CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

STN/BLA 125084

<u>PHARMACOLOGY REVIEW</u>

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:	**125084**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**8/14/03**
DRUG NAME:	**ERBITUX™ (cetuximab)**
INDICATION:	**Treatment of EGFr-expressing colorectal carcinoma in combination with irinotecan, in patients who are refractory to irinotecan-based chemotherapy**
SPONSOR:	**ImClone Systems, Incorporated**
DOCUMENTS REVIEWED:	**E-BLA Submission**
REVIEW DIVISION:	**Division of Biological Therapeutic Oncology Products (HFM-573)**
PHARM/TOX REVIEWER:	**Anne M. Pilaro, Ph.D.**
PHARM/TOX SUPERVISOR:	**M. David Green, Ph.D.**
DIVISION DIRECTOR:	**Patricia Keegan, M.D.**
PROJECT MANAGER:	**Sharon Sickafuse**

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

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EXECUTIVE SUMMARY

1. Recommendations

1.1   Recommendation on approvability

The Biologics Licensing Application STN BLA #125084/0 is approvable based on the data contained in the preclinical pharmacology and toxicology sections of the original submission. Toxicities of ERBITUX™ are extensions of the pharmacologic activity of the product, were reflected in the clinical studies, and may be monitored and treated appropriately in the clinical setting.

1.2   Recommendation for nonclinical studies

It is recommended that the sponsor conduct and submit for FDA review a nonclinical, reproductive toxicology study(ies) of ERBITUX™ in a suitable animal species to support any potential, off-label use or large-scale clinical trials in earlier stages of cancer (*i.e.* the adjuvant setting), and/or in support of any future indications for ERBITUX™ in female patients of childbearing age.

1.3   Recommendations on labeling

Modifications to the PRECAUTIONS section of the label, including revision of the language regarding potential impairment of fertility by ERBITUX™, and the Pregnancy and Nursing Mothers subsections have been communicated to and accepted by the sponsor. Additionally, a subsection to the WARNINGS section of the labeling, regarding the severe dermatologic toxicities and deaths in monkeys treated with ERBITUX™ has been added to the label and accepted by the sponsor. Copies of the final, revised language for these sections of the labeling are included as Appendix I to this review.

2. Summary of nonclinical findings

2.1   Brief overview of nonclinical findings

Cetuximab (ERBITUX™, IMC-C225, C225) was evaluated for pharmacologic activity in human tumor xenografts in nude mice, and for toxicity and pharmacokinetics in rats, mice, and cynomolgus monkeys. Tissue binding studies demonstrated that C225 bound to surface epithelial growth factor receptor (EGFr) present in the skin, tongue, mammary and salivary glands, ovaries, placenta, and urinary bladder of cynomolgus monkey and human tissues, and did not bind to tissues from rat, mouse, dog, or goat. Treatment of tumor-bearing, nude mice with cetuximab was associated with delayed tumor growth in human tumor xenografts of lung, colon, breast, or pancreatic cancers, and evidence of additive anti-tumor effects were observed when tumor-bearing mice were treated with C225 in combination with irinotecan, cis-platinum, or fluorouracil. Pharmacokinetic profiles in cynomolgus monkeys following single, intravenous infusions of 7.5, 24, or 75 mg/kg or 9.84, 31, or 98.4 mg/kg cetuximab demonstrated dose-related increases in C_{max} and AUC_{last}, dose-related decreases in clearance with an increase in apparent half-life, and a volume of distribution at steady state that was approximately equal to the vascular space. On repeat administration of cetuximab at 7.5, 24, or 75 mg/kg twice weekly, toxicokinetic evaluation confirmed that exposure to C225 was continuous over the duration of the study, with

comparable values for AUC_{last} and C_{max} at the 4, 13, 26, and 39 week time points. These data suggest that no significant accumulation of the antibody is occurring. Anti-cetuximab antibody development was observed in one monkey over the duration of the study, resulting in a decrease in serum C225 concentration as compared to the other animals in this dose group. Mutagenic activity of ERBITUX™ was not observed in the *in vitro* bacterial reversion (Ames) assay, or in an *in vivo* mammalian micronucleus assay in rats. No toxicities were observed in mice after a single dose of 300 mg C225, i/v or in Sprague-Dawley rats after single i/v dose of 200 mg/kg, or repeated intravenous infusions of up to 40 mg /kg cetuximab twice weekly for 4 weeks. Severe toxicities related to ERBITUX™ were observed in cynomolgus monkeys, following repeated weekly infusion of 7.5, 24, and 75 mg/kg/dose, i/v for up to 39 weeks; these doses represent approximately 0.4 to 4 times the labeled dose of cetuximab, when adjusted for total body surface area. Toxicities in this study included decreases in body weights, food consumption, anemia, decreases in leukocytes and platelet counts, alterations in menstrual cyclicity in the female animals, dose-related elevations in ALT, GLDH, and γ-glutamyl transpeptidase, and dose-dependent dermatological toxicities. Skin lesions included reddening and scale formation on the extremities, trunk, and inguinal areas, acneform pustules, hair thinning or loss, exanthema, dermatitis and wounds. These findings occurred at all dose levels of cetuximab, and were only partially reversible following interruption or discontinuation of dosing, so that no NOAEL could be defined for this ERBITUX™ in the preclinical safety program. Early mortalities occurred in 5/10 monkeys that were treated with 75 mg/kg/week cetuximab beginning after approximately 12 weeks on treatment, and resulting in early discontinuation of dosing in this group after 36 weeks on study. Mortalities in the high dose animals were related to excessive dermatologic toxicity of ERBITUX™ following inhibition of the EGFr by cetuximab, and the subsequent defect in maturation of epidermal cells. Prior to deaths in these animals, hyper- and parakeratosis, acanthosis and acantholysis resulting in desquamation of the external integuement, and sloughing of the epithelial mucosa of the nasal passage, esophagus, and tongue, were observed. Secondary bacterial infections of the affected skin resulted in erosive to ulcerative dermatitis with subsequent septicemia, involvement of the major organs, and death. The dose of ERBITUX™ at which these early mortalities occurred was approximately 4 times greater than the clinical dose, when scaled by total body surface area. Of note, the cynomolgus monkey was the only species to demonstrate significant tissue cross-reactivity with cetuximab in a similar distribution to human tissues, and the hematologic and dermatologic toxicities were also observed clinically and considered dose limiting after ERBITUX™ treatment.

2.2   Pharmacologic activity

ERBITUX™ 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, induction of apoptosis, decreased matrix metalloproteinase production, and decreased vascular endothelial 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 ERBITUX™, alone or in combination with irinotecan, fluorouracil, or cis-platin chemotherapy inhibits the growth and survival of human tumor cells that over-express the EGFr. No anti-tumor effects of cetuximab were observed in immune deficient mouse models bearing human tumor xenografts lacking EGFR expression.

2.3   Nonclinical safety issues relevant to clinical use

Dermatologic toxicity following ERBITUX™ treatment was observed both in preclinical studies in cynomolgus monkeys after repeated, weekly treatment with the drug, and in clinical trials in patients with metastatic colorectal carcinoma. Severe erythema, skin scaling and sloughing, pustule formation, infections, sepsis, and death in 5/10 monkeys at the highest dose group were observed in animals treated with 7.5, 24, or 75 mg/kg/week cetuximab, and were only partially reversible in the highest dose group at 9 weeks after discontinuation of the biologic. Acneform rash and other dermatologic toxicities were observed clinically, were generally Grade 2-3 in severity, and occasionally resulted in either dose reduction or dose interruption until resolved. Three clinical cases of sepsis were reported in the pivotal clinical study, with no fatalities. The potential for ERBITUX™ to induce severe dermatologic toxicity is related to its mechanism of action, through inhibition of critical cellular pathways associated with activation of the EGFr and subsequent epithelial cell maturation. Dose modification in case of acneform rash is provided in the package insert for ERBITUX™. Additionally, the dermatologic toxicities, sepsis, and deaths in the animals will be identified in the label under the WARNINGS section; recommended language for inclusion in the label is provided in Appendix I, below.

Clinical toxicities not predicted by the animal studies included severe infusion reactions, which were characterized by rapid onset of airway obstruction (bronchospasm, stridor, hoarseness), urticaria, and hypotension and were reported for approximately 3% of the patients in the phase 3 clinical study. Death secondary to severe infusion reaction occurred in one subject enrolled in the clinical study. In 90% of the incidences, the infusion reactions occurred on the first infusion of ERBITUX™. No similar airway reactivity or hypotension findings were observed in the cynomolgus monkey toxicity study, after either single or repeated cetuximab infusion of up to 4 times the clinical dose, when scaled by total body surface area. The infusion reactions have been identified in a black box WARNING in the label, as well as in the WARNINGS section of the package insert.

Pulmonary toxicities not observed in the cynomolgus monkey studies included severe interstitial lung disease, and interstitial pneumonitis resulting in death in 3 and one, respectively, of 633 patients with advanced colorectal cancer during ERBITUX™ treatment. An additional case of interstitial pneumonitis was reported in a patient with head and neck cancer, treated in an investigative study with cetuximab in combination with cisplatin. The onset of symptoms occurred between the fourth and eleventh doses of treatment in all reported cases. There were no effects of ERBITUX™ treatment on respiratory safety pharmacology in cynomolgus monkeys. Additionally, no histopathological evidence of either interstitial lung disease or pneumonitis was observed in the lungs of cynomolgus monkeys treated for up to 39 weeks with cetuximab at doses of 0.4 to 4 times the clinical dose, as scaled by total body surface area.

PHARMACOLOGY/TOXICOLOGY REVIEW

3.1   INTRODUCTION AND DRUG HISTORY

STN BLA number: 125084/0
Review number: 1
Sequence number/date/type of submission: 000/8-14-03/original licensing application
Information to sponsor: Yes (X) No ( ) (request for Segment II reproductive toxicology study as post-marketing commitment)
Sponsor and/or agent: IMCLONE SYSTEMS, INC.
Manufacturer for drug substance: ImClone Systems, Incorporated (BB36), 36 Chubb Way, Somerville, NJ, 08876; Lonza Biologics Incorporated (Lonza; pivotal trial material and all C225 used in preclinical studies manufactured here), 101 International Drive, Pease International Tradeport, Portsmouth, NH 03801

Reviewer name: Anne M. Pilaro, Ph.D.
Division name: Division of Therapeutic Biologic Oncology Products, ODE VI
HFM #: 573
Review completion date: February 11, 2004

Drug:

Trade name: ERBITUX™
Generic name: cetuximab
Code name: C225, IMC-C225, BMS-564717, EMD-271786
Chemical name: not applicable
CAS registry number: not available
Molecular formula/molecular weight: No molecular formula was provided; MW 151.8 kDa. For the amino acid sequence of the heavy and light chains of ERBITUX™, please see Figures 3.2.S.1.2-6 and 3.2.S.1.2-7, respectively, in section 3.2.S.1.2.2 of the Chemistry, Manufacturing, and Controls section of the original BLA submission.

Structure: ERBITUX™ is a chimeric mouse/human monoclonal antibody of the IgG1 isotype, with a molecular weight of 151.8 kDa. It is composed of — polypeptide chains, — — heavy (γ) chains ——————————————————————— light chains (κ)

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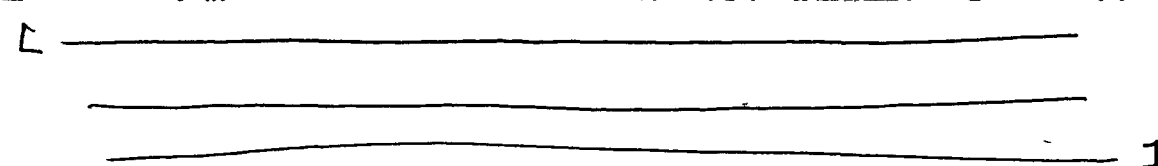
The structure of cetuximab is abstracted from the Chemistry, Manufacturing, and Controls section of the BLA submission (Figure 3.2.S.1.2-1, Section 3.2.S.1.2), and is included, below as Figure 1.

Figure 1.

Relevant INDs/NDAs/DMFs: BB IND #5804

Drug class: monoclonal antibody

Indication: ERBITUX™, used in combination with irinotecan, is indicated for the treatment of EGFr-expressing, metastatic colorectal carcinoma in patients who are refractory to irinotecan-based chemotherapy.

Clinical formulation: sterile, preservative-free, clear, colorless liquid containing 2.0 mg/ml cetuximab, 8.48 mg/ml NaCl, 0.42 mg/ml sodium phosphate monobasic, monohydrate, 1.88 mg/ml sodium phosphate dibasic heptahydrate, pH 7.0 to 7.4.

Route of administration: i/v infusion over 1-2 hours

Proposed use: initial loading dose of 400 mg/m^2, administered over 2 hour infusion, followed by weekly i/v infusions of —— mg/m^2, over a 1 hour period. ERBITUX™ is to be administered weekly until disease progression or intolerable toxicity occurs.

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

Studies reviewed within this submission:

Pharmacology Studies:

1. Cross-reactivity of anti-epidermal growth factor receptor (EGFr) chimeric monoclonal antibody with hepatic tissues of multiple species. Study #PAI IM108 (PRBC0294-03, ImClone Study #930004250 v1.0)

2. Cross-reactivity of biotinylated chimeric monoclonal antibody C225 with cryosections of normal cynomolgus monkey tissue. Study #GRA00406 (ImClone Study #930004248 v1.0).

3. Cross-reactivity of C225 with normal cynomolgus monkey and goat tissues. Study #PAI IM748 (ImClone Study #930004253 v1.0).

4. Immunoanatomic distribution and immunopathologic analysis of monoclonal antibody M225. Study # —— IMC04 (ImClone Study #930004259 v1.0).

5. Cross-reactivity of anti-epidermal growth factor receptor (EGFr) chimeric monoclonal antibody with human tissues. Study #PAI IM112R (PRBC-0294-04, ImClone Study #930004272 v1.0).

6. Cross-reactivity of C225 with normal human tissues. Study #PAI IM747 (ImClone Study #930004273 v1.0).

7. In vitro binding properties and specificity of C225 (Cetuximab). Study #Merck HGK 01-02 (ImClone Study #930004299 v1.0).

8. Comparative tissue binding study with mouse, rat, rabbit, monkey, and human tissue. Study #BMS DS02124 (ImClone Study #930003578 v 2.0).

9. Pre-clinical pharmacology of Erbitux (IMC-C225, BMS-564717 and BMS-576365), and anti-EGF receptor antibody. (ImClone Study #930003217 v1.0).

10. Evaluation of C225 monoclonal antibody (Erbitux) alone or in combination with irinotecan (CPT-11) against —— colon carcinoma xenografts. Study #SJ350501 (ImClone Study #930004236 v1.0).

11. Inhibition of human pancreatic carcinoma cell growth in vitro and in vivo by chimeric anti-EGFR monoclonal antibody C225. Study #RR0298-12 (ImClone Study #930004289 v1.0).

12. Inhibition of renal cell carcinoma cell growth in vitro and in vivo by chimeric anti-EGFR monoclonal antibody C225. Study #RR0297-20 (ImClone Study #930004280 v1.0).

13. Treatment of EGFr negative human tumor xenografts with a chimeric anti-EGFr monoclonal antibody IMC-225. Study #RR0201-10 (ImClone Study #930004295 v 1.0).

14. IMC-C225 activity in human tumor cell lines with various levels of EGFr expression. Study #RR0201-14 (ImClone Study #930004263 v1.0).

15. Pre-clinical studies with IMC-C225 and irinotecan (CPT-11) in human colon carcinoma models. Study #RR0201-08 (ImClone Study #930004261 v 1.0).

16. CPT-11/IMC-C225 combination therapy on , —— colon carcinoma xenografts , —— ——, Study #2001-0607 (ImClone Study #930004256 v1.0).

17. Irinotecan/IMC-C225 combination therapy on , —— colon carcinoma xenografts —— Study #2001-1120 (ImClone Study #930004287 v1.0).

18. CPT-11/Erbitux combination therapy on HT-29 xenografts refractory to single agent CPT-11 (HT-29 #27). Study #1175-02 (ImClone Study #930004286 v1.0).

19. CPT-11/Erbitux combination therapy on DLD-1 xenografts refractory to single agent CPT-11 (DLD-1 #21). Study #1177-02 (ImClone Study #930004285 v1.0).

20. Combination therapy with Erbitux and CPT-11 in the BxPC-3 human pancreatic carcinoma xenograft model (Panc No. 19). Study #1101-02 (ImClone Study #930004288 v1.0).

21. Conventional 5-FU/leucovorin therapy combined with Erbitux on three colon carcinoma xenograft models (DLD-1 #14, HT-29 #16, and ⌐‾‾‾‾⌐). Study #2001-1115 (ImClone Study #930004282 v1.0).

22. CPT-11/5-FU/leucovorin combination therapy with Erbitux on DLD-1 intra-hepatic colon carcinoma tumors ⌐‾‾‾⌐ Study #2001-1127 (ImClone Study #930004361 v1.0).

23. CPT-11/5-FU/leucovorin combination therapy with Erbitux on HT-29 intra-hepatic colon carcinoma tumors ⌐‾‾⌐ . Study #1105-02 (ImClone Study #930004278 v1.0).

24. CPT-11/5-FU/leucovorin combination therapy with Erbitux on colon carcinoma xenografts (DLD-1 #24, HT-29 #16, ‾‾‾‾). Study #2001-1116 (ImClone Study #930004365 v1.0).

25. CPT-11/5-FU/leucovorin combination therapy with Erbitux on colon carcinoma xenografts (DLD-1 #18, HT-29 #22). Study #2002-0220 (ImClone Study #930004277 v1.0).

26. Study of irinotecan antitumor activity in the ⌐ ‾‾ human colon tumor xenograft model ⌐‾‾‾‾ Study #2008-03 (ImClone Study #930004269 v1.0).

27. Study of irinotecan antitumor activity in the ‾‾ human colon tumor xenograft model (T-84 #2EC). Study #2027-03 (ImClone Study #930004258 v1.0).

28. Study of irinotecan and cetuximab combination therapy in an SN-38 resistant colon tumor xenograft model (HT-29 #34). Study #2008-03 (29.38R10, ImClone Study #930004254 v1.0).

29. Study of combination therapy with cetuximab and irinotecan in a model of GEO colon carcinoma xenografts refractory to single agent irinotecan (GEO #1). Study #2026-03 (ImClone Study #930004255 v1.0).

Secondary Pharmacodynamic Studies: No studies of this type were included in the submission.

Safety Pharmacology Studies:
1. C225 (EMD 271 786) cardiovascular and respiratory effects in the anaesthetized cynomolgus monkey following intravenous administration. Study #0070-100-d6146 (ImClone Study #930004468 v1.0).

Pharmacokinetics Studies:
1. C225 (EMD 271786) pharmacokinetics after single intravenous infusion in the cynomolgus monkey. Study #221-014. (ImClone Study #930004388 v1.0).
2. Analytical report for C225 pharmacokinetics after single intravenous infusion in the cynomolgus monkey. Study #PKM 11-01 (ImClone Study #930004367 v1.0).
3. C225 (EMD 271786) pharmacokinetics after single intravenous infusion in the cynomolgus monkey. Study #PKM 09-01 C225 (ImClone Study #930004369 v1.0).
4. C225 (EMD 271786) cardiovascular and respiratory effects in the anaesthetized cynomolgus monkey following intravenous administration. Study #PKM 22-02 (ImClone Study #930004374 v1.0).

Comment: Studies 1-3 listed above in the Pharmacokinetics section are all parts of Study #221-014. The analyses of the serum samples and of the resulting data were included in the BLA submission as separate reports. These three studies will be reviewed together, in Section 3.3, Pharmacokinetics/Toxicokinetics, below.

Toxicology Studies:

1. Single dose intravenous toxicity study of C225 (anti-EGFr chimeric MAb) in CD-1 mice. Study #. 2525-101 (ImClone Study #930004375 v1.0).

2. Single dose intravenous toxicity study of C225 (anti-EGFr chimeric MAb) in CD-1 mice. Study #. 2525-102 (ImClone Study #930004376 v1.0).

3. An acute intravenous infusion toxicity study and pharmacokinetic study of C225 in albino rats. Study 354165 (ImClone Study #930004389 v1.0).

4. Serology report on rat acute toxicity and pharmacokinetics of C225 in albino rats. Study #ARBC0294-09 (ImClone Study #930004386 v2.0).

5. An intravenous infusion toxicity study of C225 in the albino rat for up to 28 days. Study #54167 (ImClone Study #930004377 v1.0).

6. Serology report of 28 day rat subacute toxicity and pharmacokinetics of C225 in albino rats. Study #ARBC0294-09 (ImClone Study #930004386 v2.0).

7. C225 (EMD 271 786) - 39-week intravenous (infusion) toxicity study in the cynomolgus monkey with a 6-week treatment-free period. Study #070-087 (ImClone Study #930004381 v1.0).

8. EMD 271786 - 39-week intravenous (infusion) toxicity study in the cynomolgus monkey with a 6-week treatment-free period - toxicokinetics. Study #PKM 45-01 (ImClone Study #930004387 v1.0).

9. Analytical report for C225 serum determinations in study 070-087: C225 (EMD 271 786) 39-week intravenous (infusion) study in the cynomolgus monkey with a 6-week treatment-free period. Study #SR0201-11 (ImClone Study #930004398 v1.0).

10. EMD 271 786 – Bacterial mutagenicity assay, *Salmonella typhimurium* and *Escherichia coli*. Merck Study #T15368 (ImClone Study #930004390 v1.0).

11. EMD 271 786 – Micronucleus test in rats after intravenous administration. Merck Study #T15361 (ImClone Study #930004399 v1.0).

12. EMD 271 786 – Local tolerance study in rabbits after intravenous, intraarterial, paravenous, intramuscular, and subcutaneous treatment. Study #T15386 (ImClone Study #930004392 v1.0).

13. EMD 271 786 (Batch 204485) – Local tolerance study in rabbits after intravenous, intraarterial, paravenous, intramuscular, and subcutaneous treatment. Study #T15509 (ImClone Study #930004393 v1.0).

14. EMD 271 786 (Batch E2622LO02). – Local tolerance study in rabbits after intravenous, intraarterial, paravenous, intramuscular, and subcutaneous treatment. Study #T15510 (ImClone Study #930004394 v1.0).

15. Serology report of 28 day rat subacute toxicity and pharmacokinetics of C225 in albino rats – anti-C225 antibody determination. Study #ARF-C0294-11 (ImClone Study #930004395 v1.0).

16. Incidence of anti-C225 antibodies in study #070-087: C225 (EMD 271 786) 39-week intravenous (infusion) toxicity study in the cynomolgus monkey with a 6-week treatment-free period. Study #SR0201-12 (ImClone Study #930004496 v1.0).

Studies not reviewed within this submission: No additional non-clinical studies conducted by the sponsor have been identified that have not been included in the present BLA submission (*i.e.* submitted to the IND file but not to the BLA). Numerous publications from the open literature, describing the pharmacologic effects of C225 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 I. Unless noted in the

summary of the study evaluations below, data from these published studies were not included in this review.

3.2   PHARMACOLOGY

3.2.1   Brief summary

ERBITUX™ binds to the epidermal growth factor receptor (EGFr), which is expressed on the surface of normal human epithelial and tumor cells. The EGFr is constitutively expressed in many normal epithelial tissues, including the skin follicle, placenta, esophageal mucosa, and salivary and mammary glands. Over-expression of EGFR is also detected in many human cancers including those of the colon and rectum.   .

Cetuximab 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. ERBITUX™ binding to the EGFr also stimulates receptor internalization, leading to a reduction of EGFr expression on the cell surface, and a decreased responsiveness of tumor and other cells to EGF and related ligands. The antagonistic action of ERBITUX™ includes inhibition of phosphorylation and activation of EGFr-associated kinases and subsequent blockade of receptor signal transduction, resulting in cell cycle arrest, suppression of tumor cell growth, induction of apoptosis, decreased production of enzymes associated with tumor metastasis (*e.g.* matrix metalloproteinase), and decreased secretion of other growth factors, *e.g.* vascular endothelial growth factor associated with tumor growth and angiogenesis.

In vitro assays and *in vivo* animal studies have shown that ERBITUX™, alone or in combination with irinotecan with or without 5-fluorouracil/leukovorin chemotherapy inhibits the growth and survival of human tumor cells that over-express the EGFr. No anti-tumor effects of cetuximab were observed in immune deficient mouse models bearing human tumor xenografts lacking EGFR expression.

3.2.2   Primary pharmacodynamics
Mechanism of action: 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. The growth of EGFr positive cancer cells *in vitro* is enhanced by the binding of EGF, or other growth factors that bind the EGFr. ERBITUX™ (cetuximab) inhibits the growth of cancer cells *in vitro* and *in vivo* by binding the extracellular domain of the EGFr and competitively inhibiting the binding of EGF and other ligands of the EGFr (*i.e.* transforming growth factor-α). Binding of ERBITUX™ to the EGFr also stimulates receptor internalization, leading to a reduction of EGFr expression on the tumor cell surface. Blockade of EGFr function by ERBITUX™ inhibits phosphorylation and activation of EGFr-associated kinases, resulting in inhibition of cell growth, induction of apoptosis, decreased matrix metalloproteinase production, and decreased vascular endothelial growth factor production.

In vitro assays and *in vivo* animal studies have shown that ERBITUX™, alone or in combination with irinotecan, fluorouracil, or cis-platin chemotherapy inhibits the growth and survival of human tumor cells that over-express the EGFr. No anti-tumor effects of cetuximab were

observed in immune deficient mouse models bearing human tumor xenografts lacking EGFR expression.

Drug activity related to proposed indication: **Comment:** A list of the preclinical studies conducted in support of the pharmacologic activity of ERBITUX™ is provided, above ("Studies reviewed within this submission, Pharmacology Studies"). A total of 29 preclinical 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* pharmacologic actions of ERBITUX™ were reviewed for this BLA submission. The data from these studies will be summarized and reported below, with the exception of Studies #RR0201-10 and #RR-0201-14 demonstrating the effects of ERBITUX™ treatment in human tumor xenografts with varying levels of EGFr expression. These two studies will be reviewed in more detail below, as data from these studies were included in the label for ERBITUX™, and 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 Cetuximab to EGFr
The pharmacologic activity of ERBITUX™ was evaluated in a series of *in vitro* EGFR cell surface expression, binding, receptor activation and cytotoxicity assays, and *in vivo* in established human tumor xenografts in athymic, nude mice treated with either cetuximab alone, or in combination with irinotecan, cis-platinum, methotrexate, or 5-fluorouracil. Using immobilized, purified human EGFr isolated from the human epidermoid ——ι cancer cell line A431 in a solid-phase, enzyme-linked immunosorbent assay, ERBITUX™ was demonstrated to bind EGFr with high affinity, and this binding could be competitively inhibited by the murine monoclonal anti-EGFr antibody M225, showing that both antibodies recognize identical epitopes on human EGFr. The binding interaction between EGFr and cetuximab in *in vitro* assays was predominantly hydrophobic in nature, as it was not affected by changes in either pH or ionic strength of the buffer solution. The apparent affinity of ERBITUX™ for isolated, purified human EGFr in these *in vitro* assays was not determined (Study #HGK01-02).

Flow cytometric evaluation of tumor cells following staining with ERBITUX™ or M225 was used to evaluate the cell surface expression of EGFr by a panel of human cell lines from tumors of different embryonic cell origin. Both cetuximab and M225 bound to EGFr positive, , ——
—————————— epidermoid —— cancer cells with high affinity; the EC_{50} for cetuximab binding was — μM to A431 tumor cells, and   nM for ،   —————— cells. Additional *in vitro* studies showed that C225 or M225 antibodies were approximately 6.6 and 4.5-fold more active than unlabeled EGF in competitively inhibiting binding of the fluorescein-conjugated EGF to EGFr, either in the ELISA-based assay or in A431 human tumor cells (Studies #HGK01-02, #930003217).

ERBITUX™ treatment of A431 human epidermoid —— cancer cells or —————— cancer cells *in vitro* resulted in an inhibition of progression of tumor cells through the cell cycle, and arrest and accumulation of tumor cells in the G1 phase.[1] Cell cycle arrest was accompanied by a drop in the cell cycle associated, CDK-1, -2, -4, and –6 histone kinase activities, decreases in

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[1] Prewett, M., P. Rockwell, C. Rose, and N.I. Goldstein. 1996a. Altered cell cycle distribution and cyclin-CDK protein expression in A431 epidermoid carcinoma cells treated with doxorubicin and a chimeric monoclonal antibody to the epidermal growth factor receptor. *Mol. Cell Differen.*, 4:167-186; Prewett, M., P. Rockwell, C. Rose, and N.I. Goldstein. 1996b. Anti-tumor and cell cycle responses in KB cells treated with a chimeric anti-EGFR monoclonal antibody in combination with cisplatin. *Internat. J. Oncol.*, 9:217-224.

expression and/or activity of the cyclins A, D, or E, and an accumulation of hypophosphorylated retinobastoma protein. Reduction of tumor cell proliferation was also observed after cetuximab treatment in several different human, EGFr expressing tumor cell lines either using *in vitro* cellular proliferation assays, or *in vivo* using immunohistochemical analysis of anti-PCNA (proliferating cell nuclear antigen) staining of human pancreatic carcinoma xenografts. Binding of cetuximab to the EGFr and subsequent blockade of EGFr signal transduction in human tumor cells were accompanied by inhibition of EGFr-mediated co-activation of the nuclear transcription factors NF-κB and activator-protein-1, reductions in EGFr- and STAT-3 kinase phosphorylation, and increased phosphorylation and functional inactivation of bcl-2, and induction of programmed cell death or apoptosis.

Data from studies published in the open literature have shown that cetuximab treatment can increase the sensitivity of some human tumor cell lines or xenografts in immunodeficient mice to radiotherapy with ionizing radiation, through suppression of DNA repair mechanisms secondary to inhibition of the EGFr signaling pathway(s).[2] Other potential mechanisms of cetuximab inhibition of tumor cell growth may include down-regulation of EGFr on the tumor cell surface, induction of antibody-dependent cellular cytoxicity through interaction of cetuximab bound tumor cells to Fc receptor present on activated macrophages or NK cells, alterations in tumor cell invasion, migration and metastasis, and decreases in tumor angiogenesis *in vivo*.

In vitro Cytotoxicity of Cetuximab

The *in vitro* anti-tumor activity of cetuximab was evaluated against a panel of human tumor cell lines, which had previously been demonstrated to have either high, low, or no EGFr expression (Study #930003217). A summary table of the cell surface EGFr expression on these tumor cell lines, after either *in vitro* or *in vivo* passage is included in Table 1, below (abstracted from Study Report #930003217).

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[2] Huang, S.M. and P.M. Harari. 2000. Modulation of radiation response after epidermal growth factor receptor blockade in squamous cell carcinomas: Inhibition of damage repair, cell cycle kinetics, and tumor angiogenesis. *Clin. Cancer Res.*, 6:2166-2174; Bandyopadhyay, D., M. Mandal, L. Adam, J. Mendelsohn, and R. Kumar. 1998. Physical interaction between epidermal growth factor receptor and DNA-dependent protein kinase in mammalian cells. *J. Biol. Chem.*, 273:1568-1573.

Table 1: **EGF Receptor Expression Levels and Activation Status for Various Human Tumor Cells and Human Tumor Xenografts**

Tumor -propagation	Histology	Total EGFr Levels	Activated EGFr Levels
————	Ovary		
In vitro[a,b]		-	-
In vivo		NA	NA
A431	Skin		
In vitro[a]		+++	+++
In vivo		NA	NA
PC-3	Prostate		
In vitro[a]		++	-
In vivo		NA	NA
————	Prostate		
In vitro[a]		+	+++
In vivo		NA	NA
———	Colon		
In vitro[a]		++	NA
In vivo		NA	NA
———	Colon		
In vitro[a]		++	-
In vivo		NA	NA
HT29	Colon		
In vitro[a]		++	++
In vivo[b]		+	ND
GEO	Colon		
In vitro[a]		+++	+++
In vivo[b]		+	ND
———	Colon		
In vitro		NA	NA
In vivo[b]		++	ND
———	Lung		
In vitro[a]		+++	+++
In vivo[b]		+++	ND
———	Breast		
In vitro[a]		+	+
In vivo[b]		-	ND

- , negative. +, low. ++, medium. +++, high. NA, Not available; ND, Not detected by pY 1068 EGFR ELISA kit. [a] as determined by Western Blot; [b] as determined by ELISA

In vitro exposure of human colon or colorectal tumor (cell lines GEO, .— ١, pancreatic
carcinoma (Bx-PC3, ————————— , renal cell carcinoma ¡ ——————————
—————————————————————————————— (ZR-75-1), prostate cancer
———— DU-145, and PC-3), or epidermoid   carcinoma cells to cetuximab resulted in dose-
related inhibition of tumor cell proliferation, as demonstrated by decreased ^{3}H-thymidine
incorporation, cell counting and/or viability by MTS staining, colony formation in soft agar, and
assays for receptor phosphorylation. The range of cetuximab concentrations tested was between
——————— The extent of inhibition of tumor cell growth by cetuximab exposure varied
depending on the model used, but generally ranging between 15 and > —— of control values,
with IC_{50} values (where determined) in the range of ———— (Studies #RR0298-12,
#RR0297-20, #RR0201-14, and Appendix I, literature citations #13, #55, and #57).

Comment: Of note, in study #930003217 no *in vitro* cytotoxic effects of cetuximab were
observed on the A431, HT29, ————————————' tumor cell lines, at concentrations as
high as 50 µg/ml (320 nM). The reason for this discrepancy was not identified in the final study
report, nor were these data identified in the written Pharmacology Summary in the BLA.

In vivo Anti-tumor Effects of Cetuximab Monotherapy
The anti-tumor effects of cetuximab treatment *in vivo* were evaluated using established, EGFr
expressing, s/c human colon, pancreatic, .—— epidermoiä —— and breast tumor xenografts in
athymic, nude mice (Studies #SJ350501, #RR0201-8, #2001-0607, #2001-1120, #1175-02,
#1177-02, #1101-02, #2001-1115, #2001-1127, #1105-02, #2001-1116, #2002-0220, #2022-03,
#2027-03, #2008-03, and #2026-03). In these studies, 0.25, 0.5, or 1.0 mg/mouse cetuximab was
given by intraperitoneal injections every three days, for durations of 3 to eight weeks. In some
studies, tumor bearing mice were treated concomitantly with irinotecan (CPT-11) alone or in
combination with 5-fluorouracil and leukovorin, either as chemotherapy alone or together with
ERBITUX™.

Delayed tumor growth was observed in GEO human colon cancer (Study #930003217), or Bx-
PC3 pancreatic carcinoma (study #RR0298-12) s/c xenografts in nude mice, following twice
weekly, i/p treatment with 0.5 to 1.0 mg cetuximab/mouse for 5 to 8 weeks. No complete
regressions of tumor were reported with single agent cetuximab therapy, and survival was not
measured in these studies. By contrast, cetuximab monotherapy of A431 tumor xenografts, with
high levels of EGFr expression resulted in dose-related anti-tumor effects, including regression of
tumor in 9/10 animals following 1 mg/mouse C225 twice weekly for 5 weeks, with 4 of these
mice tumor-free (referenced in Appendix I, literature citation #22, and Study #RR0201-14).
Similarly, data cited from the open literature for human, ¡ ———— TCC bladder tumor bearing
mice treated with 1.0 mg/mouse ERBITUX™, twice weekly for 5 weeks demonstrated strong
inhibition of tumor growth, regression of established tumor, and prevention of metastasis as
compared to mice treated with saline control[3].

Comment: The following two studies show the anti-tumor activity of ERBITUX™ monotherapy
in established, human tumor xenografts in nude mice with different levels of cell surface EGFr
expression. These studies were reviewed and detailed results are reported, below.

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[3] Perrotte, P., T. Matsumoto, K. Inoue, H. Kuniyasu, B.J. Eve, D.J. Hicklin, R. Radinsky, and C.P.N.
Dinney. 1999. Anti-epidermal growth factor receptor antibody C225 inhibits angiogenesis in human
transitional carcinomal growing orthotopically in nude mice. *Clin. Cancer Res.*, 5:257-264.

Study title: Treatment of EGFr negative human tumor xenografts with a chimeric anti-EGFr monoclonal antibody IMC-225.

Key findings: No antitumor activity of ERBITUX™ was observed in two murine xenograft models with established, EGFr negative, human tumors.

Study #: RR0201-10 (ImClone Study #930004295 v 1.0)

Methods: Athymic, nude mice were implanted s/c with A431 human epidermoid carcinoma, ——————— carcinoma, or ———— colon adenocarcinoma cells and tumors allowed to establish to a volume of approximately ———— Mice were then treated with either sterile saline (control) or 1 mg/mouse cetuximab, i/p, every three days for eight weeks, or until tumor burdens were so great that the animals were euthanized for humane reasons.

Comment: The A431 cell line has previously been demonstrated to express high levels of EGFr (Study #RR0201-14, below, and referenced in literature citation #2), and respond *in vivo* in a mouse xenograft model to ERBITUX™ treatment (*ibid.*). ———— is an EGFr negative clone of the ——— human colon adenocarcinoma cell line, which was established in the sponsor's laboratory. The . ————— carcinoma is also negative for EGFr expression on the tumor cell surface. The level of EGFr expression was confirmed using either flow cytometric evaluation of cetuximab stained tumor cells, or by immunohistochemical analysis of cryosections from tumors established in nude mice.

Results: *In situ* C225 immunoperoxidase staining of tumors excised from mice, and flow cytometric evaluation of cetuximab binding *in vitro* to ———— and ———— tumor cells confirmed that there was no expression of EGFr on the cell surface membrane. Treatment of mice with ERBITUX™ at a dose of 1 mg/mouse, i/p every three days for three or eight weeks had no effect on ———— or . ————— · tumor cell growth, respectively, as compared to animals injected with saline by the same schedule as a control group. Mean tumor volumes in the ————tumor bearing animals after eight weeks of cetuximab treatment were 2299 ± 838 mm³, as compared to 2885 ± 306 mm³ in the mice injected with saline. Similarly, mean ——— tumor volumes in the saline control treated group were   These data are summarized in the figures, below, that were extracted from the final report for this study submitted to the BLA.

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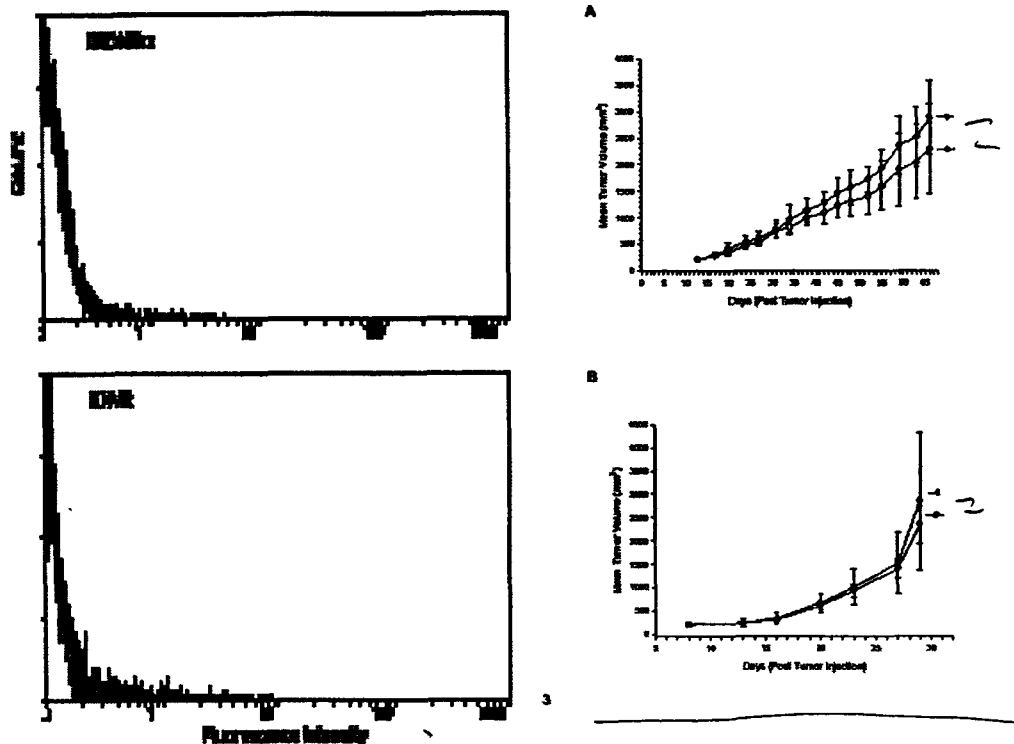
Figure 2. Correlation of cell surface EGFr expression (left) and anti-tumor activity of cetuximab (right) in human tumor xenograft models. Left panels: Both ——— human colon adenocarcinoma tumor (top) and ——— (lower) human gastric carcinoma tumor cell lines are negative for EGFr expression, as determined by flow cytometry. Black lines represent histograms from cetuximab-stained cells; grey lines represent tumor cells with no primary antibody as a negative control. Right panels: No inhibition of tumor growth is observed following ERBITUX™ treatment of nude mice bearing xenografts of either EGFr-negative tumor (top panel A, ——— lower panel B, ———

Comment: Data regarding the effects of C225 treatment on growth of A431 tumors in immunodeficient mice were not included in the final study report. Previous information published in the open literature with this cell line demonstrated dose-related effects of cetuximab in this model, with tumor regression observed in 90% of mice treated with 1.0 mg/mouse C225, i/p, twice weekly for 5 weeks (referenced in Appendix 1, Study #22, and Study #RR0201-14, below).

Study conclusion: No anti-tumor effects of ERBITUX™ were observed in human tumor xenografts of EGFr negative cells in athymic, nude mice.

Study title: IMC-C225 activity in human tumor cell lines with various levels of EGFr expression.

Key findings: Cetuximab treatment of EGFr expressing human tumor xenografts in nude mice significantly inhibited tumor growth as compared to mice injected with saline, regardless of the level of cell surface EGFr expression on the different tumor lines.

Study #: RR0201-14 (ImClone Study #930004263 v1.0)

Methods: ——— A431 (human epidermoid ——carcinoma), BxPC-3 (human pancreatic carcinoma) or . ___ human —— carcinoma tumor cells suspended in 400 µl of 50% Matrigel® solution were injected s/c into NIH *nu/nu* athymic, nude mice. When tumors had reached an established volume of approximately ———— five mice per group were treated with i/p injections of either sterile saline (control) or 1 mg/mouse cetuximab, every three days for up to ten weeks, or until animals had to be euthanized due to excessive tumor burdens.

Results: Figure 3, below shows the anti-tumor response of cetuximab treatment in each of the human tumor models, expressing different levels of EGFr. Cell surface EGFr expression was demonstrated by flow cytometric evaluation of cetuximab stained cells, and is shown in the inset panels to each graph. The A431 tumor expressed the highest level of EGFr on the cell surface, and was the most responsive to treatment with C225. At day 28 of treatment, statistically significant reductions in tumor burden were observed in mice treated with cetuximab, as compared to those animals injected with the saline control. Mean A431 tumor volumes at this point were 32 ± 20 mm3 in the ERBITUX™ treated mice, as compared to 1355 ± 455 in the saline control group (Figure 3A, p = 0.01, Student's *t*-test). Significant inhibition of BxPC-3 and ——— tumor growth, with intermediate and low levels of EGFr expression, respectively, was · also observed after cetuximab treatment. Figure 3B shows the response for BxPC-3 cells over the duration of the treatment period. At study d 63 of treatment, mean tumor volume in the saline treated mice was 1368 ± 440 mm^3, as compared to 440 ± 69 mm^3 in the ERBITUX™ treated mice (p = 0.03, Student's *t*-test). Similarly, mean . ——— tumor volume in the cetuximab treated group at treatment d 37 was 748 ± 66 mm3, as compared to 1744 ± 391 mm3 in the saline control injected group (p = 0.03, Student's *t*-test).

Comment: The flow cytometry histograms and the tumor growth curves in Figure 3, below seem to suggest that the anti-tumor activity of ERBITUX® is greater in the high EGFr expressing, A431 cell line than in the intermediate EGFr expressing, BxPC-3 pancreatic tumor over a longer duration of treatment. However, the apparent inhibition of ——— tumor growth, with the lowest level of EGFr expression per cell does not appear qualitatively different from that of the BxPC-3 tumor. No data for tumor measurements in the individual animals were included in the final study report, so the statistical analyses, and the percent of inhibition of tumor growth over time, and between the different tumor types cannot be independently verified.

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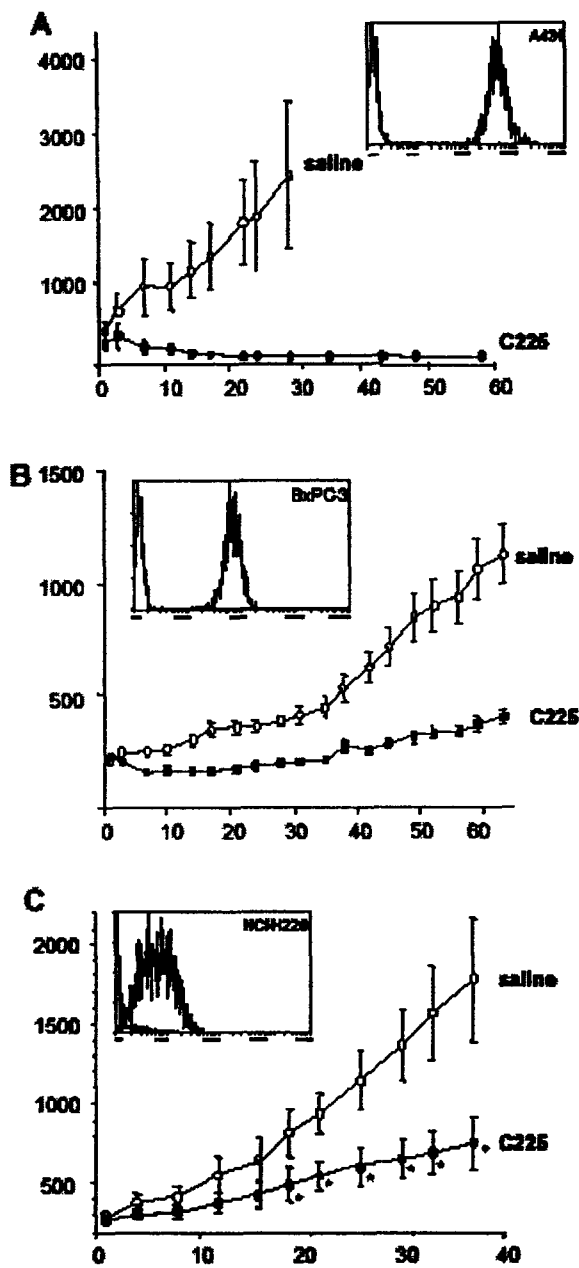
Figure 3. Tumor growth curves for A431 epidermoid carcinoma (panel A), BxPC-3 pancreatic carcinoma (panel B), and NCI-H226 lung carcinoma xenografts in nude mice. Curves represent the mean tumor volume, ± S.E.M. for each measurement on study. Insets are histograms of flow cytometry results for cetuximab staining of each tumor cell line. Peak height on the Y-axis represents the number of cells positive for EGFr expression, and distance along the X-axis of the histogram represents the level of EGFr expression per cell (*i.e.*, right shift = greater number of EGF receptors per cell).

Study conclusion: Cetuximab treatment of human A431, BxPC-3, or NCI-H226 tumor xenografts in athymic, nude mice resulted in significant inhibition of tumor growth, regardless of the level of EGFr expression on the tumor cell surface.

Anti-tumor Effects of Cetuximab in Combination with Chemotherapy
The effects of ERBITUX™ in combination with irinotecan, 5-fluorouracil and leukovorin, or with the two chemotherapeutic agents together were evaluated in athymic, nude mice bearing human tumor xenografts of various colon, or pancreatic carcinoma cell lines (Studies #SJ35-0501, RR0201-08, #2001-0607, #2001-1115, #2001-1116, and #2002-0220, colon cancer xenografts; #2001-1127 and #1105-02, intrahepatic colon tumor metastasis model; #2022-03 and #HT29-27, irinotecan-refractory colon cancer; #1101-02 and #RR0298-12, pancreatic cancer).

Athymic nude mice were implanted subcutaneously with human tumor cells suspended in 50% Matrigel® solution, and allowed to establish to measurable size, usually ⎯ In the intrahepatic metastasis models, mice were injected directly into the liver following laparotomy with either HT-29 of DLD-1 tumor cells, and the animals were allowed to recover for 3 d prior to initiating treatment. In most studies, the dose of cetuximab was 1 mg/mouse, i/p administered every three days for up to 9 weeks, or until tumor burdens became so large that the mice were euthanized for humane reasons. Concomitant chemotherapy with irinotecan (CPT-11, 42-100 mg/kg, Q7d), 5-fluorouracil and leukovorin (5-FU, 125 mg/kg and LV, 6.7 mg/kg, respectively, Q7d), or a combination of the two chemotherapy agents was initiated at the same time as C225. Saline was used as a vehicle control in all studies, and additional groups of animals receiving either cetuximab monotherapy or chemotherapy alone were included as the appropriate controls.

In all studies evaluated, there was a wide degree of anti-tumor response between the different xenograft models, and in the same tumor cell line growing either as a subcutaneous, or as an intrahepatic/intraperitoneal tumor model. In two studies, colon tumors that failed to demonstrated significant reduction following treatment of mice with high-dose irinotecan were selected and treated with cetuximab, alone or in combination with CPT-11 as a model of irinotecan-refractory tumor.

Using the HT-29, ⎯ DLD-1, or ⎯ colon carcinoma xenograft models, cetuximab monotherapy was demonstrated to delay tumor growth and result in decreased tumor burdens by 20 to 77%, as compared to saline control. As was observed in the studies using cetuximab monotherapy against different tumor xenografts *in vivo,* the response of the individual tumor cell lines was variable, both between different colon tumor lines in the same study, and between different studies with the same tumor line. Similar decreases in anti-tumor response were observed with combination irinotecan and 5-FU/LV chemotherapy, with maximal tumor inhibition (83% decrease in tumor burden, as compared to saline control) observed in ⎯ tumor bearing mice after 51 d of treatment (Study #2001-1115). In this same study, evidence of synergy between ERBITUX™ and chemotherapy was demonstrated in the HT-29 xenograft model, at study d 51. The results of this study are included in Table 2, below.

Table 2: Anti-tumor Response to Cetuximab in Combination with Irinotecan and 5-FU Chemotherapy in Human Colon Tumor Xenografts in Athymic, Nude Mice			
Treatment Group	**Mean Tumor Volume (Percent Inhibition of Growth vs. Control)**		
	DLD-1	**HT-29**	—
Saline control	2229 mm^3	2365 mm^3	861 mm^3
ERBITUX™	1861 mm^3 (17%)	1915 mm^3 (19%)	201mm^3 (77%)
5-FU/LV	1267 mm^3 (43%)	1686 mm^3(29%)	147 mm^3 (83%)
5-FU/LV + ERBITUX™	845 mm^{3a} (62%)	862 mm^{3a,b} (64%)	39mm^{3a,c} (95%)

[a] p < 0.05 vs. saline control
[b] p < 0.05 vs. 5-FU/LV alone
[c] p < 0.05 vs. ERBITUX™ alone

Similar results were observed in Study #2001-1116, in which a 99% inhibition of tumor growth was observed in —— bearing mice treated with the combination of ERBITUX™ and irinotecan, 5-FU, and leukovorin at study d 51. One mouse in this group had no evidence of tumor (complete regression) at time of sacrifice in study d 51; no complete regressions were observed in either the saline, cetuximab, or chemotherapy control groups. Inhibition of tumor growth by the combination of cetuximab and chemotherapy was significantly different from saline control for the DLD-1 tumor beginning from d 19 of treatment onward, and from d 18 of treatment onward for both the HT-29 and —— tumors in this study. These data are represented graphically in Figures 4-6, below.

DLD-1#14
CPT-11 (42 mg/kg), 5-FU (166 mg/kg), LV (6.7 mg/kg) + ERBITUX™ (1mg)
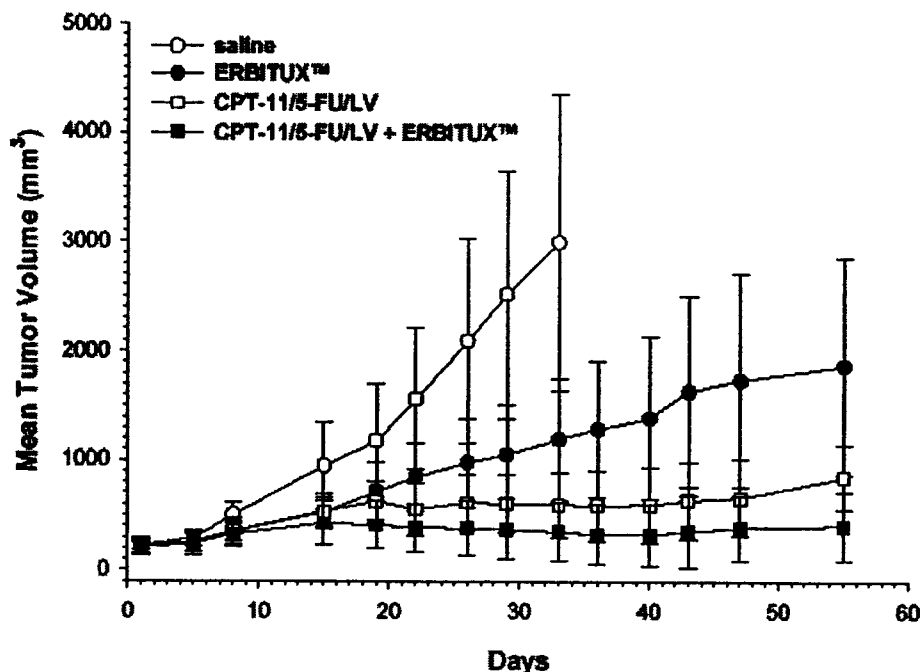
Figure 4. Mean tumor volumes for human DLD-1 colon tumor xenograft in athymic nude mice after treatment with cetuximab, 5-FU/leukovorin and irinotecan, or combination chemotherapy and ERBITUX ™.

HT-29 #16
CPT-11 (42 mg/kg), 5-FU (166 mg/kg), LV (6.7 mg/kg) + ERBITUX™ (1mg)
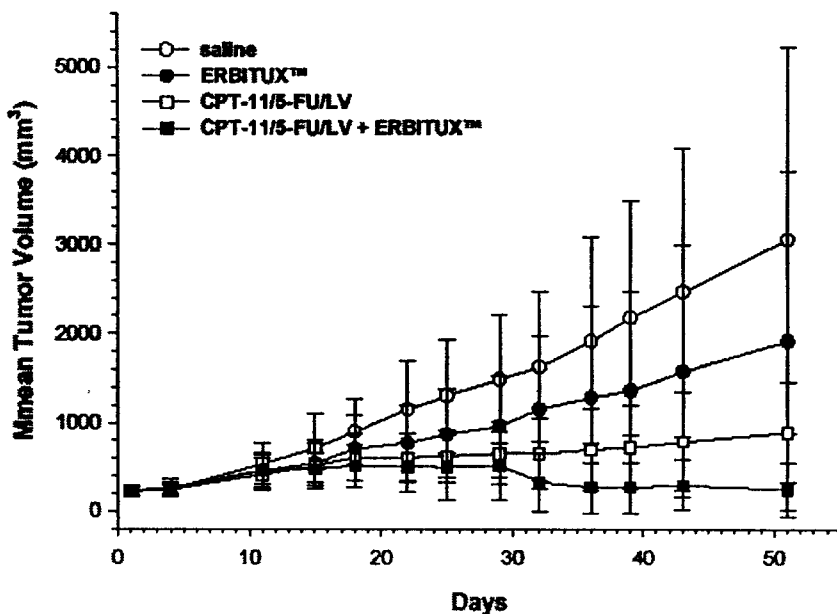

Figure 5. Mean tumor volumes for human HT-29 colon tumor xenograft in athymic nude mice after treatment with cetuximab, 5-FU/leukovorin and irinotecan, or combination chemotherapy and ERBITUX ™.

CPT-11 (42 mg/kg), 5-FU (166 mg/kg), LV (6.7 mg/kg) + ERBITUX™ (1mg)

Figure 6. Mean tumor volumes for . ————— colon tumor xenograft in athymic nude mice after treatment with cetuximab, 5-FU/leukovorin and irinotecan, or combination chemotherapy and ERBITUX ™.
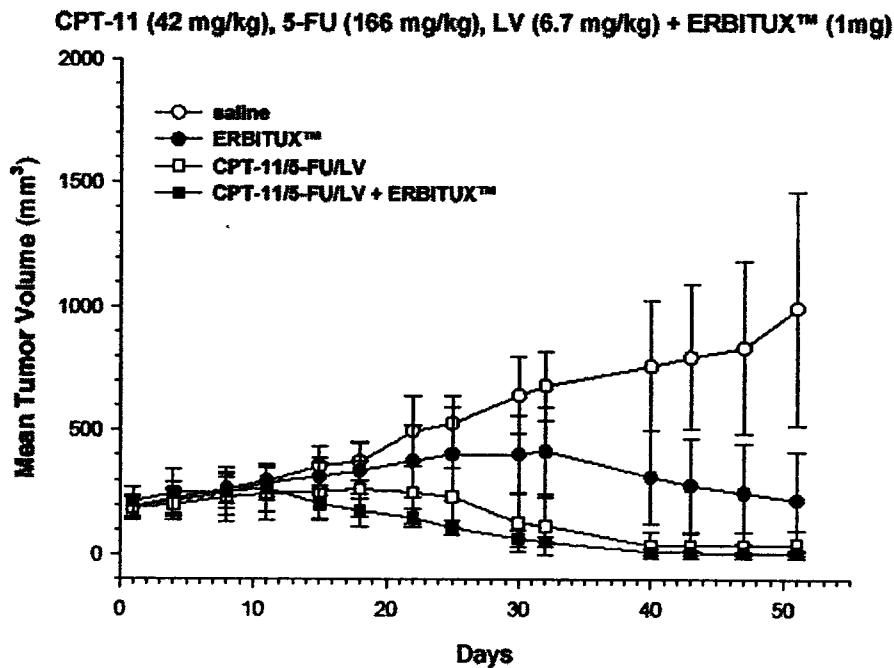
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Comment: None of the preclinical pharmacology studies submitted to the BLA included the individual animal data. The sponsor has claimed statistically significant differences from the saline control group in the ERBITUX™ alone, chemotherapy alone, and combination groups. However, the error bars on these graphs appear to show significant overlap between the groups. Without the individual values for each animal's tumor measurements at each time point, independent verification of these findings cannot be performed.

Comment: In both of these studies, and in Studies #2002-020 and #2001-0607, the addition of cetuximab to irinotecan chemotherapy, either alone or in combination with 5-FU and leukovorin rescue appears to have at best an additive effect, with the exception of the HT-29 tumors in Study #2001-1115. The sponsor has calculated their estimation of synergy based on a "predicted tumor volume" at each individual time point for both the control and the treated groups, rather than using the actual tumor measurements as the basis for this calculation. Additionally, the sponsor reports the results as "log cell kill," which while appropriate for *in vitro* anti-tumor evaluations, cannot truly be determined from measurements of tumor volume *in vivo*.

Cetuximab in combination with irinotecan alone also demonstrated significant anti-tumor activity in the BxPC-3 pancreatic carcinoma cell line, both *in vitro* and *in vivo*. Mice were treated for 9 weeks with 1 mg/mouse C225 i/p every three days, either alone or in combination with 50 mg/kg CPT-11 Q7d, followed by a 4 week, treatment-free observation period. Animals in the combination chemotherapy and ERBITUX™ treated group showed significant reductions in tumor burden at study termination on d 92, as compared to mice treated with saline as a control (p < 0.05, Mann-Whitney U-test; Study #1101-02). Differences were observed between saline and ERBITUX™ monotherapy beginning d 13 of treatment onward, and between CPT-11 alone and saline from d 26 onward. The combination of irinotecan and ERBITUX™ resulted in significant reductions in tumor burden from study d 8 onward; these reductions were also significantly different from the CPT-11 alone group beginning d 34, and from the cetuximab alone group beginning d 57. These data confirm that the human pancreatic carcinoma BxPC-3 is sensitive to ERBITUX™ monotherapy in an *in vivo* model of pancreatic cancer.

In the HT-29 intrahepatic metastasis model, the relative tumor burden in mice receiving combination treatment with C225 and CPR-11/5-FU/LV triple chemotherapy was reduced to 7% of that observed in the saline control (p = 0.002, Student's *t*-test), but was not significantly different from either chemotherapy alone (15% of control volume) or cetuximab alone (13% of control volume; Study #1105-02). No extrahepatic tumor was detected in 6/9 mice in the C225/chemotherapy combination group, as compared to 2/9 mice treated with ERBITUX™ alone and 2/6 surviving mice treated with chemotherapy as a control. Similarly, using the DLD-1 colon tumor cell line in the intrahepatic metastasis model, 5/9 mice (56%) treated with both cetuximab and CPT-11/5-FU/LV chemotherapy had no evaluable disease at study termination at 47 d in either the liver or in the extrahepatic sites, as compared to 100% incidence of tumor in the chemotherapy alone group, and one mouse each with evaluable tumor in the saline and C225 control groups.

Comment: The low incidence of tumor burden in the saline and cetuximab monotherapy groups in this study suggests an incomplete tumor "take" following injection of the DLD-1 tumor in these animals. The sponsor has used a model in which tumor cells are directly injected into the liver, and establish tumor in the liver and surrounding peritoneal cavity. This model does not truly represent the distribution of tumor within the liver in a metastatic setting, *i.e.* following injection into and subsequent hematogenous spread from the splenic or portal vein(s).

The human colon tumors ‎ were used in two different studies (Studies #2022-03 and #2027-03, respectively) to evaluate the anti-tumor effects of cetuximab alone or in combination with irinotecan in an irinotecan-refractory model. Briefly, mice were inoculated s/c with tumor suspended in 50% Matrigel® and the tumors were allowed to establish before treating with 100 mg/kg CPT-11 weekly, for 3 weeks. Animals bearing tumors that were at least 2-fold greater in volume than at time of irinotecan initiation were re-randomized to treatment with saline, continued CPT-11 at 100 mg/kg/week, or 1 mg/dose cetuximab, every three days for up to 7 weeks. Additionally, in Study #2027-03 lower doses of 25 and 40 mg/kg CPT-11 alone were also evaluated for anti-tumor activity. In both studies, the saline-treated control groups had to be sacrificed early due to excessive tumor burdens. The ‎ model (Study #A2022-03) demonstrated no difference in tumor growth inhibition between the CPT-11 alone or in combination with ERBITUX™ groups. Cetuximab monotherapy in this model had some evidence of tumor suppression as compared to saline control, but was not as dramatic as the response to irinotecan alone. In Study #2027-03, all three dose levels of continued irinotecan treatment suppressed ‎ human colon tumor growth in nude mice, as compared to saline control. However, there were no differences in the response observed with continued irinotecan treatment at the two lower dose levels than that observed with ERBITUX™ alone. The results of this study are included in Figure 7, below.
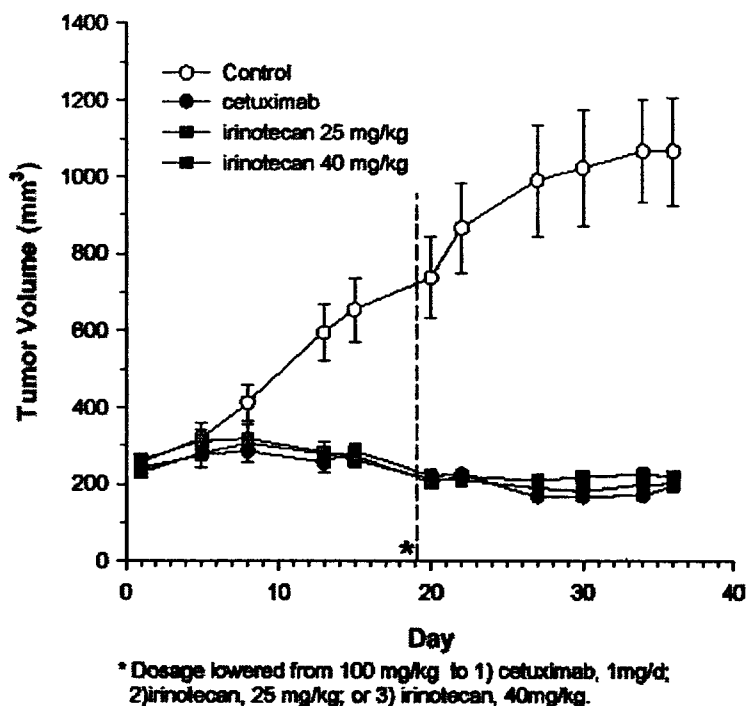
Figure 7. Growth inhibition of ‎ colon tumor xenografts refractory to 100 mg/kg/week irinotecan treatment, by continued irinotecan at lower doses, or by ERBITUX™ monotherapy.

Comment: Early mortalities were observed in several of the combination chemotherapy and ERBITUX™ studies in human tumor xenograft models. These findings resulted in a decrease in doses of 5-FU from 166 mg/kg to 125 mg/kg for subsequent studies, and identification of 50 mg/kg CPT-11 as a dose associated with acceptable toxicity and anti-tumor activity.

In summary, the pharmacologic activity of ERBITUX™ 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 cetuximab leads to inhibition of EGFr function, via receptor phosphorylation, receptor internalization and down-modulation, inhibition of EGFr-associated tyrosine kinases and subsequent signal transduction, induction of apoptotic pathways, and direct suppression of tumor cell growth. Cetuximab treatment of human tumor lines *in vitro* can also decrease metalloproteinase production associated with tumor cell metastasis, increase production of growth factors associated with tumor angiogenesis, and bind to macrophages and other activated effector cells via the F_c receptor, thereby activating antibody-dependent cellular cytotoxicity. *In vivo*, ERBITUX™ treatment, either alone or in combination with irinotecan-based chemotherapy can significantly delay tumor growth in human xenograft models with varying levels of EGFr expression. No anti-tumor activity of ERBITUX™ was observed in human tumor xenografts lacking EGFr expression. Taken together, these proof-of-concept data provide the rationale for the use of ERBITUX™ singly or in combination with irinotecan in irinotecan-refractory, metastatic colon cancer.

3.2.3   Secondary pharmacodynamics

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

3.2.4   Safety pharmacology

Study title: C225 (EMD 271 786): Cardiovascular and respiratory effects in the anaesthetized cynomolgus monkey following intravenous administration.

Key findings: Transient hypotension, increased heart rate and respirations, with decreased tidal and minute volumes were observed in anesthetized cynomolgus monkeys over a 3 hour period, after intravenous infusion of a single dose of 9.84, 31, or 98.4 mg/kg cetuximab. No prolongation of either Q-T or R-R intervals were observed on ECG evaluation. Blood samples obtained for toxicokinetic evaluation (analyzed separately) confirmed that exposure, as determined by both C_{max} and AUC were dose-related, although non-linear.

Study number: Study #0070-100-d6146 (ImClone Study #93004468 v1.0)

Volume # and page #: EDR file: pharmtox\pharm\0070-100-d6146.pdf

Conducting laboratory and location: ╲

Date of study initiation: January 22, 2002 (dosing initiated February 6, 2002)

GLP compliance: Yes

QAU statement: yes (X) no ( )

Drug, lot #, and % purity:

Methods

>   Doses: vehicle, 0.5, 1.0% morphine sulfate (positive control), cetuximab 9.8, 31, 98.4 mg/kg

>   Species/strain: *Macaca fasicularis* (cynomologus monkey); purpose-bred ╲

>   Number/sex/group or time point (main study): 4 males/group

Route, formulation, volume, and infusion rate: intravenous infusion; cetuximab formulated in phosphate buffered saline; 10 ml/kg infused; infusion rate 200 ml/h
Satellite groups used for toxicokinetics or recovery: no additional animals were included in this study for T/K evaluation. Blood samples for measurement of cetuximab serum concentrations were obtained from treated animals on this study at 10 minutes prior to and immediately following completion of infusion, and at 60, 120, and 180 minutes after dosing, and were shipped to the study sponsor for analysis.
Age: 34 to 47 months
Weight (nonrodents only): 3.5 – 5.5 kg
Unique study design or methodology (if any):

Comment: Toxicokinetic evaluation of cetuximab serum levels from this study was submitted as a separate report to the BLA (Study #PKM-022), and was reviewed separately. The study results are summarized under the Pharmacokinetics, Other section of this review (3.3.7), below.

Results:
Neurological effects: Not tested in this system.

Cardiovascular effects: Electrocardiograms were measured off lead II using subcutaneous needle electrodes. Blood pressure was measured using a blood pressure transducer affixed to a cannula placed in the femoral vein of each monkey. Transient increases in heart rate were observed in animals in the 9.8 and 31 mg/kg cetuximab dose groups, lasting for approximately 5 minutes after the end of dosing. No increased heart rate was observed in monkeys treated with either the vehicle control or the 98.4 mg/kg cetuximab dose. Transient decreases in mean arterial pressure were observed in 2/4 monkeys (animals #9M and #12M) approximately 10 minutes after dosing with 31 mg/kg C225, and had returned to baseline by approximately 40 minutes after the end of the infusion. There were no remarkable effects of the low or high-dose cetuximab treatment on blood pressure in anesthetized monkeys.

Transient decreases in R-R and Q-T intervals were observed in individual animals in the 9.8 and 31 mg/kg cetuximab dose groups, lasting approximately 5 to 10 minutes and corresponding to the increases in heart rate observed following completion of dosing. There were no ECG findings in the vehicle control and 98.4 mg/kg cetuximab dose groups.

Pulmonary effects: A pulse oximeter was used to monitor blood oxygen saturation levels, and respiratory rates, peak inspiratory and expiratory flows, minute volumes, and tidal volumes were measured for each animal. Animal #6M in the low-dose cetuximab treated group required supplemental oxygen during the experiment, since the blood oxygen saturation fell below 80%. Two monkeys, animal #8M in the 9.8 mg/kg C225 dose group and animal #12M treated with 31 mg/kg cetuximab stopped breathing towards the end or approximately 6 minutes after the end of the infusion, respectively. Both animals were placed on a ventilator with supplementary oxygen until spontaneous breathing returned.

Increases in peak inspiratory flow were noted over the 3 hour observation period following completion of dosing for animals in all three groups treated with cetuximab, and were statistically significantly higher than the vehicle control group in the monkeys treated with 9.84 mg/kg C225. Increases in minute volume, peak expiratory flow, and respiratory rate for this dose group were

also significantly different from the control group. Monkeys treated with the highest dose of cetuximab (98.4 mg/kg) also had gradual increases in minute volume and respiratory rate over the three hour observation period, but were not significantly elevated over control values. The animals in the intermediate dose group demonstrated a statistically significant increase in respiratory rate as compared to the control group, for the 5-10 minute period immediately following dosing. Gradual increases, although not statistically significant, in minute volume and peak expiratory flow were also observed in the monkeys after dosing with 31 mg/kg cetuximab.

Renal effects: Not tested in this system.

Gastrointestinal effects: Not tested in this system.

Abuse liability: Not tested in this system.

Other: Retreatment of the vehicle control animals with morphine at the end of the 3 hour observation period was used as the positive control for this experiment. Morphine administration produced the expected changes of hypotension, and decreased depth of respiration, as evidenced by decreases in both minute volumes and tidal volumes.

Study Conclusion: Intravenous administration of 98.4 mg/kg cetuximab had no effects on mean arterial pressure, ECG, or heart rate. Transient hypotension and increased heart rates were observed in 2/4 animals treated with 31 mg/kg ERBITUX™, and increases in heart rate were also observed in the group treated with 9.84 mg/kg C225. These increases were not statistically significantly different from the values in the vehicle control group. Increases in both the rate and depth of respiration were observed in all cetuximab treated groups as compared to the control, but were transient and not considered to be biologically relevant.

3.2.5 Pharmacodynamic drug interactions

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

3.3 PHARMACOKINETICS/TOXICOKINETICS

3.3.1 Brief summary

In vitro tissue binding studies with ERBITUX™ demonstrated significant cross-reactivity only with EGFr on the surface of human and cynomolgus monkey tissues, and not in tissues from mouse, rat, rabbit, dog, or goat. Tissues reacting positively for cetuximab binding included corneal epithelium in the eye, the placenta, prostate, liver hepatocytes, bronchial epithelia in the lung, mucosal and some submucosal cells in the esophagus, glandular and ductular epithelia of the mammary and salivary glands, and follicular and squamous epithelia of the skin. The greatest level of tissue cross-reactivity in both human and monkey epithelia was observed in placenta, skin, esophagus, and salivary gland.

The pharmacokinetics of cetuximab in cynomolgus monkeys following a single, 1 h i/v infusion of 7.5, 24, or 75 mg/kg ERBITUX™ showed dose-related, although non-linear increases in C_{max} and AUC_{last}. Clearance decreased with increasing dose, leading to a dose-related increase in

terminal half-life from 3 d to approximately 7 d between the low and the high-dose groups, respectively. The V_{dss} was approximately equivalent to the plasma space, and no differences in pharmacokinetic profiles of cetuximab were observed between male and female monkeys. A toxicokinetic evaluation incorporated into a single dose, cardiovascular safety study of ERBITUX™ in cynomolgus monkeys showed very similar findings for C_{max}, AUC_{last}, and Vdss as in the initial monkey study; however, an elimination half-life for cetuximab could not be determined due to the duration of the sampling time.

Repeat administration of cetuximab to cynomolgus monkeys for 35 to 39 weeks resulted in dose-dependent increases in AUC_{last}, C_{max}, V_{dss}, and elimination half-life, although the dose-related decreases in clearance on repeat administration were not as evident as following a single treatment with C225 in this species. Toxicokinetic evaluation confirmed that exposure to cetuximab was continuous throughout the treatment period. Mean values for peak and trough serum cetuximab concentrations and AUC were not remarkably different for each of the dose groups between the week 4 and week 36 or 39 time points, suggesting that no accumulation of ERBITUX™ was occurring. Neutralizing antibody activity was observed in only one female animal for the latter half of the study, and in two other monkeys at sporadic instances on study.

Single and repeat-dose toxicokinetic evaluations in rats also demonstrated dose-related increases in C_{max} and AUC_{last}; however, due to errors in sampling a true elimination half life could not be determined.

3.3.3   Absorption
No studies to determine the extent of C225 absorption were conducted in support of this application. The proposed indication is for intravenous administration in cancer patients with EGFr-positive tumors; therefore, 100% of the drug is expected to be available for binding to the receptor.

3.3.4   Distribution
Six studies were conducted to determine the potential for C225 cross-reactivity with human and animal tissues. ERBITUX™ was found to bind to EGFr on the surface of only human and cynomolgus monkey tissues, including corneal epithelium in the eye, the placenta, prostate, liver hepatocytes, bronchial epithelia in the lung, mucosal and some submucosal cells in the esophagus, glandular and ductular epithelia of the mammary and salivary glands, and follicular and squamous epithelia of the skin.

A review of each of the individual studies is provided, below.

Study title: Cross-reactivity of anti-epidermal growth factor receptor (EGFr) chimeric monoclonal antibody with hepatic tissues of multiple species.
Key findings: Cetuximab binding to EGFr was not detected in frozen liver sections from rat, mouse, dog, cynomolgus and Rhesus macaques, and baboon using concentrations as high as 12 mg/ml C225.
Study #:   PAI IM-108

Methods: The binding of C225 was evaluated using sections of liver from different test animal species, to identify an appropriate animal model for additional safety testing of the product. Frozen sections of liver from cynomolgus monkey, dog, Sprague-Dawley rat, CD-1 mouse,

Rhesus monkey and baboon were evaluated for C225 cross-reactivity by immunoperoxidase staining. Indirect immunohistochemistry techniques were employed for the rat, mouse, and dog tissues, using 5 μ sections of liver post-fixed in absolute acetone. Liver sections from one animal per sex were incubated with concentrations of 6 or 12 μg/ml C225 (lot #285-96) at the primary antibody labeling step. In the non-human primate species a biotinylated version of C225 was used (C225 — lot #306-145), to minimize background staining of endogenous IgG. Liver samples from one female baboon, and one cynomolgus or Rhesus macaque per sex were labeled with either 3 or 6 μg/ml C22: — Following incubation with C225, dog, rat, and mouse liver sections were indirectly labeled with biotinylated sheep anti-human IgG1, then all sections were stained with avidin-conjugated peroxidase and diaminobenzidine as capture reagent. Cryosections of human placenta ——— were labeled with either C225 or C225-— and stained as described as a positive control. All samples were evaluated for staining by direct visualization under light microscopy.

Results: No C225 staining was detected in any of the animal liver sections tested, at concentrations of C225 as high as 12 μg/ml in the rat, mouse, and dog samples, and at concentrations of 3 and 6 μg/ml in the baboon, cynomolgus, and Rhesus macaque tissues. Control, human placental samples were labeled with C225 at an intensity of 2^+ after incubation with either the unconjugated, or biotinylated C225 —antibody. C225 staining of human placenta was localized on the trophoblastic epithelial cell surface, consistent with cell membrane staining. Slight background staining in the interstitium of the placenta was attributed to residual endogenous IgG in the samples. No difference was noted in the intensity of C225 staining between samples labeled with unconjugated C225 and stained indirectly with biotinylated sheep anti-human IgG1 and avidin peroxidase, or those samples of human placental tissue labeled with biotinylated C225 — and stained directly with avidin-peroxidase conjugate.

Study conclusion: No cross-reactivity of C225 or C225— with EGFr on the surface of liver samples from rat, mouse, cynomolgus or Rhesus macaque, dog, or baboon was detectable using immunohistochemistry, at concentrations of C225 as high as 12 μg/ml.

Study title: Cross-reactivity study of biotinylated chimeric monoclonal antibody C225 with cryosections of normal cynomolgus monkey tissue.

Key findings: ERBITUX™ binding to EGFr was detected in cynomolgus monkey tissues, including skin, urinary bladder, esophagus, and liver, as well as in frozen sections from human head and neck squamous cell carcinomas. The most intense staining was present in skin (3^+) and esophageal (3-4^+) tissue sections from the monkey, and in human head and neck cancer (3^+) samples.

Study #: GRA00406

Methods: The purpose of this study was to evaluate the binding of C225 to a limited panel of frozen tissue sections from normal cynomolgus monkeys, to determine whether C225 can cross-react with the EGFr of this species and therefore identify the cynomolgus macaque as a relevant species for additional toxicology and pharmacology testing. Five micron sections of a panel of cynomolgus monkey tissues were prepared and fixed in absolute acetone, then labeled with biotinylated C225 (C225 — lot #O007791) at concentrations of 4 and 16 μg/ml. Following incubation with an anti-biotin secondary antibody, the sections were further processed using either the ﹍ ————— ' (for liver) or the ' ——————————— kits for final staining. The murine anti-EGFr antibody — was used as a positive control for the primary antibody

labeling, and frozen sections of human head and neck cancer tissues were labeled with C225· — and stained as described as a positive control for the staining procedure. All samples were counterstained with Mayer's hematoxylin and evaluated for staining by two independent examiners, using light microscopy.

Results: The C225 — antibody stained sections of human head and neck cancer at an intensity of 3^+, as compared to — which stained with an intensity of 4^+, when detected with the ——————————— ' kit. Tissue sections of cynomolgus monkey esophagus, skin, and urinary bladder stained positively with C225 or ˌ — with intensities ranging from 1^+ to 2^+ for bladder, 3^+ to 4^+ for esophagus, and 3^+ for skin (male animal only) with C225. Localization of the signal was highly specific for the cellular surface. No staining of these tissues or of the human head and neck tumor samples was observed when the primary antibody was omitted from the procedure. Strong background staining was observed in liver sections from both the male and female monkey in the absence of a primary antibody when the ' ———————————— method was used; therefore the ˌ ———— kit was used to stain these sections. Strong (3^+) staining of cynomolgus monkey liver was detected using either C22: — or — antibody as the primary antibody, and this test method. In the absence of primary antibody labeling, a low level of background stain was detected in the livers; however, the difference in intensity of staining following C225 — or — labeling was appropriately increased.

Study conclusion: The chimeric human-murine monoclonal antibody cetuximab was capable of detecting cell surface EGFr expression in tissue sections of skin, urinary bladder, esophagus, and liver from cynomolgus monkeys, and the results were comparable to those obtained using sections of human head and neck tumor. Cross-reactivity of C225 was similar in both distribution and intensity to that previously observed with the murine anti-EGFr antibody — Therefore, the cynomolgus macaque is identified as a pharmacologically relevant species in which to further study the toxicity of C225.

Study title: Cross-reactivity of C225 with normal cynomolgus monkey and goat tissues.
Key findings: Moderate to strong, surface membrane cross-reactivity of cetuximab was detected in epithelial cells from cynomolgus monkey cornea, esophagus, hepatic bile duct, thymus, ureter, and uterine cervix, and in the squamous, glandular, and/or ductular epithelia of the tonsil, prostate, parathyroid, mammary, and salivary glands. These findings are consistent with the known localization of EGFr in glandular and surface epithelial cells.
Study #: PAI IM-748

Methods: ' ┌ ————————————————————————————————

Results: Positive staining of ⸺ human placenta samples was detected at the membranes of the trophoblastic epithelium, with an intensity of 3-4$^+$ (please see Table 3, below for description of intensity) after incubation with either 2 or 10 µg/ml C225⸺ No C225⸺ cross-reactivity was detected in the white matter of the human ⸺ cerebellum sections. However, diffuse, cytoplasmic staining of 1-2+ intensity was detected in the molecular and granular cell layers of the cerebellum at both concentrations of C225 ₋. Due to the cytoplasmic location of the staining, this reactivity is likely unrelated to EGFr expression and may represent non-specific uptake of any of the antibodies used in the detection system by the tissues in the cerebellum.

No binding of C225 was detected by immunohistochemistry in the panel of goat tissues tested, including esophagus, heart, liver, lung, kidney, and skin. In the cynomolgus monkey tissues, samples of adrenal gland, blood vessel endothelium, bone marrow, small intestine, heart, kidney, lymph node, pituitary, spleen, skeletal muscle, and thyroid were all negative for C225 cross-reactivity, at concentrations of antibody as high as 10 µg/ml. Cytoplasmic staining of variable intensity was also noted in several of the cynomolgus monkey tissues evaluated in the panel. Those samples with cytoplasmic staining at intensities of 2$^+$ or greater are listed in Table X, below. Other tissues, with demonstrated equivocal to weak cytoplasmic staining with C225 ⸺ included the smooth muscle in the esophagus and the muscularis mucosa and muscle layers of the fundic region of the stomach, lung pneumocytes, the theca folliculi (stroma) of the ovary and the epithelium, stroma, and vascular smooth muscle of the oviducts, ureter, and uterus, the follicular and parafollicular epithelia and the perineural sheaths surrounding the parathyroid glands, the perineural connective tissue surrounding peripheral nerve, meninges of the spinal column, the Leydig cells and peritubular myofibroblasts and fibroblasts of the testis, and the capsular fibrocytes of the thymus.

Positive membrane binding of C225 ⸺was detected in several of the cynomolgus monkey tissues examined, including brain, eye, esophagus, stomach, skin, mammary gland, liver, ovary, parathyroid, placenta, prostate, salivary gland, thymus, tonsil, and uterine cervix. The intensity of C225 membrane staining was variable across the different tissues, and is likely related to expression of the EGF receptor by the different tissue types. The tissues identified as positively staining with C225 ⸺ as well as the intensity of the staining are presented in Table 3, below.

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