) when compared to the IgG2 control group, but not to either CDDP or panitumumab monotherapy groups.

Comment: The results for Studies #R20045181 and #R2005428 appear to be identical, even to the percent inhibition of tumor growth observed in the panitumumab monotherapy and combination dose groups. It is unclear from the way the study reports are written as to whether

these effects are accurate, or if the report was inadvertently duplicated. Clarification will be requested from the sponsor.

Additional studies regarding the molecular mechanism of panitumumab inhibition of tumor cell function and growth

To evaluate whether ABX-EGF treatment could inhibit the growth of EGFr-negative tumors, U87 MG glioblastoma tumor cells were transfected with a lentiviral vector system to express the vIII truncated, mutant EGFr, or with a control (empty) vector (Study #R2003550). Tumor-bearing mice were injected twice weekly for two weeks with either 500 μg/dose control IgG2a or panitumumab, and tumor measurements obtained. Under the conditions of this study, neither the EGFr-negative U87 control vector-transduced, nor the U87-vIII truncated EGFr expressing cell line showed any anti-tumor effects following panitumumab treatment.

Comment: The ability of ABX-EGF to inhibit phosphorylation of EGFr-associated tyrosine kinases in the U87-vIII transfected cell line was confirmed in a separate, *in vitro* experiment (Study #R2004440). Treatment of cultured U87-vIII cells with 2 mM panitumumab for 72 h completely inhibited the phosphorylation of EGFr-associated p1068 kinase, and completely down-regulated EGFr cell surface expression after 13 d of treatment, as compared to cells treated with the isotype matched, human IgG2 control antibody. There was no detectable p1068 phosphorylation in either the parental U87 cells, or in U87 cells transfected with a control (green fluorescent protein expressing) vector.

The effects of panitumumab treatment on tumor cell proliferation, ABX-EGF penetration into tumor, and intracellular kinase signaling and downstream EGFr-mediated events were evaluated in nude mice bearing xenografts of human A431 vulvar squamous cell, SK-MES human non-small cell lung, MCF-tb human breast, and BxPC-3 and Capan-1 human pancreatic carcinomas (Studies # R2003104, #R20044499, and #R2004501, respectively).

In all three studies, *in vivo* treatment of human A431, Capan-1, BxPC3, and MCF-tb tumor-bearing nude mice with doses of 200 μg to 1000 μg/mouse, i/p twice weekly for 1 to 4 weeks resulted in inhibition of tumor growth, and improvement in survival over tumor-bearing animals injected with vehicle control. Consistent with previous results observed in primary pharmacology studies, there were no anti-tumor effects observed in the SK-MES, EGFr-non-responsive tumor bearing animals (data not shown; Study #R2003104). Immunohistochemical staining of isolated, recovered tumor from MCF-tb tumor-bearing animals demonstrated weak, to slightly positive *in vivo* penetration and staining after dosing with ABX-EGF twice weekly for 4 weeks with either 200 or 500 μg/dose (Study #R2004449). Similar penetration of panitumumab staining was observed in Capan-1 human pancreatic tumor bearing mice treated with this same regimen of ABX-EGF, while there was no dose-dependence in antibody penetration and staining in mice bearing BxPC-3 pancreatic human tumor xenografts (Study #R2004501). Evaluation at the molecular level of EGFr-mediated, downstream events showed that ABX-EGF treatment could inhibit both endogenous and exogenous EGF-induced phosphorylation, resulting in reductions in the levels of expression of the downstream kinases cyclin D1, Ki-67, pERK, pMAPK, and to a lesser extent pAKT in A431, MCF-tb cells, and Capan-1 or BxPC-3 human pancreatic carcinoma cells, but not in EFGr-non-responsive SK-MES cells (Study #R2003104). Taken together, these studies confirm both the *in vivo* and molecular anti-tumor events associated with ABX-EGF treatment, which have been previously demonstrated in other human, colorectal tumor cell models.

Samples of human SK-MES and H1299 lung carcinoma, and MiaPaCa and Panc-1 pancreatic tumors were obtained from xenografts growing in athymic nude mice, and cryosections were evaluated *ex vivo* for ABX-EGF binding after incubation with 10 µg/ml biotinylated panitumumab, and subsequent immunoperoxidase staining. In this study, SK-MES and Panc-1 tumor xenografts exhibited strong (3^+) staining with panitumumab, while MiaPaCa tumor sections exhibited weak (1+) staining after the same dosing regimen. There was no detectable ABX-EGF staining in sections from panitumumab-treated, H1299 tumor-bearing mice. Taken together, these data suggest that the apparent lack of response to ABX-EGF in some tumor xenografts such as H1299 and MiaPaCa may be due to the loss, or reduction of EGFr expression by these tumors.

2.6.2.4 Safety pharmacology

The cardiovascular, respiratory, and central nervous system effects of ABX-EGF were evaluated in a safety pharmacology study in cynomolgus monkeys following a single, intravenous dose of panitumumab. The study results are reviewed, below.

Study title: Cardiovascular, respiratory *and* central, nervous system assessment of ABX-EGF (panitumumab) administered as a single intravenous dose to conscious cynomolgus monkeys.
Key findings: A single i/v administration of 7.5, 30 or 60 mg/kg of panitumumab showed no test article-related effects on the cardiovascular, respiratory, or central nervous systems of conscious, telemterized monkeys.
Study number: 104119 (Abgenix Study #ABX-P0307, — Study #04-6567)
Volume # and page #: EDR file: STN BLA 125147\000\module4\pharmacology studies\safety pharm\104119.pdf
Conducting laboratory and location:

Date of study initiation: April 16, 2004 (in-life, 5/27 – 6/10/04; final study report dated October 19, 2005)
GLP compliance: Yes
QAU statement: Yes (X) No ( )
Drug, lot #, and % purity: ABX-EGF placebo (50 mM sodium acetate in 100 mM sterile sodium chloride, pH 5.8), lot #A0306040000, % purity not specified; ABX-EGF, lot #954A023447, — mg/ml, purity — (size exclusion HPLC)

Methods

Doses: ABX-EGF placebo, 7.5, 30, 60 mg/kg ABX-EGF antibody
Species/strain: *Macaca fasicularis* (cynomolgus monkey); purpose-bred (—)
Number/sex/group or time point (main study): 4 males/group
Route, formulation, volume, and infusion rate: intravenous injection; placebo or ABX-EGF formulated in 50 mM sodium acetate, 100 mM NaCl buffer, pH 5.9; volume 3 ml/kg; infusion rate 4 ml/min
Satellite groups used for toxicokinetics or recovery: No additional animals were included in this study for T/K evaluation. Blood samples for measurement of ABX-EGF

serum concentrations were obtained from the main study animals. A single blood sample was collected from each animal approximately 24 h after ABX-EGF dosing, for confirmation of panitumumab exposure.
Age: approximately 3 to 5.5 years old
Weight (non-rodents only): mean 3.2 kg; range 2.7 – 3.9 kg
Unique study design or methodology (if any): Prior to ABX-EGF or placebo treatment, all animals were implanted with telemetry transmitter devices [redacted] for measurement of EKG, heart rate, and blood pressure. Monkeys were allowed to recover from the surgery for device implantation for at least 4 weeks prior to administration of panitumumab.

Lactated Ringers Solution (100 ml/kg) was administered daily by s/c injection to maintain fluid and electrolyte balance, beginning 24 h after ABX-EGF dosing and continuing until SD 15. When necessary, topical antibiotic/cortisone ointment was applied to affected skin to treat and/or minimize dermal inflammation and infection.

Results

Clinical effects: There were no mortalities on study, and the only clinical sign of toxicity was watery stool in one monkey (animal #9091) in the 60 mg/kg dose group on SD 15. Since this time point was approximately 2-3 half-lives following ABX-EGF injection, the finding is considered unrelated to panitumumab treatment. Decreased food consumption was noted at various time points over the monitoring period in 1 to 2 monkeys in each dose group, including the placebo control and was therefore considered unrelated to ABX-EGF. All animals were returned to the colony following completion of the study.

Neurological effects: Neurobehavioral evaluations were performed by personnel who were blinded to treatment group. Each monkey was evaluated under restraint in the primate chair, and in the home cage twice pre-study, at 2-5 hours following dosing on SD 1, and on SD 7 and SD 14. Monkeys were observed while chair-restrained for changes in mental status (activity levels, agitation), muscle tone and movements, convulsions, eyelid position, pupil size, eye movement and lacrimation, patellar reflex, and salivation. In addition to these parameters, in the home cage animals were observed for posture, locomotor activity, and motor function (interest in and ability to accept, handle, and consume a food treat).

Based on the assessments performed, a single, i/v dose of panitumumab had no remarkable effects on the neurobehavioral condition of the ABX-EGF treated animals.

Cardiovascular effects: Ten-lead electrocardiograms were measured manually for each animal once pre-study, and once following the last monitoring period (SD 15) using a Cambridge apparatus. The leads included standard limb leads I, II and III, augmented limb leads (aVR, aVL, aVF), and chest leads (MV_1, MV_2, MV_3, and V_{10}) rhythm strips. Cardiovascular parameters (axial lead AKG and blood pressure waveforms, heart rate, systoic, diastolic, and mean blood pressures) and body temperature were collected radiotelemetrically for 30 sec every 10 min for two, 24-h monitoring periods pre-study, and for a minimum of two hours prior to ABX-EGF dosing on SD 1. Following placebo or panitumumab administration, each animal was monitored every 10 minutes for 24 hours post-dosing on SD 1, then hourly for each day afterwards until SD 15.

Selected, telemetered EKG tracings (16 timepoints from each animal for each pre-dose EKG interval on SD 1, and approximately 8-25 telemetered EKG waveforms per animal per day as chosen by the Study Director, and all 12 pre-study timepoints) were qualitatively evaluated and interpreted by a veterinary cardiovascular physiologist employed by the contracting laboratory. Additionally, each multi-lead EKG tracing collected manually for each monkey at the pre-study and post-dosing EKG sessions was analyzed quantitatively for PR, QRS, QT and RR intervals using a computerized software package (EMKA Technologies ECG-Auto Software version 1.5.11), and the results were interpreted by the veterinary cardiologist.

There were no remarkable effects of a single, i/v dose of 7.5, 30, or 60 mg/kg ABX-EGF on EKG activity, including PR interval, QRS interval, RR interval, QT interval, QTc interval and heart rate, as compared to either pre-study measures, or to placebo control animals. Sporadic changes in systolic, diastolic, and mean blood pressure were observed in ABX-EGF treated animals during the 14-d monitoring period, and were occasionally statistically significantly different from the control group at that same time point. However, there were no significant differences in the values obtained for the ABX-EGF treated monkeys as compared to either pre-study monitoring, or pre-dose values on SD 1, and the findings were therefore considered within normal limits for this strain of macaques. There were no remarkable effects of ABX-EGF treatment at any time post-dosing on either heart rate or body temperature, as compared to either pre-study baseline, or control animal values.

Pulmonary effects: Chair-restrained monkeys were fitted with facemasks and allowed to breathe room air through a series of low resistance, one-way valves for a timed period. Exhaled air was collected during this time through a pneumotach airflow-measuring device, and capture of airlow and breath frequency data were collected. Respiratory rate, tidal and minute volumes were calculated using the Biosystem XA software integral to the system. Respiratory parameters from chair-restrained monkeys were obtained 2 to 3 times pre-study, then at 3-6 hours after ABX-EGF dosing on SD 1, and on SD 7 and SD 14.

Comment: The collection of respiration rate and tidal and minute volumes were performed following the collection of cardiovascular data on SD 1.

For most of the time points evaluated, respiratory rates, tidal volumes and minute volumes obtained from ABX-EGF treated monkeys were comparable to control animals. On SD 1, mean respiratory rated for the ABX-EGF treated groups were higher than the control values; however, comparison of each individual monkey's pre-dose and post-injection values showed no overall changes related to panitumumab. No ABX-EGF related effects were evident.

Renal effects: The present study did not measure the renal effects of panitumumab treatment. No additional safety pharmacology studies that specifically evaluate this parameter were included in the present BLA submission.

Gastrointestinal effects: The present study did not measure the gastrointestinal effects of panitumumab treatment, other than clinical observation of animals for changes in fecal consistency and frequency. No additional studies that specifically evaluate this parameter were included in the present BLA submission.

Abuse liability: No studies of that specifically evaluate this parameter were included in the present BLA submission.

Other: Toxicokinetic evaluation of ABX-EGF serum levels obtained 24 h following dosing confirmed a dose-related exposure to panitumumab. Using a previously validated ELISA assay with a lower limit of quantitation of 39 ng/ml panitumumab, no ABX-EGF was detected in serum samples from any of the monkeys treated with the placebo control. Mean ABX-EGF serum levels (± SD) for the panitumumab treated monkeys at 24 h post-dosing were 104 ± 9 pg/ml for the 7.5 mg/kg dose group, 436 ± 32 pg/ml for the 30 mg/kg dose group, and 803 ± 51 pg/ml for the 60 mg ABX-EGF/kg dose group.

Study conclusion: Under the conditions of this study, there were no remarkable effects of a single, i/v dose of 7.5, 30, or 60 mg/kg of panitumumab on the neurobehavioral, cardiovascular, or respiratory parameters measured. Toxicokinetic measurements confirmed dose-related exposure to ABX-EGF 24 h after injection on SD 1. All animals remained clinically healthy for the duration of the treatment and observation periods, with no remarkable differences in clinical signs or food consumption between the control and ABX-EGF treated groups.

2.6.2.5 Pharmacodynamic drug interactions

No studies of this type were included in the present BLA submission.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

A tabulated summary of all preclinical pharmacology studies included in the BLA was provided by the sponsor in Module 2, Section 2.6.3 of the electronic CTD submission, and is attached to this review as Appendix 3.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The pharmacokinetics of panitumumab were evaluated in cynomolgus monkeys, and in non-tumor bearing, athymic, nude mice to support ABX-EGF anti-tumor efficacy studies using murine tumor xenograft models. In mice, panitumumab exhibited an elimination half-life ranging from 10 to 16 days, and clearance was independent of dose at doses of 0.2to 20 mg/kg (approximately 5 to 500 µg/mouse). By contrast, dose-dependent clearance of panitumumab was observed in monkeys, with increasing exposure (Cmax and AUC_{0-t}) observed that was more than dose-proportional following repeated administration. At low doses of panitumumab (3 mg/kg), the elimination half-life of ABX-EGF was approximately 1 day, and increased after repeated injection of 30 mg/kg/dose to approximately 4 to 5 days. The non-linear elimination of panitumumab in monkeys is likely related to progressive saturation of EGFr, which is part of the mechanism of ABX-EGF clearance in this species, as well as in humans. No accumulation of ABX-EGF exposure was observed following repeated administration of panitumumab to cynomolgus monkeys for 1 to 6 months. However, although the increases in both C_{max} and AUC_{0-t} were less than 2-fold over the study durations, the variability of the pharmacokinetic parameters after the final dose was much greater than after the first dose. This increase in variability was attributed to the presence of monkey anti-human antibody (MAHA) responses,

which in individual animals resulted in decreased serum levels, and accelerated clearance of ABX-EGF.

2.6.4.2 Methods of Analysis

Please see under individual study reviews.

2.6.4.3 Absorption

Comment: A single study was conducted to evaluate the absorption and pharmacokinetic profile of ABX-EGF in athymic, nude mice following i/p injection. This study was conducted after the majority of primary and secondary pharmacokinetic studies were completed (in-life phase of study, 5/11 – 6/22/2003). It is presumed that the data from the present study were intended to provide information regarding the level of panitumumab exposure correlating with the therapeutic effect in the human tumor xenograft models.

Study title: A pharmacokinetic study of ABX-EGF in male nude mice following a single intraperitoneal injection.

Key findings: Intraperitoneal injection of normal (non-tumor bearing) nude mice with panitumumab resulted linear, dose-related increases in C_{max}, AUC_{0-t} and AUC_{0-inf}. There was no apparent difference in clearance (CL/F) between the two dose levels, and the elimination half-life was approximately 16 days.

Study #: 104275 (Abgenix Study #ABX-P0306. – Study # – 243.14)

Methods: Male :CD-1-*nu*BR, athymic nude mice (15/group) were injected on SD1 with either 5.22 or 522 µg/mouse ABX-EGF (lot #9099-58G), i/p. Blood samples were collected by retro-orbital plexus puncture from 5 mice/time point (0.1 ml/mouse; sparse sampling schedule) at pre-dosing, 4, 8, 16, 24, 36, 48, 60, 72, 96, 120, and 168 h after injection, and on SD10, SD12, SD15, SD17, SD19, SD22, SD24, SD26, SD29, SD31, SD33, SD36, SD38, SD40, and SD43. Serum ABX-EGF levels were measured using an electrochemiluminescence (ECL) assay with an anti-idiotype, anti-panitumumab antibody as the capture reagent, and ruthenium-labeled, anti-idiotype anti-panitumumab antibody used as the detection reagent. ECL counts for the test samples were measured and converted to panitumumab concentration by comparison against a standard curve generated with known concentrations of ABX-EGF. The limits of detection for this assay (as validated in monkey serum as the matrix) were 7.8 – 1000 ng/ml in 20% serum, and the lower limit of quantitation in 100% serum was 39 ng/ml. Non-compartmental analysis of the pharmacokinetic profiles was calculated on the average concentration for each group at each time point by least squares regression, using the WinNonlin software package.

Comment: The final report states that the ECL assay had been validated using monkey serum as the matrix; however, this assay was performed in mice, and there was no information in the BLA submission as to whether the ECL assay had been validated, or even qualified with mouse serum as the matrix. Differences in binding proteins (*i.e.* albumin), and potential inhibitory or other factors in mouse serum may interfere with this assay, and result in changes in the expected serum concentrations of panitumumab. The sponsor will be requested to provide information that addresses these issues as part of the discipline review letter.

Results: Serum concentrations after i/p injection of panitumumab showed a dose-related increase in both C_{max} and exposure (AUC_{0-t}, AUC_{0-inf}). Mean, maximal serum concentrations of 1.7 µg/ml and 181 µg/ml were detected 8 h after injection in the 5.22 µg dose group, and at the 16 h time point in mice treated with 522 mg/mouse ABX-EGF, respectively. Panitumumab serum levels remained at slightly less than the peak values for up to 7 days after injection, at which time they began to decrease in a linear fashion. There was no apparent difference in clearance between the two dose groups, and the estimated elimination half-life for ABX-EGF in mice was approximately 16 days. These data are presented in Table 10, which was abstracted from the sponsor's final study report, below.

Table 10. Study #104275. Pharmacokinetic Profile of Panitumumab in Athymic Nude Mice Following Intraperitoneal Injection

Pharmacokinetic Parameter	Dose of Panitumumab on SD1	
	5.22 µg/mouse	522 µg/mouse
t_{max} (h)	8	16
C_{max} (µg/ml)	1.73	181
AUC_{0-t} (µg*d/ml)	32.2	3110
AUC_{0-inf} (µg*d/ml)	39.7	3840
CL/F (ml/d)	0.132	0.136
$t^{1}/_{2elim}$ (d)	16.2	15.7

Comment: There were no raw data included in the final report for this study, to allow independent calculation, verification, and statistical analysis of the pharmacokinetic parameters of panitumumab in the mouse model used for the tumor xenografts.

Comment: There was still significant panitumumab detectable in mice in both dose groups at SD42, with mean values of 266 ± 207 ng/ml and 32,135 ± 13,916 ng/ml obtained for mice in the 5.22 and 522 µg/ml dose groups, respectively.

Comment: Panitumumab does not interact with murine EGFr. Therefore, the serum levels obtained in this study do not accurately represent those anticipated in athymic nude mice bearing human tumor xenografts, where binding and internalization of ABX-EGF to EGFr expressed on tumor cells is expected to contribute significantly to the clearance, and subsequent elimination half-life of panitumumab. This point will be communicated to the sponsor as part of the discipline review letter.

Study conclusion: Panitumumab exhibited linear pharmacokinetics in normal, athymic nude mice following i/p injection of 5.22 or 522 µg/mouse ABX-EGF. There were no apparent differences between the dose levels in either clearance or elimination half-life; however, the apparent persistence of panitumumab likely relates to its lack of interaction with murine EGFr. The data from this study therefore cannot be used to support the anticipated exposures in athymic nude mice bearing xenografts of human EGFr expressing tumors.

2.6.4.4 Distribution

Comment: Three studies were conducted to evaluate the distribution and excretion of ^{125}I-labeled ABX-EGF or unlabeled panitumumab, following a single, i/v dose in cynomolgus monkeys. The BLA sponsor has submitted all three studies to both the Distribution (Section 2.6.4.4) and Excretion (2.6.4.6) sections of the eCTD-BLA submission. Since there were no apparent differences between the three studies' conduct and conclusions, the data from all three studies will be reviewed and summarized, below.

Whole-body distribution and excretion of ^{125}I-panitumumab in cynomolgus monkeys

Study #: #103619, #103620, and #104274

Key findings: Distribution of ^{125}I-panitumumab to major organs was observed predominantly in those with relatively high blood flow (liver, lung, kidney, adrenal), followed by later distribution to target organs that potentially express high levels of EGFr (eye, large intestine, skin). Elimination of radiolabel from the peripheral organs and tissues paralleled that of elimination from serum. Excretion of ABX-EGF occured primarily via the urine, with > 90% of the dose of radioactivity recovered between 216 and 240 h after injection of a single dose of ^{125}I-labeled panitumumab.

Methods: The *in vivo* distribution, pharmacokinetics, and excretion of ^{125}I-ABX were evaluated in cynomolgus monkeys in Studies #103619, #103620, and #104274. For all three studies, whole body autoradiography, and quantitative assessment of ^{125}I radiolabel distribution in blood, urine, feces, and cage pan rinses/wipes were performed following a single, i/v dose of ABX-EGF. Animals (5/sex) in Study #104274 received a total dose of 6 mg/kg of a mixture of unlabeled ABX-EGF (lot #3737/TFP-99059A) and ^{125}I-ABX-EGF (lot #00-0379; specific activity, 11.1 μCi/μg; ^{125}I dose approximately 120 μCi/kg). Four monkeys/sex each in Studies #103619 and #103620 received a total dose of 7.5 mg/kg of a mixture of unlabeled, CHO-derived ABX-EGF (lot #954A021224) and ^{125}I-ABX-EGF (lot #44025-39; specific activity, 10.9 μCi/μg; ^{125}I dose approximately 120 μCi/kg). For all three studies, blood was collected from surviving animals at 2, 24, 48, 72, 96, and 120 h, and from the 2 remaining animals in Study #104274 at 240 h post-dose, and all samples were processed to serum. Radioactivity was quantitated by solid scintillation (SSC) counting of samples of both blood and serum either as total radioactivity, or following precipitation with trichloroacetic acid (TCA). Urine and feces were collected in metabolic cages every 24 h post-dose from the animals in each study designated for the last two sacrifice time points, and daily collections of residual cage debris, and trisodium phosphate cage rinses were also evaluated for ^{125}I-radiolabel by SSC. Pharmacokinetic evaluation of blood and serum radioactivity levels were performed for all three studies, and parameters calculated included C_{max}, T_{max}, AUC_{0-t}, AUC_{0-inf}, and elimination half-life using nonparametric analyses with the WinNonlin software package.

Quantitative, whole body autoradiography was performed on the carcass of each animal, following euthanasia at the scheduled time points after dosing. For all three studies, one male and one female monkey each were euthanized at 2, and 48 h after dosing. In Study #104274, an additional one/sex/time point were euthanized at 96 and 168 h, while additional monkeys (1/sex/time point/each study) were euthanized in Studies #103619 and #103620 at 120 and 216 h after dosing. Following euthanasia and cryopreservation, 40 micron, sagittal sections were cut from 5 to 6 levels of interest such that all major organs, tissues, and body fluids were represented for each animal, the sections were mounted, and exposed to autoradiographic film for detection of ^{125}I distribution. Tissue concentrations of ^{125}I-radiolabel were interpolated from standard curves

prepared for each section, and then converted to µg equivalents/g of tissue based on the specific activity of the test material.

Comment: An additional male and female monkey were planned for evaluation at 240 h post-dosing in Study #104274 but were not analyzed, since the total amount of recovered radioactivity in urine, feces, and cage wash/cage debris by this time point was > 96%.

Results: The pharmacokinetic profiles for ^{125}I-radiolabeled, ABX-EGF in serum are presented in Table 11, below. In all three studies, the maximal counts for ^{125}I radiolabel in both blood and serum were achieved at 2 h after dosing, with mean values of 85.1 and 152 mg equivalents/g achieved for blood and serum, respectively in Study #104274. Studies #103619 and #10620 yielded mean blood radiolabel concentrations of 95.0, and 92.6 µg equivalents/ml, respectively. Radioactivity in both blood and serum declined over the duration of each study, but was still detectable at study termination at 216 or 240 h. Mean values for pharmacokinetic parameters derived from serum levels in all animals on each study presented in Table 11 below.

Table 11. Pharmacokinetic Profile of ^{125}I-labeled ABX-EGF in Cynomolgus Monkeys Following a Single, Intravenous Dose

Pharmacokinetic Parameter	Mean Value, ± S.D.		
	Study #104274	Study #103619	#Study #103620
Dose of ABX-EGF	7.5 mg/kg	6 mg/kg	6 mg/kg
C_{max} (µg equiv/ml)	151 ± 10	172 ± 12	168 ± 40
T_{max} (h)	2.02 ± 0.04	2.0 ± 0	2.0 ± 0
AUC_{0-t} (µg equiv*h/ml)	7960 ± 341	7077 ± 1508[a]	7142 ± 1941
AUC_{0-inf} (µg equiv*h/ml)	8418 ± 742	8455 ± 954	8670 ± 1487
$t½_{elim}$ (h)	47.1 ± 5.8	44.4 ± 15.4	49.6 ± 19.2

[a] parameters were calculated by pooling values obtained from animals (1/sex/time point) euthanized at 48, 120, and 216 h post-dose.

Whole body autoradiography of ^{125}I-ABX-EGF tissue distribution for all three studies revealed the highest levels of radiolabel detectable at 2 h after dosing, with blood, liver, lung, spleen, adrenal gland, and kidney showing the highest levels (range, 20 to >60 µg equivalents/g tissue) at this time point. Eye, skin, large intestine, and thymus all showed demonstrable radiolabel uptake as well, with T_{max} values of 48 h after dosing (data not shown). Tissue levels of radioactivity declined over time in all three studies; however, detectable radiolabel was still present in major organs at the final sacrifice time points. In all three studies, quantifiable levels of ^{125}I radiolabel were detectable in the testes, cerebellum, cerebrum, medulla, and/or spinal cord, suggesting that ^{125}I-ABX-EGF derived radioactivity could cross the blood:testis and blood:brain barriers.

Excretion of ^{125}I radiolabel from ABX-EGF occurred mainly via the kidney for all three studies, with approximately 50% of the dose excreted by 48 h after injection (Study #104274), and ranging from 63.2 to > 90% of the dose excreted by study termination at 216 or 240 h post-dose. By contrast, the dose of ^{125}I radiolabel excreted in feces was minimal, ranging from <2% to approximately 10% for all three studies. The remainder of excreted radiolabel was recovered in the daily cage rinses, wipes and collected cage debris, for overall excretion of between 84.4% and 99.4% of the injected dose, over the duration of the measurement period.

Study conclusion: Similar patterns of tissue distribution, elimination, and excretion of ^{125}I radiolabel were obtained for both male and female monkeys, and between different lots of ABX-EGF. For all three studies, distribution of ^{125}I-panitumumab to major organs was observed

predominantly in those with relatively high blood flow (liver, lung, kidney, adrenal), followed by later distribution to target organs that potentially express high levels of EGFr (eye, large intestine, skin). Elimination of radiolabel from the peripheral organs and tissues paralleled that of elimination from serum, and excretion was predominantly via the kidney and urine. Taken together, these studies provide a pattern of distribution that should aid in identification of the target organs for panitumumab toxicity *in vivo*.

2.6.4.5 Metabolism

No studies of this type were included in the present submission.

2.6.4.6 Excretion

Non-clinical studies evaluating the excretion of ^{125}I-labeled panitumumab (Studies #103619, #103620, and #104274) were reviewed under Section 2.6.4.4, above. Excretion of ABX-EGF occurs primarily via the urine, and > 90% of the dose of radioactivity was recovered between 216 and 240 h after injection of a single dose of ^{125}I-labeled panitumumab. No additional, stand-alone studies to evaluate excretion of ABX-EGF were submitted to the BLA.

2.6.4.7 Pharmacokinetic drug interactions

No studies of this type were included in the present submission.

2.6.4.8 Other Pharmacokinetic Studies

Two additional pharmacokinetic studies were conducted to evaluate the comparability of ABX-EGF in either mice (Study #104273) or cynomolgus monkeys (Study #102876), following a change in manufacturing from hybridoma to CHO cell lines. The two studies will be summarized and reviewed together, below.

Comparability of hybridoma- and CHO cell-derived ABX-EGF in mice and cynomolgus monkeys

Study #: Study #102876, Study #104273
Key findings: In cynomolgus macaques, the pharmacokinetic profiles of the hybridoma- and CHO cell-derived materials are comparable, as determined by comparison of $AUC_{0\text{-}336h}$ and C_{max} values. No comparability of the two products could be determined in athymic nude mice, due to a high degree of inter-animal variability.
Methods: Pharmacokinetic profiles of ABX-EGF derived from two different manufacturing methods were obtained after i/v injection in female, CD-1-*nu*BR mice, and in cynomolgus macaques. Mice (36/group; Study #A104273) were dosed by tail vein injection on SD1 with either 0.1, or 0.5 mg/mouse of ABX-EGF, from either hybridoma (lot #7334,) or CHO cell (lot #8977-47, Immunex) origin. Cynomolgus monkeys (12 naïve males/group; Study #102876) were injected i/v on SD1 with 7.5 mg/kg of either hybridoma-derived panitumumab (lot #P01007F), or ABX-EGF (lot #9099-53F) from CHO cells. Blood samples were collected from mice at pre-dose (10 animals, total), and terminally from 3 mice/time point at 1, 4, 24, 48, 72, 120, 168, 240, 336, 408, and 504 h after dosing. Blood samples were collected from individual monkeys prior to dosing, and at 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, and 336 h after injection, processed to serum, and stored frozen at -70°C prior to analysis. Serum panitumumab levels in the mouse study were quantitated by ELISA, while serum ABX-EGF levels from i/v injected monkeys were measured by biosensor, using electrochemiluminescence

(ECL) as the readout, with a lower limit of quantitation for this assay of 19.5 ng/ml. Anti-panitumumab antibody levels were also measured in monkey serum samples obtained prior to treatment, and at the final sampling time point at 336 h after injection. For both studies pharmacokinetic parameters for the hybridoma-derived and CHO cell-derived ABX-EGF preparations were calculated using the WinNonlin software package, following non-compartmental analysis.

Monkeys were also monitored for in-life signs of panitumumab toxicity, including clinical observations of skin rash, flakiness, dermatitis, and changes body weights and in fecal consistency that have previously been observed with ABX-EGF treatment.

Results: All mice survived until scheduled termination. All monkeys also survived for the duration of the study; however, clinical signs of toxicity consistent with previous panitumumab treatment were noted in these animals. Skin changes, including flaky skin, dandruff, irritation, and reddening were noted in 11/12 monkeys treated with CHO-derived ABX-EGF, and all 12 monkeys treated with panitumumab derived from the hybridoma cell line, beginning as early as SD7, and continuing until approximately SD16. Changes in fecal consistency (soft feces) were also first noted beginning on SD7 and peaked in incidence in 8/12 monkeys in the group treated with CHO-derived ABX-EGF, and in 4/12 monkeys treated with hybridoma-derived panitumumab. There were no remarkable differences in body weights between the two treated groups, either at baseline or at the end of the 21 d study period.

Pharmacokinetic evaluation of panitumumab serum levels showed some minor differences between hybridoma- and CHO cell-derived materials in athymic nude mice after i/v dosing. At the lower concentration (0.1 mg/mouse), the mean C_{max} and AUC values were approximately 7% and 15% lower for CHO-derived ABX-EGF than for hybridoma-derived material, while at the 0.5 mg/mouse dose level, the C_{max} and AUC values for CHO-derived panitumumab were approximately 28% and 15% higher, respectively, than for antibody of hybridoma origin. The data are presented in Table 12, below.

Table 12. Study #104273. Summary of P/K Parameters of Hybridoma vs. CHO Cell-Derived Panitumumab in Female, Athymic Nude Mice[a]

Pharmacokinetic Parameter	0.1 mg Panitumumab/mouse		0.5 mg Panitumumab/mouse	
	Hybridoma	CHO-derived	Hybridoma	CHO-derived
C_{max} (µg/ml)	58.8	54.8	179	229
AUC_{0-t} (µg*h/ml)	15992	13603	46694	53636
AUC_{0-inf} (µg*h/ml)	24667	18986	66111	76290
V_d (ml)	1.8	2.1	2.7	3.1
CL (ml/h)	0.0041	0.0053	0.0076	0.0066
$t^{1}/_{2elim}$ (h)	302	273	248	323

[a] data are as provided by the sponsor in the final study report for Study #104274; no statistics, including SD for each of the mean values, above were calculated for this study

Comment: The sponsor reported that there was a high degree of variability within the data for the two different dose levels; therefore, a statistical evaluation of the comparability of the two preparations cannot be performed. No independent evaluation of these data was conducted by the reviewer, since panitumumab pharmacokinetics depend in part on interaction with EGFr, and ABX-EGF does not bind EGFr in this species.

In the monkeys (Study #102876), minimal differences in the values for C_{max} and AUC between the two preparations were noted. The mean C_{max} value for the CHO-derived lot of ABX-EGF was approximately 11% lower than that obtained for the hybridoma-derived antibody, while the difference in mean AUC_{0-336h} was approximately 6%. The calculations for these values are presented in Table 13, below.

Table 13. Study #102876. Comparison of Pharmacokinetic Parameters for ABX-EGF from Hybridoma or CHO Cell Origins in Cynomolgus Macaques

Pharmacokinetic Parameter	Mean Value, ± SD		Ratio CHO:Hybridoma [90% CI]
	Hybridoma-derived	CHO cell-derived	
C_{max} (µg/ml)	256 ± 56	227 ± 31	89.4 [79.4 – 100.7]
AUC_{0-336h} (µg*d/ml)	664 ± 143	619 ± 83	94.4 [83.6 – 106.5]

Statistically, there was a moderate degree of variability in both C_{max} and AUC_{0-t} between animals treated with the two preparations of ABX-EGF, as evidenced by coefficients of variation ranging between 13.4% and 21.9%. When the ratio of these values and their confidence intervals were calculated, the confidence interval for C_{max} was found to be just outside the 80:125 level considered acceptable for demonstration of bioequivalence (90% confidence intervals, 79.4 – 100.7).

Comment: Evaluation of the raw data revealed that in a single monkey treated with the hybridoma-derived ABX-EGF (animal #020571), the C_{max} value was approximately 50% higher than the mean value obtained for the group. Although the value for this animal did not meet the statistical test to be considered a true outlier (Studentized value $\geq$ 3.0; Studentized value for this monkey was 2.8), recalculation of C_{max} without this animal's measurement gave a mean value of 241 ± 93, with a ratio of CHO:Hybridoma of 93.2, and a 90% confidence interval of 84.5 – 103.0.

No anti-panitumumab antibody was detected in monkeys treated with the CHO-derived ABX-EGF at either time point measured on study. By contrast, two monkeys in the group treated with hybridoma-derived ABX-EGF (animals #020578 and #020591) were both positive for MAHA response at 336 h after dosing. One monkey (animal #020847) in this same group showed a positive MAHA response in this assay at the pre-dose sampling time, but was negative for anti-panitumumab antibody at the end of study time point.

Study Conclusions: Due to a high degree of inter-animal variability in serum ABX-EGF levels and the lack of interaction with murine EGFr in athymic, nude mice, the pharmacokinetic comparability of hybridoma- and CHO cell-derived panitumumab could not be demonstrated. In cynomolgus macaques, the pharmacokinetic profiles of the hybridoma- and CHO cell-derived materials are comparable, as determined by comparison of AUC_{0-336h} and C_{max} values.

2.6.4.9 Discussion and Conclusions

Please see Study Conclusions under individual study reviews, above.

2.6.4.10 Tables and figures to include comparative TK summary

Tables for results from the individual pharmacokinetic and toxicokinetic evaluations are integral to the study reviews (Please see Section 2.6.4, above and Sections 2.6.6.3 and 2.6.6.6 through 2.6.6.8, below). A comparative PK and TK summary table, as provided by the sponsor in Module 2, Section 2.5 is included in Appendix 4 of this review.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

A tabulated summary of all preclinical pharmacokinetic studies included in the BLA, as provided by the sponsor in Module 2, Section 2.6.5 of the electronic CTD submission is attached to this review as Appendix 4.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: Administration of panitumumab to cynomolgus monkeys for up to 26 weeks was associated with mild to severe dermatologic toxicities, epidermal sloughing, septicemia, and deaths in animals in all dose groups, with macroscopic, clinical pathologic, and histopathologic evidence of cellular and tissue damage in the epidermis and dermis of the skin. No NOAEL could be identified for ABX-EGF in the cynomolgus monkey; of note, this is the only species other than human, which demonstrated cross-reactivity of panitumumab with a selected panel of tissues in the initial *in vitro* tissue binding study, although a later study did show a similar pattern of tissue cross-reactivity of panitumumab in rabbits.

Genetic toxicology: No genetic toxicology studies were conducted for this product. Panitumumab is a recombinant, therapeutic protein (monoclonal antibody) and as such is not anticipated to interact with DNA, nor intracellular DNA binding proteins; therefore, the standard ICH S2 battery of genotoxicity testing is not considered appropriate, or useful for this product.

Carcinogenicity: No carcinogenicity studies were conducted for this product. Tissue cross-reactivity and pharmacodynamic studies have demonstrated that panitumumab is pharmacologically active only in humans and non-human primates, which are not appropriate species in which to evaluate tumorigenic potential of ABX-EGF.

Reproductive toxicology: Panitumumab inhibits ovulation and menstrual cycling in non-pregnant female cynomolgus macaques and is abortifactient in pregnant females dosed during the period of organogenesis, at doses 1.25 to 6-fold higher than the recommended dose planned for clinical use after marketing approval.

Special toxicology: Tissue cross-reactivity studies of panitumumab in cynomolgus monkey tissues have demonstrated moderate to high levels of ABX-EGF binding to known EGFr-expressing targets, including eye, skin, tonsil, prostate, breast, and urothelium in the bladder.

2.6.6.2 Single-dose toxicity

No single-dose toxicity studies were conducted for this panitumumab.

2.6.6.3 Repeat-dose toxicity

Comment: There were five different, repeat-dose toxicity studies submitted by the sponsor to this section of the BLA. The toxicity profiles of panitumumab were similar in all five studies, as well as in two additional, repeat-dose toxicity studies submitted under "Other Toxicity Studies" (Section 2.6.6.8, below). However, only three studies (Studies #BQAW-102, #BQAW-103, and #103419) submitted to this section of the BLA were performed using the CHO-derived, ABX-EGF produced at either the — or — (commercial) scale. Although all studies were reviewed as part of the pharm/tox section of the BLA, only these three studies will be included in the written review.

Study #BQAW-102:

- Species - cynomolgus monkey (*Macaca fasicularis*); 6 males/group with 5 males, 1 female monkey in control group
- Dose, duration and regimen – vehicle (Group 1), 6 or 60 mg/kg ABX-EGF, i/v bolus (Groups 2, 3, and 4) or i/v infusion (Group 5) on SD1, followed by vehicle, 3 or 30 mg/kg/dose, i/v bolus or i/v infusion SD 8, 15/16, and 22; plus 2-week, treatment-free recovery
- NOAEL = not defined; toxicities were observed in all treatment groups
- Toxicities – early mortality in 4 animals, with 2 early deaths (SD 19 and SD 20) in monkeys treated at 60/30 mg/kg/dose by i/v bolus, and 2 animals euthanized moribund in the group treated with 60/30 mg/kg/dose by i/v infusion on SD 15 and SD 22.
- persistent, soft to liquid feces present in 5/6 monkeys treated with 6/3 mg/kg/dose ABX-EGF, and in all animals in all 60/30 mg/kg/dose ABX-EGF treated groups, beginning SD 3 – SD 5 and persisting for duration of treatment
- skin changes (erythema, dryness, flakiness, swelling), rough coat, and/or scratching present in 0/6 control animals, 1/6 low-dose monkeys, and 3/6, 2/6, and 3/6 monkeys in Groups 3, 4, and 5, respectively, beginning SD 14 and persisting for duration of treatment and recovery periods
- decreased food consumption, body weights, body weight gain in all ABX-EGF groupshematology – decreased red cell counts, hemoglobin, hematocrit; indicative of anemia, secondary to blood sampling for T/K
- progressive decreases in total leukocyte, absolute PMN, and absolute lymphocyte counts in all groups, including vehicle control as compared to baseline on SD 15/16, 29, and 43
- increased fibrinogen in all ABX-EGF treated groups at SD 15/16, SD 29 as compared to both baseline values, and to mean values for control group at each time point
- clin path changes including hyponatremia, hypochloremia, hypocalcemia, hypomagnesemia, hyperkalemia, hyperphosphatemia at SD 15/16 and SD 29 in all ABX-EGF groups
    - decreased albumin, increased globulin in individual ABX-EGF treated animals as compared to both baseline and control at SD 15/16 and SD 29, with resultant decreases in A:G ratio
    - increased BUN, hyperglycemia noted in individual animals treated with 60/30 mg/kg/dose ABX-EGF at SD 15/16 and SD 29

- histopathology – mucosal hyperplasia of the large intestines, correlating with clinical findings of soft feces/diarrhea in individual animals at SD 29
    - mucosal hyperplasia still apparent in animals from all ABX-EGF dose groups at recovery sacrifice on SD 43, but of lesser severity than SD 29
- adrenocortical hyperplasia of the zona glomerulosa in individual animals in the groups treated with 60/30 mg/kg/dose ABX-EGF, at end of treatment (SD 29) sacrifice
    - finding not present at recovery sacrifice on SD 43
- dermatitis associated with serocellular crusts in multiple skin sites in animals in all ABX-EGF dose groups, without apparent relationship to dose for either incidence or severity
- myocardial degeneration present in 1/3 monkeys in 6/3 mg/kg/dose group, and none of the higher-dose ABX-EGF treated monkeys at SD 29
- T/K data – serum concentrations confirm continuous exposure to panitumumab over duration of the study
    - approximate 2-fold higher C_{max}, AUC on SD 1 vs. SD 22 explained by loading dose of ABX-EGF (6 or 60 mg/kg) vs. maintenance doses (3 or 30 mg/kg/dose)
    - decreased serum levels noted in animals with MAHA positive response, beginning SD 14 – SD 21
- immunogenicity – monkey-anti-human antibody (MAHA) observed in 0/6 control, 3/6 low-dose, and in 1/4, 0/6, and 3/4 surviving monkeys on SD 29 in groups treated with 60/30 mg/kg/dose ABX-EGF, by i/v bolus (Groups 3 and 4) or i/v infusion, respectively
    - MAHA response detected as early as SD 14

Study #BQAW-103:

- Species - cynomolgus monkey (*Macaca fasicularis*)
- Dose, duration and regimen – placebo (vehicle; 50 mM sodium acetate in 100 mM NaCl, pH 5.8), 3, 7.5, 15 mg/kg/dose ABX-EGF, i/v bolus once weekly x 13 weeks; plus 6-week, treatment-free recovery
- NOAEL = not defined; toxicities were observed in all treatment groups
- toxicities – no early mortality or unscheduled sacrifices in this study
- no remarkable effects of ABX-EGF treatment on electrocardiogram, ophthalmologic (slit-lamp, biomicroscopy examinations) results with the following exceptions:
    - loss of cilia, erythema, and/or crusting or swelling of eyelids of 3/5 female monkeys in 15 mg/kg/dose group at SD 80
    - multiple, pin-point lesions on the fovea of the fundus of the eye in one control male recovery animal on SD 122 (monkey #1004M)
- dose-related (incidence and severity), persistent, soft feces/diarrhea, abdominal swelling, dehydration in all ABX-EGF treated groups
    - fecal blood noted at multiple observations in male monkeys in 7.5 mg/kg/dose group, and in both male and female monkeys in 15 mg/kg/dose group
    - mucoid feces noted at multiple observations in both male and female monkeys in mid-dose group, and male animals in high dose group
    - diarrhea persisted through week 1 of recovery in all ABX-EGF dose groups, with incidence higher for monkeys in 7.5 and 15 mg/kg/dose recovery groups than control or 3 mg/kg/dose groups
- skin changes (erythema, dry/flakiness), rough coat, and/or hair thinning, alopecia present all groups of male monkeys, including vehicle control and in all groups of female monkeys treated with ABX-EGF

  - incidence and severity of skin changes in 3 mg/kg/dose group approximately equivalent to control animals in males, slightly higher in females for this group
  - 3 to 5-fold higher incidence, increased severity of skin and coat changes for monkeys in mid- and high-dose groups as compared to control group
- no remarkable differences in food consumption, body weights, body weight gain in all ABX-EGF groups as compared to control
- hematology – platelet counts significantly decreased from both control group, baseline for females in 15 mg/kg/dose group at SD 15
  - platelet changes not seen at any other time point, dose group on study
- increased differential, absolute PMN counts and decreased percent and absolute lymphocyte counts in female monkeys in 7.5, 15 mg/kg/dose groups at SD 29
  - decreased absolute lymphocyte counts also noted in male monkeys in 15 mg/kg/dose group at SD 43, and in females in this group on SD 57, as compared to control
- decreased PT in low-dose female monkeys, increased fibrinogen in 15 mg/kg/dose ABX-EGF treated females at SD 29, as compared to control group
- clin path – significant decreases in total protein, serum albumin, calcium, and phosphate levels at SD 15 in female monkeys treated with 7.5 or 15 mg/kg/dose ABX-EGF, as compared to control group
  - SD 29 - significant decrease in A:G ratio on SD 29 in 15 mg/kg/dose male monkeys as compared to controls, persisting to SD 43
  - SD 43 - serum albumin, A:G ratios decreased, serum globulin increased as well in female monkeys treated with 7.5 or 15 mg/kg ABX-EGF/dose
  - SD 57 - serum albumin, A:G ratios decreased, serum globulin increased in high-dose male monkeys, and in females treated with 7.5 or 15 mg/kg ABX-EGF/dose as compared to control animals
    - serum calcium, blood glucose significantly decreased in mid-dose females, and decreased blood glucose in high-dose females compared to controls
  - SD 71 – increased serum globulin in 15 mg/kg/dose monkeys of both sexes, and decreased serum albumin and A:G ratios in females treated with 7.5 or 15 mg/kg ABX-EGF/dose as compared to control animals
    - significant elevations in serum chloride in all groups ofABX-EGF treated male monkeys as compared to vehicle control group
    - decreased serum phosphate in males treated with 7.5 or 15 mg/kg ABX-EGF/dose, as compared to control animals
  - SD 85 – decreased serum A:G ratios in females treated with 7.5 or 15 mg/kg ABX-EGF/dose, high-dose males as compared to control animals
    - increased serum globulin in high-dose females as compared to control
    - decreased serum creatinine, blood glucose in female monkeys treated with 7.5 or 15 mg/kg/dose ABX-EGF compared to control group
  - no remarkable changes in serum chemistries for any of the ABX-EGF groups as compared to the vehicle control group during the recovery period
  - no remarkable changes in serum troponin, or total or fractionated CPK in the ABX-EGF treated monkeys versus control during either the treatment or recovery periods
- histopathology – minimal to mild hyperkeratosis, accompanied by epidermal acanthosis in the skin of both male and female monkeys in 7.5, 15 mg/kg/dose groups at SD 85 necropsy

- hyperkeratosis accompanied by macro- and microscopic evidence of serocellular crusts, acute and/or chronic dermal inflammation, dermal edema, ulcers, or intracorporeal pustules
- minimal to mild follicular atrophy in skin from monkeys in all ABX-EGF dose groups, but with no correlation to severity of alopecia in clinical observations
- sporadic, mild to minimal inflammatory, edema changes around intravenous catheter site(s) in all dose groups, including vehicle control
- skin lesions only partially resolved in recovery animals at SD 133 sacrifice

- T/K data – serum concentrations confirm continuous exposure to panitumumab over duration of the study in mid-, high-dose animals, with loss of exposure in low dose group at SD 78 correlating with development of MAHA response
  - approximate 20-25% higher C_{max}, AUC on SD 78 vs. SD 1 in the 7.5, 15 mg/kg/dose groups, suggesting non-linear kinetics, saturation of EGFR-mediated clearance mechanisms at the two higher dose levels
  - decreased serum levels noted in animals with MAHA positive response, beginning as early as SD 28
    - exposures (C_{max}, AUC, C_{avg}) significantly lower (approximately 5 to 6-fold) on SD 78 in 3 mg/kg/dose group as compared to SD 1
- immunogenicity – monkey-anti-human antibody (MAHA) observed in 0/10 control, 9/10 low-dose, 2/10 mid-dose, and 0/10 monkeys at SD 78 (prior to final dose of ABX-EGF)
  - MAHA response in 0/4 control, 4/4 monkeys treated with 3 mg/kg/dose ABX-EGF, 2/4 monkeys in the mid-dose recovery group, and 1/4 recovery group animals from the 15 mg/kg/dose group at SD 126
  - MAHA response detected as early as SD 29 in 8/10 low dose monkeys

Study #103419:

- Species - cynomolgus monkey (*Macaca fasicularis*)
- Dose, duration and regimen – placebo (vehicle; 50 mM sodium acetate in 100 mM NaCl, pH 5.8), 7.5, 15, 30 mg/kg/dose ABX-EGF, i/v bolus once weekly x 13 weeks; plus 2-month, treatment-free recovery
- NOAEL = not defined; toxicities, early mortalities were observed in all ABX-EGF treatment groups
- toxicities –early mortality and/or unscheduled sacrifices in fifteen animals on study
  - one in 7.5 mg/kg/dose group, 3 each from 15, 30 mg/kg/dose ABX-EGF groups euthanized on SD 32 (3 d after administration of 5th dose) due to severe skin rash, overall poor clinical condition
  - one monkey in 30 mg/kg/dose group euthanized SD 75 due to skin rash, body weight loss
  - six monkeys (one in 7.5 mg/kg/dose group, 3 from 15 mg/kg/dose ABX-EGF group, 2 from 30 mg/kg/dose group euthanized on SD 96 due to skin rash
  - one female monkey in 15 mg/kg/dose group died immediately after dosing on SD 134
  - one additional monkey each in the 15, 30 mg/kg/dose groups required dose discontinuation at SD 30 and SD 135, respectively
- infusion reactions – dose-related peri-infusion emesis, lethargy, prostration, excessive salivation, pallor to skin, gums, and/or muscle spasms observed in 5 monkeys
  - one monkey in 7.5 mg/kg/dose group, beginning SD 29 and continuing to SD 141, at which time pre-treated with prophylactic diphenhydramine

  - two monkeys each in the 15 mg/kg and 30 mg/kg ABX-EGF/dose group with 2 or fewer infusion reactions each
  - MAHA detected in the low-dose monkey, and in both monkeys from the mid-dose group that developed infusion reactions
  - monkey #33F in the 15 mg/kg/dose group died of apparent anaphylactic reaction on SD 134, shortly after completion of dosing
    - serum obtained near time of death reactive for presence of MAHA, but specific IgE immunoreactivity to the product was not detected
- skin toxicity – erythema, irritation, crust (often with secondary skin infections), flaky skin (dandruff-like), loss of fur, abrasions and/or eyelid swelling and eye redness (with associated secondary conjunctivitis
  - papules, and/or ulcerations/necrosis sporadically observed in monkeys in all ABX-EGF dose groups
  - onset of skin changes typically occurred after administration of two or three weekly doses of ABX-EGF
  - skin changes for surviving monkeys showed resolution during the treatment-free recovery period
- dose-related (frequency and severity) soft, loose, or liquid stool, diarrhea noted at various time points during treatment in all three ABX-EGF treatment groups
  - monkeys in this study did receive fluid (lactated Ringer's solution) and anti-diarrheal medication (sodium bismuth sulfate, lactobacillus) as necessary
  - fecal abnormalities were not observed during recovery period after ABX-EGF
- no remarkable effects of ABX-EGF treatment on electrocardiogram, blood pressure, or heart rate, or rectal body temperature at any time point measured
- no visible lesions observed on ophthalmologic (slit-lamp, biomicroscopy) examinations, with the following exceptions:
  - bilateral, marginal blepharitis observed at Week 26 examination in 2 monkeys in 7.5 mg/kg ABE-EGF/dose group, and 1 monkey in 30 mg/kg/dose group
- mean food consumption decreased in all ABX-EGF treated groups as compared to control throughout the duration of the study
  - decrease of up to 60% from controls for male monkeys, up to 85% of controls for female monkeys in ABX-EGF treated groups
  - corresponded to decreases of 10 to 20% in mean body weights, body weight gain in all ABX-EGF groups as compared to control
- hematology – no clinically significant changes in erythrocyte, platelet parameters in any of the ABX-EGF dose groups as compared to the vehicle control, with the following exceptions
  - elevations in absolute PMN counts in all 7 monkeys euthanized at SD 32, and in 3/6 monkeys euthanized at SD 96
  - related to stress and/or secondary bacterial infection 2° to ABX-EGF skin toxicity
  - elevated PMN in surviving monkeys resolved to baseline by end of recovery
- increased fibrinogen (by 212% to 322% of baseline) in all 7 ABX-EGF treated monkeys euthanized at SD 32
  - changes likely secondary to bacterial infection, dermatologic toxicities
- clin path – no remarkable, treatment-related changes in urinalysis profiles in ABX-EGF treated groups, as compared to vehicle control group
- serum chemistry changes are summarized in Table 14 (Table J, from the sponsor's final study report), below

Table 14: Summary of serum biochemistry changes in cynomolgus monkeys treated for 6 months with ABX-EGF.

Table J. Statistically Significant Decreases in Albumin, BUN, Calcium, Creatinine, Sodium, and A/G

Parameter	Dose Group (mg/kg)	Time point (Study Day)	% Decrease from the Control Mean
Albumin	7.5	50, 85, 113, and 183	83% – 89%
	30	50, 85, 113, 141, and 183	74% - 86%
BUN	7.5	22	60%
	15	22	60%
	30	22 and 183	41% - 57%
Calcium	30	50, 113, 141, and 183	90% - 95%
Creatinine	7.5	183	71%
	30	183	59%
Sodium	7.5	183	98%
A/G	7.5	50, 85, 113, 141, 176, and 183	65% - 72%
	15	50, 85, 113, 141, and 183	67% - 70%
	30	50, 85, 113, 141, 176, and 183	56% - 69%

  - no evidence of hypomagnesemia, hypophosphatemia in any ABX-EGF groups at any time point on study, as compared to controls
  - no remarkable changes in serum chemistries for any of the ABX-EGF groups as compared to the vehicle control group during the recovery period
- histopathology – mild to marked hyperkeratosis, accompanied by epidermal acanthosis, parakeratosis in the skin of both male and female monkeys in all ABX-EGF dose groups at SD 183 necropsy
  - acanthosis accompanied by inflammatory cell infiltration of the dermis and epidermis, small foci of ulceration with scab formation, and epidermal pustules
  - in some animals, folliculitis and perifolliculitis, necrosis and/or erosion of the epidermis noted at terminal sacrifice on SD 183
  - severity of skin lesions was similar in 15 and 30 mg/kg/dose groups, reduced slightly in 7.5 mg/kg/dose group
  - skin lesions only partially resolved in recovery animals at SD 239 sacrifice
  - other histopathologic findings in ABX-EGF treated animals that were considered related to treatment included hypercellularity with increased granulopoietic cells in the bone marrow, and granulocytosis in the spleen
  - lymphoid hyperplasia, plasmacytosis, and neutrophilic infiltration into lymph nodes also observed both microscopically, and correlated with enlarged lymph nodes observed grossly
  - findings can be attributed to the secondary skin infections related to ABX-EGF skin toxicity
  - no other treatment-related, histopathologic findings noted in any other organs
- T/K data – serum concentrations show reduction in exposure to panitumumab over duration of the study all three ABX-EGF dose groups, with loss of exposure correlating with development of MAHA response
  - SD 1, C_{max} values were 261 ± 66, 676 ± 253, and 1080 ± 406 µg/mL for the 7.5, 15, and 30 mg/kg ABX-EGF dose groups, respectively
  - SD 1, $AUC_{(0\text{-}7d)}$ values were 649 ± 133, 1650 ± 440, and 3440 ± 993 day*µg/mL for the 7.5, 15, and 30 mg/kg groups, respectively
  - Week 26, C_{max} values were 157 ± 125, 378 ± 255, and 793 ± 433 µg/mL for the 7.5, 15, and 30 mg/kg groups, respectively

- Week 26, $AUC_{(0-7)}$ values were calculated only from animals without MAHA response, and were 774 ± 259, 1660 ± 266, and 3260 ± 1300 day*µg/mL for the 7.5, 15, and 30 mg/kg groups, respectively
- decreased peak/trough serum levels noted in animals with MAHA positive response, beginning as early as SD 49

- immunogenicity – MAHA response in 0/12 control, 5/12 monkeys treated with 7.5 mg/kg/dose ABX-EGF, 2/12 monkeys in the mid-dose group, and 1/12 high-dose group animals during the treatment period (up to SD 183)
  - MAHA response detected as early as SD 50 in low dose monkeys
  - MAHA response persisted in all surviving recovery animals to SD 239 necropsy
    - no additional animals seroconverted to MAHA positive during this time

Histopathology inventory (optional)

Study	BQAW-102	BQAW-103	103419
Species	cyno	cyno	cyno
Adrenals	X*	X*	X*
Aorta	X	X	X
Bone Marrow smear	X	X	X
Bone (femur)	X	X	X
Brain	X*	X*	X*
Cecum	X	X	X
Cervix	X	X	X
Colon	X	X	X
Duodenum	X	X	X
Epididymis	X*	X*	X*
Esophagus	X	X	X
Eye	X	X	X
Fallopian tube	X	X	X
Gall bladder	X	X	X
Gross lesions	X	X	X
Harderian gland	N.A.	N.A.	N.A.
Heart	X*	X*	X*
Ileum	X	X	X
Injection site	X	X	X
Jejunum	X	X	X
Kidneys	X*	X*	X*
Lachrymal gland	N.D.	N.D.	N.D.
Larynx	N.D.	N.D.	N.D.
Liver	X*	X*	X*
Lungs	X*	X*	X*
Lymph nodes, cervical			
Lymph nodes mandibular			
Lymph nodes, mesenteric	X	X	X
Mammary Gland	X	X	X
Nasal cavity	X	X	X
Optic nerves	X	X	X
Ovaries	X*	X*	X*

Pancreas	X	X	X
Parathyroid	X	X	X
Peripheral nerve	X	X	X
Pharynx	X	X	X
Pituitary	X	X	X
Prostate	X	X	X
Rectum	X	X	X
Salivary gland	X	X	X
Sciatic nerve	X	X	X
Seminal vesicles	X*	X*	X*
Skeletal muscle	X	X	X
Skin	X	X	X
Spinal cord	X	X	X
Spleen	X*	X*	X*
Sternum	X	X	X
Stomach	X	X	X
Testes	X*	X*	X*
Thymus	X	X	X
Thyroid	X	X	X
Tongue	X	X	X
Trachea	X	X	X
Urinary bladder	X	X	X
Uterus	X	X	X
Vagina	X	X	X
Zymbal gland	N.A.	N.A.	N.A.

X, histopathology performed
*, organ weight obtained

2.6.6.4 Genetic toxicology

No studies of this type were included in the present submission. Panitumumab (ABX-EGF) is a monoclonal antibody, protein therapeutic and as such is not anticipated to directly interact with, or damage DNA.

2.6.6.5 Carcinogenicity

No studies of this type were included in the present submission. Panitumumab (ABX-EGF) is a monoclonal antibody, protein therapeutic and is not pharmacologically active in the test species (rat, mouse) traditionally used for carcinogenicity studies.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: An assessment of the effects of ABX-EGF on female fertility and early embryonic development to implantation when administered by weekly intravenous injection to cynomolgus monkeys.

Key study findings: Panitumumab treatment inhibited ovarian function during the administration period, resulting in dose-related irregularities in the duration of menstrual cycle (prolonged menstrual cycles and/or amenorrhea), decreased pregnancy rates, and decreases in serum 17β-estradiol and progesterone levels. These effects were observed at all dose levels tested, which ranged from approximately 1.25 to 5-fold greater than the human ABX-EGF dose of 6 mg/kg. Under the conditions of this study, no NOAEL for panitumumab effects on reproductive function in cynomolgus monkeys can be defined.

Study no.: 103409 (Abgenix Study #ABX-T0309, — , Study # — .026.56)
Volume #, and page #: EDR file: STN BLA 125147\000\module4\toxicology studies\reprotox\103409.pdf
Conducting laboratory and location:

Date of study initiation: August 8, 2003 (in-life, 10/ 6/03 – 4/21/04; final study report dated November 10, 2005)
GLP compliance: Yes
QA statement: yes (X) no ( )
Drug, lot #, and % purity: control article, ABX-EGF placebo (vehicle), lot #ABX-EGF 9099-61, % purity not provided (no detectable protein in sample, by high-performance liquid chromatography); ABX-EGF, lot #954A021224 — pure by size-exclusion high-performance liquid chromatography

Methods

Doses: 0 (vehicle), 7.5, 15, 30 mg ABX-EGF/kg/dose, once weekly
Species/strain: *Macaca fasicularis*, purpose-bred; country of origin China (source, number obtained not specified) and USA(source, number obtained not specified); sexually mature females, 3-9 years old; weight range 2.52 – 6.80 kg
Number/sex/group: 12 females/group in control, low dose groups, 11 females/high-dose group, and 9 females/mid-dose group
Route, formulation, volume, and infusion rate: intravenous bolus; ABX-EGF, 20 mg/mL in 50 mM sodium acetate, 100 mM sodium chloride solution, pH 5.8; volume 1.5, 0.375, 0.75, 1.5 ml/kg (for vehicle, 7.5, 15, 30 mg/kg, respectively); injection rate, approximately 4 ml/min
Satellite groups used for toxicokinetics: none (T/K samples were obtained from main study animals)
Study design: Female macaques were dosed with vehicle control, 7.5, 15, or 30 mg/kg/dose ABX-EGF once weekly over a period of two menstrual cycles prior to mating (PMC 1 and PMC2), during the mating period (maximum two menstrual cycles for females not bred in first cycle, MC1 and MC2), and up to approximately GD20 or GD25 (total of 7 – 23 doses). The total number of doses, dosing duration, and last day ABX-EGF was administered for each individual animal is summarized in Table 15 below, which was abstracted directly from the contracting laboratory's final study report.

Table 15. Study #103409. Study Design, Dosing Duration, and Pregnancy Results in Female Cynomolgus Monkeys Following Weekly Dosing with ABX-EGF

Group/ Dose	Animal No.	Total # of Doses	Dose Interruption (Study Day)	Termination of Dose, Duration of Dosing Period, and the Last Dose Day
Group 1 Vehicle 0 mg/kg	101	14	NA	Pregnant at the 1st mating. The last dose was MC1-37 (GD25, Day 92).
	102	23	NA	Not pregnant. No 2nd mating. Dose was terminated on MC2-15 (Day 156).
	103	17	NA	Pregnant at the 2nd mating. The last dose was MC2-39 (GD24, Day 113).
	104	18	NA	Pregnant at the 2nd mating. The last dose was MC2-38 (GD26, Day 120).
	105	13	NA	Pregnant at the 1st mating. The last dose was MC1-34 (GD22, Day 85).
	107	20	NA	Not pregnant. The last dose was MC3-4 [a] (i.e. MC2-36, Day 135).
	109	21	NA	Not pregnant. The last dose was MC3-1 [a] (i.e. MC2-35, Day 142).
	110	18	NA	Not pregnant. The last dose was MC3-6 [a] (i.e. MC2-37, Day 120).
	111	14	NA	Pregnant at the 1st mating. The last dose was MC1-38 (GD26, Day 92).
	112	15	NA	Pregnant at the 1st mating. The last dose was MC1-33 (GD21, Day 99).
	115	23	NA	Not pregnant. The last dose was MC2-38 (Day 156).
	117	16	NA	Not pregnant. No 2nd mating. Dose was terminated on MC1-34 (Day 106).

[a] MC3 = The third mating cycle (the third menstrual cycle started before completion of observations and examinations).

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Group/ Dose	Animal No.	Total # of Doses	Dose Interruption (Study Day)	Termination of Dose, Duration of Dosing Period, and the Last Dose Day
Group 2 ABX-EGF 7.5 mg/kg	201	17	71, 78, 106, 113	Not pregnant. No 2nd mating. Dose was terminated on MC1-7 (Day 142).
	202	17	NA	Pregnant at the 2nd mating. Dose was terminated on MC2-6 (Day 113).
	203	11	50, 71	No 1st or 2nd mating. Dose was terminated on PMC2-61 (Day 85).
	204	17	50, 57	No 1st or 2nd mating. Dose was terminated on PMC2-54 (Day 127).
	205	13	64, 78, 85, 106	No 1st or 2nd mating. Dose was terminated on MC1-1 (Day 113).
	206	12	NA	No 1st or 2nd mating. Dose was terminated on PMC1-80 (Day 78).
	207	15	NA	No 1st or 2nd mating. Dose was terminated on PMC1-101 (Day 99).
	208	19	NA	Not pregnant at 1st mating. Animal died on MC2-23 (GD11). Pregnancy result of the 2nd mating could not be determined. The last dose was MC2-23 (Day 127).
	209	21	135	Not pregnant. The last dose was MC2-37 (Day 149).
	210	15	85, 92	No 1st or 2nd mating. Dose was terminated on PMC2-79 (Day 113).
	211	14	64	No 1st or 2nd mating. Dose was terminated on PMC1-101 (Day 99).
	212	19	NA	Pregnant at the 2nd mating. Dose was terminated on MC2-3 (Day 127).
Group 3 ABX-EGF 15 mg/kg	304	14	NA	Not pregnant. The last dose was MC2-33 (Day 92).
	305	21	NA	Pregnant at the 2nd mating. Dose was terminated on MC2-26 (i.e., GD14, Day 142).
	306	16	43, 85, 113, 120, 135, 142	Not pregnant. No 2nd mating. The last dose was MC2-10 (Day 149).
	308	19	NA	Not pregnant. No 2nd mating. Dose was terminated on MC1-51 (Day 127).
	309	21	NA	Not pregnant. No 2nd mating. Dose was terminated on MC1-93 (Day 142).
	310	17	113	Not pregnant. No 2nd mating. Dose was terminated on MC1-52 (Day 120).
	311	8	NA	No 1st or 2nd mating. Animal was euthanized on MC1-13. The last dose was MC1-8 (Day 50).
	312	11	NA	No 1st or 2nd mating. Dose was terminated on PMC2-47 (Day 71).
	410	11	57, 71	No 1st or 2nd mating. Dose was terminated on PMC2-45 (Day 85).

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Group/ Dose	Animal No.	Total # of Doses	Dose Interruption (Study Day)	Termination of Dose, Duration of Dosing Period, and the Last Dose Day
Group 4 ABX-EGF 30 mg/kg	401	8	NA	No 1st or 2nd mating. Animal was euthanized on MC1-5. The last dose was MC1-2 (Day 50).
	402	7	NA	No 1st or 2nd mating. Dose was terminated on PMC2-23 (Day 43).
	403	15	57, 64	Pregnant at the 1st mating. The last dose was MC1-33 (GD21, Day 113).
	404	21	NA	Pregnant at the 1st mating. Dose was terminated on MC1-24 (GD12, Day 142).
	405	12	50, 57, 64, 78, 85, 99	Not pregnant. No 2nd mating. Dose was terminated on MC1-31 (Day 120).
	406	8	50, 57	No 1st or 2nd mating. Dose was terminated on PMC1-73 (Day 71).
	407	21	36	Not pregnant. No 2nd mating. Dose was terminated on MC1-35 (Day 149).
	408	12	NA	No 1st or 2nd mating. Dose was terminated on PMC1-80 (Day 78).
	409	21	NA	Not pregnant. Dose was terminated on MC2-31 (Day 142).
	411	15	50, 57, 78, 99, 127, 135	Not pregnant. Dose was terminated on MC2-23 (Day 142).
	412	12	NA	No 1st or 2nd mating. Dose was terminated on PMC1-80 (Day 78).

Parameters and endpoints evaluated: Maternal toxicity (clinical observations and mortality, food consumption, body weight and body weight gains, clinical pathology), menstrual cycle duration and frequency, mating and confirmation of coitus, pregnancy diagnosis and monitoring by fetal ultrasound, serum hormone levels, ABX-EGF toxicokinetics and immunogenicity (MAHA levels) in maternal blood.

Unique study design or methodology (if any): Lacatated Ringers Solution (approximately 100 ml/day, total) was administered twice daily by s/c injection to maintain fluid and electrolyte balance, beginning 24 h after ABX-EGF dosing and continuing until two days following the last dose of panitumumab. When necessary, oral anti-diarrheal treatment (*e.g.*, Pepto-Bismol, lactobacillus) was administered for palliation of continuous diarrhea, and Ketofen®, cefazolin, or Nolvasan baths were administered for treatment of moderate to severe dermatitis.

Observation Times and Results

Mortality: Mortality checks were performed at least once daily for the duration of the study. Early mortalities were noted in all three groups of monkeys treated with panitumumab. Female monkey #401F in the 30 mg/kg/dose group and female monkey #311F in the 15 mg/kg/dose group were euthanized on SD 53 (MC1, MD13) and SD 55 (MC1, MD5), respectively, due to poor clinical condition. Prior to unscheduled death, these animals demonstrated inappetence, decreases in body weight from baseline of 14% and 22%, respectively, and lethargy or hunched posture. Dermatologic toxicities (skin rash requiring intervention; please see below) were observed in both of these animals beginning in the first cycle of treatment (PMC1) for monkey #311F, and during the both PMC1 and PMC2 for animal #401F in the 30 mg/kg/dose group.

A third monkey (animal #208F, in the 7.5 mg/kg/dose group) died following dosing on SD 127, during the second mating cycle (MC2, MD23), and was necropsied as soon as possible after death. Clinical signs noted after ABX-EGF dosing in this animal included shaking/twitching, respiratory abnormalities (tachypnea, gasping, abnormal respiratory sound), ataxia, and red discharge from the nose and mouth. Prior to dosing on SD 127, no remarkable clinical signs had been recorded for this animal with the exception of mild skin changes (alopecia, and abrasion), and no abnormalities in food consumption or body weight were noted. Based on these signs, the cause of death for this monkey may have been anaphylactic shock, or an anaphylactic-like response following repeated exposure to panitumumab.

Clinical signs: Clinical observations were recorded for all female monkeys prior to, and at approximately 2 to 3 h after ABX-EGF injection on dosing days, and in the morning of all non-dosing days during the acclimation and recovery periods. During the dosing periods, each animal and cage were checked twice daily for any evidence of diarrhea. When diarrhea was observed, possible dehydration was checked in the affected animal(s) by pulling the skin, and any dehydration signs were recorded. Additional clinical observations were performed and recorded as necessary.

The most commonly noted clinical signs included changes in behavior including hunched or balled posture, scratching and/or shivering, as well as abnormalities in the skin, integument, and fur in all panitumumab treated monkeys. Skin rash characterized as erythema, irritation, crust, flaky dandruff and/or eye-lid swelling, and redness was observed at least once in all animals in the ABX-EGF-treated groups beginning after the second or third doses of panitumumab, and are considered related to the pharmacologic action of ABX-EGF on epidermal cell turnover. Skin changes worsened in some animals due to secondary infections, but generally resolved in most of the monkeys after completion of the dosing period. However, for many of the monkeys in the highest (30 mg/kg/dose) group, complete recovery of the skin rash was not observed during the observation period. Sporadic fecal abnormalities, including changes in stool consistency and/or frequency were also seen in the majority of monkeys in the ABX-EGF treated groups, although several of these changes were also present in the placebo control treated monkeys as well. A table summarizing the clinical signs observed in the present study was abstracted from the contracting laboratory's final study report, and is presented as Table 16, below.

Table 16. Study #103409. Frequency of clinical findings in pregnant female cynomolgus monkeys after panitumumab (ABX-EGF) treatment

Affected Condition	Findings	Panitumumab Dose (mg/kg/dose) Number of Animals with Findings			
		Control	7.5	15	30
Behavior	Scratching, increased grooming and/or shivering	0/12	10/12	9/9	11/11
Position	Ball position / hunched	1/12	9/12	6/9	10/11
Feces	Soft / loose feces	7/12	9/12	5/9	8/11
	Diarrhea/liquid feces	1/12	6/12	4/9	6/11
	Dry / hard feces	4/12	5/12	5/9	8/11
	Abnormal feces (color, small piece, etc.)	1/12	5/12	2/9	5/11
Emesis/Retching		0/12	5/12	1/9	1/11
Skin / Fur	Erythema, irritation, abrasion, crust, scab, flaky skin, hair loss, etc.	2/12	12/12	9/9	11/11
Eyes	Abnormal appearance (red, swollen eyelids, etc.)	0/12	10/12	8/9	10/11

Other changes noted in panitumumab included prostration and/or disorientation in two monkeys in the 7.5 mg/kg dose group following the 8th (animal #202F) or the 13th and 15th doses of ABX-

EGF (animal #212F), and in one female animal (monkey #404F) following the 21st dose of 30 mg/kg panitumumab. Later doses in the two low-dose animals were accompanied by excessive salivation immediately after the 14th and 16th doses for female #202F, and after the 18th dose in monkey #212F, and vomiting after both doses in animal #202F. In the 15 mg/kg/dose group, excessive salivation was also noted in female monkey #305F either during, or immediately following dose administration for the 18th, 19th, 20th, and 21st doses. Hyperactivity, including stereotypic hand-wiping, rubbing, or slapping hands and/or feet on the cage bars was also noted after this animal was returned to her cage after these four injections, and excessive scratching was noted following the 20th and 21st panitumumab doses. Twitching (lasting approximately 20 minutes) was also observed in monkey #404F following the 8th dose of 30 mg/kg panitumumab.

Body weight: Individual animal body weights were obtained every 2 weeks during the acclimation period, and once a week during the pre-mating and mating periods. After pregnancy was confirmed, the pregnant females were weighed on GD25, GD30 and GD35 (± 1 day).

Dose-dependent, statistically significant differences in both body weight and body weight gains were noted for all three groups of panitumumab treated monkeys, when compared to either baseline pre-study values, or to the vehicle control group. Significant body weight loss was reported in all ABX-EGF treated groups, as compared to animals treated with the placebo control. More than 10% body weight loss was noted in 0/12, 6/12, 4/9, and 7/11 individual animals in the control, 7.5, 15, and 30 mg panitumumab/kg/dose groups, respectively over the duration of the dosing period. The difference between the mean body weights during the acclimation and pre-dosing cycles, and the mean of the lowest recorded weights during the dosing period was −3.65%, −18.48%, −14.47%, and −18.81% in the control, 7.5, 15, and 30 mg ABX-EGF/kg/dose groups, respectively. From the start of dosing through the last recorded measurement during the dosing period, the mean body weight gain was increased by approximately 5% in the vehicle control group, and decreased by approximately 11%, 8%, and 11% for animals in the 7/5, 15, and 30 mg/kg/dose panitumumab groups, respectively. Following completion of dosing, animals tended to recover from the weight loss such that at the end of the recovery period, the mean body weigh losses from the start of dosing through the final weight measurement were 3.6%, 1.5%, and 5.3% for the 7.5, 15, and 30 mg/kg/dose panitumumab groups, respectively. These differences were statistically significant from control at both the end of treatment, and the end of recovery periods for all ABX-EGF treated groups ($p \leq 0.05$, ANOVA with Dunnett's test).

Food consumption: Food consumption was measured qualitatively by daily, direct observation of biscuit count during the acclimation, pre-mating and mating periods (excluding the duration of cohabitation with the male monkey for mating), and throughout either the pregnancy (for pregnant animals only), or recovery periods. There were no remarkable differences in the mean number of biscuits consumed per day between animals assigned to the control and the ABX-EGF dose groups during the acclimation period.

During the dosing period, biscuit consumption was decreased in the ABX-EGF treated groups of monkeys as compared to the control group; however, there were no statistical differences in food consumption between each ABX-EGF treated group at any recorded time point. In individual animals, slight to marked increases in the number of uneaten biscuits were noted in 3/12 (animals #101F, #104F, and #115F), 9/12 (animals #201F-#203F, #205F-#207F, #209F-#211F), 7/9 (animals #306F, #308F-#312F, and #410F), and 9/11(animals #401F-#403F, #405F-#408F, #411F and #412F) female monkeys in the control, 7.5, 15, and 30 mg/kg ABX-EGF dose groups, respectively. During the recovery period, there was an apparent recovery in food consumption in the ABX-EGF treated monkeys, although the number of uneaten biscuits was still high for

approximately 2 weeks in some monkeys across all dose groups, including the placebo control (control monkeys #101F, #104F, and #115F, as well as animals #205F, #310F, #311F, #401F, #402F, #403F, and #412D). The mean number of uneaten biscuits per day was similar across all groups, so the effect was considered reversible.

Clinical pathology: Peripheral blood samples for clinical pathology evaluations were collected from each female monkey once pre-dosing on SD 1 [*i.e.*, PMC1, menstrual day (MD) 3], and on GD35 for confirmed pregnant females (see below), or the equivalent study day for non-pregnant or recovery animals. Standard veterinary serum chemistry parameters were determined using an — chemistry analyzer. Hematology profiles were not determined in this study, with the exception of the early decedent animals.

There were no remarkable, statistically significant or biologically relevant differences in any of the mean values for the serum biochemistry parameters measured at either PMC1-MD3 (pre-dose), or at GD35, with the exception of a slight increase in total protein for the monkeys in the 7.5 mg/kg/dose group at GD35 ($p \leq 0.05$ as compared to the placebo control group; ANOVA with Dunnett's test). Although not statistically significant, decreases in serum albumin (83% to 94%), BUN (72% to 94%), and A/G (62% to 79%) levels were also noted at GD35 in the control and all ABX-EGF treated groups, as compared to of the pre-dose values on PMC1-MD3. These changes were considered related to the twice daily, s/c injections of Lactated Ringer's solution (50 ml/animal, twice daily) for maintenance of fluid and electrolyte balance.

Marked decreases in serum albumin were noted in the individual animals who were early decedents on study; serum albumin levels were $\leq$ 39% of the control mean values for both animals #311, and #401F at unscheduled necropsy. Total protein levels were also slightly decreased in these two monkeys to 77% and 76% of control mean value, respectively. The A:G ratio was not calculated for animal #401F due to lacking globulin measurements; however, the A:G ratio for animal #311F at necropsy was decreased by 29% from the control level. At necropsy, gross pathologic findings for these animals included marked atrophy of the pancreas in monkey #401F, and and liver changes (enlarged, friable, pale and/or soft) in both animals #311F and #401F (see below). Because there were no similar abnormalities in the serum chemistry parameters for remaining animals, it is considered that these two monkeys had underlying hepatic and/or pancreatic disorders which were not apparent during the pre-dose serum chemistry examination, but may also have been related to panitumumab treatment.

Menstrual cycles: Each monkey was checked at least once daily by vaginal swab for menstrual bleeding, and the length of the menstrual cycle was calculated. For evaluation of the effects of ABX-EGF on menstrual cyclicity, a normal menstrual cycle was defined as being between 20 and 40 days. Cycles longer than 40 days and 80 days were considered to be a prolonged cycle and amenorrhea, respectively. Also, menstrual cycles during the dosing period were considered to be prolonged or shortened when the difference in duration was greater than 20% of the mean value of the two acclimation cycles.

No prolongation of menstrual cycles or amenorrhea was observed for female monkeys in the placebo control groups at any of the cycles evaluated. Mean menstrual cycle durations were consistent for this group across the study, and ranged from 30.6 to 34.0 days each cycle. During the two acclimation cycles, no prolongation of menstrual cycles or amenorrhea was reported for the animals assigned to the three ABX-EGF dose groups. By contrast, female monkeys in the groups treated with all three dose levels of panitumumab demonstrated delayed menstruation and/or amenorrhea during the treatment cycles with ABX-EGF. During the dosing or recovery

periods, prolonged cycles and/or amenorrhea were seen in 12/12 (100.0%), 7/9 (77.8%), and 10/11 (90.9%) animals in the 7.5, 15, and 30 mg/kg/dosegroups, respectively. Mean values for menstrual cycle duration in monkeys receiving either panitumumab or the placebo control are presented in Table 17, below.

Table 17. Study #103409. Menstrual Cycle Duration in Female Cynomolgus Monkeys Prior to and Following Weekly Intravenous Injections of Panitumumab

Observation Cycles	Mean Menstrual Cycle Duration (days), ± SD			
	Weekly Dose of Panitumumab			
	Control	7.5 mg/kg	15 mg/kg	30 mg/kg
AC1	32.2 ± 6.4	30.0 ± 3.4	31.0 ± 1.6	28.3 ± 2.1
AC2	30.6 ± 4.7	30.8 ± 3.4	30.9 ± 5.1	28.7 ± 2.2
Treatment Cycles				
PMC1	33.2 ± 10.3[a]	70.0 ± 42.1[c]	37.4 ± 17.1	59.6 ± 44.5[h]
PMC2	34.0 ± 8.8[b]	63.9 ± 35.1	45.8 ± 31.3[e]	40.3 ± 20.6[i]
MC1	32.0 ± 6.7	31.4 ± 4.6	55.0 ± 45.7[f]	46.6 ± 8.1[j]
MC2	32.5 ± 1.3	n.a.[d]	38.0[g]	47.0[k]

[a] menstrual cycle was prolonged (> 40 d) in one female (animal #102F) in this dose group during this period

[b] menstrual cycle was prolonged (> 40 d) in two females in this dose group during this period (animals #102F, #115F)

[c] significantly different from vehicle control group at this cycle ($p \leq 0.05$, ANOVA with Dunnett's test)

[d] n.a., not available

[e] cycle PMC2 duration was prolonged in female monkeys #312F (103 days) and #410F (95 days) in this dose group

[f] cycle MC1 was prolonged in female monkeys #308F (57 days), #309F (143 days), and #310F (53 days) in this dose group

[g] n = 1

[h] cycle PMC1 was prolonged in female monkeys #404F (80 days), #406F (129 days), #408F (102 days), and #412F (138 days) in this dose group

[i] PMC2 was prolonged in female monkeys #402F (41 days), #403F (45 days), #405F (61 days), and #407F (92 days) in this dose group

[j] cycle MC1 was prolonged in female monkeys #405F (56 days), #407F (49 days), #409F (49 days), and #411F (45 days) in this dose group

[k] n = 2; cycle MC2 was prolonged in both animals (#409F and #411F; both 47 days) remaining on treatment during this cycle

In some animals with prolonged cycles or amenorrhea, menstruation returned during the dosing period, or within 64 days following completion of dosing (recovery period). The frequency of prolonged cycles and/or amenorrhea is presented in Table 18 below which was abstracted from the sponsor's final study report.

Table 18. Study #103409. Frequency of Prolonged Menstrual Cycles and/or Amenorrhea in Female Cynomolgus Monkeys Following Weekly Dosing with ABX-EGF

Group /Dose	Number of Animals								Total (Number of Cycles)
	Prolonged Cycle				Amenorrhea				
	PMC1	PMC2	MC1	MC2	PMC1	PMC2	MC1	MC2	
Group 1 Vehicle 0 mg/kg	1/12 8.3%	2/12 16.7%	0/8 0.0%	0/4 0.0%	0/12 0.0%	0/12 0.0%	0/8 0.0%	0/4 0.0%	3 / 36 8.3%
Group 2 ABX-EGF 7.5 mg/kg	6/12 50.0%	1/9 12.5%	0/5 0.0%	1/1 100.0%	3/12 25.0%	4/9 44.4%	0/5 0.0%	0/1 0.0%	15 / 27 55.6%
Group 3 ABX-EGF 15 mg/kg	4/9 44.4%	1/9 11.1%	2/6 33.3%	0/1 0.0%	0/9 0.0%	2/9 22.2%	1/6 16.7%	0/1 0.0%	10 / 25 40.0%
Group 4 ABX-EGF 30 mg/kg	2/11 18.2%	3/11 27.3%	4/5 80.0%	2/2 100.0%	3/11 27.3%	1/11 9.1%	0/5 0.0%	0/2 0.0%	15 / 29 51.7%

Mating and confirmation of coitus: Following panitumumab or placebo dosing for two consecutive menstrual cycles (PMC1 and PMC2), females monkeys were mated with untreated, sexually mature males for up to five consecutive days [range, MD10 to MD15 of the third menstrual cycle of the dosing period (MC1)]. Coitus was confirmed visually and/or by the presence of sperm in a vaginal smear. When coitus was not confirmed or the female was diagnosed as non-pregnant following mating during MC1, the female was allowed to mate during the next menstrual cycle (MC2) in the same manner as MC1.

Copulation was confirmed for all mated animals at least once during each mating cycle, with the exception of female monkey #411F in the 30 mg/kg/dose group. For this animal, the second mating session was conducted for only 30 min/day for two days because of the severe skin changes noted on clinical observation. Because of this deviation, the results of this session for monkey #411F were excluded from the calculation of copulation and pregnancy indices.

By the completion of MC1, most of the panitumumab monkeys that were not yet pregnant were experiencing amenorrhea, and dosing was stopped for animals that did not become pregnant in the first mating session (these animals did not go through the second mating session). Only 3/5 animals in the 7.5 mg/kg group, 2/6 animals in the 15 mg/kg group, and 1/4 (excluding two animals that became pregnant at the first mating session) animals in the 30 mg/kg group went through two mating sessions. The final pregnancy rates (number of successful matings/number of total mating sessions) in the control, 7.5, 15, and 30 mg/kg groups were 33.3% (6/18 mating sessions), 25.0% (2/8 mating sessions), 12.5% (1/8 mating sessions), and 25.0% (2/8 mating sessions), respectively.

Pregnancy diagnosis and monitoring: Pregnancy was confirmed in mated female monkeys by ultrasound evaluation on presumed GD20 ($\pm$ 1 day), under ketamine sedation. If the ultrasound examination did not provide definitive confirmation of pregnancy on GD20, it was repeated on GD25 $\pm$ 1. For confirmed pregnant females, embryonic viability was monitored by ultrasound GD25, GD30, and GD35 ($\pm$ 1 day).

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): The mating index [(number of copulated animals/number of paired animals) x 100] and the fertility index [(number of pregnant females/number of copulated females) x 100] were calculated for each ABX-EGF treated group, following confirmation of both coitus and pregnancy, respectively.

In the first mating session, 4/12 (33.3%), 0/5 (0%), 0/6 (0%), and 2/6 (33.3%) of animals became pregnant in the control, 7.5, 15, and 30 mg/kg ABX-EGF dose groups, respectively. During the second mating session, 2/6 (33.3%), 2/3 (66.7%), 1/2 (50.0%), and 0/2 (0%) of animals became pregnant in the control, low, mid-, and high-dose panitumumab groups, respectively. The final pregnancy rates for this study were therefore 50.0% (6/12 animals), 40.0% (2/5 animals), 16.7% (1/6 animals), and 33.3% (2/6 animals), from female monkeys in the placebo control, 7.5, 15, and 30 mg ABX-EGF/kg/dose groups, respectively. There were no statistically significant differences in the pregnancy rates between the control group, and each of the panitumumab treatment groups; however, the pregnancy rate based on the total number of matings was notably lower in the ABX-EGF treated groups compared to the contracting laboratory's historical control background data (39.1%, as reported in the final study report).

Serum hormone levels: Peripheral blood samples (approximately 3.5 ml/monkey/time point) were collected from all animals on MD2, MD7, MD9, MD11, MD13, MD16, MD19, MD23, MD27, and MD30 of PMC1, and every five days thereafter until the onset of the next menstrual cycle, or at the end of the observation or recovery period. Aliquots of serum were stored frozen at -70°C until analysis for reproductive hormone levels. Serum levels of progesterone and 17β-estradiol were measured by validated ELISA methods under GLP conditions; blood samples for serum prolactin levels were also obtained, but not measured and were subsequently discarded. Selected serum samples from the first acclimation (AC1) and pre-mating (PMC1) dosing cycles from the first four female monkeys in the vehicle control and 30 mg ABX-EGF/kg/dose groups were also evaluated for progesterone and 17β-estradiol levels using validated, radioimmunoassay techniques; however, only the results of the ELISA assays are presented in the Tables, below.

All three dose levels of panitumumab resulted in changes in reproductive hormonal status, which appeared to be related to the irregularities in menstrual cyclicity. In general, peak serum progesterone and 17β-estradiol were not significantly changed from the pre-treatment (acclimation cycles) in animals treated with the placebo control during the two pre-mating cycles. There were no statistically significant differences in peak serum 17β-estradiol levels in the ABX-EGF treated groups either as compared to the pre-treatment cycles, or to the placebo control group during PMC1 and PMC2. Interestingly, biphasic peaks for 17β-estradiol were noted for female monkeys in the 7.5 and 30 mg/kg/dose groups during PMC1, and in all three ABX-EGF treated groups during PMC2 (Table 19, below). Weekly i/v injection of panitumumab, regardless of dose level resulted in a statistically significant blunting of serum progesterone levels at MD19 during the first treatment cycle (PMC1), as compared to both the mean control group value for this time point, and the group means during the acclimation cycles (AC1 and AC2). Peak progesterone levels also occurred significantly later for all three ABX-EGF treated groups during PMC1 (Table 20, below). By the second pre-mating cycle, there was a dose-related, although not statistically significant blunting of peak progesterone levels; however, the time to peak value did not differ appreciably in the panitumumab treated groups as compared to the placebo group. Mean peak values for the two reproductive hormones are presented in Tables 19, and 20 below:

Table 19. Study #103409. Effect of Panitumumab Treatment on Peak 17β-Estradiol Levels in Non-Pregnant Female Cynomolgus Monkeys				
Weekly Dose of Panitumumab	Mean Peak Serum 17β-Estradiol (pg/ml) ± SD			
	Observation Cycle		Treatment Cycle	
	AC1	AC2	PMC1	PMC2
Control	222 ± 121	253 ± 117	270 ± 150	281 ± 174
7.5 mg/kg	233 ± 89	277 ± 97	319 ± 94[a]	286 ± 66[c]
15 mg/kg	213 ± 99	191 ± 99	289 ± 141	293 ± 231[d]
30 mg/kg	262 ± 155	281 ± 177	373 ± 218[b]	159 ± 159[e]

[a] biphasic peak seen, with first peak of 319 ± 94 pg/ml observed at PMC1-MD11, and second peak of 334 ± 136 pg/ml observed at PMC-MD105
[b] biphasic peak seen, with first peak of 373 ± 218 pg/ml observed at PMC1-MD11 on treatment, and second peak of 365 ± 134 pg/ml observed at PMC1-MD70 on treatment
[c] biphasic peak seen, with first peak of 286 ± 266 pg/ml observed at PMC2-MD13 on treatment, and second peak of 306 ± 393 pg/ml observed at PMC2-MD70
[d] biphasic peak seen, with first peak of 293 ± 231 pg/ml observed at PMC2-MD7, and second peak of 300 ± 166 pg/ml observed at PMC2-MD30
[e] biphasic peak seen, with first peak of 159 ± 1591 pg/ml observed at PMC2-MD9, and second peak of 225 ± 286 pg/ml observed at PMC2-MD30

Table 20. Study #103409. Effect of Panitumumab Treatment on Peak Progesterone Levels in Non-Pregnant Female Cynomolgus Monkeys				
Weekly Dose of Panitumumab	Mean Peak Serum Progesterone (pg/ml) ± SD (Cycle Day of Peak)			
	Observation Cycle		Treatment Cycle	
	AC1	AC2	PMC1	PMC2
Control	4.2 ± 3.2 (MD30)	2.9 ± 4.2 (MD30)	4.0 ± 4.2 (MD19)[b]	3.2 ± 3.2
7.5 mg/kg	4.5 ± 3.7 (MD23)	4.4 ± 3.7 (MD23)	4.9 ± 2.5 (MD130)	3.3 ± 6.1
15 mg/kg	3.7 ± 3.3 (MD23)	2.6 ± 1.7(MD19)	3.9 ± 1.5 (MD125)	2.1 ± 5.5
30 mg/kg	6.0 ± 3.7 (MD23)	5.1 ± 3.4[a] (MD23)	6.7 ± 12.8 (MD60)	1.3 ± 1.8

[a] significantly different from placebo control group value for this time point (control = 2.1 ± 1.7 pg/ml; $p \leq 0.05$, ANOVA with Dunnett's test)
[b] significantly different from all three ABX-EGF treated groups at this time point (mean values = 0.13 ± 0.15, 0.07 ± 0.05, and 0.09 ± 0.08 pg/ml on PMC1-MD19 for the 7.5, 15, and 30 mg/kg dose groups, respectively; $p \leq 0.05$, ANOVA with Dunnett's test)

Toxicokinetics: Serum samples for toxicokinetic analyses were collected from all animals on SD1 (PMC1, MD3), on the first dosing day of MC1 (except for animals that showed amenorrhea in PMC1 and/or PMC2), the final dosing day, and from confirmed pregnant females on GD35. Blood samples were collected prior to ABX-EGF dose administration, and at 30 min, 96 h, and 168 h after panitumumab injection (*i.e.*, before the next dosing). On GD35 or any day that an ABX-EGF dose was missed, a single blood collection was performed at approximately the same time of day as the previous dose administration. All samples were processed to serum, stored frozen at –70°C in aliquots, and transferred to the study sponsor for analysis by a validated ELISA. The lower limit of quantitation of this assay is 19.5 ng ABX-EGF/ml.

All monkeys in the panitumumab treated groups were confirmed exposed to ABX-EGF during the pre-mating cycle, and exposure (as defined by $AUC_{0\text{-}168h}$ and C_{max} measured at the 30 min post-dose sample) increased dose-proportionally. However, the mean values for both parameters were decreased in all three dose groups following the first dose of the mating cycle. When the

parameters were calculated for individual monkeys, decreases in exposure with repeated dosing were observed over time, which correlated with the development of MAHA responses. Panitumumab exposure during the mating cycle(s) for MAHA-negative animals was similar to that observed during the pre-mating cycle for these animals, indicating a lack of accumulation of the antibody (data not shown). No definitive information could be obtained in this study regarding the toxicokinetic profile of panitumumab during pregnancy, due to the limited T/K data from pregnant animals. The data for ABX-EGF exposure in this study, as calculated for all animals (both MAHA-positive and MAHA-negative) are presented in Table 21, below.

Table 21. Study #103409. Exposure of Female Cynomolgus Monkeys to Panitumumab after Weekly Dosing During Pre-Mating and Mating Cycles

Pharmacokinetic Parameter and Dosing Cycle	Mean Value, ± SD		
	Weekly Dose of Panitumumab		
	7.5 mg/kg	15 mg/kg	30 mg/kg
Pre-Mating Cycle			
C_{max} (µg/ml)	230 ± 31	455 ± 87	951 ± 199
$AUC_{0\text{-}168h}$ (µg*d/ml)	719 ± 124	1466 ± 422	3668 ± 569
First dose of Mating Cycle			
C_{max} (µg/ml)	112 ± 122	396 ± 191	631 ± 308
$AUC_{0\text{-}168h}$ (µg*d/ml)	333 ± 409	1240 ± 656	1322 ± 1506
Last dose of Mating Cycle			
C_{max} (µg/ml)	273 (n = 1)	410 (n = 2)	150 (n = 1)
$AUC_{0\text{-}168h}$ (µg*d/ml)	958 (n = 1)	1445 (n = 2)	2 (n = 1)

Immunogenicity: Blood samples for serum MAHA analysis were collected from all female monkeys once before dosing on MD3, PMC1, before the first dose during MC1, and in confirmed pregnant animals on GD35, or on the final day of collection for T/K sampling in the non-pregnant or recovery females. Samples were stored frozen in aliquots at -70°C, shipped on dry ice to the study sponsor and analyzed for MAHA using a validated, qualitative ELISA assay. Samples were considered positive for anti-ABX-EGF MAHA response if the optical density reading at 450 nm (OD_{450}) for the test sample was ≥ 2-fold of the average OD_{450} reading for the negative control replicates.

Two female monkeys in the control group (animals #105F and #117F) yielded positive MAHA readings during the pre-mating period, at the PMC-MD3 time point. However, both monkeys were negative for MAHA response at the later time points measured, and no explanation was provided for the apparent false-positive reading. Positive MAHA responses were detected in 5/12 (41.7%), 2/9 (22.2%), and 6/11 (54.5%) animals in the 7.5, 15, or 30 mg ABX-EGF dose groups, respectively, during the treatment cycles (PMC1 and PMC2, and MC1 and MC2 where applicable).

Necropsy: Female monkeys that survived the dosing and observation periods without becoming moribund were not euthanized, and were returned to the study colony following completion of the observation or recovery periods. Complete necropsies were perfomed on all three early decedents and included gross pathological examination of the carcass and musculo-skeletal systems, external orifices and surfaces, neck with associated organs and tissues, and cranial, thoracic, abdominal, and pelvic cavities and their organs and contents. Blood collections for hematology, coagulation, serum chemistry, serum hormone assay, toxicokinetics, and MAHA analyses were also obtained from all three early decedents. A limited number of tissues and organs were removed, weighed, and preserved in buffered formalin for microscopic evaluation.

Necropsy findings for animal #401 (30 mg/kg dose group) on SD53 (MC1-5) included alopecia, redness, and thinness of the skin in the abdominal, axillary, inguinal, and interscapular areas, whitish coloring and hardening of abdominal and subcutaneous fat, enlargement of the kidneys and spleen, liver changes (enlargement, pale, soft, and friable), and atrophy of the thymus and pancreas. Liver weight was also higher than expected for this animal at the unscheduled sacrifice.

Monkey #311F (15 mg/kg/dose ABX-EGF dose group) was euthanized on Day 55 (MC1-13), and gross necropsy observations included multi-focal necrosis of abdominal adipose tissue, enlargement and paleness of the kidney, liver changes (enlargement, friable and pale), and atrophy of the thymus. High liver weight was also reported for this animal. No skin changes were reported for this animal in the sponsor's final study report.

Gross necropsy observations for monkey #208F in the 7.5 mg/kg/dose group who died shortly after dosing on SD127 (MC2-MD23) included edema and dark, red areas present in the lungs, clear whitish foamy fluid from bronchi, and atrophy of the thymus. The lung weight was also higher than expected in this animal, likely from the presence of pulmonary edema. The lung changes suggest the cause of death for this animal may have been anaphylactic shock or an anaphylactic-like response following repeated administrations of panitumumab.

Increased platelet and reticulocytes were noted for monkeys #311F and #401F at the moribund necropsy evaluations. Additionally, decreased erythrocyte counts, hemoglobin concentration, and hematocrit values were noted for animal #401F only. For coagulation parameters, shortened prothrombin time and prolonged activated partial thromboplastin time were noted in animal #401F. Changes in clinical chemistry parameters at unscheduled sacrifice included decreases in serum albumin, calcium, total protein, and BUN in both animals, decreased total cholesterol in monkey #311F only, and decreased A:G ratio in this animal as well (A:G ratio could not be calculated for animal #411, due to lack of globulin value). Increased serum alkaline phosphatase levels were also noted in both animals at moribund necropsy. No clinical pathology measurements were obtained for monkey #208F in the low dose group following its death on study.

Although tissue samples were reported as obtained for microscopic evaluation, no histopathology results were included in the final study report.

Study conclusion: In summary, treatment of non-pregnant, female cynomolgus monkeys with weekly i/v injections of 7.5, 15, or 30 mg/kg/dose panitumumab inhibited ovarian function during the administration period, resulting in prolonged menstrual cycles and/or amenorrhea, and decreased pregnancy rates as compared to either the placebo control group, or the historical control values for the test facility. The effects on fertility were also evidenced by decreases in serum 17β-estradiol and progesterone levels in all animals in the ABX-EGF treated dose groups during the administration period and by irregularities in the duration of menstrual cycle, which were dose-related in incidence. Under the conditions of this study, no NOAEL for panitumumab effects on reproductive function in cynomolgus monkeys can be defined. The mechanism by which ABX-EGF exerts negative effects on ovarian function cannot be determined from these studies.

Embryofetal development

Study title: An assessment of the effects of ABX-EGF on embryo-fetal development when administered weekly by intravenous injection to pregnant cynomolgus monkeys.

Key study findings: Panitumumab was abortifacient at all dose levels tested in pregnant female cynomolgus monkeys, following weekly injection with 7.5, 15, or 30 mg/kg/dose (approximately 1.25 to 5-fold greater than the human dose of 6 mg/kg/dose) from GD20 through GD48. There were no gross anomalies observed in the surviving fetuses at Cesarean section, and no evidence of soft tissue or skeletal malformations. Toxicokinetic data confirmed exposure of the dams to ABX-EGF during the phase of organogenesis. Because of the incidence of fetal losses, no NOAEL can be defined for this study.

Study no.: #103410 (Abgenix Study #ABX-T0310, Study # .026.57)
Volume #, and page #: EDR file: STN BLA 125147\000\module4\toxicology studies\reprotox\103410.pdf
Conducting laboratory and location:

Date of study initiation: July 11, 2003 (in-life, 8/15/03 – 3/29/04; final study report dated November 4, 2005)
GLP compliance: **Yes**
QA statement: yes (X) no ( )
Drug, lot #, and % purity: control article, ABX-EGF placebo (vehicle), lot #ABX-EGF 9099-61, % purity not provided (no detectable protein in sample, by high-performance liquid chromatography); ABX-EGF, lot #954A021224, — pure by size-exclusion high-performance liquid chromatography (—

Methods

Doses: 0 (vehicle), 7.5, 15, 30 mg ABX-EGF/kg/dose, once weekly from GD20 through GD48

Species/strain: *Macaca fasicularis*, purpose-bred; country of origin China (sources:); sexually mature females, 3-12 years old; weight range 2.44 – 4.98 kg

Number/sex/group: 12 pregnant females/group in control, 15 pregnant females/group in the 7.5 mg/kg dose group, 18 females/mid-dose group, and 5 females/group in the 30 mg/kd/dose group

Route, formulation, volume, and infusion rate: intravenous bolus; ABX-EGF, 20 mg/mL in 50 mM sodium acetate, 100 mM sodium chloride solution, pH 5.8; volume 1.5, 0.375, 0.75, 1.5 ml/kg (for vehicle, 7.5, 15, 30 mg/kg, respectively); injection rate, approximately 4 ml/min dose volume

Satellite groups used for toxicokinetics: none (T/K samples were obtained from main study animals)

Study design: Presumed pregnant female cynomolgus macaques were injected i/v with vehicle control, 7.5, 15, or 30 mg/kg/dose ABX-EGF once weekly beginning on GD20 and continuing until GD48 (*i.e.*, GD20, GD27, GD34, GD41, and GF48, for a total of

five doses per animal), or until confirmed abortion/fetal death. This dosing schedule was selected to maintain panitumumab plasma levels (peak:trough ratios) throughout the period of major organogenesis that are similar to those achieved in humans following dosing every other week.

Parameters and endpoints evaluated: Pregnancies were terminated by Cesarean section between GD100 and GD103. Fetal observations included fetal and placental weights, and fetal external, visceral and skeletal examinations, as well as histopathology of fetal organ and placentas. For each fetus recovered, parameters evaluated included fetal viability, fetal and placental weights, measurment of crown:rump length, fetal head width and circumference, distance between the eyes, chest circumference, tail length, right paw and foot length, anogenital distance, amniotic fluid volume, and the diameters of both primary and secondary placentas. Other recorded, gross observations for each offspring included body form, symmetry of head, facial form, mandibular formation, eyes and eyelids, hair of head, nipple formation, anus, fingers, toes, finger and toe nails, ears, tail, upper and lower extremities, external genitalia, vertebral column, umbilical cord, umbilical cord length, and examination for cleft palate.

Each surviving fetus was necropsied following sodium pentobarbital euthanasia, for macroscopic observations of organs and tissues. Adrenal glands, ovaries or testes, heart, lungs, spleen, thymus, mesenteric lymph nodes, liver, kidneys, uterus, pancreas, and brain were removed and weighed, and tissue samples fixed in 10% neutral buffered formalin. Paired organs were weighed separately, and the combined weight was calculated. The eyes, stomach, small and large intestines, the skin of the head, ears, trachea (with thyroids), esophagus, femur (left, with bone marrow) and any abnormal organs noted during the gross observation were removed and fixed in formalin without weighing. Histopathologic evaluation was performed for sections of lung, liver, brain, thymus, pancreas, heart, small and large intestine, adrenal glands, testes or ovaries, uterus, mesenteric lymph nodes, kidney, spleen, and placenta following hematoxylin and eosin staining, from fetuses in the vehicle control, and 15 and 30 mg/kg/dose ABX-EGF groups only.

The external carcass of each fetus was fixed in 95% ethyl alcohol and stained with Alizarin red for evaluation of any skeletal variations or abnormalities, including malformations, skeletal development (number of bones with ossification centers of the vertebral centrum), and skeletal length (right side) of ossified parts of the humerus, radius, ulna, femur, tibia, and fibula.

Unique study design or methodology (if any): Lactated Ringers Solution (approximately 100 ml/day, total) was administered twice daily by s/c injection to maintain fluid and electrolyte balance, starting on the first day of ABX-EGF dosing (GD20), and continuing until seven days following the last dose of panitumumab (GD55). When necessary, oral anti-diarrheal treatment (*e.g.*, Pepto-Bismol, lactobacillus) was administered for palliation of continuous diarrhea, and Ketofen®, cefazolin, or Nolvasan baths were administered for treatment of moderate to severe dermatitis.

Results

Mortality (dams): Mortality checks were performed at least once daily for the duration of the study. No maternal deaths or moribund necropsies occurred on study.

There was a dose-related increase in both spontaneous abortions, and embryo-fetal deaths in the panitumumab-treated monkeys as compared to the placebo control group and to the historical background abortion rates for the contracting laboratory. Fetal deaths were noted in 1/12 monkeys in the control group (animal #112F, GD50), and 3/18 pregnant monkeys in the 15 mg/kg dose group (animals #407F, #305F, and #301F at GD30, GD44, and GD51, respectively). There were four spontaneous abortions noted in female monkeys #204F, #210F, #213F, and #214F, and one fetal death at GD51 (animal #211F) in the 7.5 mg/kg/dose group. Two spontaneous abortions (animals #401F and #405F) and one fetal death at GD51 (animal #403F) were also noted in the 30 mg/kg ABX-EGF dose group. Overall, the incidence of embryo-fetal losses was 1/12 (8.3%), 5/15 (33.3%), 3/18 (16.7%), and 3/5 (60.0%) for pregnant dams treated weekly from GD20 through GD48 with the placebo control, 7.5, 15, or 30 mg/kg/dose panitumumab, respectively.

Comment: Under the listed deviations from protocol, it was noted that female monkey #406F in the 30 mg/kg/dose group was mistakenly administered 15 mg/kg of ABX-EGF on GD41, instead of the full dose of panitumumab. This one female in the highest dose group did not either spontaneously abort the conceptus, nor did fetal death occur after completion of dosing as was noted for other animals in this dose group. This animal was also the only dam in the group that developed a positive MAHA response, which was not detectable until Cesarean section at GD100. It is not known whether the reason for maintenance of the pregnancy in this animal was due to a decrease in exposure to panitumumab resulting from the 50% decrease in dose on GD41, the development of the MAHA response, or a combination of both factors.

Clinical signs (dams): Skin toxicities including irritation, erythema, crusted or flaking skin, and abnormal swelling and/or redness of the eyelids was noted on at least one observation in all animals in the ABX-EGF-treated groups beginning after the second or third doses of panitumumab, and are considered related to the pharmacologic action of ABX-EGF on epidermal cell turnover. Skin changes worsened in some animals due to secondary infections, but generally began to lessen after completion of the dosing period on GD48. However, complete recovery of the skin rash was not observed in 2/4, 5/15, and 2/2 female monkeys in the 7.5, 15, and 30 mg/kg panitumumab dose groups at the time of Cesarean section on GD99-GD103.

Sporadic changes in fecal consistency and/or frequency were noted in animals in all three ABX-EGF dose groups as well as the controls during the treatment period, and included soft, loose or watery stool, and/or diarrhea on several incidences. However, because the incidence of stool changes was increased in proportion to the dose of ABX-EGF, these changes were considered related to panitumumab treatment. The changes in fecal consistency resolved during the post-dosing period in all dose groups/

A summary table of the incidence of adverse clinical observations, as provided in the sponsor's final study report, is included as Table 22, below.

Table 22. Study #103410. Incidence of Clinical Findings in Pregnant Cynomolgus Monkeys Following Weekly Treatment with ABX-EGF from GD20 to GD48

Classification	Findings	Number of animals with the finding				Comments
		Control	7.5 mg/kg	15 mg/kg	30 mg/kg	
		N=12	N=15	N=18	N=5	
Behavior	Scratching	0	7	9	1	Sporadic changes
	Increased grooming	0	3	1	0	Sporadic changes
	Shivering	0	0	1	1	Sporadic changes
Position	Ball position / Hunched	0	10	9	1	Mostly sporadic changes
Feces	Soft/Loose feces	2	11	10	4	Mostly sporadic changes
	Diarrhea/Liquid feces	3	6	3	3	Mostly sporadic changes
	Dry/Hard feces	11	10	13	4	Frequent changes
	Abnormal feces (color, small piece, etc.)	3	3	5	3	Sporadic changes
Emesis / Retching		0	5	4	1	Sporadic changes
Skin / Fur	Erythema / Irritation	2	14	18	5	Consistent changes
	Abrasion	0	0	3	0	Sporadic changes
	Crust	0	4	7	1	Consistent changes
	Flaky / Dandruff	1	11	12	1	Consistent changes
Eyes	Abnormal appearance (red, swollen eyelids, etc.)	0	9	10	2	Consistent changes associated with skin rash
Non-menstrual bleeding		7	10	11	4	Early gestation period. Placental sign

Body weight (dams): Dams were weighed on presumed GD1 (at the end of the 3-day co-habitation period), and on GD19, GD26, GD33, GD40, and GD47 prior to ABX-EGF dosing, and weekly thereafter until scheduled Cesarean section on GD100 $\pm$ 1 day. Initially, there was a >10% loss in body weight from pre-dose values at GD19 in 5/12, 6/18, and 3/5 pregnant dams in the 7.5, 15, and 30 mg/kg groups, respectively. Additionally, several animals in each of the ABX-EGF dose groups (monkeys #210, #211, #214, #301, #305, #401, and #405) that had either spontaneously aborted or underwent early Cesarean section for embryofetal death also had decreases of more than 10% in body weight from the pre-dose values. Decreases in body weights were observed throughout the dosing period and for most of the females had recovered following the final dose on GD48, with the exception of one individual dam in the 30 mg ABX-EGF/kg dose group (animal #402), whose body weight losses continued until GD75. However, group mean values for the panitumumab-treated animals were not statistically different from the control group at any time point measured.

Food consumption (dams): Estimated food consumption was evaluated qualitatively beginning at the time of confirmation of pregnancy through the day before Cesarean section, by counting the number of biscuits remaining from each day's ration at the time of the next feeding. Decreased food consumption was noted sporadically in all groups including the control during the dosing period; however, the magnitude and frequency of the individual decreases were greater in the 30 mg/kg group. Decreased food consumption was statistically lower than the control group ($p \leq 0.05$, ANOVA) for dams in the 30 mg/kg group on GD33, GD36, GD41, GD42, GD44, GD45, and GD70.

Toxicokinetics: Blood collection for T/K analyses was performed pre-dose and at 30 min and 96 h post-dsoe on GD20 and GD48, pre-dose on GD27, GD34, GD41, and GD38 for measurement of trough ABX-EGF serum levels, and once on GD55, GD62, GD69, GD76, GD83, and prior to scheduled Cesarean section. Blood samples were also collected from those female

monkeys who either spontaneously aborted or underwent emergency Cesarean section, when embryo-fetal death was confirmed.

Fetal blood was collected from the umbilical vein, and approximately 4 ml of amniotic fluid was collected from each fetus at scheduled Cesarean section. All samples were stored frozen at -70°C until shipped to the sponsor for evaluation of serum drug and antibody levels. Measurements of serum ABX-EGF from the dams and fetuses were perfomed by electrochemiluminescence assay as previously described, with a lower limit of quantitation of 39.1 ng/ml. Anti-panitumumab (MAHA) assays were performed

Comment: The final study report states on page 559 that "Fetal TK samples were inadvertently analyzed (a protocol deviation). No data analysis or interpretation will be made from these samples, and the data will not be present in the report." However, the final study protocol does state that fetal blood samples will be evaluated for serum levels of panitumumab and MAHA response in those females that are positive for MAHA. The sponsor has provided the raw data from this assay in a spreadsheet format in Module 4 of the BLA submission. Independent evaluation of the data by this reviewer revealed that all fetal samples, regardless of dose group were below the limits of quantitation for detection of ABX-EGF. Therefore, the lack of data analysis for these samples has no impact on the final conclusions for this study.

All females in the vehicle control group had undetectable serum levels of panitumumab at all time points on study. On GD20, there was a dose-related, approximately linear increase in both C_{max} (0.5 h after completion of injection) and $AUC_{0\text{-}96h}$ between the females treated with 7.5, 15, or 30 mg/kg ABX-EGF/dose. When the data were re-evaluated excluding those females who developed anti-panitumumab antibody responses, there were no statistically significant differences in either C_{max} or $AUC_{0\text{-}96h}$ at any dose level, for the same time point. These data are presented in Table 23, below.

Overall, there was very little systemic accumulation of panitumumab following 5 doses in pregnant female cynomolgus macaques. However, when female monkeys that had developed anti-ABX-EGF antibody were excluded from the calculations, evidence of slight, but still less than 2-fold systemic accumulation was noted, and was related to the dose of panitumumab. On GD48, there were no significant differences in either mean C_{max} or $AUC_{0\text{-}96h}$ values as compared to GD20 for all pregnant females in the 7.5 mg/kg dose group; therefore, no apparent accumulation of ABX-EGF occurred at this dose level. When the data were recalculated excluding the MAHA-positive females, there was a slight increase in the pharmacokinetic parameters in this dose group, with an increase in mean C_{max} of approximately 17% and an increase in $AUC_{0\text{-}96h}$ of 12% at GD48, as compared to GD20 mean values. In the 15 mg/kg dose group at GD48, there was a 29% increase in mean $AUC_{0\text{-}96h}$, and a 25% increase in C_{max} for all animals as compared to mean values at GD20; when these values were recalculated excluding the MAHA-positive dams, the increase in mean C_{max} was only 27% at GD48 as compared to GD20. However, the mean $AUC_{0\text{-}96h}$ was increased by 59.7% as compared to GD20 in the two monkeys that were MAHA-negative at GD48. Similarly, for dams in the 30 mg/kg/dose group the mean C_{max} was increased by 31% in all animals, and by 58% in the single, MAHA-negative monkey at GD48 as compared to the mean C_{max} values at GD20. The value for $AUC_{0\text{-}96h}$ in this one monkey was also increased at GD48 by 90% as compared to the mean value for the MAHA-negative animals in this dose group at GD20. A summary of the data is presented in Table 23, below.

Table 23. Study #103410. Toxicokinetic Evaluation of Serum ABX-EGF Levels in Pregnant Cynomolgus Monkeys Treated by Weekly i/v Injections During Organogenesis				
Weekly Dose of Panitumumab	**GD20**		**GD48**	
	Mean Value, ± SD		**Mean Value, ± SD**	
All animals	**C_{max} (µg/ml)**	**$AUC_{0\text{-}96h}$ (µg*d/ml)**	**C_{max} (µg/ml)**	**$AUC_{0\text{-}96h}$ (µg*d/ml)**
7.5 mg/kg	256 ± 50[a]	808 ± 145	284 ± 44	736 ± 330
15 mg/kg	461 ± 91[b]	1626 ± 347	577 ± 176	2098 ± 1183
30 mg/kg	1048 ± 204[d]	3743 ± 973	1369 ± 391	5360 ± 3074
MAHA-negative	**C_{max} (µg/ml)**	**$AUC_{0\text{-}96h}$ (µg*d/ml)**	**C_{max} (µg/ml)**	**$AUC_{0\text{-}96h}$ (µg*d/ml)**
7.5 mg/kg	261 ± 54[a]	818 ± 166	305 ± 39	917 ± 228
15 mg/kg	502 ± 71[b]	1736 ± 181	640[c]	2773[c]
30 mg/kg	1043 ± 250[d]	3965 ± 1060	1645[e]	7534[e]

[a] GD20, total n = 15/group, MAHA-negative n = 10; GD48, total n = 11, MAHA-negative n = 6
[b] GD20, total n = 18/group, MAHA-negative n = 5; GD48, total n = 15, MAHA-negative n = 2
[c] SD not calculated for this group at GD48 (MAHA-negative n = 2)
[d] GD20, total n = 4/group, MAHA-negative n = 3; GD48 total n = 2, MAHA-negative n = 1
[e] SD not calculated for this group at GD48 (MAHA-negative n = 1)

Serum panitumumab levels were obtained at the time of pregnancy termination from all dams who either spontaneously aborted, or underwent emergency Cesarean section following confirmation of embryo-fetal death. While serum ABX-EGF levels were variable in individual animals within and between the dosing groups, all dams had detectable panitumumab present at the time of fetal loss with the exception of monkey #214F in the 7.5 mg/kg dose group. This animal was reported to have undetectable ABX-EGF in serum samples obtained at the time of spontaneous abortion on GD58. The data for these animals is presented in Table 24, below.

Table 24. Study #103419. Correlation of Serum ABX-EGF and MAHA Response in Pregnant, Panitumumab-Treated Dams at Time of Fetal Losses				
Dose of ABX-EGF	**Animal #**	**Day of Fetal Loss**	**Serum ABX-EGF Level (µg/ml)**	**MAHA Response (day)**
Vehicle control	112	GD50[a]	BLQ[c]	(-)
7.5 mg/kg/dose	204	GD51[b]		(-)
	210	GD44[b]		(-)
	211	GD51[a]		(-)
	213	GD25[b]		(-)
	214	GD58[b]	BLQ	(-)
15 mg/kg/dose	301	GD51[a]		(-)
	305	GD44[a]		(-)
	407	GD30[a]		(-)
30 mg/kg/dose	401	GD50[b]		(-)
	405	GD37[b]		(-)
	403	GD51[a]		(-)

While anti-panitumumab antibody was not detectable in any of the dams that either spontaneously aborted or underwent emergency Cesarean sections after fetal death, a total of 19/38 females on study developed positive MAHA responses, with 5/15 positive in the 7.5 mg/kg/dose group, 13/18 in the 15 mg/kg/dose group, and 1/5 in the 30 mg/kg dose group. Fourteen of the fetal serum samples from the MAHA-positive females also had detectable

MAHA levels, with 4/5, 9/13, and 1/1 fetuses positive at Cesarean section from the MAHA-positive dams in the 7.5, 15, and 30 mg/kg/dose groups, respectively. Time to onset of MAHA responses in the dams was unrelated to the dose of panitumumab, with 2/5 and 3/9 of the MAHA-positive females in the 7.5 and 15 mg/kg/dose groups, respectively, positive at GD55. The only dam in the 30 mg/kg/dose group to develop the MAHA response did not show detectable anti-panitumumab antibody until GD100, at the time of Cesarean section.

Comment: One dam (animal #206, in the 7.5 mg/kg/dose group) had a reported, positive MAHA serum level on GD20, at the sample obtained prior to ABX-EGF dosing initiation. This animal subsequently tested negative for MAHA response at all later time points, including at Cesarean section on GD100; therefore, the initial sample is considered a false positive reading under the conditions of this assay.

Comment: The apparent, inverse dose-response for anti-ABX-EGF antibody suggests that the assay to detect MAHA response is not sufficiently robust as to be able to detect antibody presence while significant levels of the product are still present in serum. This has been an ongoing issue with this assay; however, the clinical assay has proven to be more robust, and should allow detection of anti-panitumumab antibodies even with levels of drug present in the serum samples.

In summary, the toxicokinetic data confirmed exposure of the dams to ABX-EGF during the phase of organogenesis. Antibody against panitumumab was not detected until after the completion of dosing on GD48. There was no detectable ABX-EGF present in serum from fetuses of treated dams at the time of Cesarean section. However, 14/19 fetuses from dams that were positive for MAHA response also had detectable anti-panitumumab antibody present in serum samples obtained at delivery; presumably, this was due to transplacental transfer of MAHA.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): Cesarean section was performed on all dams with surviving fetuses on GD100-GD103 (± 1 day), and fetuses evaluated for viability, limb and external organ measurements, gross malformations, body and placental weights, and gender. All fetuses were viable, with no external anomalies reported. No statistically significant, ABX-EGF related differences were noted in fetal weight, placental weight, and fetal external organ measurements. Pregnancies yielded three male and eight female fetuses in control group animals, six male and four female fetuses in the 7.5 mg/kg group, five male and ten female fetuses in the 15 mg/kg group, and one male and one female fetus in the 30 mg/kg group. A single placenta was observed in 1/11 (9.1%), and 2/15 (13.3%) fetuses in the control and 15 mg/kg groups, respectively.

Comment: The historical rate of single placenta in cynomolgus monkeys at this test facility was cited as approximately 10 – 13% in the final study report. Because the incidence of offspring with single placentas in the control and 15 mg/kg/dose ABX-EGF groups in this study was within this range, these findings are considered incidental to panitumumab treatment.

Offspring (malformations, variations, etc.): Cesarean section was performed on all dams with surviving fetuses on GD100-GD103 (± 1 day), and fetuses evaluated for skeletal and soft tissue malformations as described above. No visceral anomalies or variations were observed in any of the fetuses from dams in the ABX-EGF treated groups. The left thyroid was absent in one female fetus (offspring #105F) of the control group. No skeletal abnormalities were observed in fetuses from dams treated with 15 or 30 mg/kg/dose panitumumab from GD20 through GF28; however, the 12th rib was absent bilaterally in one fetus from the 7.5 mg/kg ABX-EGF dose group.

Lumbar ribs (skeletal variant) were present either unilaterally or bilaterally in 2/11 control fetuses (18.2%), and in 2/10 (20%), 1/15 (6.7%), and 1/2 fetuses from dams in the 7.5, 15, and 30 mg/kg panitumumab groups, respectively. Both the absence of ribs (skeletal anomaly) and the presence of lumbar ribs (skeletal variant) were reported by the test facility as incidental to ABX-EGF treatment.

Comment: Historical control data for this test facility show that the spontaneous rate of bilateral rib absence is approximately 2.1%, and presence of lumbar ribs is 13.3%. Therefore, these findings (absence of ribs, 1/10 or 10% of fetuses in the low dose group; lumbar ribs, 20%, 6.7%, and 50% in low, mid- and high-dose groups, respectively) cannot be truly considered incidental to the test article, as the contracting facility reports.

Both the absolute and relative mean adrenal gland weights (right, left, and paired) were increased in offspring from dams treated with 30 mg/kg/dose ABX-EGF as compared to the control group. Additionally, the mean absolute, but not the mean relative brain weight was lower in offspring from dams in the 15 mg/kg/dose panitumumab group; however, no similar changes were noted for offspring in the 30 mg/kg dose group. While these changes did reach statistical significance ($p \leq 0.05$, ANOVA), all values were within the reported historical control levels for this test facility, and were therefore not considered related to panitumumab treatment.

There were no histopathological findings present in any of the fetuses that were related to ABX-EGF treatment. Infarction and neutrophilic infiltrates were present in placentas from those animals which spontaneously aborted the pregnancies; however, there was no microscopic evidence of any toxicities or soft tissue anomalies in the conceptuses.

Study conclusion: Weekly, i/v treatment of pregnant female cynomolgus monkeys with 7.5, 15, or 30 mg/kg panitumumab (approximately 1.25 to 5-fold greater than the human dose of 6 mg/kg/dose resulted in spontaneous abortions and/or fetal deaths at all dose levels, in the absence of any teratogenic effects. Dose-related maternal toxicities, consisting of decreased food consumption, decreased body weights, and skin changes consistent with ABX-EGF induced dermatitis were also observed. The incidence of embryo-fetal losses was 1/12 (8.3%), 5/15 (33.3%), 3/18 (16.7%), and 3/5 (60.0%) for pregnant dams treated weekly from GD20 through GD48 with the placebo control, 7.5, 15, or 30 mg/kg/dose panitumumab, respectively. In the fetuses that survived until Cesarean section on GD100-GD103, there were no gross anomalies observed, and no evidence of soft tissue or skeletal malformations. Toxicokinetic data confirmed exposure of the dams to ABX-EGF during the phase of organogenesis; however, anti-panitumumab antibodies were detected after the completion of dosing on GD48. Because of the incidence of fetal losses, no NOAEL can be defined for this study.

Prenatal and postnatal development

No studies of this type were included in the BLA submission.

2.6.6.7 Local tolerance

No studies of this type were included in the BLA submission. Local irritation and inflammation at the intravenous injection site(s) was evaluated as part of the standard list of tissues examined microscopically in all repeat-dose toxicity studies of ABX-EGF.

2.6.6.8 Other toxicology studies

This section of the BLA contains six non-clinical *in vitro* and *in vivo* toxicity studies of panitumumab (ABX-EGF) from either the hybridoma or CHO cell culture manufacturing processes. Three *in vitro* studies were conducted to determine the potential for ABX-EGF tissue cross-reactivity with cynomolgus monkey tissues as part of the identification of this species as pharmacologically relevant for further toxicity testing and to demonstrate the *in vivo* localization of ABX-EGF binding following panitumumab treatment. An additional two studies were included in this section of the BLA that evaluated the toxicity of two different lots of panitumumab in cynomolgus monkeys for either 4 or 13 weeks of treatment. Finally, a single study was conducted in which the EGFr was cloned and sequenced from cynomolgus monkeys, and the sequence homology compared to human EGFr.

Each of these studies will be reviewed individually, below.

In vitro and in vivo tissue cross-reactivity of panitumumab

Study title: EGFr expression in normal cynomolgus monkey tissues: a specificity analysis.
Key study findings: Panitumumab binding to EGFr was detected in normal cynomolgus monkey tissues, including skin, eye, ovary and Fallopian tube, ureter, and tonsil, the bronchial epithelium in the lung, and glandular epithelium in the prostate, breast, and salivary gland. The most intense staining was present in the skin ($2^+ - 3^+$), eye (3^+) and prostate (3^+), with moderate staining (2^+) observed in the ureter, ovary and Fallopian tube, and lung.
Study no.: ABG-02
Volume #, and page #: EDR file: STN BLA 125147\000\module4\other toxicology studies\abg02.pdf
Conducting laboratory and location:

Date of study initiation: September 17, 1998
GLP compliance: Yes
QA statement: yes (X) no ( )
Drug, lot #, and % purity: biotinylated ABX-EGF, lot #098-068-01; biotinylated PK 16.3.1 (negative control antibody), lot #004-186-2; percent purity not specified for either primary antibody

Methods: The tissue cross-reactivity of panitumumab against normal cynomolgus monkey tissues was evaluated using sections obtained from necropsy specimens snap-frozen in liquid isopentane, and cryopreserved in OCT compound. Tissue sections were evaluated for ABX-EGF cross-reactivity by immunoperoxidase staining using indirect immunohistochemistry. Five micron sections were post-fixed in 50:50 methyl alcohol and acetone, and stained with 20 µg/ml of either biotinylated ABX-EGF, or the isotype matched, biotinylated human IgG_2 antibody PK 16.3.1 as a negative control. Sections of cryopreserved, normal human and cynomolgus monkey skin were used as the positive control tissues, and normal human and cynomolgus monkey heart sections were used as the negative tissue controls. Following incubation of the tissue sections with the primary or control biotinylated antibodies, samples were stained with steptavidin-conjugated peroxidase, incubated in hydrogen peroxide and diaminobenzidine as the capture reagent, and counterstained with hematoxylin. All sections were evaluated for panitumumab staining by direct visualization under light microscopy.

Results: The positive control tissue, normal monkey skin demonstrated a high degree of specific, membrane-associated ABX-EGF staining, with 3/3 samples staining positively in approximately 60% to 90% of the cells, at an intensity of $2^+ - 3^+$ (on a scale of $0 - 3^+$). Both squamous and glandular epithelial cells demonstrated high levels of ABX-EGF binding, consistent with the known distribution of EGFr in skin cells. No membrane-associated staining was observed in normal cynomolgus monkey heart tissue; however, occasional, non-specific staining of fibroblasts was reported. Other tissues reported as staining positively for ABX-EGF at the cell surface membrane (2^+ or greater intensity) included corneal epithelium in the eye, glandular and follicular epithelium in the ovary and Fallopian tube (glandular epithelium only), bronchial epithelium in the lung, glandular epithelia in the prostate (3^+ intensity), tonsillar epithelia, and urothelium in the ureter. Weaker, but specific ABX-EGF binding (1^+ intensity) was detected in ductal epithelial cells in the pancreas, breast and kidney, and in the salivary gland. The results of this tissue cross-reactivity study of ABX-EGF are presented in Table 25, below.

Table 25. Study #ABG02. Cross-Reactivity of ABX-EGF with Cynomolgus Monkey Tissues

Tissue Sample	Incidence	% Positive	Staining Intensity[a]	Comments
Human skin (positive control)	2/2	80, 50	3^+ (C, M)	(2/2) Strong, specific staining of squamous epithelium; non-specific staining of fibroblasts (1/2) Non-specific staining of perineurium
Monkey skin (positive control)	3/3	60 – 90	2^+ - 3^+ (C, M)	Strong, specific staining of epithelium
Human heart (negative control)	0/1	0	0	None
Monkey heart (negative control)	0/2	0	0	None
Adrenal	0/1	0	0	Non-specific staining of fibroblasts, stroma, and perineurium
Bladder	0/1	0	0	Non-specific cytoplasmic staining of smooth muscle
Blood cells	0/1	0	0	None
Bone marrow	0/1	0	0	None
Brain (cerebellum)	0/1	0	0	Non-specific staining of fibroblasts in the dura
Brain (cerebrum)	0/1	0	0	None
Breast	1/1	80	1^+ (C)	Weak, specific staining of ductal epithelial cells Non-specific, cytoplasmic staining of stromal basement membrane and perineurium
Esophagus	0/1	0	0	Non-specific cytoplasmic staining of smooth muscle
Eye	1/1	80	2^+ (M)	Specific, membrane-associated staining of corneal epithelial cells
Fallopian tube	1/1	90	2^+ (M)	Specific staining of glandular epithelial cells only
Heart)	0/1	0	0	Non-specific staining of fibroblasts
Kidney	1/1	80	1^+ (C, M)	Weak, specific staining of ductal epithelial cells Non-specific staining of smooth muscle, stromal basement membrane
Large intestine	0/1	0	0	Non-specific staining of smooth muscle
Liver	0/1	0	0	None

Lung	1/1	80	2^+ (M)	Specific staining of bronchial epithelial cells Non-specific staining of fibroblasts and stroma
Lymph node	0/1	0	0	None
Muscle, skeletal	0/1	0	0	None
Ovary	1/1	70	1^+ - 2^+ (M)	Specific staining of epithelial cells and follicles
Pancreas	1/1	80	1^+ (C, M)	Weak specific staining of ductal epithelial cells only Weak non-specific staining of fibroblasts
Pituitary	0/1	0	0	None
Prostate	1/1	90	3^+ (M)	Specific staining of glandular epithelial cells
Skin (positive control)	3/3	60 – 90	2^+ - 3^+ (C, M)	Specific staining of squamous and glandular epithelial cells
Small intestine	0/1	0	0	Weak non-specific staining of smooth muscle
Spleen	0/1	0	0	None
Spinal cord	0/1	0	0	Weak non-specific staining of fibroblasts in dura
Stomach	0/1	0	0	Non-specific staining of smooth muscle and basement membrane of fibroblasts
Testis	0/1	0	0	None
Thymus	0/1	0	0	None
Thyroid	0/1	0	0	Non-specific staining of fibroblasts
Tonsil	1/1	> 90	1^+, 2^+ (M)	Weak, specific staining of salivary gland and moderate, specific staining of epithelium
Ureter	1/1	50	2^+ (M)	Specific staining of urothelium Non-specific staining of smooth muscle
Uterus	0/1	0	0	Non-specific staining of smooth muscle, fibroblasts, and stroma

[a] staining intensity: 0 = negative; 1^+ = weak; 2^+ = moderate; 3^+ = strong; C = cytoplasmic; M = membrane

Study conclusion: Panitumumab was capable of detecting cell surface EGFr expression in tissue sections of skin, eye, ovary and Fallopian tube, prostate, tonsil and salivary gland, ureter, and in the bronchial epithelium of the lung. The pattern of ABX-EGF staining is consistent with the known distribution of EGFr in human tissues; therefore, the cynomolgus macaque is identified as a pharmacologically relevant species in which to further study the toxicity of panitumumab.

Study title: Binding of ABX-EGF to normal cynomolgus monkey tissues.
Key study findings: The *in vivo* distribution of ABX-EGF was measured following treatment in cynomolgus monkeys. Positive tissues included the adrenal gland, cecum, colon, duodenum, esophagus, heart, hair follicles, liver, pancreas, skin, and thymus. Weaker, but specific ABX-EGF binding was also detected in endocardium and interstitial spaces in the heart in animals from the Group 4 dose group, and in the germinal centers of the lymph node and the zona reticularis of the adrenal gland in animals from all four dosing groups.
Study no.: ABG-09
Volume #, and page #: EDR file: STN BLA 125147\000\module4\other toxicology studies\abg09.pdf

Conducting laboratory and location:

Date of study initiation: July 6, 1999 (final report dated February 17, 2000)
GLP compliance: Yes
QA statement: yes (X) no ( )
Drug, lot #, and % purity: no information regarding the lot number or specifications for the ABX-EGF test article was provided in the final study report.

Methods: The *in vivo* tissue cross-reactivity of panitumumab against normal cynomolgus monkey tissues was evaluated using frozen specimens obtained at necropsy, by immunoperoxidase staining using an amplified, indirect immunohistochemistry technique. Five micron sections were post-fixed in 95% ethanol and incubated with 1.25 µg/ml of either goat anti-human IgG-Fc antibody (to detect ABX-EGF bound to tissues), or an irrelevant, goat anti-mouse IgG-Fc antibody as the negative antibody control. Sections of cryopreserved, normal human tonsil were used as the positive tissue control for IgG-mediated, non-specific binding, and normal cynomolgus monkey tonsil sections were used as the negative IgG tissue control. Following incubation of the tissue sections with the goat anti-human IgG-Fc or control antibodies, samples were incubated with biotinylated donkey anti-goat antibody, then stained with steptavidin-conjugated peroxidase. The peroxidase reaction was developed in hydrogen peroxide and 3, 3'-diaminobenzidine tetahydrochloride, and sections were counterstained with hematoxylin. All sections were evaluated for panitumumab staining by direct visualization under light microscopy.

Comment: The tissue samples in the following study were presumably obtained from one or more of the completed, GLP toxicity studies of panitumumab in cynomolgus macaques. The Discussion section of the final study report states that the immunohistochemistry portion of the study was "blinded" such that the personnel at the contracting laboratory were unaware of the animal treatment conditions. However, there was no information provided in the final study report regarding the source (*i.e.* study number) from which these samples were derived, and as such, it is very difficult to determine the exposure to ABX-EGF that these animals achieved, and correlate that with both the tissue distribution patterns observed in the present study, and the previous toxicology results. The sponsor will be requested to provide the source of the tissues for the present study as part of the comments at the issuance of the discipline review letter.

Results: The *in vivo*, tissue cross-reactivity results following ABX-EGF treatment of cynomolgus monkeys are presented in Table 26, below. The positive control tissue, normal human tonsil demonstrated a high degree of specific, anti-human IgG-Fc staining, with 16/16 samples staining positively in approximately 80% to > 90% of the cells, at an intensity of $2^+ - 3^+$ (on a scale of $0 - 3^+$). However, focal areas of both cytoplasmic and membrane staining were also observed in 7/16 of the cynomolgus monkey tonsil samples; therefore, this tissue is not representative of a truly negative tissue control.

An apparent, dose-related increase in ABX-EGF binding, as evidenced by increases in incidence, staining intensity, and the percentage of positive cells in a tissue sample was noted across all tissues examined. The majority of tissues reported as staining positively for ABX-EGF at the cell surface membrane (2^+ or greater intensity) were observed in animals from Groups 3 and 4 from the *in vivo* portion of the study, and positive tissues included adrenal, cecum, colon, duodenum, esophagus, heart, hair follicles, pancreas, skin, and thymus. Canalicular staining (2^+ intensity) in

the liver was detected in 1/10 monkeys in Group 4, sinusoidal endothelial cell staining (2^+ intensity) was detected in 2/10 Group 4 monkeys, and sinusoidal staining (cell type not specified) was observed in 3/10 monkeys in this same dose group. Weaker, but specific ABX-EGF binding (1^+ - 2^+ intensity) was also detected in endocardium and interstitial spaces in the heart in animals from the Group 4 dose group, and in the germinal centers of the lymph node and the zona reticularis of the adrenal gland in animals from all four dosing groups.

Comment: The sinusoidal staining observed in the livers of 5/10 monkeys in Group 4 may be related to Fc receptor-mediated clearance of ABX-EGF by Kupffer cells and other sinusoidal lining cells. However, no data were provided in the BLA submission that address whether panitumumab can bind to monkey Fc receptor. No staining of these cell types was reported for liver sections from the other three dose groups, suggesting that Group 4 received the highest, *in vivo* exposure to ABX-EGF, and that saturation of EGFr was achieved at that dose, leaving more panitumumab available for non-specific interaction with Fc receptors.

Table 26. Study #ABG09. *In Vivo* Binding and Distribution of ABX-EGF in Normal Cynomolgus Monkey Tissues.

Tissue Sample	Group 1		Group 2		Group 3		Group 4		Comments
	Incidence	% Positive	Incidence	% Positive	Incidence	% Positive	Incidence	% Positive	
Human tonsil (positive control)	16/16 (2^+-3^+)[a]	80% – >90%							Staining localized to cellular membrane, cytoplasm
Monkey tonsil (negative control)	7/16 (0 - 1^+)	< 10% – 40%							Focal areas of staining localized to cytoplasm, cellular membrane
Adrenal	2/10 (1^+)	10%, 30%	3/6 (1^+)	<10% (1) 20%(1) 30% (1)	2/6 (1^+) 1/6 (2^+) 1/6 (3^+)	20% 30% 20%	7/10 (2^+) 1/10 (3^+)	10% - 40% < 10%	Specific staining of zona reticularis (1^+) in 1/10 in Group 1, 1/6 in Group 2, 4/6 in Group 3; and 6/10 (1^+ - 3^+) in Group 4
Cecum	4/10 (1^+)	10% (1) 30% (1) 80% (2)	3/6 (1^+)	20% (1) 40% (2)	3/6 (1^+) 1/6 (2^+)	20% (1) 80% (2) 40%	6/10 (1^+) 4/10 (2^+)	10% (1) 20% (1) 80% (4) 20% (1) 80% (3)	Staining observed in endothelium, stroma, fibroblasts, smooth muscle, and perineurium
Colon	3/10 (1^+)	< 10% (1) 60% (2)	1/6 (1+)	20%	6/6 (1^+)	20% (2) 30% (1) 60% (1) 80% (2)	1/10 (1^+) 7/10 (2^+)	30% 30% (3) 80% (3) > 90% (1)	Staining observed in endothelium, stroma, fibroblasts, smooth muscle, and nerve Staining (1^+) also observed in inflammatory cells in 1/6 monkeys from Group 3
Duodenum	3/9 (1^+)	10% (2) 30% (1)	1/6 (1^+)	40%	3/6 (1^+) 1/6 (2^+)	20% (1) 80% (2) 60%	7/10 (1^+) 2/10 (2^+)	< 10% (1) 40% (1) 50% (1) 80% (4) 40%, 80%	Staining observed in endothelium, stroma, fibroblasts, smooth muscle, and perineurium Staining (1^+) also observed in inflammatory cells (germinal

									center) in 1/6 from Group 2
Esophagus	2/10 (1^+)	20%, 40%	1/6 (1^+)	30%	1/5 (1^+) 2/5 (3^+)	< 10% 25%, 50%	2/9 (2^+) 5/9 (3^+)	30%, 50% 20% (2) 50% (2) 80% (1)	Staining observed in endothelium, stroma, fibroblasts, smooth muscle, and perineurium
Heart	3/10 (1^+) 2/10 (2^+)	< 10% (1) 30% > 90% (1) < 10%	2/6 (1+) 3/6 (2+)	< 10% < 10%	1/6 (1^+) 2/6 (2^+)	10% 10%	3/10 (1^+) 6/10 (2^+)	< 10% (1) 30% (1) 80% (1) < 10% (1) 30% (3) 40% (1) 80% (1)	Specific staining of endocardium and interstitial spaces; 1^+-2^+ in 4/10, 2^+ in 2/10 monkeys in Group 4 Staining of endocardium (1^+-2^+) only in 3/10 Group 1, 4/6 Group 2, 2/6 Group 3 monkeys Staining observed in endothelium, stroma, smooth muscle, ganglion cells and perineurium
Ileum	2/9 (1^+)	20%, 30%	3/6 (1^+)	20% (1) 30% (2)	4/6 (1^+)	10% (2) 80% (2)	4/10 (1^+) 3/10 (2^+) 1/10 (3^+)	80% (3) >90% (1) 30% (1) 40% (1) 80% (1) 30%	Staining observed in endothelium, stroma, fibroblasts, smooth muscle, and perineurium Staining (1^+) also observed in inflammatory cells (germinal center) in 1/6 from Group 2
Jejunum	3/9 (1^+)	< 10% (1) 20% (1) 30% (1)	2/6 (1^+)	20%, 30%	3/6 (1^+)	20% (1) 80% (2)	4/10 (1^+) 4/10 (2^+)	80% (4) 30% (3) 40% (4)	Staining observed in endothelium, stroma, fibroblasts, smooth muscle, and perineurium
Liver	0/10	0	0/6	0	0/6	0	4/10 (1^+) 1/10 (2^+)	20% 40%	Canalicular staining (2^+) in 1/10 from Group 4 Sinusoidal staining (1^+) in 3/10 from Group 4 Staining of endothelial cells in sinusoids (2^+) in 2/10 monkeys in Group 4
Lymph node	2/9 (1^+)	10%, 20%	4/6 (1^+)	10% (3) 30% (1)	4/6 (1^+)	10% (3) 20% (1)	3/10 (1^+) 3/10 (2^+)	10% (2) 20% (1) <10% (1) 10% (1) 20% (1)	Specific staining in germinal centers of 2/9 Group 1, 3/6 Group 2, 4/6 Group 3, and 4/10 Group 4 monkeys; all 1^+ - 2^+ intensity Staining observed in endothelium, stroma, fibroblasts,

									smooth muscle, and perineurium
Pancreas	0/10	0	2/6 (1+)	30%, 40%	3/6 (1+) 1/6 (2+)	< 10% (1) 10% (1) 30% (1) 10%	4/10 (1+) 4/10 (2+)	10% (1) 30% (1) 80% (2) 10% (2) 20% (1) 30% (1)	Specific staining of ducts in 2/6 Group 3 (1+) and 3/10 Group 4 (2+) monkeys Staining of small ducts, acini, and ductal acinar cells in 1/10 Group 4 monkeys (1+) Staining observed in endothelium, stroma, fibroblasts, smooth muscle, and perineurium
Rectum	4/10 (1+)	<10% (1) 20% (1) 30% (2)	3/6 (1+)	30% (1) 40% (1) 80% (1)	4/6 (1+)	20% (1) 30% (1) 60% (1) 80% (1)	5/10 (1+) 4/10 (2+)	20% (2) 80% (3) 20% (1) 40% (1) 80% (2)	Staining observed in endothelium, stroma, fibroblasts, smooth muscle, and perineurium Staining (1+) also observed in inflammatory cells (germinal center) in 1/6 from Group 2
Skin	6/10 (1+)	< 10% (3) 20% (3)	2/6 (1+)	10%, 20%	2/6 (1+) 2/6 (2+) 1/6 (3+)	40%, 80% <10%, 40% > 90%	1/10 (1+) 3/10 (2+) 5/10 (3+)	20% 20% - 80% 50% (4) 80% (1)	Specific staining of hair follicles (1+ - 2+) in 1/10 Group 1, 0/6 Group 2, 1/6 Group 3 and 1/10 Group 4 monkeys Staining observed in endothelium, stroma, fibroblasts, smooth muscle, and perineurium
Stomach	3/10 (1+)	20%	2/6 (1+)	30%, 60%	3/6 (1+)	20% (1) 80%(1) >90% (1)	7/10 (1+)	20% (2) 30%(2) 60% (1) 80% (2)	Staining observed in endothelium, stroma, fibroblasts, smooth muscle, and perineurium
Thymus	2/10 (1+)	< 10%	5/6 (1+)	<10% - 20%	2/6 (1+) 1/6 (2+) 1/6 (3+)	< 10% 10% 10%	1/10 (1+) 6/10 (2+)	10% < 10% (3) 10% (3)	Specific staining of epithelial cells (1+ - 2+) in 2/10 Group 1, 2/6 Group 2, 4/6 Group 3, and 7/10 Group 4 monkeys

[a] staining intensity: 0 = negative; 1+ = weak; 2+ = moderate; 3+ = strong; C = cytoplasmic; M = membrane; F - focal

Study conclusion: The *in vivo* binding and tissue distribution of panitumumab were evaluated following immunohistochemical staining of tissues from normal cynomolgus monkeys treated with ABX-EGF. Panitumumab treatment resulted in detectable ABX-EGF cell surface binding in tissue sections of skin, adrenal, cecum, colon, duodenum, esophagus, heart, hair follicles,

pancreas, and thymus, with weaker but detectable specific staining observed in the heart, and zona reticularis of the adrenal gland. These data demonstrate that ABX-EGF treatment results in *in vivo* localization and binding of the antibody to the EGFr, and identify specific target organs for panitumumab toxicity following exposure in repeat-dose toxicity studies.

Study title: Cross-reactivity of ABX-EGF (CHO) and ABX-EGF (hybridoma) with human and cynomolgus monkey tissue *ex vivo* and ABX-EGF (CHO) with rat, mouse and rabbit tissue *ex vivo*.

Key findings: Panitumumab manufactured in either CHO cells or in hybridoma cell lines bound to a panel of tissues from human and cynomolgus monkeys with similar distribution patterns, frequency, and intensity. These data indicate that there are no remarkable differences in the binding properties of ABX-EGF to either human or cynomolgus monkey tissues when the antibody is derived from CHO cells, as compared to the initial, hybridoma-derived product. There was no binding of ABX-EGF from CHO cells detected in the same panel of tissues from rats or mice; however, CHO cell-derived panitumumab could bind to EGFr in samples of urothelium in the urinary bladder and prostate, the cornea, cervix, and ductal epithelial cells in the pancreas, tubular epithelial cells in the kidney, ductular epithelium in the liver, and in the follicular epithelium of the thyroid of rabbits.

Study #: 102920 (Abgenix Study #ABX-P0305. – Study #1473-31).

Volume #, and page #: EDR file: STN BLA 125147\000\module4\other toxicology studies\102920.pdf

Conducting laboratory and location:

Date of study initiation: December 20, 2002 (final report dated May 19, 2004)

GLP compliance: Yes

QA statement: yes (X) no ( )

Drug, lot #, and % purity: ABX-EGF, CHO cell-derived, lot #9099-53F, % purity = — by size exclusion HPLC; ABX-EGF, hybridoma-derived, lot #N10004F, % purity

Methods: The binding of ABX-EGF from two different manufacturing processes to cell surface EGFr was evaluated using a panel of cryopreserved tissue sections from human, cynomolgus monkey, rat, mouse, and rabbits. Indirect immunohistochemistry techniques were employed using 5 to 7 micron tissue sections post-fixed in acetone, and incubated with either 1.0 or 5.0 µg/ml of panitumumab from the two different manufacturing sources. Following incubation with ABX-EGF, the sections were indirectly labeled with biotinylated anti-human IgG, and all sections were stained with avidin-conjugated peroxidase and diaminobenzidine as the capture reagent. Samples of A431 human vulvar squamous cell carcinoma and SW707 human lung tumor cells from xenografts in athymic, nude mice were used as the positive and negative, EGFr-expressing controls, respectively, for this assay. All samples were evaluated for ABX-EGF staining by direct visualization under light microscopy.

Results: In all experiments, the A431 human tumor xenograft samples strongly bound ABX-EGF from either the CHO cell or the hybridoma sources, with $2^+ - 3^+$ staining intensities at both the 1.0 and 5.0 µg/ml antibody concentrations. No binding of ABX-EGF from either CHO cells

or hybridoma cells was detected in the EGFr-negative, SW707 tumor xenograft samples at either antibody concentration tested. An isotype-matched, human IgG2 control antibody also did not stain any of the tissues in the panels from either the human, or the four test animal species when used at the same concentrations of 1.0 and 5.0 μg/ml.

There was no tissue-specific binding of the CHO-derived, ABX-EGF in any tissues in the panel tested from either rats or mice (data not shown). Surprisingly, ABX-EGF from CHO cells demonstrated specific binding to EGFr in samples of urothelium in the urinary bladder and prostate, the cornea, cervix, and ductal epithelial cells in the pancreas of rabbits, at concentrations of both 1.0 and 5.0 μg/ml. Overall, staining intensity was greater in rabbit tissues incubated with the higher concentration of panitumumab. Positive tissue binding was also present in the tubular epithelial cells in the kidney and ductular epithelium in the liver, and in the follicular epithelium of the thyroid in rabbit samples at the 5.0 μg/ml, but not the lower dose. Non-epithelial cell staining was present in vascular endothelial cells of the renal papilla at both the 1.0 and 5.0 μg/ml concentrations, and in the perifollicular stroma surrounding follicles in the ovary at the 5.0 μg/ml concentration only.

In both human and cynomolgus monkey tissues, comparable and specific binding of ABX-EGF from either CHO or hybridoma cells was observed at concentrations of both 1.0 and 5.0 μg/ml. Data comparing the pattern, frequency, and intensity of cell surface, specific binding of the 5.0 μg/ml panitumumab concentration for CHO-derived and hybridoma-derived ABX-EGF to both human and cynomolgus tissues are presented in Table 27, below. In general, the pattern of tissue cross-reactivity between human and monkey tissues was very similar for either ABX-EGF preparation, with cell surface binding of panitumumab from either source detected at the highest levels in epithelial cells in the skin, eye, breast, prostate, tonsil, and uterine cervix, in glomerular and tubular epithelia in the kidney and follicular epithelia in the thyroid gland, and in urothelium in the urinary bladder, and uterine endometrium. There appeared to be a slight, but consistent increase in staining intensity on these tissues from both species when hybridoma-derived ABX-EGF was used, as compared to the CHO cell-derived product. Comparing human and cynomolgus monkey tissues, the distribution of positive epithelial cells in most tissues was similar; however, panitumumab staining was more intense and consistent in cynomolgus monkey cells than in the corresponding human tissues. Differences in panitumumab cell surface binding between human and cynomolgus monkey were identified in several tissues. In human samples, positive ABX-EGF staining was identified in parathyroid epithelial cells, germinal center cells in lymph nodes (specific cell type unknown), stromal cells in the splenic red pulp and in trophoblastic cells in the placenta; these cells were unlabeled or unavailable (placenta) in cynomolgus monkeys. By contrast, follicular epithelial cells in secondary follicles in the ovary, bone marrow stromal cells, ductular epithelial cells in the liver, and lymphoid and stromal cells in the medulla of the thymus were positive in cynomolgus monkey tissues, while these same cell types were either not labeled, or presented equivocal results in the corresponding human tissues. Additional cells that stained positively with ABX-EGF in tissues from both human and cynomolgus monkeys, but are not of epithelial origin included interstitial cells in the testes, stromal cells in the uterine cervix, uterine endometrium, and tonsil. These data indicate that there are no remarkable differences in the binding properties of ABX-EGF to either human or cynomolgus monkey tissues when the antibody is derived from CHO cells, as compared to the initial, hybridoma-derived product.

Table 27: Cross-Reactivity of Panitumumab (ABX-EGF) from CHO cells or Hybridoma Cells with Normal Human and Cynomolgus Monkey Tissues

Tissue Samples	Human Samples		Cynomolgus Samples	
	ABX-EGF (CHO cell-derived)	ABX-EGF (hybridoma)	ABX-EGF (CHO cell-derived)	ABX-EGF (hybridoma)
Adrenal	1/3 (2^+)[a]	1/3 (2^+)	3/3 (1^+)	3/3 (1^+)
Bladder (urinary)	1/3 (1^+)	2/3 (2^+)	3/3 (2^+ - 3^+)	3/3 (3^+)
Blood cells	(-)	(-)	(-)	(-)
Bone marrow (perivascular stromal cells)	(-)	(-)	2/3 (1^+)	3/3 (1^+)
Brain (cerebral cortex)	1/3 (1^+)	1/3 (2^+)	(-)	(-)
Brain (cerebellum)	3/3 (1^+)	3/3 (1^+)	3/3 (1^+)	3/3 (1^+ - 2^+)
Breast (gland/ductular epi)	1/3 (1^+)	2/3 (1^+, 2^+)	3/3 (1^+)	3/3 (1^+ - 2^+)
Colon (mucosal epi)	3/3 (1^+)	3/3 (1^+ - 2^+)	3/3 (2^+)	3/3 (2^+ - 3^+)
Endothelium (various)	(-)	(-)	(-)	(-)
Eye	2/3 (1^+, 2^+)	2/3 (1^+, 2^+)	3/3 (1^+ - 2^+)	3/3 (2^+)
Fallopian tube	3/3 (1^+)	3/3 (1^+ - 2^+)	3/3 (1^+ - 2^+)	3/3 (2^+ - 3^+)
Gastrointestinal Tract	3/3 (1^+ - 2^+)	3/3 (1^+ - 2^+)	3/3 (1^+)	3/3 (2^+)
Heart	(-)	1/3 (1^+)[b]	(-)	(-)
Kidney (glomerulus and tubular epithelium)	2/3 (1^+, 2^+)	2/3 (1^+, 3^+)	3/3 (2^+ - 3^+)	3/3 (2^+ - 3^+)
Liver (ductular epithelium)	(-)	(-)	3/3 (1^+)	3/3 (2^+)
Lung (alveolar epithelium)	3/3 (2^+)	3/3 (2^+ - 3^+)	3/3 (1^+ - 2^+)	3/3 (2^+ - 3^+)
Lymph node	2/3 (1^+, 2^+)	2/3 (2^+)	(-)	1/3 (1^+)
Ovary	(-)	(-)	3/3 (3^+)	3/3 (3^+- 4^+)
Pancreas	3/3 (2^+)	3/3 (2^+)	3/3 (2^+)	3/3 (1^+ - 3^+)
Parathyroid (glandular epi)	3/3 (1^+ - 2^+)	2/3 (2^+)	(-)[c]	(-)[c]
Pituitary	3/3 (1^+ - 2^+)	3/3 (1^+ - 2^+)	2/3 (1^+)	3/3 (1^+ - 3^+)
Placenta (trophoblast epi)	3/3 (3^+)	3/3 (3^+)	N.D.[d]	N.D.[d]
Prostate (glandular and ductular epithelium)	3/3 (2^+ - 3^+)	3/3 (3^+)	3/3 (2^+)	3/3 (2^+)
Skeletal muscle	(-)	1/3 (1^+)	(-)	(-)
Skin	3/3 (2^+ - 3^+)	3/3 (2^+ - 3^+)	3/3 (1^+ - 2^+)	3/3 (1^+ - 2^+)
Spinal cord	1/3 (±)[b]	1/3 (±)[b]	(-)	(-)
Spleen (stroma, red pulp)	3/3 (1^+ - 2^+)	3/3 (1^+ - 2^+)	3/3 (1^+)	3/3 (1^+)
Testes (interstitial cells)	3/3 (2^+)	3/3 (2^+ - 3^+)	3/3 (2^+)	3/3 (2^+)
Thymus	3/4 (1^+ - 2^+)	3/4 (2^+ - 3^+)	3/3 (1^+ - 2^+)	3/3 (2^+ - 3^+)
Thyroid (follicular epi)	2/3 (1^+, 2^+)	2/3 (1^+, 2^+)	3/3 (1^+)	3/3 (1^+ - 2^+)
Tonsil (basal epi, mucosa)	3/3 (2^+ - 3^+)	3/3 (2^+ - 3^+)	3/3 (2^+ - 3^+)	3/3 (3^+- 4^+)
Ureter (urothelium)	2/3 (2^+)	2/3 (2^+)	3/3 (3^+)	3/3 (3^+- 4^+)
Uterus (cervix)	3/3 (3^+)	3/3 (3^+)	3/3 (2^+- 3^+)	3/3 (2^+- 4^+)
Uterus (endometrium)	3/3 (2^+ - 3^+)	3/3 (3^+)	3/3 (2^+- 4^+)	3/3 (2^+- 4^+)
Human A431 tumor (positive control)	1/1 (1^+)	1/1 (3^+)	1/1 (3^+)	1/1 (3^+)
Human SW706 tumor (negative control)	(-)	(-)	(-)	(-)

[a] (-) = negative staining; ± = equivocal staining; 1^+ = weak; 2^+ = moderate; 3^+ = strong; 4^+ = intense; N.A. = not applicable; N.D. = not done

[b] potential freeze artifact

[c] n = 2; no parathyroid tissue present in third sample

[d] N.D. = not done

Study conclusion: Panitumumab manufactured in either CHO cells or in hybridoma cell lines bound to a panel of tissues from human and cynomolgus monkeys with similar distribution patterns, frequency, and intensity. These data indicate that there are no remarkable differences in the binding properties of ABX-EGF to either human or cynomolgus monkey tissues when the antibody is derived from CHO cells, as compared to the initial, hybridoma-derived product. There was no binding of ABX-EGF from CHO cells detected in the same panel of tissues from

rats or mice; however, CHO cell-derived panitumumab could bind to EGFr in samples of urothelium in the urinary bladder and prostate, the cornea, cervix, and ductal epithelial cells in the pancreas, tubular epithelial cells in the kidney, ductular epithelium in the liver, and in the follicular epithelium of the thyroid of rabbits.

Comparability of toxicity of CHO-derived ABX-EGF with ABX-EGF produced in hybridoma cells

Two nonclinical toxicology studies in cynomolgus monkeys were conducted to evaluate the safety, pharmacokinetics, and immunogenicity of ABX-EGF produced in either the initial hybridoma cell line, or in CHO cells for the commercial product. They were reviewed, but will only be briefly summarized here.

Study title: A 4-week comparison study of two forms of ABX-EGF administered by intravenous injection once per week to cynomolgus monkeys with a 4-week recovery period.
Key findings: There were no apparent differences in toxicities, toxicokinetic profiles, or immunogenicity in cynomolgus monkeys treated for 4 weeks with panitumumab produced in either hybridoma cells, or in CHO cells.
Study #: 102906 (Abgenix Study #ABX-T0307, Study #02-3032)
Volume #, and page #: EDR file: STN BLA 125147\000\module4\other toxicology studies\102906.pdf
Conducting laboratory and location:

Date of study initiation: January 14, 2003 (in-life, 1/23 – 3/21/2003; final report dated December 24, 2003)
GLP compliance: Yes
QA statement: yes (X) no ( )
Drug, lot #, and % purity: ABX-EGF, CHO cell-derived, lot #9099-58G, % purity = [redacted] by size exclusion HPLC; ABX-EGF, hybridoma-derived, lot #P01007F, % purity

Methods: Cynomolgus monkeys (4/sex/group) were injected once weekly for 4 weeks with vehicle (Group 1; 50 mM sodium acetate in normal saline), or 7.5 or 30 mg/kg/dose ABX-EGF from either the hybridoma production process (Groups 2 and 3, respectively), or the same two dose levels of ABX-EGF derived from CHO cells (Groups 4 and 5). Safety evaluations included clinical observations and mortality checks, electrocardiography at pre-study and during Week 4 (5 or 6 days after dosing), body weights and clinical pathology, and organ weights and gross and microscopic pathology evaluation at necropsy. Samples for clinical pathology were also obtained at SD-8, SD8, SD22, and terminal or recovery sacrifices. At the end of the treatment period, 3 monkeys/sex per group were necropsied, and the remaining animals allowed to recover for 4 weeks befor the recovery sacrifice. Peripheral blood samples for toxicokinetic evaluations of comparability were obtained prior to treatment on Study Days -8 and SD0, then on SD4, SD7, SD11, SD14, SD18, SD21, SD25, and SD28.

Results: There were no unscheduled deaths during this study, and there were no effects of treatment with ABX-EGF from either manufacturing method on body weights, or body weight gains. Electrocardiograms for all animals were within normal limits at both pre-study and

completion of dosing evaluations. Clinical toxicities related to panitumumab treatment included skin flaking and dryness, dermatitis, and erythema beginning in the second week of dosing over various areas of the body (limbs, axillary/inguinal areas, head, neck, and/or trunk) in 3/8 monkeys in the group dosed with 7.5 mg/kg hybridoma-derived panitumumab, 2/8 monkeys dosed with 7.5 mg/kg/dose of CHO-derived ABX-EGF, and in 6/8 monkeys each dosed with 30 mg/kg/dose of ABX-EGF from either manufacturing source. By the end of the 4-week recovery period, the skin changes had resolved in all monkeys except one female in the group treated with 30 mg/kg of hybridoma-derived ABX-EGF, who had dry, flaky skin still present in the axillary area (animal #3899F). This monkey was also the only ABX-EGF treated monkey to develop the erythema and flaky skin over the entire body during the treatment period. Changes in fecal consistency (*i.e.*, unformed or watery stool) were also noted in all animals dosed with either dose level of ABX-EGF from either source, beginning in the first week and continuing for the duration of treatment. Upon histologic evaluation of the skin samples, the lesions consisted of minimal to slight, epidermal hyperkeratosis, acanthosis, and parakeratosis, with accompanying inflammatory cell infiltrates. Microscopically, skin lesions were occasionally accompanied with subcutaneous inflammation and hemorrhage. There were no correlating, microscopic findings to explain the intestinal toxicities.

No changes in the hematologic profiles were noted for monkeys treated with either formulation of panitumumab, as compared to the placebo control group. A decrease in erythrocyte parameters was noted at both the SD8 and SD22 time points in all groups as compared to baseline; however, these changes are attributed to the amount of blood removed for toxicokinetic sampling, and are not considered related to ABX-EGF treatment. As compared to the control group, mean fibrinogen levels were increased in across panitumumab treated groups by 10% to 41% on SD8 and SD22, regardless of the source of ABX-EGF. Mean serum albumin was decreased by 8% to 15% as compared to controls in male monkeys in both 7.5 mg/kg dose groups, and in female monkeys in the 30 mg/kg CHO-derived ABX-EGF dose group at SD8. At SD22, the effect was present in all dose groups, with the exception of female monkeys treated with 7.5 mg/kg/dose of the CHO-derived panitumumab. Increases in serum globulin of 10% to 26% as compared to the control group were noted in all ABX-EGF dose groups at SD22, with the exception of the female monkeys in the 7.5 mg/kg CHO-derived ABX-EGF dose group. Corresponding decreases in serum A:G ratios were also noted in all affected groups. There were no apparent differences in the magnitude of any of these changes between either the group mean, or individual animal values for monkeys treated with ABX-EGF from either manufacturing source. During the recovery phase, serum albumin and globulin levels in the panitumumab-treated groups returned to control levels, with the exception of both 30 mg/kg/dose groups of female monkeys treated with either hybridoma- or CHO-derived ABX-EGF. Serum globulin levels for these animals continued to increase during the recovery phase, and were likely due to development of MAHA response (below).

Toxicokinetic evaluation of hybridoma- or CHO cell-derived ABX-EGF exposures in cynomolgus monkeys showed similar values for C_{max} and $AUC_{0\text{-}168h}$ both on SD0, and after the final dose of panitumumab on SD21. However, slightly higher mean $AUC_{0\text{-}168h}$ and C_{max} values were obtained for CHO cell-derived ABX-EGF, than for hybridoma-derived material at both dose levels on SD0, and at the 7.5 mg/kg/dose level on SD21. The ratio for the mean values in the 7.5 mg/kg/dose group was either at or just slightly exceeded 125% at SD0. These data are presented in Table 28, below.

Table 28. Study #102906. Pharmacokinetic Comparability of ABX-EGF Produced in Chinese Hamster Ovary (CHO) or Hybridoma Cell lines After Weekly i/v Dosing in Monkeys						
Dose of Panitumumab	Pharmacokinetic Parameter Mean Value, ± S.D.					
	C_{max} (µg/ml)			$AUC_{0\text{-}168h}$ (mg*day/ml)		
SD 0	CHO-cell	Hybridoma	Ratio (%)	CHO-cell	Hybridoma	Ratio (%)
7.5 mg/kg	231 ± 45	184 ± 29	125.5	615 ± 123	493 ± 116	124.7
30 mg/kg	891 ± 282	794 ± 141	112.2	3056 ± 933	2735 ± 533	111.7
SD 21[a]						
7.5 mg/kg	252 ± 81	217[b]	116.1	718 ± 271	625[b]	114.9
30 mg/kg	811 ± 175	820 ± 148	98.9	1475 ± 1148	1516 ± 1456	97.3

[a] values from MAHA-positive animals were excluded from the calculations at SD21
[b] SD not calculated for this group at this time point; n = 2

Positive MAHA responses were seen on Day 28 and/or Day 56/57 (recovery) in 50% of the animals given ABX-EGF (hybridoma) and 44% of the animals given ABX-EGF (CHO). The data in Table 28 above exclude C_{max} and $AUC_{0\text{-}168h}$ values from the MAHA-positive monkeys at SD 21 only.

Comment: The final study report did not contain a calculation of the confidence intervals for the ratio of the mean C_{max} or $AUC_{0\text{-}168h}$ values, so no true assessment of bioequivalence of the two ABX-EGF preparations can be made. However, independent analysis of the data and computation of the ratios and appropriate confidence intervals was performed by the clinical pharmacology reviewer (Angela Men, Ph.D.). Her calculations show that while the ratios of both C_{max} and $AUC_{0\text{-}168h}$ values for the two preparations on Study Days 1 and 21 (all animals, both 7.5 and 30 mg/kg dose levels) do fall within the standards applied for assessment of bioequivalence as per FDA guidance drugs, the available confidence intervals around these ratios are outside of the 80:125 percent standard. When the ratios and confidence intervals for C_{max} and $AUC_{0\text{-}168h}$ values were calculated using data from only MAHA-negative monkeys, both the ratios and confidence intervals were outside of the 80:125 standard at all doses tested, and at both SD 0 and SD 21. The data as calculated by Dr. Men are presented in Table 29, below.

Table 29. Study #102906. Pharmacokinetic Comparability of ABX-EGF Produced in Chinese Hamster Ovary or Hybridoma Cell Lines After Weekly i/v Dosing in Monkeys (FDA Independent Calculation)				
All Animals				
Dose of ABX-EGF	C_{max}		$AUC_{0\text{-}168h}$ (mg*day/ml)	
SD 0	Ratio (%)	90% CI Range	Ratio (%)	90% CI Range
7.5 mg/kg	125	107 - 145	126	104 - 153
30 mg/kg	110	89 - 134	110	88 - 137
SD 21				
7.5 mg/kg	119	52 - 148	687	Not estimated
30 mg/kg	86	61 - 121	114	45 - 292
MAHA-negative animals				
SD 0	Ratio (%)	90% CI Range	Ratio (%)	90% CI Range
7.5 mg/kg	137	88 - 211	138	81 - 126
30 mg/kg	95	76 - 117	87	75 - 101
SD 21				
7.5 mg/kg	111	71 - 115	109	63 - 189
30 mg/kg	71	49 - 103	93	69 - 124

Study conclusion: The toxicity, pharmacokinetic, and immunogenicity profiles of ABX-EGF manufactured either in hybridoma or CHO cell lines were similar, although not truly comparable or bioequivalent. However, no differences in safety or efficacy are anticipated for the clinical setting, since the doses of panitumumab planned for licensure result in serum levels of ABX-EGF that exceed those that result in EGFr saturation.

Study title: A 3-month intravenous toxicity study of ABX-EGF in cynomolgus monkeys with a 6-week recovery period.
Key findings: There were no apparent differences in toxicities, toxicokinetic profiles, or immunogenicity in cynomolgus monkeys treated for 4 weeks with panitumumab produced in CHO cells using either the research scale —) or the commercial — process.
Study #: 103917 (Abgenix Study #ABX-T0311, — Study #03-3060)
Volume #, and page #: EDR file: STN BLA 125147\000\module4\other toxicology studies\1003917.pdf
Conducting laboratory and location:

Date of study initiation: January 23, 2004 (in-life, 1/26 – 6/7/2004; final report dated November 2, 2005)
GLP compliance: Yes
QA statement: yes (X) no ( )
Drug, lot #, and % purity: ABX-EGF, CHO cell-derived — scale, lot #954A022614, % purity = — by size exclusion HPLC; ABX-EGF, CHO cell-derived, — commercial) scale, lot #954A023447, % purity = — by size exclusion HPLC

Methods: The purpose of this study was to compare the toxicity, toxicokinetics, and immunogenicity profiles of ABX-EGF produced at the clinical trial (— and commercial scale — processes, and to compare the toxicity profiles of the biologic when administered through an in-line filter, or unfiltered. Control monkeys received once weekly injections of vehicle (ABX-EGF placebo, consisting of 50 mM sodium acetate in 100 mM NaCl, ph 5.8), through the in-line filter. Two different dosing regimens; once weekly for 3 months, or every other week for 3 months were also tested in this study. At the end of the treatment period, up to 3 monkeys/sex/group were necropsied for toxicity evaluations. The study design, including the group assignments, number of animals for each dosing group and each sacrifice time point, and the volume and concentration (ml/ml) of ABX-EGF administered is presented in Table 30 below, which was abstracted directly from the testing laboratory's final study report.

Table 30. Study #103917. 3-month Comparative Toxicity Study of — ABX-EGF vs. — ABX-EGF in Cynomolgus Monkeys: Study Design

Group	Dose[a]			Number of Animals			
				Total	Necropsy[d]		
					Terminal (Week 14/15)	Recovery (Week 19)	Microscopic Pathology
	Dose[b] mg/kg	Volume mL/kg	Conc. mg/mL	M/F	M/F	M/F	M/F
1 Control[c] (filtered Vehicle)	0	1.5	0	4/4	2/3	1/1	4/4
2 Low dose ABX-EGF (filtered —	7.5	1.5	5	3/3	3/3	0/0	3/3
3 High dose ABX-EGF (filtered —	30	1.5	20	5/5	3/3	2/2	5/5
4 High dose ABX-EGF (unfiltered —	30	1.5	20	3/3	3/3	0/0	3/3
5 Low dose (every other week) ABX-EGF (filtered —	7.5	1.5	5	3/3	3/3	0/0	3/3
6 Low dose ABX-EGF (filtered —	7.5	1.5	5	3/3	3/3	0/0	3/3
7 High dose ABX-EGF (filtered —	30	1.5	20	5/5	3/3	2/2	5/5
8 High dose ABX-EGF (unfiltered —	30	1.5	20	3/3	3/3	0/0	3/3

[a]Groups 1, 2, 3, 4, 6, 7, and 8 were dosed once per week. Group 5 was dosed once every two weeks.
[b]Doses represent active ingredient.
[c]Vehicle was administered to the Control animals.
[d]Necropsy was also performed for 1 control male sacrificed for humane reasons during the course of the study.

Safety evaluations included clinical observations and mortality checks, ophthalmology examinations at pre-study and at the end of the dosing and recovery periods, weekly body weights and food consumption, and organ weights and gross and microscopic pathology evaluation at necropsy. Samples for clinical pathology were obtained pre-test, at Study Weeks 5 and 10 during dosing, and at terminal and recovery sacrifices, and for urinalysis at the end of dosing and recovery periods. Peripheral blood samples for toxicokinetic evaluations of comparability of the two ABX-EGF products, and for measurement of the anti-panitumumab antibody response were obtained prior to treatment, and on SD1, SD29, SD57, and SD85. At scheduled necropsy time points, full necropsy with gross and microscopic pathologic evaluations were performed on all animals.

Supportive care for dehydration (*i.e.*, Lactated Ringer's Solution, 50 ml/monkey s/c) was administered in all ABX-EGF treatment groups, beginning on SD0 and continuing through SD30 or SD31, at which point fluid therapy was administered as a once daily, s/c injection of 100 ml/monkey Lactated Ringer's solution until the day prior to scheduled terminal necropsy, or until SD105 or AD106 for the recovery monkeys. Topical treatment for skin rash, dermatitis and infection (*i.e.* Animax topical antibiotic/corticosteroid ointment, Nolvasan® anti-bacterial bathing) and/or oral anti-diarrheal medications (*i.e.* Pepto-Bismol, *Lactobacillus sp.*) were administered as needed to minimize and/or treat these conditions.

Results: There were no unscheduled, treatment-related deaths during this study. One male monkey (animal #1433M) in the control group was euthanized on SD32 for humane reasons, following a broken tibia during Study Week 5. Ophthalmologic examinations for all animals were within normal limits at both pre-study, and completion of dosing and recovery evaluations.

Overall, decreases in body weights, and body weight gains were observed in individual animals in the groups treated with ABX-EGF as compared to the vehicle control group. Mean values for male monkeys in all ABX-EGF dose groups were decreased by approximately 2% to 10% of pre-study values, with the exception of the group treated with 7.5 mg/kg/dose of the filtered, ABX-EGF every other week. Body weight losses in panitumumab-treated female monkeys tended to be more severe than in males, with body weight losses of 3% to 16% from baseline occurring in individual animals, and persisting between 1 and 14 weeks in duration. However, there were no statistically significant differences between the groups treated with ABX-EGF from either manufacturing method on either the duration or magnitude of changes in body weights, or body weight gains.

Changes in fecal consistency (*i.e.*, unformed or watery stool) were noted in all animals dosed with either 7.5 or 30 mg/kg/dose ABX-EGF from either the — manufacturing scale, and in the groups treated with the filtered vs. unfiltered material. In general, both the onset and the incidence of these findings were similar within the same dose level for monkeys treated with ABX-EGF from either the — manufacturing process. However, the incidence of fecal changes was approximately 2 to 5-fold higher in monkeys treated with 30 mg/kg/dose of the filtered ABX-EGF from either manufacturing process, as compared to the findings in this same dose group treated with unfiltered product from either source. Additionally, both the incidence and severity of fecal changes were less in the monkeys treated with 7.5 mg/kg/dose of filtered ABX-EGF from the — process, in which the onset of changes in fecal consistency did not occur until approximately Study Week 5 in the male monkeys, and Study Week 4 in the females. Both the number of animals affected, and the number of days with findings were similar to animals in the control group, and had completely resolved by Study Week 12. There were no correlating, microscopic findings to explain the intestinal toxicities.

Dermal toxicities related to panitumumab treatment included skin rashes, characterized by erythema, scaling and/or flaking of the epidermis, cracking and/or breaking of the skin, and pustular formations or exudates, over various areas of the body (head, neck, axillary/inguinal areas, limbs, and/or trunk). These findings were noted in animals in all ABX-EGF-treated groups beginning in the second week of dosing, and were dose-related in both incidence and severity with slight to mild dermatitis observed at all dose levels, and rashes of moderate and severe severity occurring most often in the high-dose groups. The skin changes were seen as early as the second week of dosing, and in the high-dose recovery groups persisted in several animals for the treatment-free recovery period. There were no differences in either the incidence or severity of the skin toxicities observed between the — manufacturing groups, or if panitumumab was filtered or not filtered prior to i/v dosing. Microscopic evaluation of the skin lesions revealed acute to chronic epidermal changes, including inflammation, hyper/parakeratosis and acanthosis, intraepithelial pustules and/or abscesses, erosions and ulcerations, and subcutaneous hemorrhage and effusions. After the 6-week recovery period, there were histologic indications that the skin effects of ABX-EGF treatment were reversible, although not completely resolved in all dose groups. There were no apparent differences in clinically notable skin pathology following treatment with either unfiltered or filtered, or — scale product.

There were no significant differences in the hematologic profiles for monkeys treated at the same dose level with either filtered or unfiltered panitumumab, or between the — manufacturing processes. Statistically significant decreases in total leukocyte counts, absolute neutrophil and lymphocyte counts, and eosinophils and/or basophils as compared to the control group were noted at Study Week 5 for all groups of female monkeys treated with ABX-EGF from either manufacturing source. However, independent analysis by this reviewer of pooled data from both male and female monkeys in all dose groups did not show statistically significant differences either from control, or between the different ABX-EGF groups at the same dose level, regardless of the source of the product. There were no differences in hematology parameters at the end of the recovery period in the ABX-EGF treated groups as compared to either control monkeys, or between groups of animals treated with either the — scale products.

There were no differences noted in prothrombin time, APTT, or fibrinogen levels between the groups of monkeys treated at the same dose level with either the — manufactured ABX-EGF. As compared to the vehicle control group, there was a statistically significant increase in APTT at the end of dosing in female monkeys treated with 30 mg/kg/dose of the unfiltered, — process ABX-EGF, as compared to females treated with the filtered. — process product. However, the mean APTT value for the females treated with 30 mg/kg/dose of unfiltered — ABX-EGF at this time point was less than the baseline value and comparable to the control females at this time point; therefore, the biological relevance of this difference is unknown.

As compared to the vehicle control group, mean fibrinogen levels were statistically significantly increased at Study Week 10 for male monkeys treated with 30 mg/kg of the unfiltered, — process ABX-EGF, and in this same group at Study Week 14 as compared to both baseline values, and to the group treated with the same dose of the filtered, — process product. However, independent analysis by this reviewer of pooled data for both male and female monkeys in all dose groups did not show statistically significant differences from either baseline or control values, or between the different ABX-EGF dose groups treated with either formulation, or filtered or unfiltered product. At the end of the treatment-free recovery period, there were no differences in coagulation factor profiles between the control animals, and monkeys previously treated with 30 mg/kg panitumumab from either manufacturing process.

No consistent or statistically significant differences in serum biochemistry profiles were noted between groups of monkeys treated with filtered or unfiltered panitumumab, or ABX-EGF produced at either — scale. Although not statistically significant, the most prevalent changes in all dose groups, including the controls at Study Weeks 5 and 14 were decreases of 2% to 29% in mean serum albumin, and increases of 5% to 32% in serum globulin, as compared to baseline values. Corresponding decreases in serum A:G ratios were also noted in all affected groups. There were no apparent differences in the magnitude of any of these changes between either the group mean, or individual animal values for monkeys treated with ABX-EGF from either manufacturing source. During the recovery phase, serum albumin and globulin levels in the panitumumab-treated groups returned to control levels, with no differences noted between the control group, and the groups treated with ABX-EGF from either manufacturing process.

As compared to baseline values, serum magnesium decreased in all groups, including the controls over the duration of the study. At the end of dosing, there was a dose-related, although not statistically significant decrease in male monkeys treated with 30 mg/kg/dose ABX-EGF from either source (35-36.2%), as compared to male monkeys in either the control (24.7% decrease from baseline) or male monkeys in the 7.5 mg/kg/dose groups (23.7 to 29.7% decrease). In the females, statistically significant decreases in serum magnesium relative to baseline were noted at

sporadic intervals in both the 7.5 and 30 mg/kg ABX-EGF-treated groups regardless of material source; however, in female monkeys, the magnitude of these decreases (19-40%) was similar to that seen in the control group (12-38%). There were no differences in serum magnesium values between the control and the ABX-EGF treated groups at the end of the recovery period.

At necropsy, skin findings were present in all groups of animals administered panitumumab, with the lesions affecting the face, neck, abdomen, chest, limbs, hands/feet, and tail, and consisting of desquamative or exfoliative rashes, sores, scabs, and edema. Hair loss or absence was observed in several animals including one control monkey, but the other skin lesions were not observed in the control group. The groups of monkeys treated with 7.5 mg/kg ABX-EGF generally had fewer and less severe skin lesions than the animals treated for 13 weeks with the 30 mg/kg dose level. There were no consistent, or significant differences in either the incidence or severity of the skin lesions between the groups administered either the manufacturing scale product, or between unfiltered and filtered formulations at the same dose levels.

Toxicokinetic evaluation of CHO cell-derived ABX-EGF exposures in cynomolgus monkeys treated with material produced by either the scale processes showed similar values for C_{max} and $AUC_{0\text{-}168h}$ on SD 1 for animals treated with 7.5 mg/kg panitumumab. Slight, although statistically not significant decreases in both C_{max} and $AUC_{0\text{-}168h}$ at SD 1 were noted for monkeys treated with the filtered ABX-EGF produced by the process, as compared to the group treated with filtered panitumumab from the scale manufacturing procedure. However, these trends on SD 1 were reversed when the values for animals treated with 30 mg/kg of the unfiltered panitumumab were compared (Table 31: Day 1, below). The biologic relevance of these findings is currently unknown.

Decreases of approximately 25 to 35% in both C_{max} and $AUC_{0\text{-}168h}$ were observed for monkeys in the 7.5 and 30 mg/kg, filtered ABX-EGF dose groups at SD 29, as compared to animals treated with filtered panitumumab manufactured by the process. By contrast, there were no remarkable differences in either parameter between the two manufacturing processes for the 7.5 and 30 mg/kg dose groups at SD 29 when unfiltered material was used for treatment. Slightly higher mean $AUC_{0\text{-}168h}$ and C_{max} values were obtained for the both the filtered and unfiltered, CHO cell-derived ABX-EGF than for the process material at both dose levels on SD85. These data are presented in Table 31 below, which was abstracted directly from the sponsor's final study report.

Positive MAHA responses were seen on Day 29 and Day 85 in approximately the same percentage of monkeys given ABX-EGF produced by either manufacturing method (data regarding the $AUC_{0\text{-}168h}$ and C_{max} values for the MAHA-negative monkeys are included in Table 31, below). No data were included in the final study report that demonstrated whether the MAHA responses were increased in any of these animals during the treatment-free recovery period.

Comment: Item #6 in the list of protocol deviations on p. 44 of the final study report states that blood samples for evaluation of MAHA responses were not collected from any animals at the end of the recovery period. This omission is unfortunate, as experience has shown that anti-product antibody titers may not be detectable at the end of treatment period, due to interference with the assay by the presence of the monoclonal antibody itself. In many cases, the immunogenicity of a biologic product only becomes evident when the plasma (or serum) levels of the monoclonal antibody have significantly decreased from on-study levels, *i.e.* during the treatment-free recovery period.

Table 31. Study #103917. Pharmacokinetic Comparability of ABX-EGF Produced in CHO Cells by — vs. — Processes, After Weekly i/v Dosing in Cynomolgus Monkeys

Dose (mg/kg), Scale (L), Filtration, Frequency	All subjects			MAHA Negative[a]		
	n	$C_{0.5\,hr}$ (µg/mL)	$AUC_{(0\text{-}tau)}$ (µg·day/mL)	n	$C_{0.5\,hr}$ (µg/mL)	$AUC_{(0\text{-}tau)}$ (µg·day/mL)
Day 1[b]						
7.5, —, Filtered, QW	6	221 (90)	555 (130)	2	187 (NC)	497 (NC)
7.5, —, Filtered, QW	6	232 (26)	592 (67)	3	235 (32)	579 (14)
7.5, —, Filtered, Q2W	6	223 (41)	704 (129)	2	236 (NC)	771 (NC)
30, —, Filtered, QW	10	972 (119)	2971 (401)	8	970 (126)	2930 (389)
30, —, Unfiltered, QW	6	827 (172)	2792 (699)	2	621 (NC)	2021 (NC)
30, —, Filtered, QW	10	837 (73)	2545 (300)	7	855 (69)	2628 (325)
30, —, Unfiltered, QW	6	943 (224)	2893 (251)	5	957 (247)	2875 (276)
Day 29						
7.5, —, Filtered, QW	6	234 (67)	651 (381)	2	237 (NC)	887 (NC)
7.5, —, Filtered, QW	6	222 (88)	442 (379)	3	286 (25)	758 (171)
7.5, —, Filtered, Q2W	6	173 (44)	219 (239)	2	212 (NC)	509 (NC)
30, —, Filtered, QW	10	1336 (260)	4496 (1365)	8	1309 (288)	4361 (1512)
30, —, Unfiltered, QW	6	1150 (434)	2509 (1942)	2	1292 (NC)	3879 (NC)
30, —, Filtered, QW	10	964 (361)	2830 (2040)	7	1105 (337)	3867 (1432)
30, —, Unfiltered, QW	6	1179 (315)	3987 (2398)	5	1241 (308)	4677 (1903)
Day 85						
7.5, —, Filtered, QW	6	197 (117)	430 (426)	2	254 (NC)	769 (NC)
7.5, —, Filtered, QW	6	157 (142)	389 (431)	3	282 (37)	778 (107)
7.5, —, Filtered, Q2W	6	74 (95)	173 (269)	2	196 (NC)	517 (NC)
30, —, Filtered, QW	10	1313 (405)	3523 (1584)	8	1368 (413)	3799 (1343)
30, —, Unfiltered, QW	6	1106 (698)	1859 (1838)	2	1421 (NC)	3318 (NC)
30, —, Filtered, QW	10	1009 (394)	2801 (1927)	7	1221 (182)	3957 (612)
30, —, Unfiltered, QW	6	1091 (415)	3466 (1983)	5	1246 (186)	4136 (1245)

$C_{0.5\,hr}$ = Observed serum concentration at 0.5 hours post-dose

$AUC_{(0\text{-}tau)}$ = Area under the concentration-time curve from time 0 to the end of the dosing interval (168 hours for QW on all days and Q2W on day 85, or 336 hours for Q2W on days 1 and 29)

[a] Only monkeys demonstrating MAHA negative on days 29, 57, and 85

[b] Pretest results

NC=Standard Deviation not calculated when n = 2

Comment: The final study report did not contain a calculation of the confidence intervals for the ratio of the mean C_{max} or $AUC_{0\text{-}168h}$ values, so no true assessment of bioequivalence of the ABX-EGF manufactured by the two different scale processes can be made. However, independent analysis of the data and computation of the ratios and appropriate confidence intervals was performed by the clinical pharmacology reviewer (Angela Men, Ph.D.). Her calculations show that the majority of values for the ratios of C_{max} and $AUC_{0\text{-}168h}$, as well as the 90% confidence intervals for the two preparations of panitumumab on Study Days 1, 29, and 85 at both the 7.5 and 30 mg/kg dose levels fall outside of the 80:125 percent standard applied for assessment of bioequivalence as per FDA guidance. When the ratios and confidence intervals for C_{max} and $AUC_{0\text{-}168h}$ values were calculated using data from only MAHA-negative monkeys, approximately 50% of the ratios for C_{max} and $AUC_{0\text{-}168h}$ were within the 80:125 percent standard at all doses tested. However, the majority of the calculated values for the 90% confidence intervals around these ratios were outside of the 80:125 standard at all doses tested, and at all time points. Taken together, the ratios and confidence intervals for C_{max} and $AUC_{0\text{-}168h}$ between the reference — manufactured material and the — , commercial process material indicate that these products are not truly comparable. The results of these independent calculations were provided by Dr. Men in tabular format, and are presented directly from her review as Tables 32 and 33, below.

Table 32. Study #103917. Pharmacokinetic Comparability of ABX-EGF Produced by Either — or — Scale Manufacturing Processes (FDA Independent Assessment; Reference = — Process Material)

All Animals				
Dose of ABX-EGF	**Time Point, N**	**Parameters**	**Ratio**	**90% CI Range**
7.5 mg/kg/filtered	**SD 1** N=6;6	C_{max}	113	82 -- 155
		$AUC_{0\text{-}168h}$	109	90 -- 132
	SD 29 N=6;6	C_{max}	90	59 - 137
		$AUC_{0\text{-}168h}$	37	9 -- 160
	SD 85 N=6;6	C_{max}	75	20 -- 284
		$AUC_{0\text{-}168h}$	32	0.53 -- 1969
30 mg/kg/filtered	**SD 1** N=10;10	C_{max}	86	80 -- 94
		$AUC_{0\text{-}168h}$	86	78 -- 96
	SD 29 N=10;10	C_{max}	69	54 -- 88
		$AUC_{0\text{-}168h}$	43	22 -- 83
	SD 85 N=10;10	C_{max}	71	49 -- 103
		$AUC_{0\text{-}168h}$	32	8 -- 136
30 mg/kg/unfiltered	**SD 1** N=6;6	C_{max}	150	97 -- 233
		$AUC_{0\text{-}168h}$	142	121 -- 166
	SD 29 N=6;6	C_{max}	96	63 -- 146
		$AUC_{0\text{-}168h}$	121	65 -- 228
	SD 85 N=6;6	C_{max}	87	69 -- 109
		$AUC_{0\text{-}168h}$	121	79 -- 186

Table 33. Study #103917. Pharmacokinetic Comparability of ABX-EGF Produced by Either — Scale Manufacturing Processes (FDA Independent Assessment; Reference = — rocess Material)				
MAHA-Negative Animals				
Dose of ABX-EGF	Time Point, N	Parameters	Ratio	90% CI Range
7.5 mg/kg/filtered	SD 1 N=3;2	C_{max}	127	89 -- 180
		AUC_{0-168h}	119	80 -- 178
	SD 29 N=3;2	C_{max}	122	88 -- 169
		AUC_{0-168h}	86	49 -- 149
	SD 85 N=3;2	C_{max}	110	87 -- 140
		AUC_{0-168h}	107	56 -- 204
30 mg/kg/filtered	SD 1 N=7;8	C_{max}	89	80 -- 98
		AUC_{0-168h}	90	79 -- 103
	SD 29 N=7;8	C_{max}	82	63 -- 108
		AUC_{0-168h}	87	59 -- 129
	SD 85 N=10;10	C_{max}	92	74 -- 114
		AUC_{0-168h}	110	83 -- 145
30 mg/kg/unfiltered	SD1 N=6;6	C_{max}	113	88 -- 146
		AUC_{0-168h}	106	87 -- 130
	SD29 N=6;6	C_{max}	106	75 -- 151
		AUC_{0-168h}	167	66 -- 427
	SD85 N=6;6	C_{max}	120	52 -- 279
		AUC_{0-160h}	335	54 -- 2060

Study conclusion: The toxicity, pharmacokinetic, and immunogenicity profiles of ABX-EGF manufactured using CHO cell lines by either the — manufacturing process, or the — — commercial scale process are similar, although not truly comparable or bioequivalent. However, no differences in safety or efficacy are anticipated for the clinical setting, since the doses of panitumumab planned for licensure result in serum levels of ABX-EGF that exceed those that result in EGFr saturation.

2.6.6.9 Discussion and Conclusions

Please see Study Conclusions under individual study reviews, above.

2.6.6.10 Tables and Figures

Tables and Figures are integral to the study reviews, above.

2.6.7 TOXICOLOGY TABULATED SUMMARY

A tabulated summary of all preclinical repeat-dose, reproductive and developmental, and other toxicity studies included in the BLA, as provided by the sponsor in Module 2, Section 2.6.7 of the electronic CTD submission is attached to this review as Appendix 5.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Severe dermatologic and gastrointestinal toxicities were noted at all dose levels in cynomolgus monkeys treated weekly with 7.5, 15, 30, or 60 mg/kg panitumumab for 4, 13, or 26 weeks. These doses correspond to approximately 1.25 to 10-fold greater than the proposed human dose of 6 mg/kg ABX-EGF administered every two weeks, and approximately 3 to 24-fold higher than the proposed 2.5 mg/kg/week panitumumab dose, when adjusted for body weight. Observed toxicities included decreases in body weight and food consumption, decreases in serum calcium, phosphate, and magnesium, and dose-dependent clinical signs consisting of soft or watery stool, alopecia, skin rash, erythema, flaking and/or dryness, suppurative dermatitis, erosions, sloughing, and ulcerations, and in several studies, early mortalities secondary to the severity of the skin lesions. These changes occurred with increased frequency and severity as both the dose and duration of ABX-EGF increased, and were only partially reversible following discontinuation of panitumumab treatment. Panitumumab treatment of non-pregnant, female cynomolgus monkeys inhibited ovarian function, resulting in dose-related irregularities in menstrual cycling (prolonged menstrual cycles and/or amenorrhea), decreased pregnancy rates, and decreases in serum 17β-estradiol and progesterone levels, at doses corresponding to approximately 1.25 to 5-fold higher than the proposed, clinical doses when adjusted for body weight. Although no teratogenic effects were observed, panitumumab was abortifacient at all dose levels tested in pregnant female cynomolgus monkeys, following weekly injection from GD20 through GD48 (approximately 1.25 to 5-fold greater than the highest proposed human dose).

Unresolved toxicology issues (if any): None

Recommendations: No additional nonclinical pharmacology or toxicology studies of ABX-EGF are recommended at the present time.

Suggested labeling: Please see attached recommendations for labeling, Appendix 1

Signatures (optional):

Reviewer Signature Anne M. Pilaro 9/20/2006

Supervisor Signature Martin D. Green Concurrence Yes X No ___
9/21/06

APPENDIX/ATTACHMENTS

Draft language for communication (information requests) to the sponsor is included in the attachment, below. Draft recommendations for changes to the proposed package insert, citations from the published literature in support of panitumumab pharmacology and the role of EGFr in cancer and developmental biology, and copies of the tabular summaries of the completed pharmacology, toxicology, and pharmacokinetics studies (as provided by the sponsor in Module 2 of the e-CTD BLA) are included as Appendices 1-5, following the attachment.

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_ § 552(b)(4) Trade Secret / Confidential

_ § 552(b)(5) Deliberative Process

/ § 552(b)(4) Draft Labeling

APPENDIX 2 – PUBLISHED LITERATURE REFERENCES REGARDING PANITUMUMAB ACTIVITY, ROLE OF EGFr IN CANCER AND DEVELOPMENTAL BIOLOGY, AND ACTIVITY IN COMBINATION WITH CHEMOTHERAPY

The following references from the open literature were included in the pharmacology and toxicology sections (Module 4) of the original BLA submission, but unless noted in the sections above, were not included in this review.

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APPENDIX 3 - TABULATED SUMMARY OF PRECLINICAL PHARMACOLOGY STUDIES CONDUCTED WITH PANITUMUMAB (VECTIBIX ™)

The following tables were copied directly from Module 2, section 2.6.3 of the electronic CTD submission, as provided by the sponsor to the BLA application.

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Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Primary Pharmacodynamics					
EGFr Expression in Normal Human Tissues and Human Tissues	Normal human tissues and human renal and prostatic carcinomas	098-068-01	In vitro	—	R2003207 (ABG01)
Affinity Measurement of ABX-EGF Produced by Hybridoma	Human EGFr extracellular domain	1996 Research Material	In vitro	Abgenix Inc.	R2005552
Inhibition of ^{125}I-ABX-EGF Binding to Cell Surface Expressed Human EGFr by Unlabeled ABX-EGF	SKMES, RAW 264.7and A20 cells	954B019718	In vitro	Amgen Inc.	R2005581
Affinity Measurement of ABX-EGF Derived from CHO Cells	Human EGFr extracellular domain	954D025598; A0311200004	In vitro	Abgenix Inc.	R2005582
Inhibition of EGF Binding to A431 Cells by ABX-EGF	A431 cells	1998 Research Material	In vitro	Abgenix Inc.	R2003197
Inhibition of Tumor Cell Activation by ABX-EGF	A431 cells	1997 Research Material	In vitro	Abgenix Inc.	R2003199
Inhibition of EGFr Tyrosine-Phosphorylation by ABX-EGF	A431 cells	9099-58G	In vitro	Abgenix Inc.	R2003198

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Primary Pharmacodynamics					
Efficacy of Panitumumab in the Inhibition of EGFR Phosphorylation upon Stimulation with Different EGFR Ligands	A549 cells	9099-58G	In vitro	Amgen Inc.	R2005539
Evaluation of Differential Effects of Tyrosine Phosphorylation in EGFr by Mass Spectrometry	A431 cells	Not Documented	In vitro	/	R2003225
ABX-EGF Induced Internalization of EGF Receptor Expressed on A431 and HeLa Cells	A431 and HeLa cells	447-122	In vitro	Abgenix Inc.	R2005421
ABX-EGF Induced EGFR Internalization	A431 and A549 cells	172-167-1	In vitro	Abgenix Inc.	R2005530
Measurement of EGFr Internalization with — Imaging Technology	A431, SKMES, HeLa, and H1299 cells	9099-58G	In vitro	Amgen Inc.	R2004090
Epitope Mapping of AMG 954, an Anti-Human Epidermal Growth Factor Receptor (EGFR) Antibody	Human EGFr extracellular domain	9099-58G	In vitro	Amgen Inc.	R2005497
Test of Expression of EGFr on Pancreatic and Lung Tumor Cell Lines to be Used in Xenograft Experiments	H2126, SKMES-1, H1299, MV 522 cells	2000 Research Material	In vitro ,	Abgenix Inc.	R2003094

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Primary Pharmacodynamics					
Inhibition of A431 Cell Growth In Vitro by ABX-EGF	A431 cells	1997 Research Material	In vitro	Abgenix Inc.	R2003200
Effects of ABX-EGF on Activation of Human Vascular Smooth Muscle Cells In Vitro	Human aortic smooth muscle cells	Not Documented	In vitro	Amgen Inc.	R2003330
Inhibition of IL-8 and VEGF Production in Human Prostate Tumor Cells	DU145 cells	LO2531/41	In vitro	Abgenix Inc.	R2003373
Inhibition of IL-8 Production by ABX-EGF in Human Renal Cell Carcinoma (RCC) Cells	Caki-1 and Caki-2 cells	LO2531/41	In vitro	Abgenix Inc.	R2003211
Inhibition of VEGF Production by ABX-EGF in Human RCC Cells	Caki-1 and Caki-2 cells	LO2531/41	In vitro	Abgenix Inc.	R2003212
Effect of ABX-EGF or the 225 Antibody on A431 Tumor Growth in Nude Mice	Mouse (A431 xenograft model)	LO3008/36	Intraperitoneal injection	Abgenix Inc.	R2003204
Complete Eradication of Larger Tumors by ABX-EGF	Mouse (A431 xenograft model)	1997 Research Material	Intraperitoneal injection	Abgenix Inc.	R2003203
Evaluation of ABX-EGF in Athymic Nude Mice Bearing A431 Xenografts	Mouse (A431 xenograft model)	Not Documented	Intraperitoneal injection	Amgen Inc.	R2003201

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Primary Pharmacodynamics					
Eradication of Established Human Epidermoid Tumor in Nude Mice by ABX-EGF	Mouse (A431 xenograft model)	1997 Research Material	Intraperitoneal injection	Abgenix Inc.	R2003202
Evaluation of ABX-EGF, a Fully Human mAB Against the EGF Receptor, in Athymic Mice Bearing Human A431 Vulvar and SK-MES Lung Carcinoma Xenograft Derived from Tumor Cell Suspensions or Tumor Fragments	Mouse (A431 and SKMES xenograft models)	LO3008/38	Intraperitoneal injection		R2003113 — No. 7)
In Vitro Growth Inhibitory Activity and In Vivo Antitumor Activity of ABX-EGF, a Fully Human Monoclonal Antibody against the EGF Receptor, in the Human A431 Vulvar Carcinoma Model	A431 cells; Mouse (A431 xenograft model)	3737/TFP-99059A	In vitro; Intraperitoneal injection		R2003112 — No. 5)
Effect of ABX-EGF on the Growth of Multiple Human Tumors Derived from Different Tissues and Expressing Different Levels of EGFr	A431, MDA-MB-468, SK-RC-29, BxPC3, PC3, IGROV-1, Hs766T, HPAC, HT29, and SW707 cells/xenograft models; Mouse	1997-1998 Research Material	In vitro; Intraperitoneal injection	Abgenix Inc.	R2003205

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Primary Pharmacodynamics					
Effect of AMG 954 on HT29 Subcutaneous Tumor Growth (Repeat)	Mouse (HT-29 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003327
Dose Response of Panitumumab in EGFR Mutant NSCLC NCI-H1650 Xenograft	Mouse (NCI-H1650 xenograft model)	S01486R03	Intraperitoneal injection	Amgen Inc.	R2004660
ABX-EGF Prolonged Survival of SCID Mice Bearing MDA231 Human Breast Cancer	Mouse (MDA231 xenograft model)	1999 Research Material	Intraperitoneal injection	Abgenix Inc.	R2003206
Effects of ABX-EGF Antibody Against Established MDA-MB-468, Human Breast Carcinoma Model in Athymic Nude Mice	Mouse (MDA-MB-468 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003520
Effects of ABX-EGF and AMG 612153 Against Established MDA-MB-468, Human Breast Carcinoma Model	Mouse (MDA-MB-468 xenograft model)	9099-58G	Intraperitoneal injection and oral gavage	Amgen Inc.	R2004283
Effects of ABX-EGF Antibody in Established MiaPaCa-2, a Human Pancreatic Carcinoma, in Harlan Athymic Nude Mice	Mouse (MiaPaCa-2 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003281

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Primary Pharmacodynamics					
Effects of ABX-EGF Against Established BxPC-3 and Capan I, Human Pancreatic Carcinomas, in Nude Mice	Mouse (BxPC-3 and Capan I xenograft models)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004135
The Effect of ABX-EGF on Colo 205 Tumor Xenografts	Mouse (Colo 205 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003325
Evaluation of ABX-EGF Antibody in Established NCI-H1299, a Human Non-Small Cell Lung Carcinoma, in Harlan Athymic Nude Mice	Mouse (NCI-H1299 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003283
Immunohistological Evaluation of ABX-EGF Localization and Penetration in Tumors from Nude Mice with Established NCI-H1299 Xenografts	Mouse (NCI-H1299 NSCLC xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003576
Effects of ABX-EGF Antibody in Established SK-MES PD, a Human Squamous Cell Carcinoma, in CD1 Nude Mice	Mouse (SKMES xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003280

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Primary Pharmacodynamics					
Evaluation of EGFr Levels in Xenograft Tissues Using the EGFR PharmDx Kit	Colo 205, Colo 320, SKMES PD, HT-29, A431, Calu 6, HCT116, MIA PaCa, PC-3, CaPan1, AsPC1, LN 229, DU 145, A 673, Namalwa, U87, Mes-Sa/Dx5, Daudi, Raji, SK OV-3, H1299, ZR75-1, MCF-7, BT 474, CaPan1, MDA-468, H460, MDA-231, A549, T47D, SW 620, LNCaP, HL60 c15, PxPC-3, Panc-1, and MKN-45 cells	Not Documented	In vitro	Amgen Inc.	R2005548
Evaluation of Downstream Signaling Molecules in A431 After ABX-EGF Treatment	Mouse (A431 xenograft model)	LO2531/41	Intraperitoneal injection	Abgenix	R2003331

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Primary Pharmacodynamics					
Immunohistological Evaluation of ABX-EGF Administration and Penetration in Tumors from Nude Mice with Established A-431 Xenografts	A431 cells; Mouse (A431 xenograft model)	9099-58G	In vitro; Intraperitoneal injection	Amgen Inc.	R2004496 (PK Study 106194)
Immunohistological Evaluation of Cell Proliferation, Kinase Signaling, and ABX-EGF Penetration in Tumors from Nude Mice with Established A-431 Xenografts	Mouse (A431 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004503
Evaluation of Downstream Signaling Molecules in HCT-116 After ABX-EGF Treatment	Mouse (HCT-116 xenograft model)	LO2531/41	Intraperitoneal injection	Abgenix	R2003332
Immunohistological Evaluation of ABX-EGF Localization and Penetration in HT-29 Xenograft Tumors	Mouse (HT29 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004497
Immunohistological Evaluation of ABX-EGF Penetration, Cell Proliferation, and Signaling Cascade in HT-29 Xenograft Tumors Treated with Mono and Combination Therapy of ABX-EGF, Oxaliplatin and CPT-11	Mouse (HT29 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004500

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Primary Pharmacodynamics					
Efficacy of Panitumumab in Pancreatic (BXPC3 and CAPAN I), Epidermoid (A431), Breast (MCF7 and MDA MB 468), Colon (HT29) Cancer Cells and Tumors and the Analysis of the Signal Transduction Pathways in Panitumumab Responder and Non-Responder Tumors	BXPC3, CAPAN I, A431, HT29, MCF7, and MDA MD 468 cells	9099-58G	In vitro	Amgen Inc.	R2004292
Immunohistological Evaluation of ABX-EGF Penetration, Cell Proliferation, and Signaling Cascade in MDA-MB-468 Human Breast Carcinoma Xenograft Tumors from Nude Mice Treated with ABX-EGF	Mouse (MDA-MB-468 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004498
EGFr: Her2 Ratio in Panitumumab Responder and Non-Responder Xenograft Tumor Cell Lines	MDA MB 468, A431, HT-29, NCI-H1650, PC-3, NCI-H1975, U87, H1299, SKMES PD, and Colo205 cells	Not Applicable	In vitro	Amgen Inc.	R2004656

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Primary Pharmacodynamics					
Patterns of Gene Expression can Prospectively Predict Panitumumab (ABX-EGF) Monotherapy Responsiveness in Xenograft Models	Mouse (A431, PC3, MiaPaCa, HT29, NIH-H1299, SKMES, MCF7, U86, ZR75-1, and Colo205 xenograft models)	Not Documented	Intraperitoneal injection	Amgen Inc.	R2004657
Effect of Hybridoma Derived ABX-EGF in A431 Tumor Xenograft	Mouse (A431 xenograft model)	LO2531/41	Intraperitoneal injection	Amgen Inc.	R2003473
Evaluation of ABX-EGF in Athymic Nude Mice Bearing A431 Xenografts	Mouse (A431 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003366
Modulation of In Vitro Growth Inhibitory Activity of Selected Anticancer Agents by ABX-EGF, a Fully Human Monoclonal Antibody Against EGF Receptor	Panc-1, MiaPaCa, SKMES, H1299, MV522, and A431 cells	3737/TFP-99059A	In vitro	/	R2003110 No. 4)
In Vitro Analysis of ABX-EGF in Combination with Chemotherapeutic Drugs	A431, MDA-MB-468, BxPC3, and IGROVI cells	LO1616/75	In vitro	Abgenix Inc.	R2004035

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Primary Pharmacodynamics					
Effects of Combination of ABX-EGF and AMG 706 in A431 Human Epidermoid Carcinoma Xenograft Model in Nude Mice	Mouse (A431 xenograft model)	9099-58G	Intraperitoneal injection and oral gavage	Amgen Inc.	R2003538
Evaluation of Combination Therapy of ABX-EGF with 5-FU in HT-29 Tumor Xenograft Model	Mouse (HT29 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003558
Evaluation of Combination Therapy of ABX-EGF with CPT-11 in HT29 Tumor Xenograft Model	Mouse (HT29 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003559
Evaluation of Combination Therapy of ABX-EGF with Oxaliplatin in Ht-29 Tumor Xenograft Model	Mouse (HT29 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003560
Evaluation of Combination Therapy of ABX-EGF with Anti-hVEGF Antibody in HT29 Tumor Xenograft Model	Mouse (HT29 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003561
Effects of Combination of ABX-EGF and AMG 706 in HT29 Human Colon Carcinoma Xenograft Model in Nude Mice	Mouse (HT29 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003537

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Primary Pharmacodynamics					
Evaluation of Combination Therapy of Panitumumab with Rapamycin in HT-29 Tumor Xenograft Model	Mouse (HT29 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004446
Secondary Pharmacodynamics					
Inhibition of EGFr Phosphorylation by ABX-EGF in Human RCC Cells	SK-RC-29 and Caki-1 cells	LO2531/41	In vitro	Abgenix Inc.	R2003213
Inhibition of Human Renal Carcinoma Cells Growth In Vitro and In Vivo Tumor Xenograft Models by ABX-EGF	Caki-1 and Caki-2 cells; Mouse (Caki-1 and Caki-2 xenograft models)	97135	In vitro; Intraperitoneal injection	Abgenix Inc.	R2003371
Effects of ABX-EGF Against Established MDA-MB-231, a Human Breast Carcinoma in Female Nude Mice	Mouse (MDA-MB-231 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003527
Effects of ABX-EGF on Established ZR75-1 Human Breast Cancer Model in Athymic Female Nude Mice	Mouse (ZR75-1 xenograft model)	9099-58G	Intraperitoneal and subcutaneous injection	Amgen Inc.	R2004086

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Secondary Pharmacodynamics					
Effects of ABX-EGF Against Established U87 MG vIII, a Human Glioblastoma Tumor Model, in Female Nude Mice	Mouse (U-87 MG control and U-87 MG EGFr vIII mutant xenograft models)	9099-58G	Intraperitoneal injection and oral gavage	Amgen Inc.	R2003550
Test of Expression of EGFr on Pancreatic and Lung Xenografts	Panc-1, MiaPaCa-2, SKMES-1, H1299 cells	Not Documented	In vitro	Abgenix Inc.	R2003090
Evaluation of Downstream Signaling Molecules in A431 and SK-MES after ABX-EGF Treatment In Vivo	Mouse (A431 and SKMES xenograft models)	Not Documented	Intraperitoneal injection		R2003104
Effects of ABX-EGF in Established A549, a Human Lung Carcinoma, in Female Athymic Nude Mice	Mouse (A549 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004156
Effects of ABX-EGF Against Established NCI-H460, a Human Large Cell Lung Carcinoma in Female Athymic Nude Mice	Mouse (NCI-H460 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004155

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Secondary Pharmacodynamics					
Effects of ABX-EGF Against Established NCI-H82, a Human Small Cell Lung Carcinoma in Female Athymic Nude Mice	Mouse (NCI-H82 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004154
Evaluation of Panitumumab (ABX-EGF) Alone and in Combination with Anti-IGF-1R mAb, MAB 391 in Nude Mice Bearing Calu-6 Xenografts	Mouse (Calu-6 xenograft model)	ABX-1505-10	Intraperitoneal injection	Amgen Inc.	R2004013
Effects of ABX-EGF on Established MCF-7tb Human Breast Cancer Model in Female Nude Mice	Mouse (MCF7tb xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003479
The Effect of Panitumumab in the U118 Xenograft Tumor Model	Mouse (U118 glioblastoma xenograft model)	S01486R03	Intraperitoneal injection	Amgen Inc.	R2005549
Immunohistological Evaluation of Cell Proliferation, Kinase Signaling, and ABX-EGF Penetration in Tumors from Nude Mice with Established BxPC-3 and Capan-1 Human Pancreatic Carcinoma Xenografts	Mouse (BxPC3 and Capan-1 xenograft models)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004501

Table 2.6.3-1 Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Secondary Pharmacodynamics					
Immunohistological Evaluation of ABX-EGF Penetration, Cell Proliferation, and Signaling Cascade in MCF-7tb Human Breast Carcinoma Xenograft Tumors from Nude Mice Treated with ABX-EGF	Mouse (MCF7tb xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004499
Efficacy of EGFR Inhibitors in Kinase Domain EGFR Mutation in Transfected and Transduced CHO Cells and NCI H1975, NCI H1650, SKMESPD and A549 Cells and Tumors	Chinese hamster ovary cells, non-small cell lung cancer cells; NCI-H1975, NCI-H1650, SKMES-PD, A549	9099-58G	In vitro	Amgen Inc.	R2004720
Administration of Panitumumab Against Established NCI-H1975 NSCLC in Female Athymic Nude Mice	Mouse (NCI-H1975 xenograft model)	S01486R03	Intraperitoneal injection	Amgen Inc.	R2004637
Efficacy of EGFr Inhibitors in EGFr-vIII Mutation in U87 Cells	U87 transduced EGFr-vIII mutant cells	9099-58G	In vitro	Amgen Inc.	R2004440

Table 2.6.3-1 Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Secondary Pharmacodynamics					
Effects of ABX-EGF and Cisplatin Against Established SKMES PD, a Human Squamous Cell Carcinoma in CD1 Nude Mice	Mouse (SKMES xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003258
Effects of ABX-EGF and Cisplatin Against Established NCI-H1299 CD1 Nude Mice	Mouse (NCI-H1299 NSCLC xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003277
Effects of ABX-EGF and Cisplatin Combination Therapy on A431 Tumor Xenografts	Mouse (A431 xenograft model)	LO1616/75	Intraperitoneal injection	Abgenix Inc.	R2003208
The Effects of Panitumumab and Cisplatin Combination in NCI-H1975, EGFr Mutant NSCLC, Xenograft	Mouse (NCI-H1975 xenograft model)	S01486R03	Intraperitoneal injection	Amgen Inc.	R2005183
The Effects of Panitumumab and Taxotere Combination in NCI-H1975, EGFr Mutant NSCLC, Xenograft	Mouse (NCI-H1975 xenograft model)	S01486R03	Intraperitoneal injection	Amgen Inc.	R2005428
Effects of ABX-EGF and Docetaxel Combination Therapy on NSCLC A549 Tumor Xenografts	Mouse (A549 xenograft model)	LO2531/41	Intraperitoneal injection	Abgenix Inc.	R2003210

Table 2.6.3-1 Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Secondary Pharmacodynamics					
Effects of ABX-EGF and Taxotere Combination Therapy on NSCLS A549 Tumor Xenografts	Mouse (A549 xenograft model)	LO2531/41	Intraperitoneal injection	Abgenix Inc.	R2003370
Effects of ABX-EGF and Taxotere Against Established NCI-H1299 Tumors in CD1 Nude Mice	Mouse (NCI-H1299 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003278
The Effects of Panitumumab and Taxotere Combination in NCI H1975, EGFR Mutant NSCLC, Xenograft	Mouse (NCI-H1975 xenograft model)	S01486R03	Intraperitoneal injection	Amgen Inc.	R2005181
The Effects of Panitumumab and Taxotere Combination in NCI H1975 EGFR Mutant NSCLC, Xenograft	Mouse (NCI-H1975 xenograft model)	S01486R03	Intraperitoneal injection	Amgen Inc.	R2005184
The Effects of Panitumumab and Taxotere Combination in NCI H1650, EGFR Mutant NSCLC, Xenograft	Mouse (NCI-H1650 xenograft model)	S01486R03	Intraperitoneal injection	Amgen Inc.	R2005182

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Secondary Pharmacodynamics					
Evaluation of ABX-EGF Monotherapies and Combinations with Taxotere or Cisplatin Against H1299 and SKMES Human Non-Small Cell Lung Carcinoma Xenografts in Athymic Nude Mice	Mouse (SKMES and H1299 NSCLC xenograft models)	3737/TFP-99059A	Intraperitoneal injection	/	R2003092 — No. 3)
Test of Potency of ABX-EGF Used in In Vivo Studies with Pancreatic and Lung Tumors	ABX-EGF solutions	2001 Research Material	In vitro	Abgenix Inc.	R2003093
Effects of ABX-EGF and Taxotere Against Established SK-MES PD, a Human Squamous Cell Carcinoma in CD1 Nude Mice	Mouse (SKMES xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003170
Effects of ABX-EGF in Combination with Anti-VEGF mAb Against Established NCI-H1299, a Human Squamous Cell Carcinoma, in CD1 Nude Mice	Mouse (NCI-H1299 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003548
Effects of ABX-EGF in Combination with Anti-VEGF mAB Against SK-MES PD, a Human Squamous Cell Carcinoma in CD1 Nude Mice	Mouse (SKMES xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003547

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Secondary Pharmacodynamics					
Effects of ABX-EGF in Combination with Herceptin Against Established A549, a Human Lung Carcinoma in Female HSD Athymic Nude Mice	Mouse (A549 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004280
Effects of ABX-EGF in Combination with Herceptin Against Established NCI-H1299, a Human Squamous Cell Carcinoma, in HSD Athymic Nude Mice	Mouse (NCI-H1299 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004082
Effects of ABX-EGF in Combination with Herceptin Against Established SKMES PD, a Human Squamous Cell Carcinoma in CD1 Nude Mice	Mouse (SKMES xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004083
Effects of ABX-EGF in Combination with Low-Dose Rapamycin Against Established NCI-H1299, a Human Non-Small Cell Carcinoma in HSD Athymic Nude Mice	Mouse (NCI-H1299 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004281

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Secondary Pharmacodynamics					
The Effects of ABX-EGF in Combination With Rapamycin in SK-MES-PD Human Lung Cancer Tumor Bearing Athymic Nude Mice	Mouse (SKMES xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004282
Effects of ABX-EGF in Combination with Rapamycin Against Established NCI-H1299, a Squamous Cell Carcinoma in HSD Athymic Nude Mice	Mouse (NCI-H1299 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004084
Effects of ABX-EGF in Combination with Low-Dose Rapamycin Against Established NCI-H1299, a Human Non-Small Cell Carcinoma in HSD Athymic Nude Mice	Mouse (NCI-H1299 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004287
Effects of ABX-EGF Combined with Anti-VEGF Antibody Against Established MDA-MB-468, Human Breast Carcinoma Model in Athymic Nude Mice	Mouse (MDA-MB-468 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2003518
Effects of ABX-EGF and Herceptin on the Growth of Established BT-474 Mammary Carcinoma in Female Nude Mice	Mouse (BT474 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004279

Table 2.6.3-1. Pharmacology Overview
Test Article: Panitumumab

Type of Study	Test System	Test Article Lot Number	Method of Administration	Testing Facility	Study Number
Secondary Pharmacodynamics					
Effects of ABX-EGF Combined with Rapamycin Against Established MDA-MB-468 in Athymic Nude Mice, Human Breast Carcinoma Model	Mouse (MDA-MB-468 xenograft model)	9099-58G	Intraperitoneal injection	Amgen Inc.	R2004087
Evaluation of ABX-EGF Monotherapies and Combinations with Genzar Against MiaPaCa and Panc-1 Human Pancreatic Carcinoma Xenografts in Athymic Nude Mice	Mouse (MiaPaCa and Panc-1 xenograft models)	Not Documented	Intraperitoneal injection	/	R2003091 No. 2)
Effects of ABX-EGF and Doxorubicin Combination Therapy on Prostate DU145 Tumor Xenografts	Mouse (DU145 xenograft model)	LO1616/75	Intraperitoneal injection	Abgenix Inc.	R2003209
Safety Pharmacology					
Cardiovascular, Respiratory and Central Nervous System Assessment of ABX-EGF (Panitumumab) Administered as a Single Intravenous Dose to Conscious Cynomolgus Monkeys	Monkey	954A023447	Intravenous injection	/	104119

Table 2.6.3-2. Safety Pharmacology
Test Article: Panitumumab

Type of Study	Species/ Strain	Method of Administration	Doses (mg/kg)	Gender and No. per Group	Organ System Evaluated	Noteworthy Findings	GLP Compliance	Study Number
Safety Pharmacology								
	Cynomolgus Monkey	IV (single dose)	0 (Vehicle), 7.5, 30, 60	4M	Cardiovascular	No treatment-related effects on evaluated cardiovascular parameters (heart rate, systolic pressure, diastolic pressure, mean arterial pressure, PR interval, QRS interval, RR interval, QT interval, and QTc interval). NOAEL: > 60 mg/kg	Yes	104119 (04.6567)
					Respiratory	No treatment-related effects on evaluated respiratory parameters (respiratory rate, tidal volume, minute volume). NOAEL: > 60 mg/kg		
					Central Nervous System (CNS)	No treatment-related effects on evaluated CNS parameters (mental status/activity level, muscle tone, muscle movements, patellar reflex, position of eyelids, pupil size, eye movements, lacrimation, salivation, posture, locomotor activity, motor function). No treatment-related changes in body temperature. NOAEL: > 60 mg/kg		
					Toxicokinetics: Mean (SD) (preliminary unaudited data)	Dose (mg/kg)[a]: 0 (Vehicle); 7.5; 30; 60 Concentration (µg/mL)[a]: 0 (0); 104.3 (8.8); 435.7 (32.3); 803.4 (51.4)		

[a] Serum concentration measured at 24 hours postdose; n = 4. IV = intravenous; NOAEL = no observable adverse effect level; SD = standard deviation.

APPENDIX 4 – TABULATED SUMMARY OF PRECLINICAL PHARMACOKINETIC STUDIES CONDUCTED IN SUPPORT OF PANITUMUMAB (VECTIBIX ™)

The following tables were copied directly from Module 2, section 2.6.5 of the electronic CTD submission, as provided by the sponsor to the BLA application.

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Table 2.6.5-1. Overview of Pharmacokinetics Studies
Test Article: Panitumumab

Title (Study No.)	Study Type / GLP Status	Test Facility	Species	Material	Route	Study Design	PK Assay
Toxicity, Tissue Binding, and Pharmacokinetics of ABX-EGF Following Single and Multiple Intravenous Bolus Dose Administration in Cynomolgus Monkeys (BQAW-100; ABX-EGF-99-001)	Single dose and multiple dose toxicity/ PK was non-GLP	/	Monkey	Hyb	IV	• 3-5/sex/group • 0, 0.3, 3, 30 mg/kg (with LD 2x MD) QW for 4 weeks • Without supportive fluids	ELISA
Toxicity and Toxicokinetics of ABX-EGF Following Multiple Intravenous Bolus Dose Administration (via Bolus and Infusion) in Cynomolgus Monkeys (BQAW-102, ABX-0302)	Single dose and multiple dose/ PK was non-GLP	/	Monkey	Hyb	IV	• 6 males/group with the exception of the control group (5 males and 1 female) • 0, 3, 30 mg/kg (with LD 2x MD) QW for 4 weeks • With or without supportive fluids	ELISA
Toxicity and Toxicokinetics of ABX-EGF Following Intravenous Bolus Administration in Cynomolgus Monkeys for Three Months with a Six-Week Recovery (BQAW-103; ABX-0303)	Single and multiple dose/ PK was non-GLP	—	Monkey	Hyb	IV	• 5/sex/group • 0, 3, 7.5, 15 mg/kg QW for 12 weeks • With supportive fluids	ELISA

ELISA: Enzyme-linked immunosorbent assay
Hyb: Hybridoma
IV: Intravenous
LD: Loading dose
MD: Maintenance dose
QW: Once per week

Table 2.6.5-1. Overview of Pharmacokinetics Studies
Test Article: Panitumumab

Title (Study No.)	Study Type / GLP Status	Test Facility	Species	Material	Route	Study Design	PK Assay
A Four-Week Mechanistic Toxicity Study of ABX-EGF Administered Once Per Week by Intravenous Injection to Cynomolgus Monkeys Followed by a Two-Month Recovery Period (054.0401; ~ 00-3691; ABX-T0305)	Single and multiple dose/ PK was non-GLP	/	Monkey	Hyb	IV	• 28 males/group with the exception of the control group (6 males) • 0 or 30 mg/kg (with LD 2xMD) QW • With supportive fluids	ELISA
Comparison of the Pharmacokinetics of ABX-EGF derived from Hybridoma and Chinese Hamster Ovary (CHO) Expression Systems in Cynomolgus Monkeys After Intravenous Administration (102876; — .026.39; ABX-P0302)	Single dose / PK was GLP	—	Monkey	Hyb or CHO	IV	• 12 males/group • 7.5 mg/kg • With supportive fluids	ECL
A 4-Week Comparison Study of Two Forms of ABX-EGF Administered by Intravenous Injection Once Per Week to Cynomolgus Monkeys With a 4-Week Recovery Period (102906; ~ J2-3032; ABX-T0307)	Single dose and multiple dose / PK was GLP	/	Monkey	Hyb or —CHO	IV	• 4/sex/group • 0, 7.5, 30 mg/kg QW for 4 wks • With supportive fluids	ECL

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CHO: Chinese hamster ovary
ECL: Electrochemiluminescence
ELISA: Enzyme-linked immunosorbent assay
Hyb: Hybridoma
IV: Intravenous
LD: Loading dose
MD: Maintenance dose
QW: Once per week

Table 2.6.5-1. Overview of Pharmacokinetics Studies
Test Article: Panitumumab

Title (Study No.)	Study Type / GLP Status	Test Facility	Species	Material	Route	Study Design	PK Assay
An Assessment of the Effects of ABX-EGF on Female Fertility and Early Embryonic Development to Implantation When Administered by Weekly Intravenous Injection to Cynomolgus Monkeys (103409; — .026.56; ABX-T0309)	Single and multiple dose / PK was GLP	—	Monkey	— CHO	IV	• 9-12 females/group • 0, 7.5, 15, 30 mg/kg QW over 2 menstrual cycles, during the mating period, and up to gestation day 20 or 25 (early pregnancy) • With supportive fluids	ECL
An Assessment of the Effects of ABX-EGF on Embryo-Fetal Development When Administered Weekly by Intravenous Injection to Pregnant Cynomolgus Monkeys (103410: — .026.57; ABX-T0310)	Single and multiple dose / PK was GLP	—	Monkey	CHO	IV	• 5-18 females/group • 0, 7.5, 15, 30 mg/kg QW for 5 wks from gestation day 20 • With supportive fluids	ECL
A Six-Month Multiple Dose Toxicity Study of ABX-EGF Administered Intravenously to Cynomolgus Monkeys Followed by a Two-Month Recovery Period (103419; — .243.15; ABX-T0308)	Single and multiple dose / PK was GLP	—	Monkey	— CHO	IV	• 6/sex/group • 0, 7.5, 15, 30 mg/kg QW for 26 wks • With supportive fluids	ECL

CHO: Chinese hamster ovary
ECL: Electrochemiluminescence
IV: Intravenous
QW: Once per week
QWBA: Quantitative whole-body autoradiography

Table 2.6.5-1. Overview of Pharmacokinetics Studies
Test Article: Panitumumab

Title (Study No.)	Study Type / GLP Status	Test Facility	Species	Material	Route	Study Design	PK Assay
Quantitative Whole Body Autoradiography of Cynomolgus Monkeys After a Single Intravenous Administration of ^{125}I-ABX-EGF (103619; [redacted] 6271-611; ABX-T0311)	Single dose / PK was GLP	—	Monkey	— CHO	IV	• 4/sex • 7.5 mg/kg	QWBA; SSC
Distribution and Excretion of ^{125}I-ABX-EGF After a Single Intravenous Administration of Cynomolgus Monkeys (103620; [redacted] 6271-612; ABX-T0312)	Single dose / PK was GLP	—	Monkey	— CHO	IV	• 4/sex • 7.5 mg/kg	SSC
A 3- Month Intravenous Toxicology Study of ABX-EGF in Cynomolgus Monkeys with a 6- Week Recovery Period (103917; [redacted] 03-3060; ABX-T0311)	Single and multiple dose / PK was GLP	—	Monkey	— CHO or — CHO	IV	• 3-5/sex/group with exception of the Q2W group (3 males and 3 females) • 0, 7.5, 30 mg/kg QW • 7.5 mg/kg Q2W	ECL
Cardiovascular, Respiratory and Central Nervous System Assessment of ABX-EGF (Panitumumab) Administered as a Single Intravenous Dose to Conscious Cynomolgus Monkeys (104119; [redacted] 04-6567; ABX-P0307)	Single dose / PK was GLP	/	Monkey	— CHO	IV	• 4 males/group • 0, 7.5, 30, 60 mg/kg	ECL

CHO: Chinese hamster ovary
ECL: Electrochemiluminescence
ELISA: Enzyme-linked immunosorbent assay
IV: Intravenous
SSC: Solid scintillation counting
QW: Once per week
QWBA: Quantitative whole-body autoradiography

Table 2.6.5-1. Overview of Pharmacokinetics Studies
Test Article: Panitumumab

Title (Study No.)	Study Type / GLP Status	Test Facility	Species	Material	Route	Study Design	PK Assay
Comparison of the Pharmacokinetics of Hybridoma-origin versus CHO-origin ABX-EGF in Mice Following Intravenous Injection (104273; 054.0501; ABX-P0302)	Single dose / PK was non-GLP	Immunex	CD-1 Mouse	Hyb or Research CHO	IV	• 36 females/group, 10 female/pre-dose group • 0, 0.1, 0.5 mg/mouse	ELISA
Distribution (Quantitative Whole-Body Autoradiography) and Excretion of Radioactivity in Monkeys Following Administration of a Single Intravenous Dose of ABX-EGF and [^{125}I]ABX-EGF (104274; —— 7153-105; ABX-T0304)	Single dose / PK was GLP	—	Monkey	Hyb	IV	• 4/sex • 6 mg/kg	QWBA; SSC
A Pharmacokinetic Study of ABX-EGF in Male Nude Mice Following a Single Intraperitoneal Injection (104275; ABX-P0306)	Single dose / PK was non-GLP	—	Nude mouse	— CHO	IV	• 15 males/group • 5.22, 522 µg/mouse	ECL
Immunohistological Evaluation of ABX-EGF Administration and Penetration in Tumors from Nude Mice with Established A-431 Xenografts (106194; R2004496)	Single and multiple dose / PK was non-GLP	—	A-431 tumor bearing mouse	hIgG$_2$ or — CHO	IP	• 30 females/group • 0.5 mg/kg hIgG$_2$ Q2W • 0.02, 0.2, 0.5 mg/kg panitumumab Q2W	ECL

CHO: Chinese hamster ovary
ECL: Electrochemiluminescence
ELISA: Enzyme-linked immunosorbent assay
hIgG$_2$: Human immunoglobulin G$_2$ control antibody
IP: Intraperitoneal
SSC: Solid scintillation counting
Q2W: Twice per week
QWBA: Quantitative whole-body autoradiography

Table 2.6.5-3. Pharmacokinetics: Absorption After a Single Dose
Test Article: Panitumumab

Material	Dose (mg/kg)[1]	Sex	n	Mean (SD) PK Parameters			
				$C_{0.5\ hr}$ (µg/mL)[1]	$AUC_{0-\infty}$ (µg•d/mL)[1]	$t_{1/2}$ (day)	CL (mL/day/kg)[1]
102876 (Monkey)							
CHO	7.5	M	12	227 (31)	619 (83)[2]	NC	11.8 (2.4)[3]
Hyb	7.5	M	12	256 (56)	664 (143)[2]	NC	12.3 (1.4)[3]
103619 (Monkey)							
CHO	7.5	M / F	6	171 (13)[4]	352 (40)[5]	1.85 (0.64)	21.5 (2.5)
103620 (Monkey)							
CHO	7.5	M / F	6	170 (44)[4]	361 (62)[5]	2.07 (0.80)	21.3 (3.7)
104273; 054.0501 (CD-1 mouse)							
Research CHO	0.1 mg	F	36	54.8 (NC)	791 (NC)	11.4 (NC)	0.126 (NC)[6]
Hyb	0.1 mg	F	36	58.8 (NC)	1030 (NC)	12.6 (NC)	0.0972 (NC)[6]
Research CHO	0.5 mg	F	36	229 (NC)	3180 (NC)	13.5 (NC)	0.157 (NC)[6]
Hyb	0.5 mg	F	36	179 (NC)	2760 (NC)	10.3 (NC)	0.181 (NC)[6]
104274 (Monkey)							
Hyb	6	M / F	4	152 (NC)[4]	344 (NC)[5]	2.05 (NC)	17.0 (NC)
104275 (Nude mouse)							
CHO	5.22 µg	M	15	1.73 (NC)	39.7 (NC)	16.2 (NC)	0.132 (NC)[6]
CHO	522 µg	M	15	181 (NC)	3840 (NC)	15.7 (NC)	0.136 (NC)[6]

Mean values are presented to 3 significant figures; standard deviation values are presented to the same precision as the mean.
PK parameters were not calculated for Study 104119 as concentrations from sparse sampling were only available.

CHO: Chinese hamster ovary; Hyb: Hybridoma; NC: Not calculated
[1]Unless otherwise noted.
[2]AUC_{0-all}
[3]Based on AUC_{0-all}
[4](µg equivalents [^{125}I]panitumumab/g)
[5](µg equivalents [^{125}I]panitumumab•d/g)
[6](mL/day)

Table 2.6.5-4. Pharmacokinetics: Absorption in Monkeys After Repeated Doses
Test Article: Panitumumab

Material	Dose (mg/kg)	Sex	n	Mean (SD) PK Parameters		
				$C_{2.5\ hr}$ (µg/mL)	$AUC_{0\text{-}tau}$ (µg•d/mL)	$t_{1/2}$ (day)
ABX-EGF-99-001; BQAW-100						
	Day 1					
Hyb	0.6	M / F	6	16.4 (2.3)	14.0 (2.6)	0.680 (0.120)
Hyb	6	M / F	6	114 (15)	400 (91)	3.29 (1.09)
Hyb	60	M / F	10	1300 (220)	8130 (3840)	6.16 (2.77)
	Day 22					
Hyb	0.3	M / F	4	4.70 (1.30)	1.90 (1.20)	0.270 (0.0700)
Hyb	3	M / F	6	70.7 (22.5)	169 (102)	2.94 (2.22)
Hyb	30	M / F	8	1040 (300)	4130 (1100)	5.32 (1.40)
ABX-0302; BQAW-102						
	Day 1					
Hyb	6[1]	M	6	145 (22)	402 (116)	2.64 (0.41)
Hyb	60	M	6	1600 (250)	5050 (590)	5.06 (0.85)
Hyb	60[1]	M	6	1710 (280)	5350 (800)	5.35 (1.40)
Hyb	60[2]	M	6	1690 (370)	4210 (1170)	5.60 (2.02)
	Day 22					
Hyb	3[1]	M	6	61.5 (39.8)	61.0 (64.9)	0.900 (0.750)
Hyb	30	M	4	1090 (290)	3680 (1530)	3.53 (2.46)
Hyb	30[1]	M	6	1320 (310)	3680 (790)	4.16 (1.72)
Hyb	30[2]	M	4	921 (442)	1360 (1180)	1.17 (0.80)

Mean values are presented to 3 significant figures: standard deviation values are presented to the same precision as the mean.
PK parameters were not calculated for Studies J0-3691 (054.0401) and 106194 as concentrations from sparse sampling were only available.

CHO: Chinese hamster ovary; Hyb: Hybridoma; tau: 7 days.
[1]Anti-diarrhea lactated ringers and PRN severe diarrhea were given as supportive therapy.
[2]Dose was given as a 2-hour IV infusion. Anti- diarrhea lactated ringers and PRN electrolyte imbalance were given as supportive therapy.

Table 2.6.5-4. Pharmacokinetics: Absorption in Monkeys After Repeated Doses
Test Article: Panitumumab

Material	Dose (mg/kg)	Sex	n	Mean (SD) PK Parameters		
				$C_{0.5\ hr}$ (µg/mL)	$AUC_{0\text{-}tau}$ (µg•d/mL)	$t_{1/2}$ (day)
ABX-0303; BQAW-103						
	Day 1					
Hyb	3	M / F	10	53.6 (5.5)	69.8 (11.9)	0.97 (0.32)
Hyb	7.5	M / F	10	122 (15)	341 (27)	4.23 (2.33)
Hyb	15	M / F	10	307 (71)	803 (109)	5.02 (5.09)
	Day 78					
Hyb	3	M / F	10	15.6 (19.0)	11.0 (24.1)	0.89 (1.79)
Hyb	7.5	M / F	10	157 (94)	326 (183)	1.66 (1.15)
Hyb	15	M / F	10	445 (205)	1030 (430)	3.43 (2.11)

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Mean values are presented to 3 significant figures: standard deviation values are presented to the same precision as the mean.
PK parameters were not calculated for Studies J0-3691 (054.0401) and 106194 as concentrations from sparse sampling were only available.
Hyb: Hybridoma; LD: Loading dose; MD: Maintenance dose; NC: Not calculated

Table 2.6.5-4. Pharmacokinetics: Absorption in Monkeys After Repeated Doses
Test Article: Panitumumab

Material	Dose (mg/kg)	Day	Sex	Mean (SD) PK Parameters								
				All animals			MAHA negative animals			MAHA positive animals		
				n	$C_{0.5\ hr}$ (µg/mL)	$AUC_{0\text{-}tau}$ (µg·day/mL)	n	$C_{0.5\ hr}$ (µg/mL)	$AUC_{0\text{-}tau}$ (µg·day/mL)	n	$C_{0.5\ hr}$ (µg/mL)	$AUC_{0\text{-}tau}$ (µg·day/mL)
102906												
Hyb	7.5	0	M / F	8	184 (29)	493 (116)	2	179 (NC)	473 (NC)	6	186 (30)	449 (117)
CHO	7.5	0	M / F	8	231 (45)	615 (123)	3	229 (59)	608 (182)	5	231 (42)	619 (100)
Hyb	30	0	M / F	8	794 (141)	2740 (530)	4	820 (148)	2980 (317)	4	769 (151)	2490 (640)
CHO	30	0	M / F	8	891 (282)	3060 (930	6	811 (175)	2640 (397)	2	1130 (500)	4300 (1080)
Hyb	7.5	21	M / F	8	167 (39)	197 (297)	2	217 (NC)	625 (NC)	6	146 (23)	26.0 (54.8)
CHO	7.5	21	M / F	8	205 (74)	423 (355)	3	252 (81)	718 (271)	5	177 (60)	246 (282)
Hyb	30	21	M / F	8	997 (363)	2480 (1700)	4	1140 (300)	3390 (1180)	4	810 (413)	1270 (1640)
CHO	30	21	M / F	8	851 (262)	2410 (1330)	6	863 (288)	3060 (690)	2	817 (250)	485 (116)

Mean values are presented to 3 significant figures; standard deviation values are presented to the same precision as the mean.
PK parameters were not calculated for Studies 20-3691 (054.0401) and 106194 as concentrations from sparse sampling were only available.
Animal was considered positive for monkey anti-human antibody (MAHA) response if 1 or more of the MAHA samples collected after Day 1 were positive.
MAHA: Monkey anti-human antibody; CHO: Chinese hamster ovary; Hyb: Hybridoma; tau: 7 days; NC: Not calculated

Table 2.6.5-4. Pharmacokinetics: Absorption in Monkeys After Repeated Doses
Test Article: Panitumumab

Material	Dose (mg/kg)	Day	Sex	Mean (SD) PK Parameters: All animals n	All animals $C_{0.5\ hr}$ (µg/mL)	All animals AUC_{0-tau} (µg·day/mL)	MAHA negative animals n	MAHA negative animals $C_{0.5\ hr}$ (µg/mL)	MAHA negative animals AUC_{0-tau} (µg·day/mL)	MAHA positive animals n	MAHA positive animals $C_{0.5\ hr}$ (µg/mL)	MAHA positive animals AUC_{0-tau} (µg·day/mL)
103409												
Pre-mating cycle												
CHO	7.5	—	F	12	230 (31)	719 (124)	7	239 (24)	755 (126)	5	218 (37)	670 (114)
CHO	15	—	F	7[a]	455 (87)	1470 (420)	5	483 (87)	1570 (460)	2	386 (NC)	1208 (NC)
CHO	30	—	F	12	951 (199)	3670 (570)	6	1020 (190)	3830 (540)	6	881 (203)	3500 (6590)
First dose of mating cycle												
CHO	7.5	—	F	6	112 (122)	333 (409)	3	220 (52)	665 (294)	3	5.00 (4.00)	0.00 (NC)
CHO	15	—	F	6	396 (191)	1240 (660)	4	465 (81)	1520 (310)	2	257 (NC)	688 (NC)
CHO	30	—	F	6	631 (308)	1320 (1510)	2	734 (NC)	2250 (NC)	4	580 (384)	858 (1710)
Last dose of mating cycle												
CHO	7.5	—	F	1	273 (NC)	958 (NC)	1	273 (NC)	958 (NC)	0	ND	ND
CHO	15	—	F	2	410 (NC)	1450 (NC)	2	410 (NC)	1450 (NC)	0	ND	ND
CHO	30	—	F	1	150 (NC)	2.00 (NC)	0	ND	ND	1	150 (NC)	2.00 (NC)
103410												
CHO	7.5	GD20	F	15	258 (50)	808 (145)	10	261 (54)	818 (166)	5	252 (47)	788 (103)
CHO	15	GD20	F	18	461 (91)	1630 (347)	5	502 (71)	1740 (181)	13	446 (95)	1580 (390)
CHO	30	GD20	F	4[b]	1050 (200)	3740 (970)	3	1040 (250)	3970 (1060)	1	1060 (NC)	3080 (NC)
CHO	7.5	GD48	F	11	284 (45)	736 (330)	6	305 (39)	917 (228)	5	258 (40)	519 (315)
CHO	15	GD48	F	15	577 (166)	2100 (1180)	2	640 (NC)	2770 (NC)	13	567 (175)	1990 (1210)
CHO	30	GD48	F	2	1370 (NC)	5360 (NC)	1	1650 (NC)	7530 (NC)	1	1090 (NC)	3190 (NC)

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Mean values are presented to 3 significant figures; standard deviation values are presented to the same precision as the mean.
PK parameters were not calculated for Studies , 00-3691 (054.0401) and 106194 as concentrations from sparse sampling were only available.
Animal was considered positive for monkey anti-human antibody (MAHA) response if 1 or more of the MAHA samples collected after Day 1 were positive. CHO: Chinese hamster ovary; tau: 7 days; NC: Not calculated; ND: No data; GD: Gestation day
[a]Nine animals were in this group; however, 2 animals (1 that had received 30 mg/kg and 1 that had no measurable concentrations) were excluded from the toxicokinetic summary statistics for this group.
[b]Five animals had toxicokinetic samples collected; however, 1 animal had a fetal abortion or fetal death resulting in insufficient data for analysis.

Table 2.6.5-4. Pharmacokinetics: Absorption in Monkeys After Repeated Doses
Test Article: Panitumumab

Material	Dose (mg/kg)	Day	Sex	Mean (SD) PK Parameters								
				All animals			MAHA negative animals			MAHA positive animals		
				n	$C_{0.5\ hr}$ (µg/mL)	AUC_{0-tau} (µg·day/mL)	n	$C_{0.5\ hr}$ (µg/mL)	AUC_{0-tau} (µg·day/mL)	n	$C_{0.5\ hr}$ (µg/mL)	AUC_{0-tau} (µg·day/mL)
103419												
CHO	7.5	1	M / F	12	261 (66)	649 (133)	7	245 (61)	639 (162)	5	284 (72)	663 (96)
CHO	15	1	M / F	12	676 (253)	1650 (440)	10	684 (252)	1680 (440)	2	635 (362)	1510 (570)
CHO	30	1	M / F	12	1080 (410)	3440 (990)	11	1010 (330)	3310 (930)	1	1880 (NC)	4890 (NC)
CHO	7.5	176	M / F	10[a]	157 (125)	NR	6	250 (51)	774 (259)	4	19.0 (13.0)	NR
CHO	15	176	M / F	4[b]	378 (255)	NR	3	504 (53)	1660 (270)	1	0.00 (NC)	NR
CHO	30	176	M / F	6[c]	793 (433)	NR	6	793 (433)	3260 (1300)	0	ND	ND

Mean values are presented to 3 significant figures; standard deviation values are presented to the same precision as the mean.
PK parameters were not calculated for Studies J0-3691 (054.0401) and 106194 as concentrations from sparse sampling were only available.
Animal was considered positive for monkey anti-human antibody (MAHA) response if 1 or more of the MAHA samples collected after Day 1 were positive.
CHO: Chinese hamster ovary; tau: 7 days; NC: Not calculated; NR: Not reported, AUC was not calculated for MAHA-positive animals; ND: No data

[a]2 animals in the 7.5-mg/kg group were euthanized early (see Table 6 in Section 2.6.6.3.5); the data for these animals were not included in the table.

[b]8 animals in the 15-mg/kg group were euthanized early or died or placed on early recovery (see Table 6 and Table 7 in Section 2.6.6.3.5); the data for these animals were not included in the table.

[c]6 animals in the 30-mg/kg group were euthanized early (see Table 6 in Section 2.6.6.3.5); the data for these animals were not included in the table.

Table 2.6.5-4. Pharmacokinetics: Absorption in Monkeys After Repeated Doses
Test Article: Panitumumab

Material	Dose (mg/kg)	Day	Sex	Mean (SD) PK Parameters: All animals n	All animals $C_{0.5\ hr}$ (µg/mL)	All animals $AUC_{0\text{-}tau}$ (µg·day/mL)	MAHA negative animals n	MAHA negative animals $C_{0.5\ hr}$ (µg/mL)	MAHA negative animals $AUC_{0\text{-}tau}$ (µg·day/mL)	MAHA postive animals n	MAHA postive animals $C_{0.5\ hr}$ (µg/mL)	MAHA postive animals $AUC_{0\text{-}tau}$ (µg·day/mL)
103917												
CHO, filtered	7.5	1	M / F	6	221 (90)	555 (130)	2	187 (NC)	497 (NC)	4	238 (108)	585 (129)
CHO, filtered	7.5	1	M / F	6	232 (26)	592 (67)	3	235 (32)	579 (14)	3	230 (25)	606 (102)
CHO, filtered[1]	7.5	1	M / F	6	223 (41)	704 (129)	2	236 (NC)	771 (NC)	4	217 (39)	670 (131)
CHO, filtered	30	1	M / F	10	972 (119)	2970 (400)	8	970 (126)	2930 (390)	2	980 (NC)	3130 (NC)
CHO, unfiltered	30	1	M / F	6	827 (172)	2790 (700)	2	621 (NC)	2020 (NC)	4	931 (67)	3180 (460)
CHO, filtered	30	1	M / F	10	837 (73)	2540 (300)	7	855 (69.0)	2630 (330)	3	794 (77)	2350 (80)
CHO, unfiltered	30	1	M / F	6	943 (224)	2890 (250)	5	957 (247)	2880 (280)	1	874 (NC)	2980 (NC)
CHO, filtered	7.5	29	M / F	6	234 (67.4)	651 (381)	2	237 (NC)	887 (NC)	4	233 (81)	533 (407)
CHO, filtered	7.5	29	M / F	6	222 (87.5)	442 (379)	3	286 (25.0)	758 (171)	3	159 (80)	127 (180)
CHO, filtered[1]	7.5	29	M / F	6	173 (44.3)	219 (239)	2	212 (NC)	509 (NC)	4	153 (39)	74.1 (55.3)
CHO, filtered	30	29	M / F	10	1340 (260)	4500 (1370)	8	1310 (290)	4360 (1510)	2	1440 (NC)	5040 (NC)
CHO, unfiltered	30	29	M / F	6	1150 (430)	2510 (1940)	2	1290 (NC)	3880 (NC)	4	1080 (500)	1830 (1820)
CHO, filtered	30	29	M / F	10	964 (361)	2830 (2040)	7	1110 (340)	3870 (1430)	3	633 (109)	411 (153)
CHO, unfiltered	30	29	M / F	6	1180 (310)	3990 (2400)	5	1240 (310)	4680 (1900)	1	868 (NC)	538 (NC)
CHO, filtered	7.5	85	M / F	6	197 (117)	430 (426)	2	254 (NC)	769 (NC)	4	168 (139)	261 (380)
CHO, filtered	7.5	85	M / F	6	157 (142)	389 (431)	3	282 (37.0)	778 (107)	3	31.9 (46.9)	0.333 (0.493)
CHO, filtered[1]	7.5	85	M / F	6	74.4 (95.0)	173 (269)	2	196 (NC)	517 (NC)	4	13.5 (9.9)	0.125 (0.096)
CHO, filtered	30	85	M / F	10	1310 (400)	3520 (1580)	8	1370 (410)	3800 (1340)	2	1090 (NC)	2420 (NC)
CHO, unfiltered	30	85	M / F	6	1110 (700)	1860 (1940)	2	1420 (NC)	3320 (NC)	4	949 (843)	1130 (1860)
CHO, filtered	30	85	M / F	10	1010 (390)	2800 (1930)	7	1220 (180)	3960 (610)	3	516 (279)	105 (89)
CHO, unfiltered	30	85	M / F	6	1090 (420)	3470 (1980)	5	1250 (190)	4140 (1250)	1	315 (NC)	117 (NC)

Mean values are presented to 3 significant figures. Standard deviation values are presented to the same precision as the mean.
PK parameters were not calculated for Studies 00-3691 (054.0401) and 106194 as concentrations from sparse sampling were only available.
Animal was considered positive for monkey anti-human antibody (MAHA) response if 1 or more of the MAHA samples collected after Day 1 were positive.
tau: 7 days; NC: Not calculated
[1]Given Q2W

Table 2.6.5-5a. Pharmacokinetics: Organ Distribution
Test Article: Panitumumab

Study No: 103619
Species: Cynomolgus Monkeys
Sex (N): 1 male, 1 female sacrificed per time point
Radionucleotide: ^{125}I
Dose (route): 7.5 mg/kg; 11.1 µCi/mg (IV)
Method: quantitative whole-body autoradiography; solid scintillation counting

	µg Equivalents ^{125}I-panitumumab/g			
	Sacrifice Time			
Tissue	2 hr	48 hr	120 hr	216 hr
Adrenal gland	36.7	14.1	7.87	2.44
Bile	14.2	3.64	1.77	0.579
Blood	67.1	38.4	13.6	3.57
Bone	2.36	1.55	0.705	0.316
Bone marrow	22.1	5.19	2.81	1.03
Cerebellum	1.59	0.679	0.333	0.164
Cerebrum	1.20	0.473	0.234	0.125
Diaphragm	11.1	5.78	1.79	0.720
Epididymis[a]	5.38	6.35	2.89	1.34
Esophageal contents	9.37	5.13	7.88	0.528
Esophagus	17.9	10.7	9.20	1.38
Eye	1.58	1.98	1.12	0.496
Fat (abdominal)	12.0	1.74	1.54	0.534
Fat (brown)	12.2	6.58	4.88	0.734
Gall bladder	29.1	9.20	7.37	1.13
Kidney	35.1	13.0	5.80	2.09
Large intestinal contents	1.20	1.80	0.689	0.328

Values are presented to 3 significant figures.
[a] n = 1 (male only)

Table 2.6.5-5a. Pharmacokinetics: Organ Distribution
Test Article: Panitumumab

Study No: 103619
Species: Cynomolgus Monkeys
Sex (N): 1 male, 1 female sacrificed per time point
Radionucleotide: ^{125}I
Dose (route): 7.5 mg/kg; 11.1 μCi/mg (IV)
Method: quantitative whole-body autoradiography; solid scintillation counting

	μg Equivalents ^{125}I-panitumumab/g			
	Sacrifice Time			
Tissue	2 hr	48 hr	120 hr	216 hr
Large intestine	5.62	6.51	2.04	0.927
Liver	65.4	20.1	9.25	2.86
Lung	67.9	26.0	10.8	2.80
Medulla	1.53	0.502	0.341	0.163
Muscle	1.05	0.803	0.366	0.175
Myocardium	21.4	9.59	4.49	0.975
Nasal turbinates	39.4	7.33	3.97	1.15
Olfactory lobe	2.55	1.91	0.897	0.274
Ovary[b]	24.9	21.0	5.62	2.40
Pancreas	11.5	5.54	2.59	0.939
Parotid gland	4.37	4.44	2.32	0.890
Pituitary gland	7.60	5.97	2.82	0.752
Prostate[a]	3.24	5.86	2.37	1.19
Renal cortex	34.5	12.8	5.58	2.01
Renal medulla	45.8	15.0	8.01	2.88
Salivary gland (submandibular)	6.23	7.35	2.98	1.02
Seminal vesicle	7.17	8.06	5.29	1.62
Skin	4.44	3.79	2.16	0.908

Values are presented to 3 significant figures.
[a]n = 1 (male only)
[b]n = 1 (female only)

Table 2.6.5-5a. Pharmacokinetics: Organ Distribution
Test Article: Panitumumab

Study No: 103619
Species: Cynomolgus Monkeys
Sex (N): 1 male, 1 female sacrificed per time point
Radionucleotide: ^{125}I
Dose (route): 7.5 mg/kg; 11.1 µCi/mg (IV)
Method: quantitative whole-body autoradiography; solid scintillation counting

Tissue	µg Equivalents ^{125}I-panitumumab/g			
	Sacrifice Time			
	2 hr	48 hr	120 hr	216 hr
Small intestinal contents	4.07	1.04	0.912	0.256
Small intestine	6.74	7.16	3.07	1.34
Spinal cord	1.33	0.842	0.424	0.183
Spleen	44.9	11.7	6.35	2.19
Spleen (red pulp)	54.8	15.6	6.95	NS
Spleen (white pulp)	29.7	8.81	4.06	NS
Stomach	19.6	9.35	5.48	1.96
Stomach contents	16.0	6.49	5.16	1.69
Testis[a]	6.82	4.27	1.35	0.528
Thymus	15.2	7.16	4.12	0.912
Thyroid	7.17	136	123	186
Trachea	13.1	4.93	4.91	0.679
Urinary bladder	7.78	4.89	3.27	1.24
Urine	62.7	7.55	3.62	1.00
Uterus[b]	3.71	7.56	7.15	1.96
Uveal tract	7.58	8.41	4.99	1.84

Values are presented to 3 significant figures.
[a]n = 1 (male only)
[b]n = 1 (female only)
NS: No sample.

Table 2.6.5-5b. Pharmacokinetics: Organ Distribution
Test Article: Panitumumab

Study No: 103620
Species: Cynomolgus Monkeys
Sex (N): 1 male, 1 female sacrificed per time point
Radionucleotide: ^{125}I
Dose (route): 7.5 mg/kg; 11.1 µCi/mg (IV)
Method: Solid scintillation counting

	Radioactivity Conc. (µg Equivalents ^{125}I-panitumumab/g)				% TCA-precipitable of Total Radioactivity (%)				TCA-precipitable Radioactivity Conc. (µg Equivalents ^{125}I-panitumumab/g)			
	Sacrifice Time											
Tissue	2 hr	48 hr	120 hr	216 hr	2 hr	48 hr	120 hr	216 hr	2 hr	48 hr	120 hr	216 hr
Adrenal glands	18.4	7.88	2.50	1.17	80.8	85.4	87.5	89.5	14.9	6.73	2.18	1.05
Bladder (urinary)	10.5	4.20	2.20	1.13	NA	NA	NA	NA	NA	NA	NA	NA
Bone (femur)	3.68	2.32	0.642	0.368	NA	NA	NA	NA	NA	NA	NA	NA
Bone marrow (from femur)	4.01	4.33	1.64	0.579	NA	NA	NA	NA	NA	NA	NA	NA
Cecum	3.41	4.68	1.82	0.666	68.1	81.7	83.2	90.5	2.39	3.83	1.51	0.604
Cerebellum	1.49	0.66	0.274	0.128	80.5	84.3	87.1	95.3	1.20	0.556	0.238	0.122
Cerebrospinal fluid	0.0221	0.0600	0.0268	0.00446	82.1	92.9	122	166	0.0184	0.0560	0.0270	0.0071 1
Cerebrum	1.42	0.554	0.293	0.0991	82.5	82.5	106	91.8	1.17	0.457	0.304	0.0907
Colon	4.09	3.52	1.51	0.550	69.1	80.6	79.7	83.3	2.86	2.84	1.20	0.457
Duodenum	4.90	4.54	1.72	0.734	60.8	70.3	80.1	82.4	3.01	3.19	1.38	0.611
Epididymides[a]	6.93	5.26	2.25	0.959	71.5	83.3	84.3	87.3	4.95	4.38	1.90	0.837
Eyes (both)	1.28	1.32	0.678	0.312	NA	NA	NA	NA	NA	NA	NA	NA
Fat (brown)	2.82	3.47	1.40	0.593	65.6	79.0	83.2	87.1	1.80	2.74	1.16	0.516
Fat,(reproductive)	2.38	1.66	0.725	0.390	NA	NA	NA	NA	NA	NA	NA	NA
Gall bladder	9.15	5.96	2.43	0.858	NA	NA	NA	NA	NA	NA	NA	NA
Heart	8.57	5.67	2.36	1.02	84.7	87.0	88.2	91.0	7.25	4.93	2.08	0.925
Ileum	3.73	4.00	1.54	0.660	73.9	85.4	90.0	90.7	2.77	3.40	1.38	0.602

Values are presented to 3 significant figures.
[a]n = 1 (male only)
NA: Not analyzed.

Table 2.6.5-5b. Pharmacokinetics: Organ Distribution
Test Article: Panitumumab

Study No: 103620
Species: Cynomolgus Monkeys
Sex (N): 1 male, 1 female sacrificed per time point
Radionucleotide: ^{125}I
Dose (route): 7.5 mg/kg; 11.1 µCi/mg (IV)
Method: Solid scintillation counting

	Radioactivity Conc. (µg Equivalents ^{125}I-panitumumab/g)				% TCA-precipitable of Total Radioactivity (%) Sacrifice Time				TCA-precitable Radioactivity Conc. (µg Equivalents ^{125}I-panitumumab/g)			
Tissue	2 hr	48 hr	120 hr	216 hr	2 hr	48 hr	120 hr	216 hr	2 hr	48 hr	120 hr	216 hr
Jejunum	5.27	3.49	1.44	0.578	74.0	79.1	82.0	84.8	3.92	2.75	1.18	0.494
Kidneys	24.4	8.53	3.26	1.39	84.2	90.9	87.7	90.8	20.5	7.75	2.85	1.25
Liver	30.3	7.97	3.36	1.66	79.7	84.6	86.4	88.3	24.2	6.73	2.90	1.47
Lungs	17.8	11.0	4.53	1.86	78.0	85.9	88.3	91.0	13.9	9.46	4.00	1.69
Lymph nodes (mesenteric)	3.62	4.38	1.62	0.735	NA	NA	NA	NA	NA	NA	NA	NA
Mammary gland	1.52	2.33	1.56	0.751	69.6	70.4	89.1	83.5	1.02	1.59	1.39	0.629
Medulla oblongata	1.55	0.613	0.246	0.112	78.6	83.5	85.7	89.7	1.21	0.511	0.210	0.100
Muscle (thigh)	1.47	0.503	0.258	0.105	NA	NA	NA	NA	NA	NA	NA	NA
Nasal turbinates	9.08	5.46	1.74	0.685	71.0	81.6	78.6	87.1	6.36	4.50	1.37	0.591
Ovaries[c]	14.9	15.7	7.86	1.93	76.4	84.6	86.7	90.6	11.4	13.3	6.81	1.75
Pancreas	5.36	3.74	1.27	0.678	NA	NA	NA	NA	NA	NA	NA	NA
Parotid glands	3.30	2.44	1.56	0.567	NA	NA	NA	NA	NA	NA	NA	NA
Pituitary gland	10.2	3.83	2.03	0.787	74.8	83.5	81.4	84.0	7.63	3.16	1.66	0.660
Prostate[a]	4.44	4.26	2.44	1.19	NA	NA	NA	NA	NA	NA	NA	NA
Rectum	6.34	3.51	1.51	0.621	70.5	79.2	82.9	89.7	4.48	2.78	1.25	0.558
Salivary glands (mandibular)	4.93	3.39	1.78	0.876	NA	NA	NA	NA	NA	NA	NA	NA
Skin (dorsal shaved)	4.88	3.51	1.86	0.777	72.6	79.9	83.9	81.8	3.52	2.83	1.56	0.636

Values are presented to 3 significant figures.
[a]n = 1 (male only)
[b]n = 1 (female only)
NA: Not analyzed.

Table 2.6.5-5b. Pharmacokinetics: Organ Distribution
Test Article: Panitumumab

Study No: 103620
Species: Cynomolgus Monkeys
Sex (N): 1 male, 1 female sacrificed per time point
Radionucleotide: ^{125}I
Dose (route): 7.5 mg/kg; 11.1 µCi/mg (IV)
Method: Solid scintillation counting

Tissue	Radioactivity Conc. (µg Equivalents ^{125}I-panitumumab/g)				% TCA-precipitable of Total Radioactivity (%)				TCA-preciitable Radioactivity Conc. (µg Equivalents ^{125}I-panitumumab/g)			
	Sacrifice Time											
	2 hr	48 hr	120 hr	216 hr	2 hr	48 hr	120 hr	216 hr	2 hr	48 hr	120 hr	216 hr
Spinal cord	1.90	0.670	0.399	0.241	76.8	80.8	82.2	83.5	1.46	0.541	0.328	0.200
Spleen	17.7	5.15	2.19	1.10	74.8	82.8	82.2	87.3	13.2	4.26	1.80	0.956
Stomach	7.48	5.02	2.03	0.797	NA	NA	NA	NA	NA	NA	NA	NA
Testes[a]	5.74	2.77	1.09	0.631	72.0	78.5	76.0	85.1	4.13	2.17	0.828	0.537
Thymus	4.19	3.32	1.59	0.696	NA	NA	NA	NA	NA	NA	NA	NA
Thyroid	8.80	74.0	82.2	126	NA	NA	NA	NA	NA	NA	NA	NA
Uterus[b]	5.11	10.7	4.89	1.53	NA	NA	NA	NA	NA	NA	NA	NA

Values are presented to 3 significant figures.
[a]n = 1 (male only)
[b]n = 1 (female only)
NA: Not analyzed.

Table 2.6.5-5c. Pharmacokinetics: Organ Distribution
Test Article: Panitumumab

Study No: 104274
Species: Cynomolgus monkeys
Sex (N): 1 male, 1 female sacrificed per time point
Radionucleotide: ^{125}I
Dose (route): 6 mg/kg; 10.9 μCi/mg (IV)
Method: quantitative whole-body autoradiography; solid scintillation counting

	μg Equivalents ^{125}I-panitumumab/g			
	Sacrifice Time			
Tissue	2 hr	48 hr	96 hr	168 hr
Adrenal gland	42.1	17.8	8.95	5.58
Bile	5.16	2.80	0.942	0.733
Blood	78.7	28.1	12.6	6.40
Bone	2.66	1.62	1.30	0.508
Bone marrow	12.8	5.71	3.52	1.09
Cerebellum	1.13	0.512	0.297	0.154
Cerebrum	1.02	0.460	0.215	0.0950
Choroid/uvea	6.09	7.54	4.56	3.25
Diaphragm	8.71	4.64	2.68	1.14
Epididymis[3]	3.16	3.20	5.75	2.41
Esophageal contents	8.68	4.31	2.75	1.07
Esophagus	16.7	10.9	6.79	2.65
Eye	1.44	2.61	1.03	1.26
Fat (abdominal)	3.29	2.51	1.30	0.815
Fat (brown)	14.0	5.77	2.59	1.75
Gall bladder	16.4	16.8	4.02	2.22
Kidney	26.0	15.1	7.18	3.98
Large intestinal contents	0.542	0.815	0.323	0.149

Values are presented to 3 significant figures.
[3]n = 1 (male only)

Table 2.6.5-5c. Pharmacokinetics: Organ Distribution
Test Article: Panitumumab

Study No: 104274
Species: Cynomolgus monkeys
Sex (N): 1 male, 1 female sacrificed per time point
Radionucleotide: ^{125}I
Dose (route): 6 mg/kg; 10.9 μCi/mg (IV)
Method: quantitative whole-body autoradiography; solid scintillation counting

Tissue	μg Equivalents ^{125}I-panitumumab/g			
	Sacrifice Time			
	2 hr	48 hr	96 hr	168 hr
Large intestine	4.31	5.66	3.01	1.23
Liver	58.1	17.7	10.8	4.70
Lung	58.1	20.0	10.9	5.57
Medulla	0.969	0.452	0.215	0.117
Muscle	0.762	0.709	0.413	0.163
Myocardium	22.4	10.8	4.72	2.16
Nasal turbinates	15.2	9.84	3.95	2.36
Ovary[b]	19.7	5.11	5.71	5.93
Pancreas	8.57	7.21	2.61	1.85
Parotid gland	4.42	4.96	3.34	1.57
Pituitary gland	7.11	1.74	2.37	1.03
Prostate[a]	NR	NR	NR	2.03
Renal cortex	25.7	14.6	7.37	3.90
Renal medulla	28.4	17.6	7.69	4.42
Salivary gland	7.19	7.65	4.68	2.11
Seminal vesicle	NR	8.44	6.54	2.28
Skin	2.25	3.88	2.62	1.30

Values are presented to 3 significant figures.
[a]n = 1 (male only)
[b]n = 1 (female only)
NR Not represented (tissue not present in section)

Table 2.6.5-5c. Pharmacokinetics: Organ Distribution
Test Article: Panitumumab

Study No: 104274
Species: Cynomolgus monkeys
Sex (N): 1 male, 1 female sacrificed per time point
Radionucleotide: ^{125}I
Dose (route): 6 mg/kg; 10.9 μCi/mg (IV)
Method: quantitative whole-body autoradiography; solid scintillation counting

	μg Equivalents ^{125}I-panitumumab/g			
	Sacrifice Time			
Tissue	2 hr	48 hr	96 hr	168 hr
Skin	2.25	3.88	2.62	1.30
Small intestinal contents	2.13	0.878	0.655	0.255
Small intestine	9.73	6.59	4.08	2.12
Spinal cord	1.07	0.569	0.341	0.154
Spleen	34.0	14.1	6.87	3.52
Stomach	18.1	12.4	6.59	2.91
Stomach contents	13.9	7.55	4.68	2.25
Testes[a]	20.2	6.14	5.80	1.32
Thymus	9.77	6.66	3.16	2.02
Thyroid	13.4	43.5	89.2	92.1
Urinary bladder	9.37	7.01	4.18	2.42
Urine	18.4	4.58	2.84	0.707
Uterus[b]	3.53	6.02	4.69	3.06

Values are presented to 3 significant figures.
[a]n = 1 (male only)
[b]n = 1 (female only)

Table 2.6.5-7a. Pharmacokinetics: Study in Pregnant or Nursing Animals
Test Article: Panitumumab

Study No:	103409
Dose (Route):	7.5, 15 and 30 mg/kg (IV) given weekly over 2 menstrual cycles, during the mating period, and up to gestation day 20 or 25 (early pregnancy)
Material:	— CHO
Species:	Female Cynomolgus Monkey
Assay:	Electrochemiluminescence

	All animals			MAHA-negative animals			MAHA-positive animals		
Dose (mg/kg)	n	$C_{0.5\ hr}$ (µg/mL)	$AUC_{0\text{-}tau}$ (µg•day/mL)	n	$C_{0.5\ hr}$ (µg/mL)	$AUC_{0\text{-}tau}$ (µg•day/mL)	n	$C_{0.5\ hr}$ (µg/mL)	$AUC_{0\text{-}tau}$ (µg•day/mL)
Pre-mating Cycle									
7.5	12	230 (31)	719 (124)	7	239 (24)	755 (126)	5	218 (37)	670 (114)
15	7[a]	455 (87)	1470 (420)	5	483 (87)	1570 (460)	2	386 (NC)	1210 (NC)
30	12	951 (199)	3670 (570)	6	1020 (190)	3830 (540)	6	881 (203)	3500 (590)
First Dose of Mating Cycle									
7.5	6	112 (122)	333 (409)	3	220 (52)	665 (294)	3	5.00 (4.00)	0.00 (NC)
15	6	396 (191)	1240 (660)	4	465 (81)	1520 (310)	2	257 (NC)	688 (NC)
30	6	631 (308)	1320 (1510)	2	734 (NC)	2250 (NC)	4	580 (384)	858 (1710)
Last Dose of Mating Cycle									
7.5	1	273 (NC)	958 (NC)	1	273 (NC)	958 (NC)	0	ND	ND
15	2	410 (NC)	1460 (NC)	2	410 (NC)	1460 (NC)	0	ND	ND
30	1	150 (NC)	2.00 (NC)	0	ND	ND	1	150 (NC)	2.00 (NC)

Mean values are presented to 3 significant figures; standard deviation values are presented to the same precision as the mean.
Animal was considered positive for monkey anti-human antibody (MAHA) response if 1 or more of the MAHA samples collected after Day 1 were positive.

CHO: Chinese hamster ovary; tau: 7 days; ECL: Electrochemiluminescence; NC: Not calculated; ND: No data

[a]Nine animals were in this group; however, 2 animals (1 that had received 30 mg/kg and 1 that had no measurable concentrations) were excluded from the toxicokinetic summary statistics for this group.

Table 2.6.5-7b. Pharmacokinetics: Study in Pregnant or Nursing Animals
Test Article: Panitumumab

Study No:	103410
Dose (Route):	Panitumumab at 7.5, 15 and 30 mg/kg (IV) given weekly for 5 weeks from gestation day 20
Material:	— CHO
Species:	Female Cynomolgus Monkey
Assay:	Electrochemiluminescence

	All animals			MAHA-negative Animals			MAHA-positive animals		
Dose (mg/kg):	N	$C_{0.5\ hr}$ (µg/mL)	$AUC_{0\text{-}tau}$ (µg•day/mL)	n	$C_{0.5\ hr}$ (µg/mL)	$AUC_{0\text{-}tau}$ (µg•day/mL)	n	$C_{0.5\ hr}$ (µg/mL)	$AUC_{0\text{-}tau}$ (µg•day/mL)
Gestation Day 20									
7.5	15	258 (50)	808 (145)	10	261 (54)	818 (166)	5	252 (47)	788 (103)
15	18	461 (91)	1630 (350)	5	502 (71)	1740 (180)	13	446 (95)	1580 (390)
30	4[3]	1050 (200)	3740 (970)	3	1040 (250)	3970 (1060)	1	1060 (NC)	3080 (NC)
Gestation Day 48									
7.5	11	284 (45)	736 (330)	6	305 (39)	917 (228)	5	258 (40)	519 (315)
15	15	577 (166)	2100 (1180)	2	640 (NC)	2770 (NC)	13	567 (175)	1990 (1210)
30	2	1370 (NC)	5360 (NC)	1	1650 (NC)	7530 (NC)	1	1090 (NC)	3190 (NC)

Mean values are presented to 3 significant figures; standard deviation values are presented to the same precision as the mean.
Animal was considered positive for monkey anti-human antibody (MAHA) response if 1 or more of the MAHA samples collected after Day 1 were positive.
Milk excretion was not evaluated.
CHO: Chinese hamster ovary; tau: 7 days; NC: Not calculated.
[3]Five animals had toxicokinetic samples collected; however, 1 animal had a fetal abortion or fetal death resulting in insufficient data for analysis.

Table 2.6.5-13a. Pharmacokinetics: Excretion
Test Article: Panitumumab

Study No: 103619
Species: Cynomolgus monkey
Sex (N): 1 male, 1 female sacrificed per time point
Radionucleotide: ^{125}I
Material: — CHO
Dose (route): 7.5 mg/kg; 11.1 µCi/mg (IV)
Method: Solid scintillation counting

Time (hr)[a]	Cumulative % Radioactive Dose			
	Urine	Feces	Cage Rinse	Total
24	25.1	0.120	3.20	28.4
48	39.9	0.380	6.07	46.4
72	48.1	0.590	9.01	57.7
96	54.7	0.720	11.2	66.6
120	59.2	0.830	12.1	72.1
144	66.2	0.690	12.9	79.8
168	68.3	0.740	13.8	82.8
192	69.9	0.770	14.6	85.3
216	71.1	0.810	NS	71.9

Values are presented to 3 significant figures.
CHO: Chinese hamster ovary; NS = No sample.
[a]Collection from time zero to time listed.

Table 2.6.5-13b. Pharmacokinetics: Excretion
Test Article: Panitumumab

Study No: 103620
Species: Cynomolgus monkey
Sex (N): 1 male, 1 female sacrificed per time point
Radionucleotide: ^{125}I
Material: CHO
Dose (route): 7.5 mg/kg; 11.1 µCi/mg (IV)
Method: Solid scintillation counting

Collection Interval (hr)	% Radioactive Dose						
	Urine[a]	Feces[a]	Cage Rinse[a]	Subtotal	Blood	Tissue	Total
2	NS	NS	NS	NA	84.8	29.8	115
24	20.5	0.130	2.70	23.3	NS	NS	23.3
48	34.2	0.380	4.47	39.1	32.5	12.8	84.4
72	43.7	0.520	6.03	50.3	NS	NS	50.3
96	50.0	0.680	6.77	57.5	12.0	6.10	75.6
120	54.2	0.760	6.51	61.5	NS	NS	61.5
144	62.1	0.770	7.10	70.0	NS	NS	70.0
168	64.3	0.840	7.40	72.5	NS	NS	72.5
192	66.0	0.880	7.62	74.5	NS	NS	74.5
216	67.4	0.920	NS	68.3	4.36	2.90	75.6

Values are presented to 3 significant figures.
CHO: Chinese hamster ovary; NS: No sample; NA: Not applicable.
[a]Collection from time zero to time listed.

Table 2.6.5-13c. Pharmacokinetics: Excretion
Test Article: Panitumumab

Study No: 104274
Species: Cynomolgus monkey
Sex (N): 1 male, 1 female sacrificed per time point
Radionucleotide: ^{125}I
Material: Hybridoma
Dose (route): 7.5 mg/kg; 10.9 µCi/mg (IV)
Method: Solid scintillation counting

Time (hr)[a]	% Radioactive Dose			
	Urine	Feces	Cage Rinse	Total
24	21.5	0.230	4.75	26.5
48	35.8	0.630	13.8	50.2
72	44.2	0.970	19.9	65.1
96	51.5	1.34	23.7	76.5
120	57.4	1.57	26.1	85.1
144	60.8	1.75	28.7	91.3
168	63.8	1.82	NS	65.6
192	64.3	1.98	29.7	96.0
216	66.2	2.38	30.6	99.2
240	68.4	2.68	32.2	103

Values are presented to 3 significant figures.
[a]Collection from time zero to time listed.

Table 2.6.5-16. Pharmacokinetics: Other
Test Article: Panitumumab

Study No:	102876
Dose (Route):	A single dose of panitumumab at 7.5 mg/kg (IV)
Material:	Hybridoma or — CHO
Species:	Male Cynomolgus Monkey
Assay:	Electrochemiluminescence

	— CHO (test)			Hybridoma (reference)			Ratio	
Parameter	Mean	SD	n	Mean	SD	n	(test/reference)	90% CI
AUC_{all} (µg·day/mL)	619	83	12	664	143	12	94.4	83.6 to 106.5
C_{max} (µg/mL)	227	31	12	256	56	12	89.4	79.4 to 100.7

Mean values are presented to 3 significant figures; standard deviation values are presented to the same precision as the mean.
CHO: Chinese hamster ovary.

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Table 2.6.5-17. Pharmacokinetics: Lot Number for Test Article
Test Article: Panitumumab

Study No.	Species	Dose	Material	Lot No.
102876 (J26.39; ABX-P0302)	Monkey	7.5 mg/kg	Hybridoma	P01007F
			CHO	9099-53F
103619 (6271-611; ABX-T0311)	Monkey	7.5 mg/kg	CHO	954A021224
103620 (6271-612; ABX-T0312)	Monkey	7.5 mg/kg	CHO	954A021224
104273 (054.0501; ABX-P0302)	Mouse	0.1, 0.5 mg	Hybridoma	7334
			Research CHO	8977-47
104274 (7153-105; ABX-T0304)	Monkey	6 mg/kg	Hybridoma	3737/TFP-99059A
104275 (ABX-P0306)	Mouse	5.22, 522 µg	CHO	9099-58G

Lot numbers for pharmacology studies and toxicity studies in which PK was include as a component are listed in Table 2.6.3-1 and Table 2.6.7-4, respectively.

APPENDIX 5 – TABULATED SUMMARY OF PRECLINICAL TOXICOLOGY STUDIES CONDUCTED IN SUPPORT OF PANITUMUMAB (VECTIBIX™)

The following tables were copied directly from Module 2, section 2.6.7 of the electronic CTD submission, as provided by the sponsor to the BLA application.

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Table 2.6.7-1. Toxicology Overview
Test Article: Panitumumab

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg) [a]	GLP Compliance	Testing Facility	Immunex Study No. [Abgenix Study No.] (CRO Study No.)
Repeated-Dose Toxicity	Cynomolgus monkey	Intravenous	4 weeks	weekly doses: 0, 0.3, 3, 30, loading doses: 0, 0.6, 6, 60	Yes [b]	—	No Immunex no. [ABX-EGF-99-001] (BQAW-100)
	Cynomolgus monkey	Intravenous	4 weeks	weekly doses: 0, 3, 30, loading doses: 0, 6, 60	Yes [b]	—	No Immunex no. [No Abgenix no.] (BQAW-102)
	Cynomolgus monkey	Intravenous	4 weeks	weekly doses: 0, 30, loading doses: 0, 60	Yes [b]	—	054.0401 [ABX-T0305] (00-3691)
	Cynomolgus monkey	Intravenous	3 months	0, 3, 7.5, 15	Yes [b]	—	No Immunex no. [No Abgenix no.] (BQAW-103)
	Cynomolgus monkey	Intravenous	6 months	0, 7.5, 15, 30	Yes	—	103419 [ABX-T0308] (243.15)
Female Fertility & Early Embryonic Development	Cynomolgus monkey	Intravenous	[c]	0, 7.5, 15, 30	Yes	—	103409 [ABX-T0309] (026.56)

[a] Unless otherwise specified.
[b] Toxicokinetic and monkey anti-human antibody (MAHA) data non-GLP.
[c] Females: Once weekly for 2 pre-mating menstrual cycles, 1 or 2 mating menstrual cycles, and up to day 20 of gestation or final pregnancy diagnosis; Males: untreated.

Table 2.6.7-1. Toxicology Overview
Test Article: Panitumumab

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)[a]	GLP Compliance	Testing Facility	Immunex Study No. Abgenix Study No. (CRO Study No.)
Embryo-fetal Development	Cynomolgus monkey	Intravenous	5 weeks	0, 7.5, 15, 30	Yes	—	103410 [ABX-T0310] (026.57)
Other Studies – Comparability	Cynomolgus monkey	Intravenous	4 weeks	0, 7.5, 30	Yes	—	102906 [ABX-T0307] (02-3032)
Other Studies – Comparability	Cynomolgus monkey	Intravenous	3 months	0, 7.5, 30	Yes	—	103917 [ABX-T0311] (03-3060)
Other Studies – Tissue Cross-Reactivity	Human, cynomolgus monkey, rabbit, rat, mouse	In vitro	-	1.0 or 5.0 µg/mL	Yes	—	102920 [ABX-P0305] (1473-31)
Other Studies – EGFr Expression	Cynomolgus monkey	In vitro	-	20 µg/mL	No	—	No Immunex no. [No Abgenix no.] (ABG02)
Other Studies – Tissue Binding	Cynomolgus monkey	In vitro	-	1.2 µg/mL	No	—	No Immunex no. [No Abgenix no.] (ABG09)
Other Studies – Cloning and Sequence Homology	Human, cynomolgus monkey	In vitro	-	NA	No	Amgen Inc.	Amgen: 2005 IT 027 GE

[a] Unless otherwise specified.
[b] Toxicokinetic and monkey anti-human antibody (MAHA) data non-GLP.
[c] Females: Once weekly for 2 pre-mating menstrual cycles, 1 or 2 mating menstrual cycles, and up to day 20 of gestation or final pregnancy diagnosis; Males: untreated.
NA = not applicable

Table 2.6.7-2. Overview of Toxicokinetics Studies
Test Article: Panitumumab

Type of Study	Test System	Method of Administration	Doses (mg/kg) [a]	GLP Compliance	CRO Study Number
Repeated-dose Toxicity	Cynomolgus monkey	Intravenous	weekly doses: 0, 0.3, 3, 30 loading doses: 0, 0.6, 6, 60	Yes [b]	BQAW-100
Repeated-dose Toxicity	Cynomolgus monkey	Intravenous	weekly doses: 0, 3, 30 loading doses: 0, 6, 60	Yes [b]	BQAW-102
Repeated-dose Toxicity	Cynomolgus monkey	Intravenous	weekly doses: 0, 30 loading doses: 0, 60	Yes [b]	00-3691
Repeated-dose Toxicity	Cynomolgus monkey	Intravenous	0, 3, 7.5, 15	Yes [b]	BQAW-103
Repeated-dose Toxicity	Cynomolgus monkey	Intravenous	0, 7.5 15, 30/15 [c]	Yes	243.15
Female Fertility and Early Embryonic Development	Cynomolgus monkey	Intravenous	0, 7.5, 15, 30	Yes	026.56
Embryo-fetal Development	Cynomolgus monkey	Intravenous	0, 7.5, 15, 30	Yes	026.57
Other Studies - Comparability	Cynomolgus monkey	Intravenous	0, 7.5, 30 (hybridoma and CHO)	Yes	02-3032
Other Studies - Comparability	Cynomolgus monkey	Intravenous	0, 7.5, 30	Yes	03-3060
Other Studies - Tissue Cross-Reactivity		In vitro	1 or 5 µg/mL	No	1473-31
Other Studies - EGFr Expression		In vitro	20 µg/mL	Yes	ABG02

[a] Unless otherwise specified.
[b] Toxicokinetics and MAHA data non-GLP.
[c] On Day 43 only, all 30 mg/kg animals were treated with 15 mg/kg.

Table 2.6.7-3. Overview of Toxicokinetics Data
Test Article: Panitumumab

Daily Dose (mg/kg)	Steady State $AUC_{0\text{-}tau}$ (µg-day/mL), Mean (SD) PK Parameters [a]			
	Monkeys			Human
	All	MAHA negative	MAHA positive	
0.3	1.90 (1.20) [b]			
2.5				515 [d]
3	169 (102) [b]			
6				1310 [e]
7.5	NR [c]	774 (259) [c]	NR [c]	
9				2180 [e]
15	NR [c]	1660 (270) [c]	NR [c]	
30	4130 (1100) [b]	3260 (1300) [c]	NR [c]	

[a] Representative toxicokinetics data from 4-week (BQAW-100) and 6-month (103419) toxicity studies. [b] Study BQAW-100; [c] Study 103419; [d] AUC value based on simulation of steady-state PK profile after 2.5 mg/kg weekly dosing; [e] AUC value at steady-state from a clinical study (Study 20030138); $AUC_{0\text{-}tau}$ = area under the concentration-time curve during the dosing interval; SD = standard deviation; PK = pharmacokinetics; MAHA = monkey anti-human antibody; NR = not reported (due to the limited number of surviving animals in the mid- and high-dose groups at the end of the study, exposure parameters for week 26 were reported only for MAHA-negative animals).

Table 2.6.7-4. Toxicology: Drug Substance/Product
Test Article: Panitumumab

Lot #	Purity (%)	Placebo Lot #	CRO Study Number	Type of Study
L01616/75	— (SDS-PAGE, reduced)	C414045	BQAW-100	Toxicity, tissue binding, and PK (IV) in cynomolgus monkeys
2090/TFP-99027	— (SDS-PAGE, reduced) — SDS-PAGE, non-reduced)	4943	BQAW-102	4-Week toxicity and TK (IV) in cynomolgus monkeys
7096/TFP-00093	— (SDS-PAGE, reduced) — SDS-PAGE, non-reduced)	7642/TFP-01007A	00-3691	4-Week mechanistic toxicity (IV) in cynomolgus monkeys
3737/TFP-99059A	— SDS-PAGE, reduced) — SDS-PAGE, non-reduced)	4943/TFP-00017	BQAW-103	3-Month toxicity and TK (IV) in cynomolgus monkeys
F-EGF-001	— (SEC) — SDS-PAGE)	A0306040000	243.15	6-Month IV toxicity in cynomolgus monkeys
954A021224	— (SE-HPLC)	ABX-EGF 9099-61	— 026.56	Female fertility/early embryonic development (IV) in cynomolgus monkeys
			— 026.57	Embryo-fetal development (IV) in cynomolgus monkeys
1505-10; 9099-58G	— SEC)	9099-61	02-3032	4-Week IV comparison in cynomolgus monkeys
954A022614 954A023447	— (SE-HPLC) — (SE-HPLC)	A0306040000	03-3060	3-Month IV toxicity in cynomolgus monkeys
9099-53F (CHO) N10004F (hybridoma)	— SEC) — SDS-PAGE, reduced) — SDS-PAGE, non-reduced)	031K9215	1473-31	Tissue cross-reactivity
098-068-01[a]		NA	ABG02	EGFr expression in cynomolgus monkey tissue
NA [b]		NA	ABG09	Tissue binding

NA = not applicable; PK = pharmacokinetics; IV = intravenous; TK = Toxicokinetics; [a] purity data not available for this study; [b] panitumumab not used on this study

Table 2.6.7-7A. Repeated-dose Toxicity: Pivotal Studies
Test Article: Panitumumab

Repeated-dose Toxicity	Report Title: Toxicity, Tissue Binding, and Pharmacokinetics of ABX-EGF Following Single and Multiple Intravenous Bolus Dose Administration in Cynomolgus Monkeys	Test Article: Panitumumab
Species/Strain: Cynomolgus Monkey	Duration of Dosing: 4 weeks	Abgenix Study No. ABX-EGF-99-001
Initial Age: Young adult to adult	Duration of Postdose: 2 weeks	CRO Study No. BQAW-100
Date of First Dose: 2 March 1999 (males) 3 March 1999 (females)	Method of Administration: Slow intravenous injection	
	Placebo/Formulation: 0.9% saline	GLP Compliance: Yes (Toxicokinetics and MAHA data non-GLP)
Special Features: Tissue Cross-reactivity Assays [a]		
No Observed Adverse Effect Level: < 0.3 mg/kg		

Weekly Dose (mg/kg)	0 (Placebo)		0.3 (0.6 loading)		3 (6 loading)		30 (60 loading)	
Number of Animals	M: 5	F: 5	M: 3	F: 3	M: 3	F: 3	M: 5	F: 5
Toxicokinetics: Mean (SD)								
AUC_{0-tau} day 1 (µg·day/mL)	0		14.0 (2.6) n = 6		400 (91) n = 6		8130 (3840) n = 10	
AUC_{0-tau} day 22 (µg·day/mL)	0		1.9 (1.2) n = 4		169 (102) n = 6		4130 (1100) n = 8	
Incidence of MAHA [b]	0	0	3	2	1	1	1	1
Noteworthy Findings								
Died or Euthanized Moribund	0	0	0	0	0	0	1 (day 37)	1 (day 35)
Body Weight (%) [c,d]	2.64 kg	2.20 kg	-14	-3	-20	-17	-23	-12

[a] ABX-EGF staining showed variable binding to endomysial interstitial cells of the myocardium in monkey heart samples. [b] Considered MAHA positive if any postdose sample indicated the presence of MAHA. [c] At end of dosing period. [d] For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences). [e] Incidence is presented. AUC_{0-tau} = area under the concentration-time curve during the dosing interval; MAHA = monkey anti-human antibody; SD = standard deviation; NA = not applicable; - no noteworthy findings; * $p \leq 0.05$, ** $p<0.01$ by Dunnett's t-test (parametric) or Dunn's procedure (nonparametric).

Table 2.6.7-7B. Repeated-dose Toxicity: Pivotal Studies
Test Article: Panitumumab

Repeated-dose Toxicity	Report Title: Toxicity and Toxicokinetics of ABX-EGF Following Multiple Intravenous Dose Administration (via Bolus and Infusion) in Cynomolgus Monkeys	Test Article: Panitumumab
Species/Strain: Cynomolgus Monkey	Duration of Dosing: 4 weeks	CRO Study No. BQAW-102
Initial Age: Young adult	Duration of Postdose: 2 weeks	
Date of First Dose: 29 March 2000	Method of Administration: Intravenous bolus or infusion	
	Vehicle/Formulation: 50 mM sodium acetate in 100 mM NaCl	GLP Compliance: Yes (Toxicokinetics and MAHA data non-GLP)
Special Features: Supportive fluids (oral and/or subcutaneous [SC]) and antidiarrheal treatment (eg, loperamide) were administered as needed to all animals (except group 3) to prevent dehydration		
No Observed Adverse Effect Level: < 3 mg/kg		

Weekly Dose (mg/kg)	0 (Vehicle) Bolus/Concurrent therapies [a, b]	3 (6 loading) Bolus/Concurrent therapies [a, b]	30 (60 loading) Bolus/No Concurrent Therapies [a]	30 (60 loading) Bolus/Concurrent therapies [a, b]	30 (60 loading) Infusion/Concurrent therapies [b, c]
Number of Animals	M: 5 + F: 1	M: 6	M: 6	M: 6	M: 6
Toxicokinetics: Mean (SD)					
AUC_{0-tau} day 1 (µg·day/mL)	NA	402 (116)	5050 (590)	5350 (800)	4210 (1170)
AUC_{0-tau} day 22 (µg·day/mL)	NA	61.0 (64.9)	3680 (1530) [e]	3680 (790)	1360 (1180) (n = 4)
Incidence of MAHA [d]	0	3	1	0	3

[a] Test article administered by bolus intravenous (IV) injection. [b] Antidiarrheal medication and/or supportive fluids (oral, IV, or subcutaneous) administered as needed throughout the study. [c] Test article administered by 2-hour IV infusion. [d] Considered MAHA positive based on a 3-fold increase (by optical density) in any postdose sample. [e] At end of dosing period. [f] For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on percent differences). [g] Incidence is presented. [h] Gross pathology number examined includes early deaths of animals from main and recovery-designated segments of the study; histopathology for early deaths is presented separately. AUC_{0-tau} = area under the concentration-time curve during the dosing interval; MAHA = monkey anti-human antibody; SD = standard deviation; NA = not applicable; - no noteworthy findings; * $p < 0.05$, ** $p < 0.01$ compared to controls using Dunnett's t-test (parametric) or Dunn's procedure (nonparametric).

Table 2.6.7-7C. Repeated-dose Toxicity: Pivotal Studies
Test Article: Panitumumab

Repeated-dose Toxicity	Report Title: Four-Week Mechanistic Toxicity Study of ABX-EGF Administered Once per Week by Intravenous Injection to Cynomolgus Monkeys Followed by a Two-Month Recovery Period	Test Article: Panitumumab
Species/Strain: Cynomolgus monkey	Duration of Dosing: 4 weeks	Immunex Study No. 054.0401
Initial Age: Young adult (3 to 7 years)	Duration of Postdose: 8 weeks	Abgenix Study No. ABX-T0305
Date of First Dose: 12 March 2001	Method of Administration: Intravenous injection	CRO Study No. 00-3691
	Vehicle/Formulation: 50 mM sodium acetate, 100 mM sodium chloride, pH 5.8	GLP Compliance: Yes (Toxicokinetics, MAHA, and echocardiograph data non-GLP)
Special Features: Immunohistochemistry (heart); supportive SC fluids administered; animals serially terminated (days 4, 11, 18, 25, 64, 91)		
No Observed Adverse Effect Level: < 30 mg/kg		

Weekly Dose (mg/kg)	0 (Vehicle)	30 (60 loading)
Number of Animals	M: 6	M: 28
Toxicokinetics: Mean (SD)	Serum Concentration (µg/mL)	Serum Concentration (µg/mL)
Day 0 [a]	BLQ	1497.4 (452.6)
Day 4	BLQ	592.1 (55.8)
Day 7 [a]	BLQ	1260.2 (294.0)
Day 11	BLQ	598.4 (134.2)
Day 14 [a]	BLQ	1017.3 (320.4)
Day 18	BLQ	452.9 (195.9)
Day 21 [a]	BLQ	831.4 (326.7)
Day 25	BLQ	383.9 (118.5)

[a] 2 hours postdose. [b] Incidence is presented. [c] The pattern of staining was consistent with passive diffusion of panitumumab into the tissues, and specific binding of panitumumab to tissues could not be demonstrated. [d] Animals serially terminated and necropsied on days 4, 11, 18, 25, 64, and 91. [e] Considered MAHA positive if any postdose sample indicated the presence of MAHA. MAHA = monkey anti-human antibody; SC = subcutaneous; SD = standard deviation; BLQ = below the limit of quantitation; NE = not evaluated; - No noteworthy findings; * $p < 0.05$, ** $p < 0.01$ compared to control mean using Dunnett's, Williams, or Cochran and Cox's modified t-test (parametric) or by Shirley's test, Dunn's test, or Pairwise Comparison with Bonferroni Correction (nonparametric).

Table 2.6.7-7D. Repeated-dose Toxicity: Pivotal Studies
Test Article: Panitumumab

Repeated-dose Toxicity	Report Title: Toxicity and Toxicokinetics of ABX-EGF Following Intravenous Bolus Administration in Cynomolgus Monkeys For Three Months with a Six-Week Recovery	Test Article: Panitumumab
Species/Strain: Cynomolgus Monkey	Duration of Dosing: 3 months	CRO Study No. BQAW-103
Initial Age: Young adult	Duration of Postdose: 6 weeks	
Date of First Dose: 10 October 2000	Method of Administration: Intravenous	
	Vehicle/Formulation: 50 mM sodium acetate in 100 mM NaCl, pH 5.8	GLP Compliance: Yes (Toxicokinetics and MAHA data non-GLP)
Special Features: Subcutaneous fluid administered to prevent dehydration		
No Observed Adverse Effect Level: < 3 mg/kg		

Weekly Dose (mg/kg)	0 (Vehicle)		3		7.5		15	
Number of Animals	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5	M: 5	F: 5
Toxicokinetics: Mean (SD)	n = 10		n = 10		n = 10		n = 10	
$AUC_{0\text{-}tau}$ day 1 (µg·day/mL)	BLQ		69.8 (11.9)		341 (27)		803 (109)	
$AUC_{0\text{-}tau}$ day 78 (µg·day/mL)	BLQ		11.0 (24.1)		326 (183)		1030 (430)	
Incidence of MAHA [a]	0	0	4	5	2	1	1	1

[a] Considered MAHA positive if any postdose sample indicated the presence of MAHA. [b] At end of dosing period. [c] For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences). [d] Incidence is presented.
BLQ = below the limit of quantitation; MAHA = monkey anti-human antibody; SD = standard deviation; $AUC_{0\text{-}tau}$ = area under the concentration-time curve during the dosing interval; - No noteworthy findings. * - $p < 0.05$, ** - $p < 0.01$ compared to control using Dunnett's t-test (parametric) or Dunn's procedure (nonparametric).

Table 2.6.7-7E. Repeated-dose Toxicity: Pivotal Studies
Test Article: Panitumumab

Repeated-dose Toxicity	Report Title: A Six-month Multiple Dose Toxicity Study of ABX-EGF Administered Intravenously to Cynomolgus Monkeys Followed by a Two-month Recovery Period	Test Article: Panitumumab
Species/Strain: Cynomolgus monkey	Duration of Dosing: 6 months	Immunex Study No. 103419
Initial Age: ≥ 3 years (mature adults)	Duration of Postdose: 2 months	Abgenix Study No. ABX-T0308
Date of First Dose: 22 September 2003	Method of Administration: Intravenous	CRO Study No. 243.15
	Vehicle/Formulation: ABX-EGF Placebo: 50mM sodium acetate, 100 mM sodium chloride solution, pH 5.8	GLP Compliance: Yes

Special Features: Supportive treatments, including Ketofen® (nonsteroidal anti-inflammatory), antibiotics (Cephazolin, Baytril) and Nolvasan® (2% chlorohexidine) administered to all animals as needed to minimize dermal irritation and secondary infection. Dosing occasionally skipped for certain animals because of severe skin rash and/or poor clinical condition; animals in the 30 mg/kg dose group received the 15 mg/kg dose on day 43. Several animals euthanized at unscheduled intervals because of severe skin rash and/or poor clinical condition. Subcutaneous fluid administered to prevent dehydration.

No Observed Adverse Effect Level: < 7.5 mg/kg

Weekly Dose (mg/kg)	0 (Control)		7.5		15		30	
Number of Animals	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6
Toxicokinetics: Mean (SD)								
Week 1 $AUC_{0\text{-}tau}$ (µg·day/mL) [a]	BLQ, n = 12		623 (171), n = 6		1600 (360), n = 3		3530 (1230), n = 5	
Week 26 $AUC_{0\text{-}tau}$ (µg·day/mL) [a]	BLQ, n = 12		774 (259), n = 6		1660 (266), n = 3		3260 (1300), n = 5	
Incidence of MAHA	0	0	3	2	0	2	0	1
Noteworthy Findings								
Died or Euthanized Moribund	0	0	1	1	4	3	2	4
Body Weight (% [b, c])	4.699 kg	3.649 kg	+6	-16	-20	-19	-8	-17

[a] Toxicokinetics for MAHA-negative animals in week 26. [b] At end of dosing period. [c] For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences). [d] Incidence is presented. [e] Unscheduled interim necropsies on days 32 (n = 7) and 96 (n = 6); additional early deaths on days 75 (n = 1) and 134 (n = 1). [f] Terminal necropsy day 183. [g] Does not include data for 2 animals placed into early recovery and necropsied on days 89 and 197. SD = standard deviation, $AUC_{0\text{-}tau}$ = area under the concentration-time curve during the dosing interval; BLQ = below the limit of quantification; MAHA = monkey anti-human antibody; NA = not applicable; - No noteworthy findings; * $p < 0.05$.

Table 2.6.7-12. Reproductive and Developmental Toxicity: Fertility and Early Embryonic Development to Implantation (Pivotal)
Test Article: Panitumumab

Reproductive and Developmental Toxicity: Fertility and Early Embryonic Development to Implantation	Report Title: An Assessment of the Effects of ABX-EGF on Female Fertility and Early Embryonic Development to Implantation When Administered by Weekly Intravenous Injection to Cynomolgus Monkeys	Test Article: Panitumumab
Species/Strain: Cynomolgus Monkey	Duration of Dosing: Once weekly from the first pre-mating cycle through approximately gestation day 20	Immunex Study No. 103409 Abgenix Study No. ABX-T0309
Initial Age: 3 to 9 years	Method of Administration: Intravenous	CRO Study No. 026.56
Date of First Dose: 06 October 2003	Vehicle/Formulation: 50 mM sodium acetate in 100 mM NaCl, pH 5.8	GLP Compliance: Yes
Special Features: Subcutaneous fluid administered to prevent dehydration; dosing occasionally skipped for certain animals due to skin rash and poor clinical condition		
No Observed Adverse Effect Level: Maternal: < 7.5 mg/kg Fertility and implantation: < 7.5 mg/kg		

Weekly Dose (mg/kg)	0 (Control)	7.5	15	30
Toxicokinetics: Mean (SD) [a]				
$AUC_{0\text{-}tau}$ during pre-mating cycle (µg·day/mL)	0	719 (124) n = 12	1466 (422) n = 7	3668 (569) n = 12
$AUC_{0\text{-}tau}$ first dose of mating cycle (µg·day/mL)	0	333 (409) n = 6	1240 (656) n = 6	1322 (1506) n = 6
$AUC_{0\text{-}tau}$ last dose of mating cycle (µg·day/mL)	0	958 (NC) n = 1	1445 (NC) n = 2	2 (NC) n = 1
Incidence of MAHA	0	5	2	6

[a] Only animals that completed both premating cycle 1 and mating cycle 1 are presented. [b] Mean body weight gain over entire dosing period. [c] Shown as average number of biscuits consumed/day (total fed/day = 20) during dosing interval; [d] Incidence is presented. [e] Mating confirmed for all animals at least once for each mating session. [f] Gross pathology and organ weights apply only to animals that were euthanized or that died (non-terminal study). SD = standard deviation; $AUC_{0\text{-}tau}$ = area under the concentration-time curve during the dosing interval; NC = not calculated; MAHA = monkey anti-human antibody; PMC = premating cycle; MC = mating cycle; NA = not applicable; P = present; QNS = quantity not sufficient; - No noteworthy findings. * $p < 0.05$ ** $p < 0.01$ compared to controls using Dunnett's t-test (parametric)

Table 2.6.7-13. Reproductive and Developmental Toxicity: Effects on Embryo-fetal Development (Pivotal)
Test Article: Panitumumab

Reproductive and Developmental Toxicity: Effects on Embryo-Fetal Development		Report Title: An Assessment of the Effects of ABX-EGF on Embryo-Fetal Development When Administered Weekly by Intravenous Injection to Pregnant Cynomolgus Monkeys			Test Article: Panitumumab
Species/Strain: Cynomolgus Monkeys		Duration of Dosing: 5 weeks (once per week, G20-G50)			Immunex Study No. 103410
Initial Age: 3 to 12 years					Abgenix Study No. ABX-T0310
Date of First Dose: 14 August 2003		Day of C-Section: G100 to G103			CRO Study No. 026.57
		Method of Administration: Intravenous			
Special Features: Subcutaneous fluids administered to prevent dehydration		Vehicle/Formulation: 50 mM sodium acetate in 100 mM NaCl, pH 5.8			GLP Compliance: Yes
No Observed Adverse Effect Level: Dams: < 7.5 mg/kg Embryo-fetal development: < 7.5 mg/kg					
Weekly Dose (mg/kg)		0 (Control)	7.5	15	30
Dams:	Toxicokinetics: AUC Mean (SD) [a]				
	AUC $_{0\text{-}tau}$ G20 (µg·day/mL)	0	808(144) n = 15	1625 (347) n = 18	3743 (973) n = 4
	AUC $_{0\text{-}tau}$ G48 (µg·day/mL)	0	736 (330) n = 11	2098 (1183) n = 15	5360 (NC) n = 2
	Incidence of dam MAHA [b]	0/12	5/15	13/18	1/5
	Incidence of fetal MAHA	0/11	4/10	9/15	1/2
	Incidence of amniotic fluid MAHA	NA	1/5	4/13	0/1

[a] Only animals that completed through G48 are presented. [b] Considered MAHA positive if any postdose serum dam sample indicated the presence of MAHA. [c] Shown as the average number of uneaten biscuits/day (total fed/day = 20) between G20 and G55 (first day of dosing to 7 days after final dose). [d] Incidence is presented.
G = gestation day; SD = standard deviation; $AUC_{0\text{-}tau}$ = area under the concentration-time curve during the dosing interval; NC = not calculated; MAHA = monkey anti-human antibody; NA = not applicable; - No noteworthy findings; * - $p < 0.05$ ** - $p < 0.01$ compared to controls using Dunnett's t-test (parametric) or Steel's test (nonparametric).

Table 2.6.7-17A. Other Toxicity Studies
Test Article: Panitumumab

Other Toxicity Studies - Comparability	Report Title: A 4-Week Comparison Study of Two Forms of ABX-EGF Administered by Intravenous Injection Once per Week to Cynomolgus Monkeys With a 4-Week Recovery Period	Test Article: Panitumumab (hybridoma- and CHO-derived [redacted] scale])
Species/Strain: Cynomolgus Monkey	Duration of Dosing: 4 weeks	[redacted] Study No. 102906
Initial Age: ~ 3 to 5.5 years	Duration of Postdose: 4 weeks	Abgenix Study No. ABX-T0307
Date of First Dose: 23 January 2003	Method of Administration: Intravenous injection	CRO Study No. 02-3032
	Vehicle/Formulation: 50 mM sodium acetate, 100 mM sodium chloride, pH 5.8	GLP Compliance: Yes (except dose solution analysis)
Special Features: Supportive fluids administered		
No Observed Adverse Effect Level: < 7.5 mg/kg		
Comments: No apparent differences were observed in the toxicity profile and the number of MAHA responses between hybridoma- and CHO-derived material		

Weekly Dose (mg/kg)	0 (Control)		7.5 (hybridoma)		30 (hybridoma)		7.5 (CHO)		30 (CHO)	
Number of Animals	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Toxicokinetics: Mean (SD)	n = 8		n = 8		n = 8		n = 8		n = 8	
$AUC_{0\text{-}tau}$ day 1 (µg·day/mL)	0		493 (116)		2740 (530)		615 (123)		3060 (930)	
$AUC_{0\text{-}tau}$ day 22 (µg·day/mL)	0		197 (297)		2480 (1700)		423 (355)		2410 (1330)	
Incidence of MAHA [a]	0	0	3	3	2	2	3	2	2	0

[a] Considered MAHA positive if any postdose sample indicated an increase in the presence of MAHA. [b] Incidence is presented. CHO = Chinese hamster ovary; SD = standard deviation; $AUC_{0\text{-}tau}$ = area under the concentration-time curve during the dosing interval; MAHA = monkey anti-human antibody; - No noteworthy findings. * $p \leq 0.05$ compared to controls using Dunnett's, Williams, or Cochran and Cox's modified t-test (parametric) or by Shirley's or Steel's test (nonparametric).

Table 2.6.7-17B. Other Toxicity Studies
Test Article: Panitumumab

Other Toxicity Studies - Comparability	Report Title: A 3-Month Intravenous Toxicity Study of ABX-EGF in Cynomolgus Monkeys with a 6-Week Recovery Period	Test Article: Panitumumab (CHO-derived: — scale)
Species/Strain: Cynomolgus Monkey	Duration of Dosing: 3 months	Immunex Study No. 103917
Initial Age: 3 to 7.5 years	Duration of Postdose: 6 weeks	Abgenix Study No. ABX-T0311
Date of First Dose: 26 January 2004	Method of Administration: Intravenous	CRO Study No. 03-3060
	Vehicle/Formulation: 50 mM sodium acetate in 100 mM NaCl, pH 5.8	GLP Compliance: Yes

Special Features: Subcutaneous fluid administered to prevent dehydration. Supportive treatments administered as needed to minimize dermal irritation and prevent secondary infection.

No Observed Adverse Effect Level: < 7.5 mg/kg every 2 weeks

Weekly Dose (mg/kg)	Group 1: Control (filtered vehicle)		Group 2: 7.5 (filtered —		Group 3: 30 (filtered —		Group 4: 30 (unfiltered —,	
Number of Animals	M: 4	F: 4	M: 3	F: 3	M: 5	F: 5	M: 3	F: 3
Toxicokinetics: Mean (SD)	n = 8		n = 6		n = 10		n = 6	
AUC_{0-tau} day 1 (µg·day/mL)	0		555 (130)		2971 (401)		2792 (699)	
AUC_{0-tau} day 29 (µg·day/mL)	0		651 (381)		4496 (1365)		2509 (1942)	
AUC_{0-tau} day 85 (µg·day/mL)	0		430 (426)		3523 (1584)		1859 (1838)	
Incidence of MAHA [a]	4	4	1	3	1	1	2	2

[a] Considered MAHA positive if any postdose sample indicated the presence of MAHA. Animals testing positive for pre-existing heterophilic antibodies (MAHA) during pre-screening were placed in the control group (group 1). No increase in the level of MAHA in the control group was observed between days 29 and 85. [b] Euthanized during week 5 because of a fractured arm. [c] Incidence is presented. SD = standard deviation; AUC_{0-tau} = area under the concentration-time curve during the dosing interval; MAHA = monkey anti-human antibody; - No noteworthy findings ; * $p < 0.05$, ** $p < 0.01$.

Table 2.6.7-17B. Other Toxicity Studies
Test Article: Panitumumab

Study No. 103917; ABX-T0311; 03-3060

Weekly Dose (mg/kg)	Group 5: 7.5 (every other week, filtered		Group 6: 7.5 (filtered		Group 7: 30 (filtered		Group 8: 30 (unfiltered	
	M: 3	F: 3	M: 3	F: 3	M: 5	F: 5	M: 3	F: 3
Toxicokinetics: Mean (SD)	n = 6		n = 6		n = 10		n = 6	
AUC_{0-tau} day 1 (µg·day/mL)	704 (129)		592 (67)		2545 (300)		2893 (251)	
AUC_{0-tau} day 29 (µg·day/mL)	219 (239)		442 (379)		2830 (2040)		3987 (2398)	
AUC_{0-tau} day 85 (µg·day/mL)	173 (269)		389 (431)		2801 (1927)		3466 (1983)	
Incidence of MAHA [a]	3	1	1	2	1	2	1	0

[a] Considered MAHA positive if any postdose sample indicated the presence of MAHA. Animals testing positive for pre-existing heterophilic antibodies (MAHA) during pre-screening were placed in the control group (group 1). No increase in the level of MAHA in the control group was observed between days 29 and 85. [b] Euthanized during week 5 because of a fractured arm. [c] Incidence is presented. SD = standard deviation; AUC_{0-tau} = area under the concentration-time curve during the dosing interval; MAHA = monkey anti-human antibody; - No noteworthy findings; * $p < 0.05$, ** $p < 0.01$.

Table 2.6.7-17C. Other Toxicity Studies
Test Article: Panitumumab

Animal Species/ Test Model	Method of Administration	Dose Range and Schedule	Sex and No. Per Group	Noteworthy Findings	Study Number
Tissue Cross-reactivity model	In vitro	1.0 or 5.0 µg/mL ABX-EGF (CHO; — scale) or ABX-EGF (hybridoma)	NA	Tissues with positive staining: Human: skin, GI tract/colon (mucosal epithelium), parathyroid epithelial cells, eye (conjunctival mucosa), prostate (epithelium), lung (alveolar epithelium), germinal center cells in lymph nodes, spleen stromal cells in red pulp), thyroid (follicular epithelium), trophoblastic cells in placenta, kidney (tubular epithelium) Cynomolgus: skin, GI tract/colon (mucosal epithelium), eye (conjunctival epithelium), lung (alveolar epithelium), follicular epithelial cells in secondary follicles in the ovary, prostate (glandular epithelium), ductular epithelial cells in liver, lymphoid and stromal cells in medulla of thymus, thyroid (follicular epithelium), kidney (tubular epithelium) Both human and cynomolgus: Non-epithelial cells: interstitial cells in testes, stromal cells in uterus (cervix), uterus (endometrium), and tonsil Rabbit: urinary bladder (urothelium), cornea, prostate (urothelium), cervix (mucosa), ductular epithelial cells in pancreas, thyroid follicular epithelium, and tubular/ductular epithelial cells in kidney and liver Rabbit non-epithelial cells: vascular endothelial cells of renal papilla, perifollicular stroma surrounding follicles in ovary Rat: no consistent staining was identified Mouse: no consistent staining was identified	Immunex: 102920 Abgenix: ABX-P0305 CRO: 1473-31

CHO = Chinese hamster ovary; NA = not applicable

Table 2.6.7-17C. Other Toxicity Studies
Test Article: Panitumumab

Animal Species/ Test Model	Method of Administration	Dose Range and Schedule	Sex and No. Per Group	Noteworthy Findings	Study Number
EGFr Expression	In vitro	20 µg/mL for primary antibody, biotinylated panitumumab 20 µg/mL for negative control antibody, biotinylated human IgG2	NA	ABX-EGF binds to tissues with proliferating cells of epithelial origin. Specific panitumumab staining of various tissues including: breast, eye, fallopian tube, kidney, lung, ovary, pancreas, prostate, skin, tonsil, and ureter. Positive control: cynomolgus monkey skin Negative control: cynomolgus monkey heart Negative antibody control: PK 16.3.1 (biotinylated human IgG2 isotype)	CRO: ABG02

CHO = Chinese hamster ovary; NA = not applicable

Table 2.6.7-17C. Other Toxicity Studies
Test Article: Panitumumab

Animal Species/ Test Model	Method of Administration	Dose Range and Schedule	Sex and No. Per Group	Noteworthy Findings	Study Number
ABX-EGF Tissue Binding to Cynomolgus Monkey Tissues	In vitro	1.25 µg/mL for primary antibody (anti-human IgG-Fc antibody) 1.25 µg/mL for negative control antibody (goat anti-mouse IgG-Fc antibody)	NA	Certain tissues (e.g., heart, skin, liver) were collected from all animals (vehicle control, 0.3, 3.0, and 30 mg/kg dose groups) in Study BQAW-100 and shipped to — for immunohistochemical analysis. Analysis of panitumumab binding was performed using an indirect immunoperoxidase method. Positive control: normal human tonsil Negative control: tonsil obtained from vehicle control animals in Study BQAW-100 Negative or weak staining was observed in tissues obtained from animals in the control and 0.3 mg/kg groups. Stronger staining patterns (2+ or 3+) were observed in tissues obtained from animals in the 3.0 and 30 mg/kg dose groups indicating that incorporation/binding of panitumumab increased with increasing dose in most tissues. Monkey tissues with were scored with ≥ 2+ staining for 1 or more animals included: adrenal gland (e.g., endothelium, zona reticularis), large intestine, cecum, duodenum, ileum, jejunum, rectum, esophagus, stomach, liver (endothelium), lymph node (endothelium), pancreas (stroma, endothelium), heart (e.g., endothelium) thymus (epithelial cells, endothelium), and skin.	CRO: ABG09

CHO = Chinese hamster ovary; NA = not applicable

Table 2.6.7-17C. Other Toxicity Studies
Test Article: Panitumumab

Animal Species/ Test Model	Method of Administration	Dose Range and Schedule	Sex and No. Per Group	Noteworthy Findings	Study Number
Cloning and Sequence Homology Analysis of Cynomolgus EGF Receptor	In vitro	NA	NA	Human and cynomolgus EGFr protein have over 99% sequence homology, which supports the use of the cynomolgus monkey as a relevant animal species in toxicology studies with panitumumab.	Amgen: 2005 IT 027 GE

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CHO = Chinese hamster ovary; NA = not applicable

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