Table 3: Cross-Reactivity of C225 with Normal Cynomolgus Monkey Tissues				
TISSUE SAMPLE	C225, 10 µg/ml		C225, 2 µg/ml	
	MALE	FEMALE	MALE	FEMALE
Brain (cerebrum)	(-)[a]	2-3+ (C)[b]	(-)	1+ (C)
Brain (cerebellum)	(-)[c]	1+ (C)	(-)[c]	1+ (C)
Eye (corneal epi)	N.D.	2-3+	N.D.	2-3+
Eye (retina)	1+	1+	1+	1+
Colon	± - 1+	± - 1+	± - 1+	± - 1+
Small Intestine	(-)	(-)	(-)	(-)
Stomach (parietal epithelial cells)	N.D.	1-2+	N.D.	1-2+
Esophagus (squamous epi)	1-2+	1-2+	1-2+	1-2+
Liver (hepatocytes)	1+	1+	1+	1+
Liver (bile duct Epithelium)	1+	2+	1+	2+
Lung (bronchiolar epithelium)	± - 1+	N.D.	± - 1+	N.D.
Mammary gland (nipple and follicular, squamous epi)	2-3+	2-3+	2-3+	2-3+
Mammary gland (glandular and duct epithelium	1-2+	1-2+	1-2+	1-2+
Mammary gland (sebaceous epi)	1-2+	1-2+	1-2+	1-2+
Ovary (follicle)	N.A.	2-3+ (M+C)[d,e] 2-3+ (M+C)	N.A.	2-3+ (M+C) 2-3+ (M+C)
Ovary (stroma)	N.A.	± - 1+[(d)] ± - 1+	N.A.	± - 1+ ± - 1+
Pancreas	± - 1+[(d)]	± - 1+	± - 1+	± - 1+
Parathyroid (follicular epi)	1+ (M+C)[f] (-)	N.D.	1+ (M+C) (-)	N.D.
Parathyroid (ductular epi)	3+	N.D.	3+	N.D.
Placenta (trophoblastic epi)	N.A.	2-4+	N.A.	2-4+
Placenta (trophoblastic epi)	N.A.	2-4+	N.A.	2-4+
Prostate (glandular and ductular epi	2+ (M+C)[f] 2+ (M+C)	N.A.	2+ (M+C) 2+ (M+C)	N.A.
Salivary gland (ductular epi)	3+ (M+C)	3+ (M+C)	3+ (M+C)	3+ (M+C)
Salivary gland (glandular epi)	1-2+ (M+C)	1-2+ (M+C)	1-2+ (M+C)	1-2+ (M+C)
Spinal cord (meninges)	2-3+ (C)	2-3+ (C)	2-3+ (C)	(-)
Thymus (epithelial cells)	2+[(c)]	1+[(c)]	2+	1+
Thymus (capsular fibroblasts)	2+ (C)[(c)]	N.D.	2+ (C)[(c)]	N.D.
Tonsil (squamous and ductular epi)	1-2+ (M+C)[(c)]	± - 1+ (M+C)	1-2+ (M+C)[(c)]	± - 1+ (M+C)
Ureter (epithelium)	2+	2+[(c)]	2+	2+[(c)]
Uterus – cervix (squamous epi)	N.A.	(-)[(d)] 1-2+	N.A.	(-)[(d)] 1-2+
Human placenta		3-4+[(c)]		3-4+[(c)]
Human cerebellum (white matter)	(-)[(c)]		(-)[(c)]	
Human cerebellum (grey matter)	1-2+[(c)]		1-2+[(c)]	

[a] (-) = negative staining; ± = equivocal staining; 1^+ = weak; 2^+ = moderate; 3^+ = strong; 4^+ = intense; N.A. = not applicable; N.D. = not done
[b] (C) = cytoplasmic staining
[c] gender not specified
[d] both samples from female animals
[e] M+C = membrane and cytoplasmic staining
[f] both samples from male animals

Study conclusion: In summary, the cross-reactivity of cetuximab with EGFr on the cellular surface on a panel of tissues from cynomolgus monkeys and goats was evaluated by immunohistochemical staining. No tissues in the panel from goats stained positively for C225 cross-reactivity. By contrast, moderate to strong, surface membrane staining 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, and suggest that the cynomolgus monkey is an appropriate, pharmacologically relevant animal species for the conduct of additional toxicology and pharmacology testing of ERBITUX™.

Study title: Immunoanatomic distribution and immunopathologic analysis of monoclonal antibody M225.
Key findings: The murine monoclonal antibody M225, directed against the human EGFr was found to bind to the cell surface of normal human tissue samples, including liver, kidney, adrenal gland and lung, and to human tumors of epithelial origin, with moderate to strong staining intensities.
Study #: —— IMC04

Methods: [redacted]

Results: Binding of M225 antibody was detected in all of the normal human tissues of epithelial origin, including the liver, bronchiolar epithelial and alveolar cells, and the glomerulus and tubules of the kidney. Staining intensities ranged from weak (1^+) to moderate to strong ($2\text{-}3^+$) in the various tissues. Tissues of mesenchymal origin, including heart (muscle), bone marrow, and lymph nodes were negative for M225 cross-reactivity. These findings are presented in Table 4, below.

Table 4: Cross-Reactivity of Murine Monoclonal Antibody M225 with Normal Human Tissues		
Normal Human Tissue	**Number Positive/Number Tested**	**Staining Intensity**
Adrenal Cortex	3/3	1-3+
Adrenal Medulla	1/1	2-3+
Bone Marrow	0/3	0
Heart	0/2	0
Liver – hepatocytes	3/3	2-3+
Liver – bile duct epithelium	3/3	2-3+
Liver – Kupffer cells	0/1	0
Lung – bronchial epithelium	3/3	1+
Lung – alveolar cells	3/3	2-3+
Kidney – glomerulus	3/3	1-2+
Kidney – tubular epithelium	3/3	1-3+
Kidney – collecting tubules	1/1	2+
Lymph Node	0/3	0

All three tumor types evaluated were of epithelial origin, and the M225 antibody on the cellular surface membranes positively stained approximately 90% of the tumor cells. The intensity of the tumor staining was generally moderate to strong (2-3+); however, weak staining of fibroblasts (intensity = 1+) was detected in 2/3 of the ovarian carcinoma samples. The data are presented in Table 5, below.

Table 5: Cross-Reactivity of Murine Monoclonal Antibody M225 with Human Tumor Samples			
Tumor Sample	**Number Positive/ Number Tested**	**Percent of Tumor Cells Staining**	**Staining Intensity**
Breast carcinoma	3/3	90	2-3+
Lung (squamous) carcinoma	3/3	90	2-3+
Ovarian carcinoma	3/3	90	1-3+

Study conclusion: In summary, the murine monoclonal antibody M225, directed against the human EGFr was found to bind to normal human tissue samples, including liver, kidney, adrenal gland and lung, as well as to human tumors of epithelial origin, with moderate to strong staining intensities. These data are consistent with the expected distribution of EGFr in human tissue, and provide an indication of potential target organs for C225 cross-reactivity both *in vitro* in additional tissue binding studies, and *in vivo* in clinical and preclinical toxicity evaluations.

Study title: Cross-reactivity of anti-epidermal growth factor receptor (EGFr) chimeric monoclonal antibody (C225) with human tissues.

Key findings: Cetuximab binding to cell surface EGFr was demonstrated in normal human tissue samples of epithelial origin, including cornea, skin, the mucosa and submucosal glands of the esophagus, and salivary ductular epithelium, as well as to three human lung carcinomas of epithelial origin, with moderate to strong staining intensities.

Study #: PAI IM112R

Methods:

[redacted]

Results: Cross-reactivity of cetuximab with cellular EGFr was detected only in a limited number of normal human epithelial tissues, including skin, esophagus, salivary gland, and tonsillar and corneal epithelium. Intensity of the staining in these tissues was variable, with equivocal to weak staining noted in several tissues, and the strongest staining noted in the ductular epithelium of the salivary gland and the submucosal glands of the esophagus. Human placental tissue, which was used as the positive control for C225 — binding also displayed variability in staining intensity. The results of this assay are presented in Table 6, below.

Table 6: Cross-Reactivity of Monoclonal Antibody C225 with Normal Human Tissues

Normal Human Tissue	Number Positive/Number Tested	Staining Intensity
Adrenal	0/2	(-)[a]
Blood	0/1	(-)
Blood Vessel	All tissues	(-)
Bone Marrow	0/2	(-)
Brain – cerebellum	0/2	(-)
Brain - cerebrum	0/2	(-)
Brain - midbrain	0/1	(-)
Brain – medulla oblongata	0/1	(-)
Breast – mammary gland	0/2	(-)
Cervix - mucosa	1/2	$\pm$
Esophagus – mucosa	2/2	$\pm$
Esophagus – submucosal glands	1/2[b]	2-3^{+}
Eye – corneal epithelium	1/3[b]	1^{+}
Heart	0/2	(-)
Kidney	0.2	(-)
Liver	0/2	(-)
Lung	0/2	(-)
Lymph node	0/2	(-)
Ovary	0/2	(-)
Pancreas	0/2	(-)
Parathyroid	0/2	(-)
Peripheral Nerve	0/2	(-)
Pituitary	0.2	(-)
Placenta	1/1	1-3^{+}
Prostate	0/2	(-)
Salivary gland – duct epithelium	2/2	1-3^{+}
Skeletal muscle	0/3	(-)
Skin – epidermis – s. basale	3/3	$\pm$ - 2^{+}
Skin – other structures	0/3	(-)
Small intestine	0/2	(-)
Spinal cord	0/1	(-)
Spleen	0/2	(-)
Stomach	0/2	(-)
Testis	0/2	(-)
Thymus	0/3	(-)
Thyroid	0/2	(-)
Tonsil – epithelium	1/2	$\pm$ - 1$^{+(c)}$
Urinary bladder	0/3	(-)
Uterus	0/3	(-)

[a] (-) = negative staining; ± = equivocal staining; 1^+ = weak; 2^+ = moderate; 3^+ = strong
[b] tissue missing in other samples in this group

The cross-reactivity of C225 – was also evaluated against a panel of human tumor samples. Only three tumors demonstrated positive labeling with C225 – with two being squamous cell carcinomas, and the third being a large cell carcinoma. All three tumor types staining positively with C225 – were of lung origin, and approximately 20% of the tumor cells were positively stained by C225 on the cellular surface membranes. The intensity of the tumor staining was generally moderate to strong (2-3+); however, weak staining was detected in the large cell carcinoma samples at both dilutions of C225 – antibody. The data are presented in Table 7, below.

Table 7: Cross-Reactivity of Monoclonal Antibody C225 with Human Tumor Samples

Tumor Sample	Number Positive/ Number Tested	Percent of Tumor Cells Staining	Staining Intensity
Adenocarcinoma, mammary	0/2	0	(-)[a]
Adenocarcinoma, prostate	0/2	0	(-)
Adenocarcinoma, pulmonary	0/2	0	(-)
Carcinoma, large cell (lung)	1/1	20	1^+
Carcinoma, ovarian	0/2	0	(-)
Carcinoma, renal	0/2	0	(-)
Carcinoma, squamous (lung)	2/2	20	$1\text{-}3^+$
Carcinoma, transitional (lung)	0/1	0	(-)
Carcinoma, transitional (bladder)	0/1	0	(-)
Lymphoma, non-Hodgkin's	0/1	0	(-)
Lymphoma, large cell	0/1	0	(-)
Melanoma	0/2	0	(-)

[a] (-) = negative staining; ± = equivocal staining; 1^+ = weak; 2^+ = moderate; 3^+ = strong

Study conclusion: The chimeric monoclonal antibody cetuximab, directed against the human EGFr was found to bind to normal human tissue samples of epithelial origin, including cornea, skin, the mucosa and submucosal glands of the esophagus, and salivary ductular epithelium, as well as to three human lung carcinomas of epithelial origin, with moderate to strong staining intensities. These data are consistent with the expected distribution of EGFr in human tissue, and provide an indication of potential target organs for C225 cross-reactivity.

Study title: Cross-reactivity of C225 with normal human tissues.
Key findings: Moderate to strong cross-reactivity of ERBITUX™ was observed with EGFr expressed on the cell surface membrane in glandular, ductular, and/or squamous epithelial cells of human breast, eye, lung, skin, thymus, salivary gland, tonsil, and in liver hepatocytes and basal keratinocytes in the uterine cervix. Weak to moderate staining was also observed in tissue sections of human adrenal gland, stomach, kidney, colon, pancreas, prostate, ureter, and uterine endometrium.
Study #: PAI IM747

Methods: Cetuximab tissue cross-reactivity against EGF receptor was evaluated using a selected panel of normal human tissues, and indirect, immunoperoxidase staining. Previously frozen human tissue specimens were sectioned at 5 μ thickness, fixed in acetone, and stored at -70°C until staining. Samples were incubated with C225 antibody at 2 and 10 μg/ml for 1 hour

after blockade of endogenous peroxidase activity, then washed and incubated with the secondary and tertiary biotinylated anti-IgG1 antibodies. Diaminobenzidine was used as the substrate and color reagent for the horseradish peroxidase conjugate. Tissues included in this panel were brain (cerebrum and cerebellum), eye, esophagus, peripheral nerve, spinal cord, testis, and uterine endometrium. Sections of placenta, cerebellum, esophagus, peripheral nerve, spinal cord and testis were also stained in a competitive inhibition assay, or with the murine anti-EGFr antibody or an irrelevant, mouse IgG antibody as the negative control.

Results: Moderate to strong (2^+-3^+) membrane staining of EGFr with ERBITUX™ was observed on several human tissues of epithelial origin from 3/3 separate donors, including adrenal, mammary and sebaceous glandular, and ductular epithelia of the breast, squamous epithelia of the breast follicle and nipple, the corneal epithelium of the eye, squamous epithelium in the esophagus, liver hepatocytes, epidermal and follicular squamous epithelia in skin, glandular and ductular epithelia of the salivary gland, thymus, squamous epithelia in the tonsil, and basal keratinocytes in the uterine cervix in 2/3 donors. Weak to moderate (1^+-2^+) cetuximab staining was also observed at the cell membranes in adrenal, stomach, kidney, pancreas, prostate, ureter, urinary bladder, and uterus (cervix) tissue samples. Cross-reactivity of cetuximab with epithelial goblet cells in the colon (1^+), bronchiolar epithelium in the lung (2^+-3^+), C-cells and parafollicular cells in the thyroid (1^+-2^+), and uterine endometrial epithelium (1^+) was observed in 2/3 donors, and staining of the fallopian tube (oviduct) was observed in tissue sections from 1/3 donors. Weak staining ($\pm$-1^+) of tissues of mesenchymal origin with C225 was observed predominantly in the cytoplasm of fibroblasts, fibrocytes, or myofibroblasts in the cerebrum, fallopian tube, spinal cord, testes, and uterine cervix, and smooth muscle cells in the colon, small intestine, urinary bladder, and uterus in 3/3 donors, and in the smooth muscle of the esophagus or oviduct in 1/3 samples. Cetuximab staining of glial cells, neurons, and neuropil in the brain and spinal column from 3/3 donors was observed, and in 2/3 donors' sections of optic nerve layer and inner and outer plexiform layers of the retina of the eye. Staining of cells of neuronal or mesenchymal origin with ERBITUX™ was equivocal to weak ($\pm$ to 1+), was predominantly localized in the cytoplasm, and could be competed with the addition of an excess of unlabelled antibody. No staining of EGFr was observed when an irrelevant, human or murine IgG1 was used as the primary antibody, or in tissue samples incubated without any primary antibody as an assay control, confirming the specificity of cetuximab.

Study conclusion: Cetuximab staining of EGFr on cell membranes with moderate to strong cross-reactivity was observed in glandular, ductular, and/or squamous epithelial cells of human breast, eye, lung, skin, thymus, salivary gland, tonsil, and in liver hepatocytes and basal keratinocytes in the uterine cervix. Weak to moderate staining was also observed in tissue sections of human adrenal gland, stomach, kidney, colon, pancreas, prostate, ureter, and uterine endometrium. These findings are consistent with the known localization of EGFr in human tissues.

Study title: Comparative tissue binding study with mouse, rat, rabbit, monkey, and human tissue.
Key findings: ERBITUX™ binding was detected only in human and cynomolgus monkey placenta, skin, esophagus, testis, and ovary, and in rabbit basal follicular and epidermal epithelium. No cross-reactivity of cetuximab was detected with rat, mouse, or rabbit esophagus, placenta, testis, ovary, or skeletal muscle, or skin sections from rat or mouse.
Study #: BMS DS02124

Methods: Five micron thick, frozen tissue sections of placenta, esophagus, skeletal muscle, ovary, testis and skin from CD-1 mice, Sprague-Dawley rats, New Zealand white rabbits, cynomolgus monkeys and humans were incubated with 10 µg/ml biotinylated cetuximab or an irrelevant, human IgG1 antibody as a negative control. Positive antibody binding was then detected following incubation with streptavidin-conjugated, horseradish peroxidase, and development of colored product using diaminobenzidine as the substrate. Normal human placenta and skeletal muscle sections were used as the positive and negative assay controls, respectively. Immunoperoxidase staining of EGFr was then detected microscopically in a blinded fashion, and the sections were peer-reviewed.

Results: ERBITUX™ bound to both human placenta and pulmonary squamous cell carcinoma with moderate to strong intensity, and did not bind to human skeletal muscle as a negative control tissue. No binding of the irrelevant, biotinylated human IgG1 was detected in any tissues, confirming that the binding observed with biotinylated C225 to EGFr was specific. Cetuximab binding of moderate to strong intensity was observed in human and cynomolgus monkey placental trophoblastic epithelium, epidermal, follicular, and adnexal epithelium of the skin, and the mucosal and glandular (ductular) epithelium of the esophagus. Staining of the mucosal basilar epithelium in the esophagus tissue was of moderate intensity in the monkey, as compared to strong intensity in the human tissue sections. Binding of cetuximab to the interstitial and stromal cells of the testes was observed in both human and cynomolgus monkey tissues, and was of slight intensity in the monkey (1^+) and strong (3^+) intensity in the human samples. Similarly, ERBITUX™ staining of slight to moderate intensity was observed in stroma of the ovary in both human and monkey samples; however, in the monkey, cetuximab binding was also detected in the ovarian follicle, with moderate staining. Surprisingly, weak (1^+) cetuximab cross-reactivity was also observed in the basilar (less mature) epidermal and follicular epithelium of the skin in rabbits, and in the esophageal mucosal epithelium of 1/2 rabbit samples. ERBITUX™ cross-reactivity was not detected in all other tissue sections from rabbit, or in all tissue sections of mouse or rat origin.

Study conclusion: Positive binding of ERBITUX™ was detected in sections of human or cynomolgus monkey placenta, skin, or esophagus with moderate to strong intensity, and with lesser intensity to interstitial and stromal cells of the testis and ovary. Cetuximab staining of lesser intensity was also detected in the basal follicular and epidermal epithelium in rabbits. No tissue cross-reactivity of ERBITUX™ was detected in any other tissues from rabbit, or in all rat or mouse tissues.

3.3.5 Metabolism

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

3.3.6 Excretion

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

3.3.6 Pharmacokinetic drug interactions

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

3.3.7 Other Pharmacokinetic Studies

Study title: C225 (EMD 217786) Pharmacokinetics after single intravenous infusion in the cynomolgus monkey.
Key study findings: Dose-related, although non-linear increases in C_{max} and AUC_{last} were observed in monkeys following a single i/v administration of 7.5, 24, or 75 mg/kg ERBITUX™ by 1 h infusion. Clearance decreased with increasing dose, leading to a dose-related increase in terminal half-life from 3 d to approximately 7 d. 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.
Study no.: #221-014 (ImClone Study #930004388 v1.0; dosing and administration); #PKM – 101 (ImClone Study #930004367 v1.0; analytical report); and #PKM 09-01 (ImClone Study #930004369 v1.0, calculation of pharmacokinetic profiles)
Volume #, and page #: EDR files: pharmtox\pk\221-014.pdf (dosing and administration); pkm11-01.pdf (analytical report); and pharmtox\pk\pkm11-09.pdf (data analysis)
Conducting laboratory and location: [redacted] (Study #221-014); ImClone Systems, Somerville, NJ, USA (Study #PKM 11-01); Merck KGaA, Institute of Drug Metabolism and Pharmacokinetics, Grafing, Germany (Study #PKM 09-01)
Date of study initiation: June 16, 2000 (Study #221-014); December 21, 2000 (final report for Study #PKM 11-01); December 3, 2001 (final report for Study #PKM 09-01)
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: [redacted]

Methods

Doses: cetuximab 7.5, 24, 75 mg/kg
Species/strain: *Macaca fasicularis* (cynomologus monkey); purpose-bred [redacted]
Number/sex/group or time point (main study): 3/sex/dose group
Route, formulation, volume, and infusion rate: intravenous infusion; cetuximab formulated in phosphate buffered saline; 7.5 ml/kg infused; infusion rate 200 ml/h
Sampling times for pharmacokinetics: Blood samples for measurement of cetuximab serum concentrations were obtained from treated animals on this study before dosing (0 hr), at the end of infusion (1 hour), at 4, 8, and 24 h following completion of infusion, and at days 3, 5, 7, 9, 11, 13, 15, 17, and 19 after dosing.
Age: 3 to 4 years old
Weight (nonrodents only): 3.3 – 4.3 kg (males) and 3.1 – 3.7 kg (females)
Unique study design or methodology (if any): [redacted]

Comment: The ImClone Systems SOP 210-0205 (provided with the report for Study #PKM 11-01) describes this assay in detail. With this system, the amount of change in refractive index is directly proportional to the amount of C225 bound to the immobilized EGFr on the BIAcore film.

Comment: The final report for the BIAcore assay (Study #PKM 11-01) states that the theoretical limit of quantitation of this assay is —— cetuximab, and that for the purposes of this assay, the lower level of quantitation was set at the greatest concentration of C225 detected in the pre-treatment samples, which was ———— The range of cetuximab concentrations used to generate the standard curve was ——— ———— The lower level of quantitation for this assay stated in the final study report appears to be in error by —— fold, and should be verified with the product reviewer.

Observation times and results

Mortality and Clinical Observations: All monkeys were observed once daily for general health and behavioral or other clinical signs of overt toxicity. Mortality checks were performed once daily. Body weights were determined prior to dosing on study d 1, then weekly thereafter until study termination on d 20. Food consumption was determined by the amount of feed rations left or discarded, and was measured daily. Clinical findings were limited to soft feces and/or diarrhea in 1/6 monkeys in the low dose, 4/6 animals in the mid-dose, and 3/6 monkeys in the high dose group. The incidence and duration of the findings were related to the dose of ERBITUX™, with a single incidence of soft feces on study d 9 in the low dose animal, and multiple incidences in the mid- and high dose groups from study days 8 through 20 in the mid-dose and study days 4 through 20 in the animals treated with 75 mg/kg cetuximab, respectively. There were other overt toxicities noted, and no adverse effects of C225 on food consumption or body weights over the duration of the study.

One male monkey (animal #20030M) treated with 75 mg/kg C225 was sacrificed moribund on study d 15, following massive bleeding from the rectum. Gross and histopathologic evaluations were performed for this monkey, and revealed macroscopic evidence of severe hemorrhage in the colon, and hemorrhage in the submucosal vasculature, an almost complete absence of mucosal epithelium, and fresh blood and inflammatory cell infiltrates surrounding the crypts on histopathologic examination. Microscopic evaluation also revealed severe hemorrhage in the mesenteric lymph nodes; this finding was considered related to the intestinal bleeding. The cause of demise in this animal was reported as massive internal bleeding and hemorrhagic colitis resulting in subacute fulminant exsanguination, and was not considered related to ERBITUX™ treatment.

Comment: The EGFr is also expressed in low levels on normal colon mucosal and goblet epithelial cells in the monkey, and in humans. It is possible that the effects observed in this animal were due to an exaggerated pharmacologic effect of cetuximab, *i.e.* inhibition of EGFr function and disruption of normal mucosal epithelial turnover, thereby destroying the epithelial barrier. Similar effects were not observed in monkeys following repeat administration of up to 40 mg/kg/week ERBITUX™ for 39 weeks (Study #070-087; please see review, below).

Pharmacokinetics: In all groups of animals, cetuximab concentrations in serum were below the limits of quantitation of the assay prior to infusion of the biologic. Immediately following infusion, mean peak serum levels (C_{max}) were 166 µg/ml in the male monkeys and 175 µg/ml in the females in the low dose group, 949 µg/ml in the male and 936 µg/ml in the female animals

treated with 24 mg/kg C225, and 2300 µg/ml and 2460 µg/ml for the male and female monkeys, respectively, in the high dose group. Approximately 6.5-fold increases in the mean C_{max} values were observed between the dose groups, suggesting that the pharmacokinetics of cetuximab in this species are non-linear. At the final sampling point 19 days after completion of the infusion, mean serum levels of C225 were less than the lower limit of quantitation for the assay in both male and female monkeys treated with 7.5 mg/kg C225, and were 18 and 125 µg/ml for animals in the 24, and 75 mg/kg dose groups, respectively. The area under the concentration/time curve (AUC) was calculated out to the last sampling time and was also proportional, although not linear, to the dose of ERBITUX™ administered. Clearance of cetuximab decreased with increasing dose of the biologic, and the calculated volume of distribution at steady state (V_{dss}) was approximately 61 to 76 ml/kg, indicating that cetuximab is localized primarily in the vascular space. The values for each of the pharmacokinetic parameters are included in Table 8, below.

Table 8: Pharmacokinetic Profile of Cetuximab in Cynomolgus Monkeys Following a Single Intravenous Infusion

Pharmacokinetic Parameter	Dose of Cetuximab					
	7.5 mg/kg		24 mg/kg		75 mg/kg	
	Male	Female	Male	Female	Male	Female
C_{max} (ng/ml)	166 ± 12[a]	175 ± 31	949 ± 49	936 ± 22	2300 ± 10	2460 ± 23
T_{max} (min)	1 ± 0	2 ± 87[b]	1 ± 0	6 ± 100[c]	2 ± 87[b]	1 ± 0
AUC_{last} (µg/ml*h)	10933 ± 14	8854 ± 29	61113 ± 6	65523 ± 7	200753 ± 11	213637 ± 4
Cl (ml/h/kg)	0.6 ± 14	0.8 ± 30	0.4 ± 5	0.3 ± 8	0.3 ± 17	0.3 ± 6
V_{dss} (ml)	61 ± 16	76 ± 34	50 ± 22	48 ± 7	67 ± 9	64 ± 8
t½ (h)	64 ± 6	74 ± 21	97 ± 14	112 ± 5	163 ± 8	160 ± 16
AUC_{INF}/dose	1619 ± 13	1354 ± 25	2623 ± 5	2877 ± 8	3149 ± 18	3187 ± 7

[a] values presented are group means, ± % CV (n = 3/sex)

[b] the apparent increase in T_{max} and wide CV are related to an elevated T_{max} of 4 h in one animal, as compared to 1 h (*i.e.* at the end of the infusion period) in the remaining two monkeys in this group

[c] the three female monkeys had an individual T_{max} value of 12, 1, and 4 h, respectively, after dosing, resulting in the apparent increase in T_{max} and wide CV for this dose group

Study Conclusion: The pharmacokinetics of cetuximab after a single, intravenous infusion in monkeys demonstrated dose-related increases in C_{max} and AUC_{last}. Clearance of cetuximab was decreased in the mid- and high dose groups as compared to the low dose group. The V_{dss} was approximately equivalent to the plasma space. The elimination half-life was approximately 3 – 7 days, and increased with increasing dose of the monoclonal antibody. There were no differences in the pharmacokinetic parameters evaluated between male and female monkeys treated with ERBITUX™ under the conditions of this study.

Study title: C225 (EMD 217786) Cardiovascular and respiratory effects in the anaesthetized cynomologus monkey following intravenous administration.

Comment: Toxicokinetic evaluation of serum cetuximab concentrations in cynomologus monkeys after a single dose of C225 were incorporated into the design for the cardiovascular safety study (Study #0070-100-d6146, reviewed in section 3.2.4, above). The results from the toxicokinetic analyses were provided in the BLA submission as a separate final report (Study #PKM 22-01), and are reviewed here.

Key study findings: Dose-related increases in C225 C_{max} and AUC_{last} were demonstrated for animals treated with a single i/v infusion of 9.84, 31, or 98.4 mg/kg cetuximab. Clearance decreased with increasing dose of C225, and the volume of distribution was approximately equal to the vascular space. No estimation of terminal half-life could be calculated, due to the short duration of sampling.
Study no.: PKM 22-02 (ImClone Study #930004373 v1.0)
Volume #, and page #: EDR file: pharmtox\pk\pkm22-02.pdf
Conducting laboratory and location: Merck KGaA, Institute of Drug Metabolism and Pharmacokinetics, Grafing, Germany (treatment of animals and collection of serum samples)
Date of study initiation: April 19, 2002; April 26, 2002 (begin measurements)
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity:

Methods

Doses: vehicle; 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.
Age: 34 to 47 months
Weight (nonrodents only): 3.5 – 5.5 kg
Unique study design or methodology (if any):

Comment: No SOP for the ELISA assay was included in this study. The SOP is referenced in the final study report,[4] but is marked "available on request." Therefore, no information is available regarding the sensitivity, specificity, or lower level of quantitation of this assay for detection of ERBITUX™.

Observation times and results

Pharmacokinetics: In all groups of animals, cetuximab concentrations in serum were below the limits of quantitation of the assay prior to infusion of the biologic. Cetuximab was undetectable in serum samples from the control monkeys at all time points on study. Serum levels of C225 were proportional to the dose administered up to 180 hours after completion of the infusion.

[4] Theis, S. ELISA zur quantitaven Bestimmung von EMD 271786 (C225) in Affenund Humanserum, SOP AB 29 3390, Version 4, Merck KgaA, Darmstadt, Germany.

Immediately following infusion, mean peak serum levels were 246 µg/ml in the low dose group, 765 mg/ml in the animals treated with 31 µg/kg C225, and 1990 µg/ml in the high dose group. At the final sampling point 180 min after completion of the infusion, mean serum levels of C225 were 188, 531, and 1613 µg/ml for animals in the 9.84 mg/kg, 31 mg/kg, and 98.4 mg/kg dose groups, respectively. The area under the concentration/time curve (AUC) was calculated out to the last sampling time, and was also proportional to the dose. Clearance of cetuximab decreased with increasing dose of the biologic, and the calculated volume of distribution at steady state (V_{dss}) was low, indicating that cetuximab is localized primarily in the plasma space. The values for each of the pharmacokinetic parameters are included in Table 9, below.

Comment: The blood samples from C225 treated cynomologus monkeys infusion were only collected out to 3 hours after infusion. Because of the short duration of sampling, true calculation of terminal half-life and AUC is not possible.

Table 9: Pharmacokinetic Profile of Cetuximab in Cynomolgus Monkeys Following a Single Intravenous Infusion

Pharmacokinetic Parameter	Dose of Cetuximab		
	9.84 mg/kg	31 mg/kg	98.4 mg/kg
C_{max} (ng/ml)	252750 ± 7544[a]	792250 ± 198713	2040000 ± 264071
T_{max} (min)	45 ± 30	15 ± 30	60 ± 85
AUC_{last} (µg/ml*min)	39471 ± 2261	115656 ± 19707	313046 ± 22952
Cl (ml/h)	4.8 ± 2.5	4.3 ± 1.3	8.9 ± 7.8
V_{dss} (ml)	36.1 ± 3.6	41.0 ± 8.1	42.2 ± 8.4
t½ (min)	464 ± 230	429 ± 173	419 ± 157
C_{max}/dose	25686 ± 767	25557 ± 6410	20731 ± 2684
AUC_{last}/dose	4011 ± 230	3731 ± 636	3181 ± 233
Cl/dose (ml/h/kg)	0.5 ± 0.2	0.1 ± 0.04	0.09 ± 0.08
V/dose (ml/kg)	3.67 ± 0.37	1.32 ± 0.26	0.43 ± 0.09

[a] values presented are group means, ± S.D. (n = 4)

Study Conclusion: The pharmacokinetics of cetuximab after a single, intravenous infusion in monkeys demonstrated dose-related increases in C_{max} and AUC_{last}. Clearance of cetuximab decreased with increasing dose of the biologic, and the V_{dss} was approximately equivalent to the plasma space. An elimination half-life cannot be calculated from the limited time points sampled; however, an estimated half-life of 7 - 8 hours was determined for ERBITUX™ under the conditions of this study.

3.3.10 Tables and figures to include comparative TK summary

The following tables have been abstracted from the Pharmacokinetics Written Summary section of the BLA; of note, the BLA was submitted electronically in the ICH Harmonised, Common Technical Document (ICH M4S) format. The comparative T/K Summary (Table 14) was prepared by this reviewer from data included in the T/K analysis for Study #221-014 and #PKM 09-01, and from data contained in Section 2.6.7.3, Module 2 (Common Technical Document Summaries; Tabulated Toxicokinetics Summary) contained in the BLA Submission.

Table 10: Summary of Cetuximab Single Dose Pharmacokinetic Study #221-014 in Cynomolgus Monkeys

A. Study Design

Study / Report Number	Species/ Group Size	Dose (mg/kg)	Study Duration	Major Findings
221-014 (study number) PKM 11-01 (bioanalytics) PKM 9-01 (pharmacokinetics)	Cynomolgus monkeys 3 male (M) / 3 female (F)	7.5, 24, 75 IV infusion over 1 hour	Single dose, observations for 19 days	C_{max} generally occurred at the end of the infusion. A decrease in CL was observed with increasing dose and there was a corresponding increase in terminal $t_{1/2}$. In accordance with this, dose-normalized C_{max} and AUC_{inf} values were about 1.4 to 2.4 times higher at the highest dose group than at the lowest dose group. V_{ss} was generally low. No gender-dependent differences were observed.

B. Summary of Mean Pharmacokinetic Parameters

Dose (mg/kg)	7.5		24		75	
Gender	M	F	M	F	M	F
C_{max} (µg/mL)	166	175	949	936	2300	2460
C_{max}/Dose ([µg/mL]/[mg/kg])	22	23	40	39	31	33
t_{max} (hour)	1	1 to 4	1	1 to 12	1 to 4	1
$t_{1/2}$ (hour)	64	74	97	112	163	160
AUC_{last} (µg/mL x h)	10933	8854	61113	65523	200753	213637
AUC_{inf}/Dose ([µg/mL x h]/[mg/kg])	1619	1354	2623	2877	3149	3187
CL (mL/h/kg)	0.6	0.8	0.4	0.3	0.3	0.3
V_{ss} (mL/kg)	61	76	50	48	67	64

Note: t_{max}: time to reach C_{max}
AUC_{last}: AUC at the last measurement taken
Values are the mean of 3 animals/sex/group

Table 11: Summary of Cetuximab Serum Concentration Data from a Single Dose Safety Pharmacology Study #0700/100221-014 in Cynomolgus Monkeys

Dose (mg/kg)	9.84 n=4	31 n=4	98.4 n=4
C_0 (µg/mL)[a]	246	765	1990
C_{last} (µg/mL)[b]	188	581	1613

[a] concentration at end of infusion

[b] concentration at last time point measured (180 minutes after end of infusion)

Table 12: Summary of Cetuximab Toxicokinetic Parameters from Study #070-087 in Cynomolgus Monkeys

A. Study Design

Study / Report Number	Species/ Group Size	Dose (mg/kg) [a]		Study Duration	Pharmacokinetic Parameters
070-087 (study number)	Cynomolgus monkeys	1st infusion	Subsequent infusions	39 weeks	C_{max}, AUC, $t_{1/2}$, CL, V_{ss}
SR0201-11 (bioanalytics)	3M/3F (low and intermediate dose groups)	Control: 0	0		
PKM 45-01 (toxicokinetics)	5M/5F (control and high dose groups)	Low: 12	7.5		
		Intermed: 38	24		
		High: 120	75		

[a] Infusions given IV once weekly. Duration of first infusion was 2 hours. All subsequent infusions were given over 1 hour.

B. Summary of Mean Toxicokinetic Parameters

Week	4	13	26	39
Gender	M / F	M / F	M / F	M / F
12/7.5 mg/kg (mean of n=3 animals/gender)				
C_{max} (µg/mL) [a]	308 / 270	236 / 230	231 / 177	242 / 192
C_{max} / D ([µg/mL] / [mg/kg]) [a]	41.1 / 36.0	31.5 / 30.7	30.8 / 23.6	32.3 / 25.6
$t_{1/2}$ (h)	77.4 / 57.2	60.4 / 49.9	68.9 / 50.1	64 / 50.9
AUC_{LAST} (µg/mL x h)	16500 / 11800	11800 / 8800	10700 / 9570	10900 / 9320
AUC_{LAST} / D ([µg/mL x h] / [mg/kg])	2190 / 1580	1570 / 1170	1420 / 1280	1460 / 1240
CL (mL/h/kg)	0.49 / 0.638	0.69 / 0 855	0.788 / 0.784	0.706 / 0.807
V_{ss} (mL/kg)	50.3 / 49 2	55.3 / 56.9	70.0 / 54.7	60.3 / 58.7
38/24 mg/kg (mean of n=3 males and n=2 females)				
C_{max} (µg/mL) [a]	901 / 1080	921 / 434	950 / 857	757 / 943
C_{max} / D ([µg/mL] / [mg/kg]) [a]	37.5 / 44.8	38.4 / 18	39.6 / 35.8	31.6 / 39.3
$t_{1/2}$ (h)	58.5 / 93.8	72.4 / 77.6	70.2 / 64.2	58.6 / 71.7
AUC_{LAST} (µg/mL x h)	46100 / 59100	39700 / 47100	41500 / 44300	31600 / 47000
AUC_{LAST} / D ([µg/mL x h] / [mg/kg])	1920 / 2460	1650 / 1965	1730 / 1850	1320 / 1960
CL (mL/h/kg)	0.521 / 0.411	0.62 / 0.551	0.656 / 0.543	1.82 / 0.515
V_{ss} (mL/kg)	40.9 / 52.9	61.7 / 66.5	57.2 / 51.8	79.9 / 51.4
120/75 mg/kg (mean of n=5 animals/gender)				
C_{max} (µg/mL) [a]	2910 / 3640	2630 / 2240	3170 / -	-
C_{max} / D ([µg/mL] / [mg/kg]) [a]	38.8 / 48.5	35.1 / 29.9	42.3 / -	-
$t_{1/2}$ (h)	206 / 127	206 / 110	141 / -	-
AUC_{LAST} (µg/mL x h)	216000 / 229000	172000 / 154000	168000 / -	-
AUC_{LAST} / D ([µg/mL x h] / [mg/kg])	2880 / 3050	2300 / 2050	2250 / -	-
CL (mL/h/kg)	0.358 / 0.347	0.448 / 0.507	0.457 / -	-
V_{ss} (mL/kg)	100 / 59.3	143 / 77.7	83.8 / -	-

[a] C_{max} was observed at $C_{1.1\,h}$ (serum concentration 1 1 hours after start of infusion).

Note: D: Dose -. PK sampling was incomplete
All values referring to time are given before start of infusion

Table 13: Summary of Supporting Serum Cetuximab Concentration Data in Rodents

Study / Report Number	Species/ Group Size	Dose (mg/kg)	Study Duration	Major Findings
54165 (study number) ARBC0294-09 (serum concentrations)	Sprague-Dawley rats 15M/15F	0, 17, 50, 200 IV infusion over 15 minutes	Single dose, observations for 14 days	No pharmacokinetic data, only serum concentrations available. C_{max} increased approximately in proportion to the dose.
54167 (study number) ARBC0294-10 (serum concentrations)	Sprague-Dawley rats 15M/15F	0, 2.5, 10, 40 IV infusion over 15 minutes	Twice weekly for up to 28 days	No kinetic data, only serum concentrations available at pre-injection, 20 minutes and 24 hours after injection. Twice weekly dosing maintained a stable concentration of circulating cetuximab. There was no indication of cetuximab accumulation.

Table 14: Comparative Pharmacokinetic And Toxicokinetic Parameters In Humans and Cynomolgus Monkeys after Intravenous ERBITUX™ Infusion

Weekly Dose of Cetuximab (mg/kg)[a]	Mean Value For Each P/K Parameter				
	Cyno Monkey Single Dose[b]		Cyno Monkey Repeat Dose[c]		Human[d]
Mean C_{max} (µg/ml)	**Male**	**Female**	**Male**	**Female**	**M/F**
0	BLQ	BLQ	BLQ	BLQ	
12/7.5	166	175	308, 242[e]	270, 192[e]	
400/250 mg/m^2					153[f]
400 mg/m^2					168[g]
38/24	949	936	901, 757[e]	1080, 943[e]	
120/75	2300	2460	2910, N/A[e]	3640, N/A[e]	
Mean C_{min} (µg/ml)					
0	BLQ	BLQ	BLQ	BLQ	
12/7.5			45, 21	26, 15	
400/250 mg/m^2					40
400 mg/m^2					3.4
38/24			76, 122[e]	175, 123[e]	
120/75			860, N/A[e]	786, N/A[e]	
Mean AUC (µg/ml * h)[h]					
0	BLQ	BLQ	BLQ	BLQ	
12/75	10933	8854	16500	11800	
400/250 mg/m^2					13039
400 mg/m^2					12572
38/24	61113	65523	46100	59100	
120/75	200753	213637	216000	229000	
Mean Cl (ml/h/kg)[i]					
12/7.5	0.6	0.8	0.5	0.6	
400/250 mg/m^2					0.5[j]
400 mg/m^2					0.6[k]
38/24	0.4	0.3	0.5	0.4	
120/75	0.3	0.3	0.4	0.3	
Mean $t_{½}$ (h)					
12/7.5	64	74	77	57	
400/250 mg/m^2					119
400 mg/m^2					94
38/24	97	112	59	94	
120/75	163	160	206	127	
Mean V_{dss} (ml/kg)					
12/7.5	61	76	50	49	
400/250 mg/m^2 (12)					51[l]
400 mg/m^2					74[m]
38/24	50	48	41	53	
120/75	67	64	100	59	

[a] for monkey and human subjects in Study #EMR 62 202-12, first digit = initial dose, second digit = all subsequent doses
[b] Study #221-014 and #PKM 09-01
[c] Study #070-087 and #PKM 45-01
[d] data not split by gender
[e] values are shown for weeks 4 and 39 (Study #070-087)
[f] data are shown for Study Group B in Study #EMR 62 202-12, receiving weekly infusions of 400 mg/m^2 cetuximab for 3 weeks

[g] data are shown for Study #CP02-9710 in renal cell carcinoma, receiving initial dose of 400 mg/m^2 cetuximab followed by weekly infusions of 250 mg/m^2 ERBITUX™
[h] AUC_{last} (calculated for the time infusion started to the last value above the limit of quantitation) for the two monkey studies, AUC_{0-t} (calculated for the time infusion started to the last quantifiable concentration for human study CP02-9710), and AUC_τ (calculated within one complete dosing interval) for human Study #EMR 62 202-12
[i] Cl_{ss} for monkey Study #070-087 and for human Study #EMR 62 202-12
[j] data were taken from Study #EMR 62 202-12, average Cl in this study was 0.035 L/h; conversion into ml/hr/kg was based on the assumption of an average patient of 70 kg with a BSA of 1.7 m^2
[k] data were taken from Study #9710, average Cl in this study was 0.024 L/h/m^2; conversion into ml/hr/kg was based on the assumption of an average patient of 70 kg with a BSA of 1.7 m^2
[l] data were taken from Study #EMR 62 202-12, average V_{dss} in this study was 3.59 L; conversion into ml/kg was based on the assumption of an average patient of 70 kg with a BSA of 1.7 m^2
[m] data were taken from Study #9710, average V_{dss} in this study was 3.04 L/m^2; conversion into ml/hr/kg was based on the assumption of an average patient of 70 kg with a BSA of 1.7 m^2

3.4 TOXICOLOGY

3.4.1 Overall toxicology summary

General toxicology: There was no toxicity associated with a single, intravenous administration of 300 mg/kg cetuximab to outbred, CD-1 mice, or 200 mg/kg C225 to Sprague-Dawley rats. No remarkable toxicities were observed in Sprague-Dawley rats after twice weekly intravenous infusion of 2.5, 10, or 40 mg/kg cetuximab for 2 or 4 weeks. Toxicokinetic evaluations after single and repeat-dose administration of C225 to rodents demonstrated dose-related increases in C_{max} immediately upon completion of infusion. Repeat administration of cetuximab in rats did not result in significant accumulation of the protein. Administration of ERBITUX™ to cynomolgus monkeys for up to 39 weeks was associated with mild to severe dermatologic toxicities, epidermal sloughing, septicemia, and deaths in 5/10 animals in the high dose group, and macroscopic, clinical pathologic, and histopathologic evidence of cellular and tissue damage in the liver, bone marrow, spleen, and lymphoid organs. No NOAEL could be identified for ERBITUX™ in the cynomolgus monkey; of note, this is the only species other than human, which demonstrated cross-reactivity of cetuximab with a selected panel of tissues in the *in vitro* tissue binding studies.

Genetic toxicology: Cetuximab did not induce genetic damage *in vitro* using the *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay, nor *in vivo* in rats after intravenous infusion, as measured by micronucleus formation in bone marrow smears. Cetuximab is a protein, and therefore not expected to have direct mutagenic activity in either of these assays.

Carcinogenicity: No studies of this type were included in this submission.

Reproductive toxicology: No studies of this type were included in this submission.

Special toxicology: No special toxicology studies were included in this submission.

3.4.2 Single-dose toxicity

Study title: Single dose intravenous toxicity study of C225 (anti-EGFr chimeric MAb) in CD-1 mice.
Key study findings: No toxicities were observed in CD-1 mice following a single intravenous injection of 300 mg/kg ERBITUX™.
Study no.: 2525-101 (Imclone Study #930004375 v1.0)
Volume #, and page #: EDR file: pharmtox\tox\ — 2525-101.pdf
Conducting laboratory and location: ——
Date of study initiation: March 11, 1994 (in-life phase, March 23, 1994)
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: ——

Methods

Doses: 0 (vehicle), 300 mg/kg
Species/strain: *Mus muscularis*, Crl:CD-1®(—— strain
Number/sex/group or time point (main study): 5/sex/group
Route, formulation, volume, and infusion rate: single i/v (tail vein) injection; liquid formulation; 30 ml/kg volume; bolus injection
Satellite groups used for toxicokinetics or recovery: None included in this study
Age: 6 weeks old at treatment initiation
Weight (non-rodents only): 26 – 32 g (male), 22 – 25 g (female)
Unique study design or methodology (if any):

Observation times and results

Mortality: Mice were observed twice daily for signs of morbidity or mortality. One control male and four female control mice, and one male and two female mice treated with cetuximab were found dead following the blood collection on study d 3. Additionally, one control male mouse and one C225 treated female mouse died following blood collection prior to euthanasia on study d 16. These deaths were all attributed to stress during phlebotomy and were considered incidental to the cetuximab treatment. All other mice survived until scheduled sacrifice.

Clinical signs: All animals were evaluated weekly for clinical observations; daily, cageside observations for clinical signs of toxicity were also recorded. There were no remarkable, treatment-related clinical signs observed over the duration of the study.

Body weights: Body weights for all mice were measured at randomization, prior to dosing on study d 1, and on study days 8 and 15 prior to sacrifice. All mice gained weight over the duration of the study, and there were no remarkable differences in mean body weight values between the groups treated with cetuximab or vehicle control.

Food consumption: Food consumption on study was measured weekly at weeks 1 and 2. There was no effect of ERBITUX™ treatment on food consumption as compared to the control group.

Ophthalmoscopy: Not included in this study.

EKG: Not included in this study.

Hematology: Peripheral blood samples were collected under CO_2/O_2 anesthesia on study days 3 and 16 by orbital plexus puncture, following an overnight fast. Samples were analyzed for standard erythrocyte parameters, absolute and differential leukocyte counts, and platelet counts. There were no remarkable effects of cetuximab treatment on the various hematologic parameters as compared to the group treated with vehicle at either the d 3 or d 16 time point.

Clinical chemistry: Samples for serum clinical chemistry evaluation were collected at the same time point as for hematology profiles. Due to the limited amounts of serum available, only alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine, total bilirubin, gamma glutamyl transpeptidase (γ-GT) were evaluated at study d 3, with the addition of total protein, albumin, globulin, A:G ratios, and electrolytes at terminal sacrifice on study d 16. There were no remarkable differences between the individual animal, or group mean values for each analyte where available in the control and C225 treated groups, at either time point on study.

Urinalysis: Not included in this study.

Gross pathology: Complete necropsies were performed on all surviving animals at terminal sacrifice, following pentobarbital-induced euthanasia and exsanguination. The mice found dead after the d 3 phlebotomy were discarded without necropsy. The two mice that died due to complications of phlebotomy on study d 16 were necropsied at that time. Gross pathologic examination included evaluation of the external body surface, carcass, and all orifices, the nasal cavity and paranasal sinuses, the cranial cavity and external surface of the brain, and the thoracic, abdominal, and pelvic cavities and viscera. There were no remarkable, treatment related findings in the animals receiving C225 as compared to the control group. Incidental findings included bilateral distension and thickening of the uterine horns in two female mice in the cetuximab treated group (animals #A47111F and #A47112F), and one C225 treated female (animal #A47110F) with a pale, tan area identified on the median lobe of the liver. Of note, this animal was one of the two mice that did not survive the pre-euthanasia phlebotomy.

Organ weights (specify organs weighed if not in histopath table): Not included in this study.

Histopathology: Adequate Battery: yes ( ), no (X)—explain
Peer review: yes ( ), no ( )

Comment: Histopathologic evaluation of tissue samples was not included in this study design. A panel of tissues was sampled and preserved in neutral buffered formalin for potential future examination by the sponsor. Additional tissue samples were snap-frozen in OCT medium for evaluation of EGFr expression and cetuximab binding. No data for histopathologic examination or C225 binding were included in the final report for this study.

Comment: The panel of tissues sampled for this study included:

bone marrow (sternum)	liver	thymus (or remnant)
duodenum	ovaries	
jejunum	spleen	

kidney (right), cortex with glomeruli	stomach
gross lesions	testis (right)

Toxicokinetics: Not included in this study.

Study Conclusion: No treatment related toxicities and no remarkable differences in hematologic or clinical chemistry profiles were observed in CD-1 mice following a single, intravenous injection of cetuximab. The NOAEL for this study is 300 mg/kg, i/v as a single, bolus dose on study d 1.

Comment: The mouse is not a relevant species in which to evaluate the toxicity of cetuximab. Tissue binding studies conducted with samples from mouse, rat, and other species (Study #BMS DS02124) did not demonstrate any cross-reactivity of ERBITUX™ with tissues of murine origin.

Study title: Single dose toxicity study of C225 (anti-EGFr chimeric MAb) in CD-1 mice.
Key study findings: No toxicities were observed in CD-1 mice following a single intravenous injection of 282 mg/kg ERBITUX™.
Study no.: — 2525-102 (ImClone Study #930004376 v1.0)
Volume #, and page #: EDR file: pharmtox\tox\ — 2525-102.pdf
Conducting laboratory and location: —
Date of study initiation: April 4, 1994 (initiation of in-life phase, April 18, 1994)
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: —

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Methods

Doses: 0 (vehicle), 282 mg/kg
Species/strain: *Mus musculari*s, Crl:CD-1®(— strain
Number/sex/group or time point (main study): 5/sex/group
Route, formulation, volume, and infusion rate: single i/v (tail vein) injection; liquid formulation; 30 ml/kg volume; bolus injection
Satellite groups used for toxicokinetics or recovery: additional 3 mice/sex/dose group included for blood sampling at study d 3
Age: 6 weeks old at treatment initiation
Weight (non-rodents only):
Unique study design or methodology (if any):

Observation times and results

Mortality: Mice were observed twice daily for signs of morbidity or mortality. Three control female mice and one C225 treated female, and two C225 treated male mice died following blood collection prior to euthanasia on study d 16. These deaths were all attributed to stress during phlebotomy and were considered incidental to the cetuximab treatment. All other mice survived until scheduled sacrifice.

Clinical signs: Clinical observations were performed once on study d 1; daily, cageside observations for clinical signs of toxicity were also recorded. There were no remarkable, treatment-related clinical signs observed over the duration of the study.

Body weights: Body weights were obtained for all mice at randomization, prior to dosing on study d 1, and weekly thereafter. All mice gained weight over the duration of the study, and there were no remarkable differences in mean body weight values between the groups treated with ERBITUX™ or vehicle control.

Food consumption: Food consumption on study was measured weekly at weeks 1 and 2. There was no effect of ERBITUX™ treatment on food consumption as compared to the control group.

Ophthalmoscopy: Not included in this study.

EKG: Not included in this study.

Hematology: On study d 3, peripheral blood samples were collected by orbital plexus puncture from the satellite animals (3/sex/dose group) under CO_2/O_2 anesthesia, following an overnight fast. Satellite animals were then euthanized and discarded without further necropsy evaluation. Peripheral blood samples were collected from the main study animals at terminal sacrifice on study d 16, prior to euthanasia. Samples were analyzed for standard erythrocyte parameters, absolute and differential leukocyte counts, and platelet counts. Hematology profiles were not remarkably different between the cetuximab and the vehicle control groups at either the d 3 or d 16 time points.

Clinical chemistry: Samples for serum clinical chemistry evaluation were collected from individual animals at the same time point as for hematology profiles. Due to the limited amounts of serum available, only ALT, BUN, creatinine, total bilirubin, total protein, albumin, globulin, and A:G ratios could be evaluated. Not all analytes could be measured for all animals; however, where samples were analyzed, no remarkable differences were detected at either time point between the individual animal values, or group mean values for the control and C225 treated groups.

Urinalysis: Not included in this study.

Gross pathology: Complete necropsies were performed on all surviving animals at terminal sacrifice, following pentobarbital-induced euthanasia and exsanguination. The six mice that died due to complications of phlebotomy on study d 16 were necropsied at that time. Gross pathologic examination included evaluation of the external body surface, carcass, and all orifices, the nasal cavity and paranasal sinuses, the cranial cavity and external surface of the brain, and the thoracic, abdominal, and pelvic cavities and viscera. There were no remarkable, treatment related findings in the animals receiving C225 as compared to the control group. Incidental findings included bilateral distension and fluid in the lumen of the uterine horns in one female mouse (animal #A47138F) and bilateral thickening of the uterine walls, a cyst in the left ovary, and a pinpoint, dark focal area in the stomach mucosa in a second female (animal #A47141F) in the cetuximab treated group. There were no findings in any of the male mice in either group at necropsy; however, one control female mouse (animal #A47126) had a cyst on the left ovary, and a dark, focal area was present in the stomach mucosa of a second control female (animal #A47122F).

Organ weights (specify organs weighed if not in histopath table): Not included in this study.

Histopathology: Adequate Battery: yes ( ), no (X)—explain
Peer review: yes ( ), no ( )

Comment: Histopathologic evaluation of tissue samples was not included in this study design. A selected panel of tissues was sampled and preserved in neutral buffered formalin for potential future examination by the sponsor. Additional tissue samples were snap-frozen in OCT medium for evaluation of EGFr expression and cetuximab binding. No data for histopathologic examination or C225 binding were included in the final report for this study.

Comment: The panel of tissues retained for this study included:

bone marrow (sternum)	liver	thymus (or remnant)
duodenum	ovaries	
jejunum	spleen	
kidney (right), cortex with glomeruli	stomach	
gross lesions	testis (right)	

Toxicokinetics: Not included in this study.

Study Conclusion: No evidence of toxicity was observed in CD-1 mice following a single, intravenous injection of cetuximab. The NOAEL for ERBITUX™ in this study is 282 mg/kg, i/v as a single, bolus dose on study d 1.

Comment: Cetuximab binding studies conducted with mouse tissues (Study #BMS DS02124) did not demonstrate any cross-reactivity of ERBITUX™ with tissues of murine origin. Therefore, no toxicities secondary to the binding of C225 to EGFr in the mouse are expected after *in vivo* administration, and the mouse cannot be considered a relevant species in which to evaluate ERBITUX™ toxicity.

Study title: An acute intravenous toxicity and pharmacokinetic study of C225 in albino rats.
Key study findings: No treatment related toxicities were observed in rats following intravenous infusion of a single dose of 17, 50, or 200 mg/kg ERBITUX™.
Study no.: 54165 (ImClone Study #930004389 v1.0; main toxicology study) and ARFC0294-09 (ImClone Study #930004386 v2.0; toxicokinetics and immunogenicity data)

Comment: The toxicokinetic and immunogenicity analyses for this study were submitted to the BLA as a separate study report, Study #ARFC0294-09 (ImClone Study #930004386 v2.0). The data from this report will be reviewed and summarized in the report for the present study, #54165.

Volume #, and page #: EDR file: pharmtox\tox\54165.pdf (main toxicology study) and pharmtox\tot\arbc0294-09.pdf (toxicokinetics assay)
Conducting laboratory and location:
Canada (main toxicology study); ImClone Systems, Inc., Somerville, NJ (toxicokinetics assay)
Date of study initiation: May 31, 1994 (protocol signed), June 1, 1994 (dosing initiated)
GLP compliance: Yes

QAU statement: yes (X) no ( )
Drug, lot #, and % purity:

Methods

Doses: 0 (vehicle control), 15, 50, 200 mg/kg
Species/strain: *Rattus norvegicus*; Sprague-Dawley Crl:CD®(SD, —
Number/sex/group or time point (main study): 15/sex/group
Route, formulation, volume, and infusion rate: intravenous infusion, 15 minutes duration; liquid formulation; 1.3 ml/min/kg
Satellite groups used for toxicokinetics or recovery: none
Age: 6 to 7 weeks old at start of treatment
Weight (non-rodents only): 200 – 254 g (male), 150 – 184 g (female)
Unique study design or methodology (if any):

Comment: The body weight range for these animals deviated from the protocol specified weight ranges of 226 – 250 g for males and 176 – 200 g for female rats.

Comment: Rats #203M, #253F, and #258F in the 200 mg/kg dose group, and animal #314M in the 50 mg/kg C225 dose group were underdosed with cetuximab by 22%, 21%, 15%, and 18%, respectively, due to 13 min rather than 15 min infusion times. Additional rats (animal numbers or dose groups were not reported) were reported to have been underdosed by 10-15%. The contract laboratory attributes these dose errors to be due to the small volumes administered over a short period of time, and considers that they did not have an impact on the integrity of the study.

Observation times and results

Mortality: Mortality checks were performed twice daily for all animals on study. Animal #209M in the high dose group, and animal #353F, in the mid-dose group died following blood collection on study days 7 and 14, respectively. All other animals survived until scheduled termination.

Clinical signs: Animals were examined twice daily for clinical signs of toxicity, and any observations were recorded individually. Swollen and/or protruding eyes and opacities were noted in individual animals in all groups, including the control, and were attributed to the blood collection procedures. No other clinical signs of toxicity were observed that were related to the treatment with ERBITUX™.

Body weights: Body weights were measured and recorded twice during the pre-treatment period, then weekly after dosing. All animals gained weight over the duration of the study, and there were no remarkable differences in either final, fasted body weight mean values, or mean body weight gains between the vehicle control and cetuximab treated groups.

Comment: Body weights prior to dosing with cetuximab were obtained two days prior to injection, rather than the one day prior specified in the protocol. Fasted body weights were obtained at terminal sacrifice.

Food consumption: Food consumption was measured weekly during the pre-treatment periods and throughout the duration of the study. Prior to cetuximab treatment, a statistically significant increase in food consumption was observed for male and female rats assigned to the 17 mg/kg dose group as compared to those animals in the vehicle control group, and a significant decrease in mean body weight values was observed for male rats assigned to the 200 mg/kg dose group as compared to control ($p \leq 0.01$, Dunnett's test). However, following C225 treatment, food consumption was comparable between the control and the ERBITUX™ treated rats at all time points on study.

Ophthalmoscopy: Not included in this study.

EKG: Not included in this study.

Hematology: Peripheral blood samples were obtained at study termination from the abdominal aorta under light ether anesthesia, prior to euthanasia and analyzed for erythrocyte parameters, total and differential leukocyte counts, and platelet counts. Coagulation profiles (prothrombin time [PT], activated partial thromboplastin time [APTT], and thrombin time [TT]) were also measured for each animal. Bone marrow smears (femoral marrow) were also prepared but were not evaluated for effects of C225 on hematopoietic cells. There were no statistically significant differences in total or differential leukocyte counts, platelet counts, or platelet volumes between ERBITUX™ treated and control male or female animals. Slight, although statistically significant decreases were observed in mean hematocrit values for male rats treated with 200 mg/kg C225, and in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration in the low dose males, and for red cell distribution width values in both male and female rats treated with 17 mg/kg C225, as compared to their respective control groups ($p \leq 0.05$, Dunnett's test). These values however, were still within the range of normal limits for rats of this strain and age.

There was a statistically significant decrease in APTT as compared to the respective controls for both male and female rats treated with 17 mg/kg cetuximab, and significant decrease in PT for males in the low-dose group as compared to control ($p \leq 0.05$, Dunnett's test). The toxicological significance of these findings is unknown, since they were not observed in the groups treated with either 50 or 200 mg/kg dose of cetuximab.

Clinical chemistry: Serum biochemistry profiles were determined from samples obtained at study termination, following overnight fasting of the animals. Serum samples were analyzed for glucose, hepatic transaminases, γ-GT, alkaline phosphatase (ALP) and total bilirubin, BUN, creatinine, and electrolyte levels, and total protein, albumin, globulin, and A:G ratios. Mean values for all of these parameters were comparable between the male control and cetuximab treated rats. A statistically significant increase in total cholesterol was noted in female rats treated with 200 mg/kg C225 as compared to the control females, and slight but statistically significant increases in albumin and A:G ratio were observed for female in the mid- and high-dose groups, respectively. The mean values for these parameters were still within normal limits for this strain and age of rats, and are therefore not considered to be toxicologically relevant.

Urinalysis: Urinalysis was performed at the time of study termination, on samples obtained overnight from animals deprived of both food and water. There were no remarkable differences between the control or cetuximab treated groups, or between individual animals in any one group in urine volume, pH, specific gravity, color and appearance, or qualitative analysis of blood, protein, glucose, ketones, nitrates, bilirubin, or urobilinogen levels.

Gross pathology: Animals were fasted overnight prior to terminal sacrifice, and following blood collection for clinical chemistry evaluations were euthanized by exsanguination from the abdominal aorta. A complete necropsy, consisting of detailed external and internal examination and identification of all clinically recorded lesions was performed for each animal. There were no macroscopic pathological findings that were related to ERBITUX™ treatment. Opacities in the eye were observed in individual female rats in the 50 mg/kg and 17 mg/kg C225 dose groups, and were attributed to the blood collection procedure for the pharmacokinetics portion of this study. Other incidental findings included enlarged lymph nodes, dark areas in the lung, pale areas in the liver, and renal tubular dilatation, discoloration, or cysts in the kidneys in individual rats in both the control and cetuximab treated groups, with no relationship in either dose or severity to the treatment with C225 monoclonal antibody.

Organ weights (specify organs weighed if not in histopath table): Not included in this study.

Histopathology: Adequate Battery: yes ( ), no (X)—explain
Peer review: yes ( ), no ( )

Tissues were collected at terminal sacrifice, preserved in 10% neutral buffered formalin, and retained without further histopathological evaluation.

Toxicokinetics: Peripheral blood samples (0.75 ml/animal) for determination of cetuximab toxicokinetic profiles were obtained following orbital plexus puncture on study days 0, 3, 7, and 14. Sparse sampling techniques were employed such that blood was obtained from cohorts of 5 separate rats/sex/dose group. Time points sampled included immediately prior to and immediately after the end of the infusion, and at 0.5, 1, 2, 4, 8, 12, and 24 hours after infusion. The first cohort of 5 animals/sex was sampled immediately prior to infusion, and at 1 and 8 hours after completion of treatment. The second cohort of 5 rats/sex/dose level was sampled immediately at the completion of infusion, and at 2 and 12 hours after dosing. The final cohort of 5 rats/sex/dose group was sampled at 0.5, 4, and 24 hours after infusion. Following the final sampling for all three cohorts, all rats received supplemental Ringer's lactate solution (4 ml. s/c into the dorsal region) to restore volume. Sampling was originally scheduled to be repeated for five rats/dose group on study days 3, 7, and 14, with rotation of the animals in the different cohorts.

Comment: Due to an error in sampling on days 3 and 7, serum levels of cetuximab for those days cannot be determined for all dose groups. The protocol was amended to obtain samples from all 15 rats per dose group on study d 14, rather than only the sparse sampling of 5 rats/sex/dose group originally planned. This error does impact the toxicokinetics portion of the study, such that the values for the intermediate 3 and 7 day time points may not be accurate, and the terminal elimination half-life of cetixumab cannot be determined.

Serum levels of cetuximab were evaluated using a [redacted] ELISA assay with [redacted] and horseradish peroxidase conjugated, C225 as the competing antibody. Dilutions of the serum samples are added to the wells prior to the addition of the peroxidase conjugated C225. As the concentration of cetuximab in the test serum samples increases, the amount of peroxidase conjugated C225 binding decreases, leading to a decrease in the final, colored reaction product after addition of the substrate. Under these conditions, color

intensity is inversely proportional to the amount of cetuximab present in the serum sample, and was quantitated against a standard curve of unlabelled C225 antibody.

Dose-related increases in C_{max} were observed in cetuximab treated rats at the timepoint immediately following completion of the infusion, and the serum levels declined over time. Because of the errors in sample collection, a true terminal half life could not be determined; however, approximately 10 to 20% of the initial serum levels of cetuximab were still circulating at the 14 d (336 h) time point. The group mean values for the control and each of the ERBITUX™ treated dose groups have been abstracted from the final study report, and are presented in Table 15, below:

Table 15: Mean Cetuximab Serum Levels after a Single, Intravenous Infusion in Rats

Time after Infusion (hrs)	Dose of Cetuximab (mg/kg, 15 min i/v infusion)			
	Control	200 mg/kg	50 mg/kg	17 mg/kg
0	BLQ[a]	BLQ	BLQ	BLQ
0.017	BLQ	4791 µg/ml	749 µg/ml	326 µg/ml
0.5	BLQ	4732 µg/ml	632 µg/ml	252 µg/ml
1	BLQ	4175 µg/ml	528 µg/ml	287 µg/ml
2	BLQ	2914 µg/ml	602 µg/ml	222 µg/ml
4	BLQ	2946 µg/ml	542 µg/ml	225 µg/ml
8	BLQ	2091 µg/ml	663 µg/ml	262 µg/ml
12	BLQ	1950 µg/ml	438 µg/ml	185 µg/ml
24	BLQ	1107 µg/ml	391 µg/ml	156 µg/ml
72	1.1 µg/ml	n.a.[b]	305 µg/ml	n.a.
168	9.1 µg/ml	644 µg/ml	n.a.	n.a.
336	3.6 µg/ml	469 µg/ml	173 µg/ml	68 µg/ml

[a] BLQ = below level of quantitation of the assay
[b] n.a. = not available

Comment: No data were included in the final study report (Study #ARBC0294-09) for serum C225 values for individual animals at each of the sampling time points. Therefore, no independent analysis and verification of these data, or statistical evaluation of group mean values and standard deviation from the mean can be performed.

Comment: The toxicokinetic analysis for this study was submitted to the BLA as a separate study report, Study #ARFC0294-09 (ImClone Study #930004386 v2.0). The summary data from this report are included here in this review of Study #54165, and the toxicokinetics will not be reviewed separately.

Comment: The SOP for the ELISA assay, as well as the sensitivity, specificity, and lower levels of detection and quantitation for this assay were not included in the final study report.

Study Conclusion: Treatment of Sprague-Dawley rats with 17, 50, or 200 mg/kg ERBITUX™ by a single, intravenous infusion was not associated with any toxicities that were related to the protein therapeutic. Toxicokinetic evaluations on study d 1 immediately after completion of dosing demonstrated a dose-related increase in the serum levels of cetuximab, which declined over time but were still present at approximately 10 to 20% of the initial values at 14 d after dosing. The NOAEL for cetuximab under the conditions of this study was 200 mg/kg, i/v, over a 15 minute infusion.

Comment: Tissue binding studies using samples from rats (Study #BMS DS02124) failed to demonstrate cross-reactivity of ERBITUX™ with tissues of rat origin. Therefore, the rat is not a relevant model to detect toxicities related to interaction of cetuximab with the EGFr receptor.

3.4.3 Repeat-dose toxicity

Study title: An intravenous infusion toxicity study of C225 in the albino rat for up to 28 days.
Key study findings: early mortalities (not drug-related); thrombosis and phlebitis at the injection site; no treatment-related, remarkable effects of cetuximab on clinical toxicities, hematologic, serum biochemistry, or urinalysis profiles, no effects of test article on systemic organ toxicity or histopathology
Study no.: 54167 (ImClone Study #930004377 v1.0; main toxicology study), ARFC0294-10 (ImClone Study #930004378 v1.0; toxicokinetics), and ARF-C0294-11 (ImClone Study #930004395 v1.0; immunogenicity data)

Comment: The toxicokinetic and immunogenicity analyses for this study were submitted to the BLA as separate study reports. Study #ARFC0294-10 (ImClone Study #930004381 v1.0), included the toxicokinetic data from this study, and Study #ARF-C0294-11 (ImClone Study #93004395 v1.0) was the final study report for the antigenicity determination of cetuximab in the 28 d rat study. The data from these reports will be included in the review for Study #54167.

Volume #, and page #: EDR file: pharmtox\tox\54167.pdf (main toxicology study), pharmtox\arfc0294-10.pdf (T/K determinations), and pharmtox\tox\arfc-0294-11.pdf (immunogenicity analysis)
Conducting laboratory and location: [redacted]
Canada (main toxicology study); ImClone Systems, Inc., Somerville, NJ (toxicokinetics and immunogenicity assays)
Date of study initiation: June 30, 1994; July 4, 1994 (in-life phase initiated)
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: [redacted]

Comment: The analytical certificate for the test article used in this study was included as Appendix 10 of the final study report. This certificate states that the lot number for this product was #IMB-001, and that the antibody was [redacted] An explanation for this discrepancy was not provided.

Methods

Doses: vehicle control (phosphate buffered saline, pH), 2.5, 10, 40 mg/kg/dose, twice weekly x 4 weeks
Species/strain: *Rattus norvegicus*; Sprague-Dawley Crl:CD®(SD).[redacted]
Number/sex/group or time point (main study): 15/sex/dose group (please see study design, below)
Route, formulation, volume, and infusion rate: i/v infusion over 15 minutes; C225 diluted in sterile PBS; infusion rate and volume, 1.3 ml/kg/min
Satellite groups used for toxicokinetics or recovery: No additional animals were included in this study for recovery. Samples for evaluation of toxicokinetic parameters

were obtained from the main study animals at different time points after treatment (please see below).
Age: 9-11 weeks old
Weight (non-rodents only):
Unique study design or methodology (if any): Each rat was surgically implanted with a catheter into the femoral vein at least 7 days prior to initiation of test article treatment. Catheter patency was maintained by daily infusion of sterile 0.9% saline.

Ten rats/sex/group were sacrificed after 2 weeks of treatment, with the remaining 5/sex/group sacrificed after 4 weeks of treatment. No recovery group was included in this study.

Observation times and results

Mortality: All animals were checked twice daily for mortality and any findings were recorded individually. Three early mortalities were observed on study; one female rat in the vehicle control group (animal #1671F) was found dead on study d 5 following surgery to repair the catheter implant. One female rat (#4612F) in the 40 mg/kg/dose group died on study d 7 during a check of the catheter patency, and an additional female rat in the vehicle control group (animal #1642F) was euthanized moribund on study d 27 due to self-mutilation. These three deaths were not considered related to treatment with ERBITUX™. All remaining animals survived until scheduled termination.

Clinical signs: Rats were observed once daily during the pretreatment and treatment periods for clinical signs of toxicity, behavioral changes, and ill health. Following dosing, the injected animals were examined in detail. There were no clinical signs of toxicity related to cetuximab treatment.

Comment: There were no individual line listings for mortality or clinical observations included in the final, audited study report.

Comment: Animals #2641 and #2632 in the 2.5 mg/kg/dose group were replaced on study days 2 and 3, respectively, with spare animals from the same shipment, due to problems with the femoral catheter. These two animals were sacrificed and tissues retained, but no data are reported.

Body weights: Rats were weighed weekly during the pretreatment and treatment periods, and a final fasting body weights were obtained at terminal sacrifice. There were no differences between the mean body weights or body weight gains between the vehicle and the cetuximab treated groups over the duration of the study.

Food consumption: Food consumption was measured weekly during pretreatment and throughout the duration of the study. There were no effects of C225 treatment on food consumption, as compared to rats in the vehicle control group.

Ophthalmoscopy: All animals were evaluated fundoscopically and using a slit lamp (biomicroscopically) once during the pretreatment period and again at terminal sacrifice at weeks 2 and 4. There were no treatment-related ocular changes reported during the duration of the study.

EKG: Not done

Hematology: Blood samples were collected at terminal sacrifice following an overnight fast. The parameters evaluated included hematocrit, red cell count, hemoglobin, red blood cell morphology, red cell distribution width, reticulocyte count, white blood cell count (total, absolute, and percent differential), platelet count, mean platelet volume, and activated partial thromboplastin and prothrombin times. Three bone marrow smears from femoral marrow were prepared for each rat. With the exception of a decreased mean absolute lymphocyte count in male rats treated with 40 mg/kg/dose cetuximab at the d 29 sacrifice, there were no significant differences in hematologic findings between animals in the vehicle control group and those that were treated with C225 at any dose level.

Comment: Similar findings of decreased lymphocytes were not observed in the female rats in this dose group, and there was no apparent dose-relationship; therefore, the biologic relevance of this finding is unknown.

Clinical chemistry: Blood samples were collected at terminal sacrifice as described above. The parameters evaluated included serum hepatic transaminase levels, ALP, total bilirubin, BUN, creatinine, total protein, albumin, globulin and A:G ratio (calculated), serum cholesterol, and sodium, potassium, chloride, phosphorous, and calcium. Although a statistically significant ($p \leq 0.05$, Dunnett's test) elevation in mean total bilirubin was observed for male rats sacrificed at study d 15 in the 2.5 mg/kg/dose group as compared to the vehicle control, there were no statistical differences between the mean values in the low dose group and those observed for rats in the 10 or 40 mg/kg/C225 dose groups. Similarly, elevated mean total glucose values were observed in female rats in the 10 and 40 mg/kg/dose cetuximab treated groups at the study d 29 sacrifice, but not at study d 15. The mean values for both findings were still within the normal range for this strain of rats; therefore, the biologic significance of these changes is unknown.

Urinalysis: Urine samples were collected from individual animals at terminal sacrifice following overnight deprivation of water, and were evaluated for color and appearance, volume, pH, presence or absence of blood, glucose, ketone, protein, bilirubin, and urobilinogen levels, nitrites, specific gravity, and by microscopic evaluation for urinary sediment. There were no remarkable, treatment-related effects of ERBITUX™ on urinalysis parameters as compared to the vehicle control group.

Comment: The urinalysis data were found in Appendix 5 of the final study report and were not summarized elsewhere.

Gross pathology: A complete necropsy was performed on all animals at scheduled termination, following exsanguination via the abdominal aorta. Necropsy consisted of external examination including identification of all clinically recorded lesions, and a detailed internal examination under the supervision of a veterinary pathologist. There were no apparent gross pathological lesions that were related to ERBITUX™ treatment. Miscellaneous findings at the two and four week necropsies included enlargement of the bronchial, mediastinal, and/or iliac lymph nodes, pale areas in the liver, small testes in individual male rats, and thickening at the infusion site. These findings were present in control and ERBITUX™ treated rats at approximately equal incidence and severity, and were not related to toxicity of cetuximab.

Organ weights (specify organs weighed if not in histopath table): No organ weights were determined in this study.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

Full histopathological evaluation was performed on rats in the control and 40 mg/kg/dose ERBITUX™ groups only. The panel of tissues evaluated is listed in Table 16, below. There were no microscopic pathologies that were related to cetuximab toxicity. Phlebitis, with mixed cellular infiltrates and thrombosis around the catheter site were present in individual rats in both the cetuximab and vehicle control groups, were mild in severity, and were considered secondary to the catheter implantation and subsequent, repeated continuous infusions. Where enlarged lymph nodes were noted on gross pathological examination, microscopic findings included lymphoid hyperplasia, plasmacytosis, and occasional evidence of histiocytosis in the affected lymph node(s). Histological findings that were incidental to ERBITUX™ exposure included testicular atrophy and epididymal oligospermia in individual male rats in both groups, occasional, focal areas of hepatocellular vacuolization or capsulitis in the spleen, and perivascular, eosinophilic infiltrates in the lungs with occasional foreign matter granulomas present. All findings were slight in severity, and occurred at approximately equal incidence in the control and cetuximab treated groups.

Table 16: Histopathology inventory (optional)

Study	54167	070-087
Species	S/D rat	Cyno monkey
Adrenals	X	X*
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur)	X	X
Brain	X	X*
Cecum	X	X
Cervix		X
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X	X
Fallopian tube		X
Gall bladder		
Gross lesions		X
Harderian gland		
Heart	X	X*
Ileum	X	X
Injection site	X	X
Jejunum	X	X
Kidneys	X	X*
Lachrymal gland		X
Larynx		
Liver	X	X*
Lungs	X	X

Lymph nodes, cervical		
Lymph nodes mandibular	X	X
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity		X
Optic nerves	X	X
Ovaries	X	X*
Pancreas	X	X
Parathyroid	X	X*
Peripheral nerve		
Pharynx		
Pituitary	X	X*
Prostate	X	X
Rectum		X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle	X	X
Skin	X	X
Spinal cord	X	X
Spleen	X	X*
Sternum		X
Stomach	X	X
Testes	X	X*
Thymus	X	X
Thyroid	X	X*
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X	X
Vagina		X
Zymbal gland		

X, histopathology performed
*, organ weight obtained

Toxicokinetics: Peripheral blood samples (0.5 ml/animal) for determination of cetuximab toxicokinetic profiles were obtained on study days 0, 3, 7, 11, 14, 17, 22, and 24. Sparse sampling techniques were employed such that blood was obtained from 5 separate rats/sex/dose group prior to infusion, approximately 20 minutes after completion of infusion, and 24 hours after dosing for the first two weeks of treatment. Ten rats/sex/dose group were sacrificed at the end of the two week period; the remaining 5/sex/group were treated with C225 or control for an additional two weeks. Five animals/dose group were bled alternately prior to infusion, at 20 minutes after the end of treatment, and at 24 hours post-dosing on study days 14, 17, 22, and 24. Serum levels of cetuximab were evaluated using a competitive, —— ELISA assay with ——