Dose-related increases in serum cetuximab levels were observed over the duration of the study, with peak serum concentrations noted 20 min following completion of infusion in all groups. Serum C225 levels had declined by 24 h following completion of the infusion; however, in most cases the levels had not declined to baseline (below the level of quantitation of the assay) by the time of the next infusion. The data are included in Table 17, below, which was abstracted from the final report for this study.

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.

Comment: The sponsor had provided a table with in the final, audited study report for Study #ARFC-0294019m with serum levels of cetuximab for each of the different dose groups and times after dosing. Values in this table were presented as a number, ± a second number. The only identifier was a statement at the top of the table saying "units = ug/ml of C225." It is assumed that the values included in the table below represent the mean for each dose group, ± S.D. However, no values for the individual animal data were included in the report, so independent calculation of the mean and S.D. each dose group and time point is not possible.

Table 17: Toxicokinetics of Cetuximab in Sprague-Dawley Rats in a Repeat-Dose, i/v Study

C225 Dose (mg/kg)	Reported Serum Levels of Cetuximab (µg/ml; as per Table 1 of Final Study Report)							
	Day 0	Day 3	Day 7	Day 11	Day 14	Day 17	Day 21	Day 24
Pre-dose								
control	BLQ[a]	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
2.5	BLQ	22	36	42	47	48	36	31
10	BLQ	61	164	108	133	116	105	139
40	BLQ	426	445	534	601	560	482	435
20 min post								
control	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
2.5	45	49	58	57	66	84	54	57
10	189	251	375	447	275	506	271	424
40	796	824	824	823	995	1162	1396	1120
24 h post								
control	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
2.5	32	37	50	60	52	44	46	87
10	143	96	175	259	147	219	140	219
40	654	632	549	743	781	764	537	818

[a] BLQ = below limits of quantitation of the assay

Other: Immunogenicity evaluation of C225: Blood samples (0.5 ml) were collected via the tail vein on study days 7, 14, 21, and 28 prior to treatment with cetuximab, and shipped on dry ice to ImClone for evaluation of anti-C225 antibodies. Total anti-C225 antibodies in rat serum were measured by [redacted], and by an ELISA assay using a [redacted] as the secondary antibody and [redacted] as the color reagent.

Comment: Evaluation of the serum samples from cetuximab treated rats for anti-C225 antibody activity was included separately, in the final report for study #ARFC0294-11. This study will be reported in the review for the present study, #54167, and will not be reviewed separately.

Antibody development was inversely related to the dose of cetuximab. Of note, three rats in the control group had at least one positive time point for anti-C225 antibody by ELISA; however,

samples from all three animals were negative for anti-C225 antibody by immunoblot analysis. Seven rats in the group treated with 2.5 mg/kg/dose had detectable anti-C225 antibody by ELISA, with 1/7 positive at study d 14, an additional 4/7 positive at the d 21 time point, and 1/7 positive at study termination on d 28. The remaining male rat (animal #2581) tested positive for anti-cetuximab antibody by ELISA on study d 0, and with the exception of the d 21 time point, remained positive for the 28 d duration of the study. All seven rats were positive for anti-C225 activity by immunoblot analysis.

Five rats in the 10 mg/kg/dose group, and 3 rats in the 40 mg/kg/ dose group had detectable anti-C225 antibody by ELISA. All three rats in the high dose group, and 3/5 rats in the mid-dose group were positive at study d 14. No additional rats in the high dose group developed antibody after this time point, while 2/5 rats in the mid-dose group became positive at study d 21. Anti-C225 antibody was confirmed positive by immunoblotting in 4/5 ELISA positive rats in the mid-dose group, and 2/3 ELISA-positive rats after treatment with 40 mg/kg/dose ERBITUX™ twice weekly for 14 days.

Comment: Other therapeutic proteins have also demonstrated inverse dose-relationships to antibody development in test animal species. In this study, the levels of cetuximab present in the animals remained relatively high over the duration of the study, and increased in proportion to the dose of C225 administered. Experience has shown that high circulating levels of the test article may bind any antibody present in the plasma, and thereby interfere with the detection of antibody to the therapeutic by ELISA or Western blot analysis.

Study Conclusion: There were no treatment-related clinical, biochemical, hematologic, or gross or histopathological toxicities in Sprague-Dawley rats following treatment with 2.5, 10, or 40 mg/kg/dose cetuximab by i/v infusion twice weekly for two or four weeks. C225 was detected in serum samples from all rats over the duration of the study, with no apparent change in either peak or trough values over the 4 week period, and no evidence of accumulation or increased clearance of the biologic. Anti-C225 antibody developed in cetuximab treated rats in an inverse dose-related fashion, and was confirmed by immunoblot analysis. The NOAEL for ERBITUX™ in the rat is 40 mg/kg/dose, twice weekly for four weeks.

Comment: The rat is not a pharmacologically relevant model in which to evaluate the toxicity of ERBITUX™. *In vitro* binding studies of C225 monoclonal antibody to a panel of tissues from rat or mouse origin (Study #BMS DS02124) did not detect any cross-reactivity of cetuximab in these species.

Study title: C225 (EMD 271 786) 39 week intravenous (infusion) toxicity study in the cynomologus monkey with a 6-week treatment-free period.

Key study findings: ERBITUX™ treatment of cynomolgus monkeys resulted in early mortality in 5/10 (50%) of the animals in the 75 mg/kg/dose group, and significant morbidity due to development of desquamative lesions, secondary bacterial infections, and septicemia. Skin lesions were observed in all dose groups treated with cetuximab, and were dose-related in incidence and severity. No NOAEL could be identified for this study. Other toxicities associated with ERBITUX™ treatment included dose related decreases in body weights, body weight gains, and food intake, anemia, thrombocytopenia, leukopenia, and alterations in coagulation parameters, dose-related elevations in ALT, γ-GT, and GLDH, and increases in organ weights and evidence of tissue pathology in the liver, spleen, and peripheral lymph nodes.

Study no.: 070-087, 070-087a, 070-087b (ImClone Study #930004381 v1.0), #PKM 45-01 (toxicokinetics; ImClone Study #930004387 v1.0), #PKM 41-01 (immunogenicity; ImClone Study #930004398 v1.0)

Comment: The toxicokinetic and immunogenicity analyses for this study were submitted to the BLA as separate study reports. Studies #SR02-01.11 (analytical report for the BIAcore assay) and #PKM 45-01 (analysis of toxicokinetic profiles), included the toxicokinetic data from this study, and Studies SR0201-12 (analytical report) and #PKM 41-01 (antibody titer assay) were the final study reports for the antigenicity determination of cetuximab in the 39 week monkey toxicity study. The findings from these reports will be included in the review for Study #5070-087, below.

Volume #, and page #: EDR files: pharmtox\tox\070-087\070-087.pdf, pharmtox\tox\070-087\070-087a.pdf, pharmtox\tox\070-087\070-087b.pdf, pharmtox\tox\pkm45-01.pdf, pharmtox\tox\sr0201-11.pdf
Conducting laboratory and location: [redacted]
(Study #070-087); ImClone Systems, Somerville, NJ (Studies #PKM 45-01 and PKM 41-01)
Date of study initiation: May 31, 2000 (start of in-life phase)
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: [redacted]

Methods

Doses: Week 1: vehicle control (phosphate buffered saline), 12, 38, 120 mg/kg/dose (mg/m2); weeks 2-39 vehicle control, 7.5, 24, 75 mg/kg/dose (mg/m2)

Species/strain: cynomologus monkeys (*Macaca fasicularis*)

Number/sex/group or time point (main study): 3/sex/group plus 2/sex/group control, high-dose for recovery

Route, formulation, volume, and infusion rate: intravenous infusion, rate 6 ml/kg/hour; volume 12 ml/kg for week 1 (loading dose), 7.5 ml/kg for weeks 2-39 (maintenance doses)

Satellite groups used for toxicokinetics or recovery: no satellite groups; blood for T/K and antibody (immunogenicity) determinations taken from all main study animals (please see time points, below)

Age: 4 to 9 years old (sexually mature as determined by descended testicles in male and evidence of menses in female animals)

Weight (non-rodents only): 3.0 – 5.0 kg (males), 2.3 – 4.9 kg (females)

Unique study design or methodology (if any): [redacted]

Observation times and results

Mortality: All animals were observed twice daily for morbidity and mortality throughout the experimental period. Early mortalities occurred in 5/10 animals in the group treated with 120/75 mg/kg/week C225 antibody, beginning after approximately 13 weeks of treatment. A list of the animals, gender, and date of death is provided in Table 18, below.

Table 18: Early Mortality in Cynomolgus Monkeys Treated with ERBITUX™				
Animal Number	Gender	Dose of ERBITUX™	Fate	Time on Study of Death
10597	F	120/75 mg/kg/week, i/v	Euthanized	Week 14 (study d 94)
6852	F	120/75 mg/kg/week, i/v	Euthanized	Week 25 (study d 169)
9830	M	120/75 mg/kg/week, i/v	Euthanized	Week 27 (study d 185)
10328	M	120/75 mg/kg/week, i/v	Found dead	Week 30 (study d 205)
10599	F	120/75 mg/kg/week, i/v	Euthanized	Week 35 (study d 240)

The final animal sacrificed moribund was on study d 240 (week 35), at which point an amendment to the study was made to discontinue dosing in this group. All other ERBITUX™ and control animals survived until scheduled sacrifice, and the two lower dose groups completed dosing with cetuximab out to week 39.

Clinical signs: All animals were observed for behavior, appearance, and feces twice daily, and for condition of the animals at least once daily. Treatment-related findings included mild to severe lesions on the skin in all animals receiving ERBITUX™, consisting of scale formation, reddening, erythema, dermatitis, fissures, wounds and exanthema on the legs, arms, inguinal and axillary regions, and hair thinning and/or loss over the whole body. The time to appearance of these lesions, as well as the extent and severity were dose-dependent, with the first observed skin lesions at study days 64, 22, and 15 for animals treated with 7.5, 24, or 75 mg/kg/week C225 antibody, respectively. Skin lesions were so severe in the high-dose animals that dosing with ERBITUX™ was discontinued at study weeks 35/36. Although the skin lesions in this group were less pronounced at the end of the 9-week, treatment-free recovery period, they had still not completely resolved suggesting that these effects were not fully reversible following discontinuation of C225 treatment.

Behavioral changes, consisting of hypoactivity and sluggishness were observed in one male monkey (animal #10295M) in the group treated with 38/24 mg/kg/week C225 at study d 22, and in one female and two male monkeys in the high-dose group (animals #6852F, #9830M, and #10328M) on study days 155-169, d 29, and d 204, respectively. Poor physical condition, cachexia, and/or prone position were also noted in these animals and in female monkey #10597F in the group treated with 120/75 mg/kg/week ERBITUX™. Of note, these were the same monkeys that were either euthanized or found dead later in the study. Tremor during infusion was also observed in female monkey #9891F in the 38/24 mg/kg/week dose groups on study d 57, and in animals #3078/2F, #9830M, #6852F, and #10599F at various time points on study.

Macroscopic changes in the eye were observed in both male and female animals in all C225 treatment groups, and consisted of conjunctivitis, reddened, swollen, and/or encrusted eyes. Not all of these findings were included in the ophthalmologist's study report. The incidence and timing of these changes are reported in Table 19, below.

Table 19: Ocular Findings in Cynomolgus Monkeys Treated with ERBITUX™			
Animal Number	Gender	Dose of ERBITUX™	Time of Findings
10292	M	12/7.5 mg/kg/week, i/v	Study days 260-266
10291	M	38/24 mg/kg/week, i/v	Study days 160, 161
8384	F	38/24 mg/kg/week, i/v	Study days 22-35
9830	M	120/75 mg/kg/week, i/v	Study days 78-874, 148-185,
10328	M	120/75 mg/kg/week, i/v	Study d 204
6852	F	120/75 mg/kg/week, i/v	Study days 113-126, 148-169
10599	F	120/75 mg/kg/week, i/v	Study days 153, 157-170, 176-189, 197-240
10480	F	120/75 mg/kg/week, i/v	Study days 148-231

Other clinical findings including soft feces and/or diarrhea, emesis, injuries, abrasions, hematoma, and/or wounds, bloody nasal discharge, encrusted nares, and restricted movement of arms were observed in individual animals in all treatment groups, including the vehicle control group and are considered incidental to ERBITUX™ infusion.

Body weights: Individual body weights were recorded pre-dose on study d 1, then once weekly throughout the experimental period and at terminal sacrifice. There was a trend towards decreased mean body weight values in all groups of male monkeys treated with ERBITUX™, as compared to the control group; however, this change was not statistically significant. The one male monkey #10328M who was sacrificed moribund on study day 205 gained weight during the first 26 weeks on study to a maximum of 4.3 kg, then decreased in body weight to 3.6 kg at the time of euthanasia. Similarly, there was a trend towards a reduction in body weights in the female monkeys treated with C225 over the duration of the treatment period, as compared to both baseline values and the vehicle control group. Two females in the 12/7.5 mg/kg/week dose group lost 200 gm body weight between baseline and the end of the 39 week treatment period; the third animal in this group gained 400 gm over the same time period. One female monkey in the mid-dose group lost 700 gm body weight at study termination as compared to baseline; however, the remaining two animals in this group gained 100 and 300 gm each, respectively. Two of the female monkeys that were euthanized early had a body weight loss of 1500 and 400 gm from baseline, respectively; while the third animal sacrificed moribund, as well as the two surviving female monkeys gained 100 to 400 gm each over the study duration. There were no statistically significant differences in mean body weight gains between the control and the ERBITUX™ treated groups, not within the C225 treatment groups at any time.

Food consumption: Qualitative estimation of individual animal food intake was estimated daily, and food consumption of each monkey was evaluated weekly. Reduced food consumption was observed preceding death in all five monkeys that were either sacrificed moribund or died on study. Otherwise, there were no remarkable effects of ERBITUX™ treatment on food consumption when compared to animals treated with the vehicle control.

Ophthalmoscopy: Ophthalmologic examination of ocular fundus with macular lutea, pupilla, and ocular vessels by both direct and indirect ophthalmoscopy for all animals, prior to dosing, at weeks 13, 35/36 (for surviving high-dose animals), week 39, and at the end of the treatment-free recovery period for all surviving animals. There were no ocular changes in the control or ERBITUX™ treated monkeys that were related to treatment with cetuximab.

Comment: The clinical observations noted conjunctivitis, reddening and swelling of the eyelids, and encrusted eyes in eight animals over the duration of the study. However, not all of these findings were included in the ophthalmologist's report. It is not clear from the consulting ophthalmologist's report if these findings were not present on the days of examination in these animals, of if they were present and considered incidental by the evaluating ophthalmologist.

EKG: EKGs (leads I, II, III and augmented leads aVR, aVL, and aVF) were evaluated in conscious animals once pre-dosing, and at weeks 4, 13, 26, and 39 (35/36 for surviving animals in the high-dose group) approximately 1 hour after C225 infusion, and in all surviving monkeys at the end of the recovery period. Parameters measured on Lead II were heart rate (bpm), RR, PR, QRS, and QT intervals in seconds, QTc and QT dispersions, P, R, S, T voltage measurements (in mV), and systolic, diastolic, and mean arterial blood pressures. There were no remarkable, treatment-related electrocardiography findings in the groups treated with ERBITUX™ as

compared to vehicle control, although isolated cases of tachycardia, bradycardia, negative T wave, and fluctuations in blood pressure were observed in individual animals in all treatment groups.

Hematology: Blood samples were collected from all monkeys prior to dosing once pre-dose, and during weeks 4, 13, 26, and 39 (weeks 35/36 for surviving animals in the high-dose group), and from all surviving animals at terminal or recovery sacrifices. Monkeys were fasted overnight prior to blood sampling. In addition to the standard hematology parameters, blood from ERBITUX™ treated monkeys was also evaluated for coagulation profiles (PT, APTT, and TT). No remarkable changes in were observed in erythrocyte parameters, with the exception of an increase in reticulocyte counts as compared to baseline in all groups including the vehicle control beginning at week 4 on study. Reticulocytes remained elevated as compared to baseline, although to a lesser extent until terminal sacrifice. There were no remarkable effects of C225 treatment on platelet counts at any time point on study.

Although a trend towards decreased total number of leukocytes at week 39 as compared to baseline was observed in monkeys of both sexes in all treatment groups including control, these findings were not statistically significantly different from the pretreatment values. There were also no statistical differences between the mean values for the control and cetuximab treated groups. However, the mean values for total leukocyte counts at week 35 in the animals treated with 120/75 mg/kg/dose cetuximab had decreased by approximately 10% from baseline in the males, and from 25 to 40% from baseline in the surviving female animals. Four of the five monkeys with early mortality in this group showed decreases of total white blood cell counts, ranging from 28 to 57% from baseline prior to death. Differential leukocyte counts in the three female monkeys showed 2 to 3-fold increases in the percentage of neutrophils, and approximately 50 to 60% decreases in the percentage of lymphocytes from baseline to the time of the individual animals' demise. By contrast, the opposite effect was observed in the two male monkeys prior to their death, with dramatic decreases in neutrophil percentages from 27% and 37% of the differential count at baseline, to 6% and 0% of the differential count at the time of death for monkeys #9830M and 10328M, respectively. A corresponding increase in the percentage of lymphocytes, from 71% to 91 of the differential in animal #9830M and from 59% to 94% of the differential count in animal #10328M was also observed between baseline and the time of the animals' death. Differential neutrophil and lymphocyte counts were not remarkably different from baseline or from the vehicle control group in the remaining animals. There were no significant differences across the different treatment groups for the percentages of monocytes, basophils, eosinophils, blast cells, juvenile, or band neutrophilic precursors, as compared to either the vehicle control group or the baseline values for each of the ERBITUX™ treated groups.

Coagulation profiles demonstrated minor to mild, statistically significant decreases in prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin time (TT) in all cetuximab treated groups at week 39, as compared to the vehicle control. One male and two female monkeys in the group treated with 12/7.5 mg/kg/dose C225 had decreased PT, APTT, and TT times that contributed to the overall decrease observed for this group. Likewise, all cetuximab treated females and males #10288 and #10291 in the mid-dose group had decreased APTT at week 39 as compared to the vehicle control, with decreased PT also noted in males #10288 and #10295 in this dose group. Both PT and APTT were decreased, although statistical significance could not be determined due to the small number of surviving animals, in monkeys #10279M, #10248M, and #10480F in the highest cetuximab dose group at week 39. These findings in the surviving animals in this group had returned to within normal limits by the end of the 9-week recovery period.

Bone marrow smears (sternum) were prepared at necropsy from all monkeys treated with 120/75 mg/kg/week cetuximab or vehicle control at the time of sacrifice (early, terminal, or recovery). Five hundred \ ——— cells were counted microscopically under 1000X magnification, and the total number and percentages of each of the cell types, as well as the myeloid:erythroid ratio were determined for each animal. Both samples from male monkeys #9830 and #10328, who died on study were unevaluable due to the presence of excessively stained material in the marrow samples. There were no remarkable differences in the numbers and percentages of erythroblasts, myeloblasts, early, or late normoblasts, promyelocytes, and metatmyelocytes, or mature granulocytes, lymphocytes, and megakaryocytes between the vehicle control and high-dose cetuximab group. Male monkeys treated with C225 exhibited an approximate 2-fold decrease in intermediate erythroblasts as compared to the vehicle control animals (from 8.1 ± 2.0% in the control group to 4.5 ± 0.9% in the cetuximab group). An increase in myelocytes was observed in the ERBITUX™ treated female monkeys as compared to the vehicle control (9.3 ± 1.0% in the control and 13.8 ± 3.5 in the C225 group). Regardless of these changes, there were no remarkable differences in the M:E ratios between the control and cetuximab treated groups. Myeloid:erythroid ratios were 0.9 ± 0.1 and 1.0 ± 0.2 for male and female monkeys in the vehicle control group, and 1.1 ± 0.1 and 1.1 ± 0.2 for male and female monkeys treated with 120/75 mg/kg/week cetuximab for 35 weeks, respectively.

Comment: These data were contained as Appendix 11 to final study report 070-087b. The contract laboratory has evaluated samples from all animals together for the group mean values, regardless of whether the animals were sacrificed at the end of treatment, the end of the recovery period, or were euthanized moribund on study. An independent evaluation of these data revealed that for the female monkeys with early mortality after ERBITUX™ treatment, there was a two-fold decrease in the mean percentage of mature neutrophils, with a corresponding shift to increased myelocyte and metamyelocyte precursors, as compared to either the vehicle control group or the two animals sacrificed following the 9 week, treatment-free recovery period. These findings correlate with the increase in both absolute and differential total leukocyte and neutrophil counts in these animals that were observed at early sacrifice. There were no data for the male monkeys that died on study that could be evaluated.

Clinical chemistry: Analysis of serum biochemistry profiles was performed on blood samples collected from all C225-treated monkeys at the same time points as for hematology evaluation. The parameters evaluated included serum hepatic transaminase levels, alkaline phosphatase, total bilirubin, blood urea nitrogen, creatinine, total protein, albumin, globulin and A:G ratio (calculated), serum cholesterol, and sodium, potassium, chloride, phosphorous, and calcium. Increases in serum levels of γ-glutamyltranspeptidase, glutamine dehydrogenase (GLDH), aspartate aminotransferase (AST), and alanine aminotransferase were observed in individual animals in all of the ERBITUX™ treated groups, and both the incidence and severity of these findings were dose-related. The incidence of elevations or decreases in these serum protein levels of at least 2-fold over baseline at at least one time point on study is presented for each of the different cetuximab dose groups in Table 20, below:

Table 20: Incidence of Specific Clinical Chemistry Changes in Cynomolgus Monkeys Following Treatment with ERBITUX™							
Parameter	Change	Dose of Cetuximab, i/v					
		12/7.5 mg/kg/wk		38/24 mg/kg/wk		120/75 mg/kg/wk	
		Male	Female	Male	Female	Male	Female
γ-GT	Increase	0/3	2/3	2/3	1/3	3/5	4/5
GLDH	Increase	0/3	1/3	0/3	0/3	3/5	3/5
AST	Increase	0/3	0/3	0/3	0/3	4/5	4/5
ALT	Increase	0/3	0/3	1/3	0/3	2/5	5/5
Albumin	Decrease	0/3	0/3	0/3	0/3	2/5	3/5
A:G Ratio	Decrease	0/3	0/3	1/3	2/3	4/5	3/5

Although for many of the parameters, the mean values for each of the C225 groups were not statistically significantly different from the vehicle controls, significant elevations in γ-GT (> 2-fold over background and > 3-fold over vehicle control group) were observed at week 26 in the female monkeys treated with 120/75 mg/kg/week cetuximab, and the values continued to increase following discontinuation of dosing at week 35. By the end of the 9-week recovery period, the mean γ-GT values in the cetuximab treated female monkeys were approximately 3.3-fold greater than baseline, and more that 15-fold increased over the concurrent vehicle control group.

The elevations in GLDH observed in female monkeys treated with 75 mg/kg/week cetuximab were statistically significantly different from both the vehicle control group and from the mean baseline value beginning at week 26 on. These findings could be attributed to one female monkey (animal #10480F) with severely elevated GLDH beginning at this time point, that continued to escalate throughout the treatment-free recovery period. Increased GLDH levels were also observed in the two high dose male monkeys #9830M and #10328M prior to their deaths at weeks 27 and 30, respectively, on study.

The increases in AST were observed in these same two male monkeys prior to their deaths, and in the two female animals #10597F and #10599F prior to necropsy at study weeks 14 and 35, respectively. At the end of the recovery period, elevated AST levels were observed in two male monkeys (animals #10279M and #10284M) and one surviving female monkey (animal #10480F) in the group treated with 75 mg/kg/week ERBITUX™ for 36 weeks. In addition, ALT levels were markedly increased in one male monkey (animal #10295M) treated with 24 mg/kg/week cetuximab at weeks 4 and 39, and in 1/5 male and 3/5 female monkeys in the high dose group at study week 4 (animals #10284M, #3078/2F, #6852F, and #10597F, resepectively), in female monkey #10480F at study weeks 26 and 39, and at early necropsy in monkeys #9830M, #10328M, and #10599F in this same dose group. The majority of these treatment-related changes in clinical chemistry profiles did not completely recover to baseline during the 9 week treatment free recovery period.

Urinalysis: Urine was collected at the same time points as hematology and clinical biochemistry were done, following overnight use of metabolism trays inserted under each cage. Water and food were withdrawn for the duration of the collection. Samples were evaluated for volume, pH, specific gravity, and protein, blood, glucose, ketones, bilirubin, urobilinogen, and α1 microglobulin were determined semi-quantitatively (dipstick). Urine samples were also evaluated microscopically for evidence of erythrocytes, leukocytes, epithelial cells, casts, and organic and inorganic components. Positive reactions for hemoglobin and/or red cells and leukocytes were observed in individual monkeys in both the vehicle control and 75 mg/kg/week ERBITUX™ treated groups at occasional time points on study. No treatment related changes in

other parameters were observed, with most values below the detectable limit of the test strip. The exception was α1-microglobulin, which displayed a high degree of variability both between control and ERBITUX™ treated groups of monkeys, and between individual animals within a treatment group at different time points on study. No assignment of causal relationship of these findings to ERBITUX™ treatment could be made, due to this high variability.

Gross pathology: All animals were subjected to a complete necropsy evaluation following euthanasia and exsanguination. A full macroscopic examination of all tissues and organs *in situ* was performed, and all lesions were recorded. Skin lesions were documented by digital or traditional photography at the end of the treatment phase, and from all surviving recovery animals at weeks 35/36, 39/40, and 45 during the recovery period (documentation included in the BLA submission in study report #070-087a). At terminal and recovery sacrifices, skin lesions were obtained from all surviving monkeys with lesions present, and were submitted for bacteriological evaluation. In the five monkeys that died or were euthanized early on study, samples of colon, small intestine, liver, and kidney, in addition to the skin lesions and normal skin were also obtained, and submitted for bacteriological culture.

Skin lesions in all ERBITUX™ treated groups were present at both scheduled and early necropsies, and were the major gross pathological finding. Dermatologic findings included reddening and scaling of skin (reported as squamous skin), loss of hair, edema, discoloration, pustules, swelling, and wounds in the high dose animals only. These lesions were clearly dose-related and were characterized as mild in severity in the low dose group (3M/3F affected), moderate in severity in the 24 mg/kg/dose C225 group (3M/2F), and severe in the animals treated with the highest dose of ERBITUX™. The skin scaling, alopecia, and squamous changes were still present in 3/ 4 of the high dose cetuximab treated monkeys at the end of the 9 week recovery period, indicating that these changes were not readily reversible.

Additional findings at necropsy included discolored foci and irregular or mottled surface to the kidney in some individual animals in the mid dose group. One female monkey in the cetuximab treated group had an indentation present in the kidney at the 9 week recovery sacrifice.

In the animals that either died or were euthanized early, additional gross necropsy findings included enlargement of the liver, kidneys and spleen and liquefaction of the sternal bone marrow in both male monkeys (animals #9830M and #10328M), and enlarged liver in one female monkey (animal #6852F).

Evaluation for specific pathogenic bacteria was performed on skin samples from animals in all groups, and was negative in the control animals, and in all monkeys treated with either 7.5 or 24 mg/kg/week cetuximab. Animal #10328M in the high dose group that died week 30 on study was culture positive for *Staphylococcus aureus* in the small intestine, skin, liver, kidney, and colon. An additional female decedent in this group (animal #6852F, died week 25 on study) was also positive for *Staph. aureus* in the liver. All other decedents were negative for infectious bacteria in all organs tested.

Organ weights (specify organs weighed if not in histopath table): Identified in histopathology Table 16, above. Slightly to markedly increased weights of the popliteal lymph nodes were observed in 1 male and all 3 females in the low dose, and 2 male and 2 female monkeys each in the 24 mg/kg/week cetuximab dose group, and were significantly different as compared to the control. Both absolute and relative mean kidney weights in the mid-dose female animals, and relative mean kidney weights in the females treated with 7.5 mg/kg/week cetuximab

were significantly increased from control; these findings were due to increased kidney weights in all female monkeys in both the low and mid-dose groups. In male monkeys, there was a trend towards an increase in mean absolute and relative kidney weights in the low and mid-dose animals, but the mean values for these groups were not significantly different from control. Absolute kidney weights were increased on 1 low dose male and all 3 male monkeys in the mid-dose group. Less pronounced changes were observed in the relative kidney weights, where the increase was noted in the one male low dose animal (#10282M), and 2/3 monkeys in the mid-dose group (animals #10288M and #10295M).

One female monkey (animal #10627F) in the group treated with 24 mg/kg/week cetuximab had an increase in spleen weight, that resulted in a slight, although not statistically significant increase in the mean value for this group as compared to the control animals. Spleen weights were also increased in one low dose female (animal #9954F) and one low (#10322M0 and one mid-dose male monkey (animal #10291M), with no significant differences as compared to control.

The heart weight was increased in one male monkey in the 24 mg/kg/week dose group at terminal sacrifice (animal #10291M). Relative liver weights were also elevated in low and intermediate dose monkeys at terminal sacrifice, which were attributed to cetuximab-related impairment of weight gain.

Organ weights in the early decedent animals in the 75 mg/kg/week cetuximab group showed elevations in popliteal lymph node, spleen, liver, adrenal, and kidney absolute and relative weights. There were no surviving high dose monkeys at week 39, so no comparison of effects on organ weights could be performed with either the early decendents or the lower cetuximab dose groups. However, both relative and absolute kidney weights remained elevated over control on one male and one female monkey each in the 75 mg/kg/week cetuximab treated group at the end of the 9 week recovery period. Female #3072F also had increased adrenal and liver weights in addition to the increased kidney weight at this sacrifice.

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

Microscopic pathology of tissue sections from monkeys in the 7.5 and 24 mg/kg/week cetuximab treated groups demonstrated treatment-related, purulent skin lesions as the major finding in almost all of the animals at the catheter injection site and at other peripheral skin locations. Inflammatory lesions were associated with hyperkeratosis, acanthosis, and epidermal hydropic degeneration, and were dose-related in both incidence and severity. Chronic interstitial inflammation in the kidney was also observed in 2/3 male and female monkeys, each in the low dose group, and 2 male and 1 female monkey after treatment with 24 mg/kg/week cetuximab for 39 weeks. These lesions were considered secondary to bacterial infection following cetuximab mediated disruption of the skin barrier.

In the monkeys treated with 120/75 mg/kg/week ERBITUX™, histologic findings in the skin included severe ulcerations and erosion, and inflammatory changes in the tongue, esophagus, and nasal passages that were accompanied by sloughing of the mucosal epithelium. The dermatologic findings in all cetuximab dose groups were treatment related, and were attributed to disruption of the normal epidermal maturation process by EGFr inhibition following cetuximab treatment.

Histologic changes also included microscopic evidence of inflammation in the lymph nodes, liver, spleen, heart, lung, and bone marrow of the early decendent animals, and in individual

animals in all cetuximab dose groups at terminal or recovery sacrifice with no apparent dose relationship in either incidence or severity. Degenerative changes in the renal tubular epithelium, including dilatation, vacuolization, and degeneration of tubules, chronic interstitial nephritic, and massive glomerular thrombosis were also present in individual animals in all dose groups, including the control.

Comment: Of note, interstitial lung disease was observed in several patients in the pivotal clinical study of ERBITUX™ and irinotecan treatment in metastatic lung cancer. However, the microscopic lesions present in the lungs of the cetuximab treated monkeys were not representative of the findings expected for interstitial lung disease (e.g. pneumonitis), and were limited to such findings as edema, congestion, and inflammatory infiltrates in one of the early mortalities, and pigmented macrophages, inflammatory foci, and infiltrating inflammatory cells in all dose groups, with the highest incidence reported in the control animals at the week 39 sacrifice.

Toxicokinetics: Blood samples (approximately 3-5 ml) were collected prior to treatment at weeks 4, 13, 26, and 39 (weeks 35/36 for surviving high-dose group monkeys) at time 0 (*i.e.* prior to infusion), and 0.1, 4, 8, 24, 72, 120, and 167 hours after completion of infusion. Levels of cetuximab in serum were measured using the BIAcore solid phase, instrument-based system as described in section 3.3.7, above. The lower limit of quantitation of this assay was [redacted] cetuximab. Serum C225 levels in placebo control treated monkeys were below the lower limit of assay quantitation at all time points on study. At each week tested, peak serum cetuximab levels were maximal at 0.1 h after completion of dosing, and declined approximately 4 to 9-fold over the 167 hour sampling period. Mean values for maximal serum concentration of ERBITUX™ and for AUC_{last} in the three dose groups were comparable between weeks 4, 13, 26, and 36/39, suggesting that continuous exposure to the biologic had occurred for the duration of the study. No differences in toxicokinetic profiles were noted between male and female animals. The summary tables for this study have been abstracted from the final study report, and are included as Tables 21 and 22, below.

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Table 21: Toxicokinetics of Cetuximab in the Cynomolgus Monkey, following Repeated Administration by i/v Infusion for 39 weeks – Male animals

Mean (N=3 / 5) pharmacokinetic parameters of C225 (EMD 271786) after repeated intravenous (infusion) in monkeys					
Dose	Sex / Test week	MALE weeks			
[mg/kg]	Study day	4	13	26	39
7.5*	$C_{1.1\,h}$ [µg/mL]	308	236	231	242
	$C_{1.1\,h}$ / D [(µg/mL) / (mg/kg)]	41.1	31.5	30.8	32.3
	t ½ [h]	77.4	60.4	68.9	64
	AUC_{LAST} [µg/mL x h]	16500	11800	10700	10900
	AUC_{LAST} / D [µg/mL x h / (mg/kg)]	2190	1570	1420	1460
	CL_{ss} [mL/h/kg]	0.49	0.69	0.788	0.706
	Vss [mL/kg]	50.3	55.3	70.0	60.3
24*	$C_{1.1\,h}$ [µg/mL]	901	921	950	757
	$C_{1.1\,h}$ / D [(µg/mL) / (mg/kg)]	37.5	38.4	39.6	31.6
	t ½ [h]	58.5	72.4	70.2	58.6
	AUC_{LAST} [µg/mL x h]	46100	39700	41500	31600
	AUC_{LAST} / D [µg/mL x h / (mg/kg)]	1920	1650	1730	1320
	CL_{ss} [mL/h/kg]	0.521	0.62	0.656	1.82
	Vss [mL/kg]	40.9	61.7	57.2	79.9
75+	$C_{1.1\,h}$ [µg/mL]	2910	2630	3170	-
	$C_{1.1\,h}$ / D [(µg/mL) / (mg/kg)]	38.8	35.1	42.3	-
	t ½ [h]	206	206	141	-
	AUC_{LAST} [µg/mL x h]	216000	172000	168000	-
	AUC_{LAST} / D [µg/mL x h / (mg/kg)]	2880	2300	2250	-
	CL_{ss} [mL/h/kg]	0.358	0.448	0.457	-
	Vss [mL/kg]	100	143	83.8	-

*: mean of n = 3, +: mean of n = 5 -: PK sampling was incomplete
all values referring to time are given before start of infusion

Table 22: Toxicokinetics of Cetuximab in the Cynomolgus Monkey, following Repeated Administration by i/v Infusion for 39 weeks – Female animals

Mean (N=3 / 5) pharmacokinetic parameters of C225 (EMD 271786) after repeated intravenous (infusion) in monkeys					
Dose	Sex: FEMALE Test week: weeks				
[mg/kg]	Study day	4	13	26	39
7.5*	$C_{1.1\,h}$ [µg/mL]	270	230	177	192
	$C_{1.1\,h}$ / D [(µg/mL) / (mg/kg)]	36.0	30.7	23.6	25.6
	t ½ [h]	57.2	49.9	50.1	50.9
	AUC_{LAST} [µg/mL x h]	11800	8800	9570	9320
	AUC_{LAST} / D [µg/mL x h / (mg/kg)]	1580	1170	1280	1240
	CL_{ss} [mL/h/kg]	0.638	0.855	0.784	0.807
	Vss [mL/kg]	49.2	56.9	54.7	58.7
24x	$C_{1.1\,h}$ [µg/mL]	1080	434	857	943
	$C_{1.1\,h}$ / D [(µg/mL) / (mg/kg)]	44.8	18	35.8	39.3
	t ½ [h]	93.8	77.6	64.2	71.7
	AUC_{LAST} [µg/mL x h]	59100	47100	44300	47000
	AUC_{LAST} / D [µg/mL x h / (mg/kg)]	2460	1965	1850	1960
	CL_{ss} [mL/h/kg]	0.411	0.551	0.543	0.515
	Vss [mL/kg]	52.9	66.5	51.8	51.4
75+	$C_{1.1\,h}$ [µg/mL]	3640	2240	-	-
	$C_{1.1\,h}$ / D [(µg/mL) / (mg/kg)]	48.5	29.9	-	-
	t ½ [h]	127	110	-	-
	AUC_{LAST} [µg/mL x h]	229000	154000	-	-
	AUC_{LAST} / D [µg/mL x h / (mg/kg)]	3050	2050	-	-
	CL_{ss} [mL/h/kg]	0.347	0.507	-	-
	Vss [mL/kg]	59.3	77.7	-	-

*: mean of n = 3, , x: mean of n=2 +: mean of n = 5 -: PK sampling was incomplete
all values referring to time are given before start of infusion

Immunogenicity: Peripheral blood samples for antibody (immunogenicity) determination were collected at pretreatment, and at weeks 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 28, 32, and 36, and in all surviving animals in the recovery groups at weeks 2, 4, and 6 of the treatment-free period. Antibody to cetuximab was determined using the BIAcore instrument-based assay system. No anti-C225 antibodies were detected at any time point in monkeys in the vehicle control or 7.5 mg/kg/week ERBITUX™ treated groups. Two monkeys in the group treated with 24 mg/kg/week cetuximab had at least one positive anti-C225 antibody titer on study; however, male monkey #10288M had a single positive value of [redacted] at study week 6, and was below the limits of quantitation of the assay ([redacted] for all other time points, so this finding was likely not a true immunogenic response. By contrast, animal #10627F developed anti-cetuximab

antibody by week 12 on study with a titer of 878 ng/ml, which continued to rise to a final level of 3381 ng/ml at the final sample at week 36, with the exception of week 16, where the values were below the lower limit of quantitation of the assay. One monkey (animal #10391M) in the group treated with 120/75 mg/kg/week ERBITUX™ had a detectable anti-C225 antibody titer of 19 ng/ml prior to treatment with cetuximab, but all subsequent samples were below the limit of quantitation of the assay so this animal was not considered to have a positive anti-C225 response. All other serum samples in the high dose cetuximab treated animals were negative for anti-ERBITUX™ antibody at all time points during the treatment and recovery periods.

Other: Menstrual bleeding was checked daily in the mornings by inserting a cotton bud into the vagina of all female monkeys, from week 25 onward and for all surviving female animals during the treatment-free recovery period. A scale of 1 (very little bleeding) to 4 (very heavy bleeding) was used. Cycle length in cynomolgus monkeys was reported for the colony as 30 ± 5 days, and an absence of menstrual cycling was defined as more than two mean cycle lengths (60 days). Variations in duration of menstrual cycles were observed in all groups of female monkeys on study, including the control animals. Duration of menstrual cycling was unaffected in 2/5 control females. Irregular or absent menstrual cyclicity was observed in all female monkeys treated with ERBITUX™ from week 25 onward, and in all surviving females in the high dose group during the recovery period. Irregular menstrual cycles were observed in 1/3 female monkeys in the 7.5 mg/kg/dose group and in 2/3 females treated with 24 mg/kg/dose, and in 2/5 females in the control group. One control female was also partially acyclic. Lack of menstrual cycling with irregular bleeding was observed in 2/3 females in the low dose, and complete lack of cyclicity was noted in 1/3 females in the mid-dose, and in all surviving female monkeys treated with 75 mg/kg/dose cetuximab.

Comment: Although the changes in menstrual cycling appear to be related to treatment with ERBITUX™, no baseline values for menstrual cycle duration were obtained for these animals prior to study initiation, or prior to week 25 of treatment so no definitive causality can be assigned.

Sperm evaluations were performed in all surviving male monkeys once during weeks 26/27, 32/33, and 38/39. Sperm was collected following rectal probe stimulation under anesthesia, digested with trypsin, and sperm numbers, motility, and morphology were assessed microscopically. Measurement of serum testosterone levels by radioimmunoassay (RIA) was also performed on blood samples obtained from all surviving male monkeys at the same time points as for sperm analysis. Variations in sperm count, ejaculate volume, and sperm morphology were observed in individual animals in all groups including the control, and were attributable to variability in sexual maturity in these animals. There were no remarkable, treatment-related differences between sperm counts, viability, motility, or morphology or serum testosterone levels between the control and ERBITUX™ treated male monkeys.

Study conclusion: The toxicities of ERBITUX™ in cynomolgus monkeys after 39 weeks of treatment by weekly intravenous infusions were predominantly related to exaggerations of the anti-EGFr pharmacologic activity of the antibody. Dermatologic findings, including inflammation at the injection site and at the inguinal and axillary areas, trunk and peripheral limbs, accompanied by skin scaling and sloughing were observed at all dose levels of ERBITUX™, such that no NOAEL can be defined. At the highest dose level, ulcerative lesions, erosions, and desquamation of the internal integument, and of the epithelial mucosa of the nasal passage, esophagus, and tongue were observed, along with microscopic evidence of degenerative changes in the renal tubular epithelium. Deaths due to bacterial infection and/or sepsis secondary

to the severe skin lesions were observed in 50% (5/10) of the animals at the highest dose level beginning after approximately 13 weeks of treatment. The skin toxicities were only partially reversible after discontinuation of ERBITUX™ treatment for 9 weeks. Other toxicities observed in this study included inappetence, decreased food intake, and loss of body weight in the high dose animals secondary to development of ulcerative lesions in the esophageal mucosa and tongue, anemia, leukocytosis and/or leukopenia generally related to the skin lesions, elevations in hepatic γ-glutamyl transpeptidase and ALT, and abnormal or absent menstrual cycles in the female animals, beginning at approximately 26 weeks of treatment and continuing in the high dose group throughout the 9 week, treatment-free recovery period. The dermatological and mucosal epithelial toxicities of ERBITUX™ are related to its mechanism of action of inhibition of the function of EGFr, and subsequent defects in epithelial maturation. Similar dermatologic findings, including Grade 3 acneform rash and skin sloughing were observed in the pivotal clinical study. No NOAEL can be defined for this study.

3.4.4. Genetic toxicology

Study title: EMD 271 786 bacterial mutagenicity assay, *Salmonella typhimurium* and *Escherichia coli*
Key findings: EMD 271 786 (C225, cetuximab) did not cause DNA damage in this bacterial mutation assay
Study no.: Merck Study #T15368 (Imclone Study #930004390)
Volume #, and page #: EDR file:pharmtox\tox\15368.pdf
Conducting laboratory and location: Merck KGaA Institute of Toxicology, Darmstadt, Germany
Date of study initiation: April 8, 2002; April 17, 2002 (start of experimental phase)
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: [redacted]

Formulation/vehicle: cetuximab solution, 9.4 mg/ml; formulated in PBS (also used as vehicle)

Methods
Strains/species/cell line: *Salmonella typhimurium*, strains TA98, TA100, TA102, TA 1535, and TA 1537; *Escherichia coli* strain WP2 *uvr*A
Doses used in definitive study: 50, 158, 500, 1580, and 5000 µg per plate
Basis of dose selection: range-finding study demonstrating no cytotoxicity at concentrations of C225 of 5 to 5000 µg/plate
Negative controls: EMD 271 786-placebo (phosphate buffered saline), distilled water
Positive controls: 9-aminoacridine, 2-aminoanthracene, benzo(A)pyrene, cumene hydroperoxide, daunomycin, and N-ethyl, N'-nitro-N-nitrosoguanidine
Incubation and sampling times: 3 parallel plates for each test article concentration, ± 10% or 30% liver homogenate S9 fraction from Aroclor 1254-induced rats; incubate 37°C for 3 days and count number of revertant colonies manually or using an image analyzer

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Each treatment with the positive control substance, in the presence or absence of external metabolism by rat liver S9 fraction, produced an increased number of revertant bacterial colonies as compared to solvent or vehicle control.

Study outcome: No increase in the number of revertant *S. typhimurium* or *E. coli* colonies as compared to vehicle or solvent controls was detected in cultures exposed to cetuximab in the presence or absence of rat liver homogenate S9 fraction, at concentrations up to the maximum tested.

Genetic Toxicology Summary: Cetuximab treatment of bacteria at concentrations up to 5000 µg/plate did not increase the number of revertant colonies in this assay, when tested in the presence or absence of external metabolism by rat liver S9 fraction.

Study title: EMD 271 786 micronucleus test in rats after intravenous administration
Key findings: EMD 271 786 (IMC-C225, cetuximab) treatment of rats at the maximally feasible dose of 197 mg/kg, i/v did not increase the number of micronucleated, normochromatic or polychromatic erythrocytes
Study no.: Merck Study #T15361 (ImClone Study #930004399)

Volume #, and page #: EDR file:pharmtox\tox\t15361.pdf
Conducting laboratory and location: Merck KGaA Institute of Toxicology, Darmstadt, Germany
Date of study initiation: April 12, 2002; April 15, 2002 (start of experimental phase)
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: EMD 271 786 (C225, cetuximab); lot #008894; purity not specified
Formulation/vehicle: cetuximab solution, 9.84 mg/ml; formulated in PBS (also used as vehicle)

Methods

Strains/species/cell line: Wistar ——→ rats, 6-8 weeks old, 5 males/group
Doses used in definitive study: 197 mg/kg, i/v (sterile phosphate buffered saline as vehicle control)
Basis of dose selection: represents the maximal feasible dose of C225 that can be tested, based on a concentration of 9.84 mg/ml cetuximab, and a maximal volume for i/v injection of 20 ml/kg in the rat
Negative controls: EMD 271 786-placebo (phosphate buffered saline)
Positive controls: 16.5 mg/kg cyclophosphamide, p/o
Sacrifice times: Sacrifice 5 rats treated with control, IMC-C225, or cyclophosphamide at 24 hours after injection; sacrifice remaining 5 rats treated with IMC-C224 48 hours after treatment; stain femoral bone marrow smears with modified —— evaluate microscopically at 1250X magnification for evidence of micronucleated red cells

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Manually count and score 2000 polychromatic erythrocytes per animal for micronuclei, 1000 total erythrocytes counted and scored for normochromatic:polychromatic nucleated erythrocyte ratios. The positive control cyclophosphamide, produced an increase in the percentage of micronucleated, polychromatic erythrocytes to 17.9% of the cells counted.

Study outcome: No increase in the number of micronucleated, normochromatic or polychromatic erythrocytes compared to vehicle control was detected in bone marrow samples from rats treated with 197 mg/kg cetuximab 24 of 48 hours prior to sample preparation. The percentage of micronucleated, polychromatic erythrocytes was 1.6, 0.7, and 0.7% of the cells evaluated in the vehicle control, and 24 and 48 hour cetuximab treated groups, respectively.

Genetic Toxicology Summary: Using an *in vivo* assay for mutagenesis, cetuximab treatment of male rats at the maximally feasible dose of 197 mg/kg, i/v did not induce increases in micronucleated, normochromatic or polychromatic erythrocytes, under conditions where the positive control, cyclophosphamide exhibited potent mutagenic effects.

3.4.5. Carcinogenicity - N.A. no studies of this type were included in the present submission

3.4.6. Reproductive and developmental toxicology - N.A. no studies of this type were included in the present submission

3.4.7 Local tolerance

Study title: EMD 271 786 local tolerance study in rabbits after intravenous, intraarterial, paravenous, intramuscular, and subcutaneous treatment.
Key study findings: Cetuximab injection was generally very well tolerated. Local toxicities after intravenous injection were limited to transient hematoma, with mild to minimal hemorrhage microscopically at the injected site 24 hours after dosing. All findings had resolved by 96 hours after treatment.
Study no.: T15386 (ImClone Study #930004392 v1.0)
Volume #, and page #: EDR file: pharmtox\tox\t15386.pdf
Conducting laboratory and location: Merck KGaA Institute of Toxicology, Darmstadt, Germany
Date of study initiation: April 19, 2002 (protocol signed); April 22, 2002 (in-life treatment begun)
GLP compliance: Yes
QA statement: yes (X) no ( )
Drug, lot #, and % purity: EMD 271 786 placebo, lot #008914; EMD 271 786 (C225, cetuximab); lot #008616; percent purity not specified in final report
Formulation/vehicle: cetuximab, final clinical formulation in PBS, 2.01 mg/ml

Comment: The composition of the EMD 271 786 placebo was not specified anywhere in the final study report. However, the Tables 4 and 6 documenting the gross and histopathological findings list the control article as sterile water for injection.

Methods

Species/strain: New Zealand white rabbit, strain Crl: ———, 18-20 weeks old; weight range 3.22-3.97 kg (mean 3.70 kg); 6 females/group
Doses: 0.5 ml of a 2.0 mg/ml solution of cetuximab or EMD 271 786 placebo
Study design: Cetuximab (2.0 mg/ml) was administered i/v, i/a, i/m, s/c, or paravenously to rabbits by a single injection on study d 1, using an 18-gauge injection cannula. Six females were injected by the i/v, s/c, and i/m routes in the same animal, with the remaining three rabbits per group injected i/a and paravenously. Cetuximab was injected on the left, and the vehicle was tested at the contralateral (right) side of the same animal. The area of the injection site(s) is described in Table 23, below.

Table 23: Study Design for Local Tolerability of Cetuximab Injection in Rabbits

Route of Administration	Volume (ml)	Injection site
Intravenous	0.5	Ear vein (peripheral)
Intraarterial	0.5	*Arteria auricularis aboralis* (towards tip of ear)
Paravenous	0.2	Area of ear vein at the auricle base
Intramuscular	0.5	*Musculus longissimus dorsi*
Subcutaneous	0.5	Area of the chest wall

Injection sites were evaluated clinically for local irritation, tolerability, and behavioral and clinical signs of toxicity immediately and one hour after injection on study d 1, and twice daily until scheduled sacrifice. Three rabbits per group were sacrificed 24 and 96 hours later (study days 2 and 5, respectively), and histopathological evaluation of the injection sites performed following hematoxylin and eosin staining of formalin-fixed tissue sections.

Results:

There were no clinical signs of toxicity and no early mortalities observed in this study. Local toxicities consisting of hematoma, were observed in all 6 rabbits at the injection site treated with placebo by intraarterial injection, and in 5/6 rabbits at the cetuximab i/a injection site. All 6 rabbits injected with cetuximab also displayed immediate reddening of the injected ear, which had resolved by the next observation point 1 hour after treatment. At necropsy evaluation, all hematomas at the C225 i/a injected sites had resolved, with no histopathological findings at either the study d 2 or d 5 terminal sacrifice. Small hematomas were evident on gross pathologic evaluation in 2/3 rabbits in the placebo group at 24 hours after dosing, with no further findings observed at the later time point. On microscopic evaluation, the findings in the placebo injected sites were associated with focal areas on periarterial hemorrhage in all three animals. No changes were present histologically at the placebo injected sites at the terminal sacrifice on study d 5. These findings after injection of either cetuximab or placebo were considered related to the intraarterial injection technique, and not to local irritation produced by ERBITUX™.

Hematomas at the injection site, lasting from 1 to 24 hours after dosing were also observed in one rabbit at the i/v site after injection of both placebo and C225 monoclonal antibody. The hematoma on the side treated with placebo was not evident at necropsy or on microscopic evaluation at the study d 2 sacrifice (24 hours after injection). Histologic evaluation of the ERBITUX™-treated injection sites revealed mild to minimal, paravenous hemorrhage in both this animal, and a second animal dosed with C225 i/v at the study d 2 sacrifice. There were no

gross pathologic or microscopic findings at either the placebo or the cetuximab i/v injection sites at the study d 5 terminal sacrifice.

Following i/m or s/c injection of placebo, local hematomas were grossly present in 1/6 rabbits in each group. At the study d 2 necropsy, one rabbit in the had a small hematoma still present at the cetuximab i/m injected site, and a focal area of interstitial fluid at the site on histopathologic examination. A second animal had evidence of discoloration at the C225 i/m injected site, with no microscopic evidence of local tissue damage 24 hours after treatment. An additional animal injected with placebo had macroscopic findings of a small hemorrhage at the i/m injected site at terminal sacrifice on study d 5, with no histopathological correlate observed. A hematoma was observed in 1/6 rabbits at the s/c site injected with placebo and persisted for 72 hours after injection. There were no histopathologic findings at the injection site, at terminal sacrifice on study d 5 for this animal. All other animals injected s/c, or paravenously with either cetuximab or vehicle control had not macroscopic or histologic evidence of local tissue damage at the injected sites.

Study Conclusion: Cetuximab injections were generally well tolerated. Intraarterial injection of rabbits with cetuximab was associated with immediate reddening of the injected ear, and hematomas at the injected site that persisted in some animals to 96 hours after treatment. Transient, local hematoma and microscopic evidence of hemorrhage were present in one animal after intravenous injection in 24 hours after C225 dosing. Local hematoma, with associated interstitial fluid on microscopic evaluation was present in one rabbit 24 hours after i/m injection of cetixumab. These findings had resolved by 96 hours after treatment. Paravenous, or subcutaneous dosing with cetuximab was not associated with any local irritation at the injected site.

Study title: EMD 271 786 local tolerance study in rabbits after intravenous, intraarterial, paravenous, intramuscular, and subcutaneous treatment.
Key study findings: Transient, local dilatation of the injected vein, mild hemorrhage and congestion of minimal severity were present up to 24 h after intravenous injection of cetuximab. There were no remarkable local clinical or gross pathologic findings after injection of cetuximab by the i/a, s/c, or i/m routes of injection, and hematoma with mild hemorrhage present in 1/3 rabbits after paravenous injection of C225.
Study no.: T15509 (Imclone Study #930004392 v1.0)
Volume #, and page #: EDR file: pharmtox\tox\t15509.pdf
Conducting laboratory and location: Merck KGaA Institute of Toxicology, Darmstadt, Germany
Date of study initiation: November 19, 2002 (dosing initiated December 9, 2002)
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: EMD 271 786 placebo, lot #008914; cetuximab, lot #204485; percent purity not specified
Formulation/vehicle: cetuximab, final clinical formulation in phosphate buffered saline, 2.0 mg/ml

Comment: The composition of the EMD 271 786 placebo was not specified anywhere in the final study report. However, the lot number for the placebo is the same lot used in Study #T15386, above, where the control article was listed in Tables 4 and 6 as sterile water for injection.

Methods

Species/strain: New Zealand white rabbit, strain Crl:. ——————19-21 weeks old; weight range 2.89 -4.50 kg (mean 3.49 kg); 6 females/group

Doses: 0.5 ml of a 2.0 mg/ml solution of cetuximab or EMD 271 786 placebo

Study design: Rabbits were injected with a single, i/v, i/a, i/m, s/c, or paravenous injection of placebo or cetuximab (2.0 mg/ml) on study d 1, using an 18-gauge injection cannula. Six females were injected by the i/v, s/c, and i/m routes in the same animal, with the remaining three rabbits per group injected i/a and paravenously. Cetuximab was injected on the left, and the vehicle was tested at the contralateral (right) side of the same animal. The injection site areas are described in Table 24, below.

Table 24: Study Design for Local Tolerability of Cetuximab Injection in Rabbits

Route of Administration	Volume (ml)	Injection site
Intravenous	0.5	Ear vein (peripheral)
Intraarterial	0.5	*Arteria auricularis aboralis* (towards tip of ear)
Paravenous	0.2	Area of ear vein at the auricle base
Intramuscular	0.5	*Musculus longissimus dorsi*
Subcutaneous	0.5	Area of the chest wall

Observation for behavioral and clinical signs of toxicity, and examination of the injection sites were performed immediately, and one hour after injection on study d 1, and twice daily until scheduled sacrifice. Three rabbits per group were sacrificed 24 and 96 hours later (study days 2 and 5, respectively), and histopathological evaluation of the injection sites performed following hematoxylin and eosin staining of formalin-fixed tissue sections.

Results:

All animals survived until scheduled sacrifice, and no clinical signs of systemic toxicity were observed. There were no apparent effects of treatment on body weight over the duration of the study, and gross pathologic evaluation of the major organs at necropsy was unremarkable. There was no evidence of local irritation or intolerance at daily examination of either the cetuximab or placebo injected sites after i/a, i/m, s/c, or paravenous injection.

At necropsy 24 h after treatment, no gross pathologic findings were evident at the i/a or s/c cetuximab or placebo injected sites. Histologically, one rabbit in the i/a dosed group had evidence of mild hemorrhage at both the placebo and C225 injection sites at the d 2 sacrifice, and mild hemorrhage was seen at the s/c placebo injected site in an additional rabbit at this same time point. Minimal hematoma was observed in 1/3 rabbits at the paravenous cetuximab injection site on study d 2 necropsy; this animal also had microscopic evidence of hemorrhage at both the placebo and C225 injected sites. One rabbit had a focal area of discoloration at the i/m cetuximab injection site; however, there was no microscopic evidence of local tissue damage. There was no evidence of gross or microscopic pathology at the i/a, i/m, s/c, or paravenous injection sites at study termination on study d 5.

Following intravenous injection, slight dilatation of the injected vein was noted clinically in 2/6 rabbits each after either C225 or placebo, and persisted from 1 to 24 h after dosing. At terminal sacrifice 24 h later, all three rabbits dosed with cetuximab by i/v injection had congestion noted at gross pathological evaluation of the injected site. Microscopic evaluation revealed mild hemorrhage at the site in 1/3 cetuximab treated rabbits, and 2/3 placebo injected animals. No gross or histopathologic abnormalities were noted at either the placebo or the cetuximab i/v injected sites at study d 5 sacrifice.

Study Conclusion: Intravenous injection of cetuximab was well tolerated, with no irritation of the local vasculature or tissue. Transient dilatation of the injected vein, associated with congestion of minimal severity around the injected site and mild hemorrhage was present up to 24 h after dosing. There were no remarkable local clinical or gross pathologic findings after injection of cetuximab by the i/a, s/c, or i/m routes of injection, and hematoma with mild hemorrhage present in 1/3 rabbits after paravenous injection of C225.

Study title: EMD 271 786 local tolerance study in rabbits after intravenous, intraarterial, paravenous, intramuscular, and subcutaneous treatment.
Key study findings: No clinical evidence of local irritation or intolerance was observed following intravenous injection of cetuximab. Dilatation of the injected vein was observed in one of six rabbits at both the cetuximab and placebo injected sites, with no corresponding gross or histopathological findings.
Study no.: T15510 (ImClone Study #930004394 v1.0)
Volume #, and page #: EDR file: pharmtox\tox\t15510.pdf
Conducting laboratory and location: Merck KGaA Institute of Toxicology, Darmstadt, Germany
Date of study initiation: November 19, 2002 (dosing initiated December 9, 2002)
GLP compliance: Yes
QAU statement: yes (X) no ( )
Drug, lot #, and % purity: EMD 271 786 placebo, lot #008914 ; cetuximab (EMD 217 786), lot #E2622LO02; percent purity not specified
Formulation/vehicle: cetuximab, final clinical formulation in phosphate buffered saline, 2.0 mg/ml

Comment: The composition of the EMD 271 786 placebo was not specified anywhere in the final study report, but it is the same lot used in Study #T15386 and Study #T15509, above.

Comment: This study was conducted at the same time, and is identical in design to study #T15509, above. The difference between the two studies is that a different lot of ERBITUX™ was tested in the present study than that evaluated in Study #T15509.

Methods
Species/strain: New Zealand white rabbit, strain Crl ———— approximately 13 weeks old; weight range 2.48 -3.02 kg (mean 2.75 kg); 6 females/group
Doses: 0.5 ml of a 2.0 mg/ml solution of cetuximab or EMD 271 786 placebo
Study design: Rabbits were injected with a single, i/v, i/a, i/m, s/c, or paravenous injection of placebo or cetuximab (2.0 mg/ml) on study d 1, using an 18-gauge injection cannula. Six females were injected by the i/v, s/c, and i/m routes in the same animal, with the remaining three rabbits per group injected i/a and paravenously. Cetuximab was injected on the left, and the vehicle was tested at the contralateral (right) side of the same animal. The area of the injection sites is the same as described in Table 24, in Study #T15590, above.

Observation for behavioral and clinical signs of toxicity, and examination of the injection sites were performed immediately, and one hour after injection on study d 1, and twice daily until scheduled sacrifice. Three rabbits per group were sacrificed 24 and 96 hours later (study days 2 and 5, respectively), and histopathological evaluation of the injection sites performed following hematoxylin and eosin staining of formalin-fixed tissue sections.

Results:
There were no systemic toxicities, no early mortalities, and no apparent effects of cetuximab treatment on body weights or organ pathology at terminal sacrifice. Slight dilatation of both the placebo and cetuximab injected veins was evident in 1/6 rabbits beginning one hour after injection, and continuing until terminal sacrifice at 96 hours after dosing. No abnormalities were detected clinically at the i/a, i/m, s/c, or paravenous sites injected with either placebo or cetuximab, at any time point on study.

At necropsy on study d 2, gross pathological evidence of red, striped discoloration was present in one rabbit each at the cetuximab or placebo injection sites. There were no corresponding microscopic findings reported for these two animals. All other injection sites were unremarkable at gross pathologic evaluation at either study d 2 or d 5 sacrifice. Microscopic evidence of inflammatory infiltrates of minimal severity was present at the s/c cetuximab injection site on study d 2 in 1/3 rabbits, and 1/3 rabbits had evidence of hemorrhage around the puncture site of the i/a injection at this same time point. Two rabbits injected paravenously with cetuximab had histopathological findings of minimal and mild hemorrhage around the injection site. There were no remarkable abnormalities on microscopic evaluation of the i/v or i/m injection sites at either sacrifice time.

Study Conclusion: Intravenous injection of cetuximab was well tolerated, with no irritation of the local vasculature or tissue. Transient dilatation of the injected veins was present up to 96 h after dosing with either C225 or placebo. There were no remarkable local clinical or gross pathologic findings after injection of cetuximab by the i/a, s/c, or i/m routes of injection.

3.4.8 Special toxicology studies - N.A. no studies of this type were included in the present submission

3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: ERBITUX™ binding to cell surface EGFr was detected only in human and cynomolgus monkey skin, esophagus, salivary and mammary gland epithelia, bronchial epithelia in the lung, and corneal epithelium, and to a lesser degree in the adrenal, prostate, liver, urinary bladder, and uterine cervix. A panel of human tumors of epithelial cell origin, including head and neck and lung cancers, pancreatic, and colon carcinomas also expressed EGFr and demonstrated cetuximab binding. Pharmacologic evaluation of cetuximab anti-tumor activity *in vitro* and *in vivo* demonstrated multiple potential mechanisms for the effects of ERBITUX™ on tumor cells, including inhibition of pathways associated with EGFr signal transduction, down-regulation of EGFr on tumor cell surface, induction of apoptosis, and inhibition of pathways associated with autocrine and paracrine growth factor secretion and receptor interaction, and enzymes associated with tumor cell metastasis and invasion. Human tumor xenografts expressing EGFr in various levels responded to cetuximab with delays and/or inhibition of tumor growth after treatment with ERBITUX™ either alone, or in combination with the chemotherapeutic agents irinotecan or 5-fluorouracil in an additive, and sometimes synergistic fashion. Pharmacokinetic evaluation of serum cetuximab levels revealed dose-related, although non-linear increases in C_{max} and AUC for ERBITUX™ in rats and cynomolgus monkeys after single or repeated i/v administration. In cynomolgus monkeys, there was no evidence of accumulation of cetuximab at the 13, 26, or 39

week time points as compared to the 4 week time point on study (at which C225 serum levels had reached steady state) In rats, there was an inverse relationship of dose and development of anti-cetuximab antibody activity after repeated administration for 4 weeks; these animals also had high levels of circulating ERBITUX™, which may have interfered with detection of the anti-C225 antibody. One monkey treated for 39 weeks with 38/24 mg/kg/week ERBITUX™ developed anti-C225 antibody such that serum levels were no longer measurable in this animal, and toxicities had resolved. Two other animals had transient antibody development in this study. The toxicities of cetuximab in cynomolgus monkeys were cumulative, and related to both the dose of ERBITUX™ administered and the duration of treatment. Early mortalities were observed in 50% of the monkeys (5/10) in the group treated with 120/75 mg/kg/week C225, and were associated with severe skin lesions, secondary bacterial infections at the lesion, septicemia, and involvement of multiple major organs. Dermatological toxicities were observed in all cetuximab dose groups so that a NOAEL could not be defined, and were only partially resolved in the high-dose group 9 weeks after discontinuation of treatment. Other toxicities observed in this study included inappetence, decreased food intake, and loss of body weight in the high dose animals secondary to development of ulcerative lesions in the esophageal mucosa and tongue, anemia, leukocytosis and/or leukopenia generally related to the skin lesions, elevations in hepatic γ-glutamyl transpeptidase and ALT, and abnormal or absent menstrual cycles in the female animals, beginning at approximately 26 weeks of treatment and continuing in the high dose group throughout the 9 week, treatment-free recovery period. The dermatological and mucosal epithelial toxicities of ERBITUX™ are related to its mechanism of action of inhibition of the function of EGFr, and subsequent defects in epithelial maturation. Similar dermatologic findings, including Grade 3 acneform rash and skin sloughing were observed in the pivotal clinical study.

Unresolved toxicology issues (if any): The safety of ERBITUX™ for use on pregnant women is unknown. The EGFr has been implicated in the control of prenatal development and may be essential for normal organogenesis, proliferation, and differentiation in the developing embryo. In addition, human IgG1 is known to cross the placental barrier; therefore ERBITUX™ has the potential to be transmitted from the mother to the developing fetus. A nonclinical, reproductive toxicology study will be requested to evaluate the effects of ERBITUX™ on the developing fetus, in support of future use of this product in the adjuvant setting for colorectal cancer, or for use in women of childbearing age with cancers other than irinotecan-refractory, colorectal cancer.

Recommendations: The preclinical pharmacology, pharmacokinetics, and toxicology data for ERBITUX™ support the safety and biologic activity of this product for use in the treatment of late-stage cancers. Recommendation is for approval, with inclusion of language in the WARNINGS section of the label regarding the dermatologic toxicities, sepsis, and death observed in cynomolgus monkeys after repeated cetuximab administration, and the corresponding findings of Grade 3 and 4, dermatologic toxicity and sepsis in the pivotal clinical trial.

Suggested labeling: Modifications to the language in the label are included in Appendix I, below. At this time of this review, the sponsor had accepted these changes in the final package insert, with no further modification.

Signatures (optional)

Reviewer Signature /S/ Feb 11, 2004

Supervisor Signature /S/ Concurrence Yes ✓ No ___

3.7. APPENDIX/ATTACHMENTS

2 page(s) of revised draft labeling has been redacted from this portion of the review.

APPENDIX II - PUBLISHED REFERENCES REGARDING ERBITUX ACTIVITY, ROLE OF EGFR IN CANCER AND DEVELOPMENTAL BIOLOGY, AND CHEMOTHERAPY WITH IRINOTECAN

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

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