

BLA STN # 125085

**REVIEW AND EVALUATION OF
TOXICOLOGY DATA**

Bevacizumab (AVASTIN)

**Genentech, Inc.
South San Francisco, CA**

Reviewer:
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**Division of Therapeutic Internal Medicine Products (HFM-576)
Center for Drug Evaluation and Research
Food and Drug Administration**

2/25/04

PHARMACOLOGY AND TOXICOLOGY REVIEW

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1. EXECUTIVE SUMMARY

1.1. RECOMMENDATIONS

1.1.1. Recommendation on Approvability

The Biologics License Application STN BLA # 125085/0 is approvable for the proposed indication based on the data contained in the preclinical toxicology sections of the original submission. The toxicities of AVASTIN are extensions of the pharmacologic activity of the product. The clinical patient populations can be selected and/or monitored appropriately to avoid unreasonable risk.

1.1.2. Recommendations for Additional Nonclinical Studies

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1.1.3. Recommendations on Labeling

Modifications to the PRECAUTIONS section of the label, including revision of the language regarding impairment of fertility by AVASTIN, revision of the language regarding teratogenicity in the PREGNANCY CATEGORY C section, addition of information on physeal dysplasia to the PEDIATRIC USE section and appropriate cautions regarding reductions in wound healing capacity have been communicated to and agreed upon by the sponsor. Final language for these sections is provided in section 2.8.3 of this review.

1.2. BRIEF SUMMARY OF NONCLINICAL FINDINGS

1.2.1. Pharmacological Activity

Review of pharmacology and pharmacokinetics are not included in this review document.

1.2.2. Toxicological Findings

In three repeat-dose toxicity studies in cynomolgus monkeys, AVASTIN was generally well tolerated at IV doses up to 50 mg/kg twice per week for 13 weeks, 50 mg/kg for up to 26 weeks and 10 mg/kg twice per week for 26 weeks. Two studies were performed to investigate the effect on embryo-fetal development after treatment with AVASTIN during pregnancy in rabbits. Adverse events in the clinical studies prompted several non-GLP studies in two wound healing rabbit models to investigate the effect of AVASTIN treatment on wound healing.

The data provided from the three repeat-dose toxicology studies indicated that, in the intended patient population under the proposed conditions of use, AVASTIN should be reasonably safe. No apparent study drug related hematology or clinical chemistry or physical exam findings were reported with the exception of a small rise in blood pressure for female monkeys after 26 weeks of treatment at 50 mg/kg/wk. The biological significance is not clear since the male animals did not show this finding. However, careful monitoring of blood pressure for human use is recommended.

Anatomical pathology analyses revealed several findings that indicate toxic effects on general growth and skeletal development, fertility, and wound healing capacity. In general, the toxic effects revealed in these studies appear to be predictable extensions of the pharmacologic activity of AVASTIN.

Severe physal dysplasia was consistently reported in monkeys with open growth plates receiving the middle and high doses in all repeat dose studies. This physal dysplasia was characterized by a linear cessation of growth line and chondrocyte hyperplasia. This finding demonstrates a clear dose response relationship to the study drug. Fewer female animals displayed these changes. The difference in toxicity between genders may be explained by differences in ages of the two groups. The dysplasia did not resolve after 4 or 12 weeks of recovery. Severity appeared to be less after 12 weeks of recovery, indicating that some recovery may be possible. ☐

No specific studies assessing the effect of Avastin on fertility were performed. However, results of anatomical pathology from the repeat-dose toxicity studies indicate that treatment with Avastin may have an adverse effect on fertility. In all studies, a dose dependent reduction in uterine and ovarian organ weight was identified in the 10 mg/kg, and 50 mg/kg groups compared to control. After 26 weeks of treatment, the dose dependent reduction in uterine ovarian weights (up to approximately 50%) was apparent for all dose groups compared to control. This reduction in organ weight was still apparent after as long as 12 weeks of recovery (n=2) although, due to variability and the small number of recovery animals, this finding did not reach statistical significance.

The reductions in ovarian and uterine weights correlated with notable reductions in proliferation of the uterine endometrium functionalis for females receiving 10 and 50 mg/kg for 26 weeks. Follicular arrest is noted for all females in groups 4 (50 mg/kg) and 5 (10 mg/kg X2/week) treated for 26 weeks. Examination of the ovaries after 13 or 26 weeks of treatment revealed an absence of corpora lutea for the 10 mg/kg groups as well as 100% of the animals receiving 50 mg/kg. This effect is clearly study drug related. After 4 weeks of recovery absence of corpora lutea persisted for the 50 mg/kg group (n=2) but, after 12 weeks, 50% of the 50 mg/kg animals (n=2) showed this finding. Follicular arrest and the reduced endometrial proliferation

were not observed after 12 weeks of recovery (n=2) suggesting that at least partial recovery is possible. However, the persistence of the absence of corpora lutea in 1 of 2 recovery females that received 26 weeks of treatment at 50 mg/kg/wk indicates that recovery is not complete within this time period. A reduction of 67% in menstrual cycles was also reported for the 50 mg/kg females. Reduced menstrual cycles were reported for the recovery animals (50 mg/kg, n=2) but, due to individual variations and the small number of recovery animals, these data are difficult to interpret. (See Appendices for detailed tabulation of the histopathological data relating to fertility.)

Other findings that may be study drug-related are: enlarged spleens noted for 1 male and 1 female in the 50 mg/kg dose group and a statistically significant increase in organ to body weight of the pituitary (50%) for high dose females after 4 weeks of treatment twice weekly. The biological significance of these findings is not clear since they did not occur in other studies with longer exposure.

Glomerulonephritis was observed histologically in one female from the low dose group after 4 weeks of dosing and one male control animals after 13 weeks of dosing. Since these findings occurred in the lowest dose and control groups, and no other occurrences were noted in the three repeat-dose studies, this finding does not appear to be related to administration of study drug and, for the purposes of these studies does not appear to be of biological significance. In all studies there was a high occurrence of signs of chronic inflammation in multiple organ systems suggesting the presence of a pre-existing condition. However, the presence of a pre-existing inflammatory condition could possibly confound interpretation of results by masking a study drug related low level toxicity. The NOAEL for repeat dosing of AVASTIN identified in these studies was 2 mg/kg.

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Embryo-fetal development toxicity:

Two studies were performed addressing the potential for treatment with AVASTIN during pregnancy to cause fetal developmental toxicities when administered IV to pregnant rabbits during the period of organogenesis. No clinical signs of toxicity were noted for the pregnant dams with the exception of an early (DG 6 and 7) significant reduction in mean body weight gain for the low dose group and a mean body weight loss for the middle and high-dose groups. This occurred after the first dose of the test article and was temporary. All remaining body weight gains and body weights were comparable among the 0 (Placebo), 10, 30 and 100 mg/kg dose groups.

In contrast, clear evidence of a dose related teratogenic effect was identified upon examination of the 73 litters and over 600 fetuses. Multiple skeletal deformities were noted in the fetuses at all dose groups with increased incidence for the high dose group.

A clear NOAEL for developmental toxicity was not defined in this study. From the data generated in this study the best estimate of NOAEL is less than 10 mg/kg/dose. The data indicate that bevacizumab can have a teratogenic effect on developing fetuses.

Reduced wound healing:

The toxicity package included several non-GLP studies addressing the potential for AVASTIN to reduce wound healing capacity. Two rabbit models of skin wound healing were developed: a full-thickness skin incision model and a rabbit dermal wound model of incomplete skin lesion. Results of both studies demonstrated a dose-dependent reduction in wound healing capacity in rabbits treated with Avastin at doses of 2, 10 and 50 mg/kg. Reductions in wound healing capacity were noted for all doses examined.

1.2.3. Nonclinical Safety Issues Relevant for Clinical Use

Wound healing: As described above, several non-GLP studies revealed a notable reduction in wound healing capacity in rabbits treated with AVASTIN. Two models of skin wounds were developed: full-thickness linear incision to mimic a surgical wound and a partial thickness circular dermal wound. Both models revealed that treatment with AVASTIN can reduce wound healing capacity. These results are confirmed by adverse events that occurred in the clinical studies and lead to recommendations and cautions being added to the label. These instructions included the recommendation that AVASTIN treatment be stopped well in advance of any planned surgery.

Physeal dysplasia: Physeal dysplasia was noted in all repeat dose studies that included monkeys with open growth plates in long bones. This potential toxicity will not be of serious concern for AVASTIN use in mature adults. However, should use of this drug in younger (pediatric) populations be considered, additional studies should be conducted to further evaluate this effect and the potential for recovery after cessation of treatment.

Impairment of fertility: Use of AVASTIN in cynomolgous monkeys revealed histopathological findings that indicate probable impairment in fertility in females. These findings include dose-dependent reductions in ovarian and uterine weight, arrest of follicular development, absence of corpora lutea, reduced numbers of menstrual cycles and severe reductions in endometrial proliferation. These fertility-related findings showed incomplete recovery after up to 12 weeks without drug. If use of AVASTIN in patient populations that include women of child-bearing potential are considered, further investigation of the effect of AVASTIN on fertility and subsequent recovery are recommended.

Teratogenicity: Definite dose-dependent teratogenic effects of AVSTIN treatment during pregnancy were identified in embryo-fetal studies conducted in rabbits. Use of AVASTIN in women of child-bearing potential should be accompanied by concurrent use of appropriate effective contraceptive measures.

2. PHARMACOLOGY AND TOXICOLOGY REVIEW

2.1. BASIC INFORMATION

NDA Number:

Sequence Number/Date/Type of Submission:

Sponsor and/or Agent: Genentech, Inc.

Reviewer Name: Barbara J. Wilcox, Ph.D.

Division Name: Division of Biological Internal Medicine Products

HFD #: HFM-579

Review Completion Date: 2/24/04

Drug Product Trade Name: Avastin

Drug Substance:

Generic Name: Bevacizumab

Chemical Name: recombinant human Mab Vascular Endothelial Growth Factor

Molecular Weight: Approximately 149,000 dalton

Structure: Bevacizumab is a full length, IgG1 antibody composed of

The  of bevacizumab are provided in the Appendix.

human IgG1.

all human IgG1 antibodies.

Relevant INDs/NDAs/DMFs: IND 7023

Pharmacological Class: Monoclonal antibody

Clinical Indication: First line treatment of metastatic colorectal cancer in combination with 5-fluorouracil based chemotherapy

Clinical Formulation: Information on the clinical formulation is provided in the appendix.

Route of Administration: Intravenous

Material Reviewed: Toxicology

Material Not Reviewed: Pharmacology and Pharmacokinetics

[Note: Tables and graphs in this review have been produced by the reviewer, unless stated otherwise that they were obtained from the sponsor's submission.

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2.4. TOXICOLOGY

2.4.1. Single-Dose Toxicity

No single-dose toxicology studies were performed.

2.4.2. Repeat-Dose Toxicity

2.4.2.1. Study Title: 4-week Intravenous Toxicity Study with rhuMAb VEGF in Cynomolgus Monkeys with a 4-week Recovery

Testing Facility and Location: _____
Study Number: 96-181-1751
Date of Study Initiation: 9/10/96
Drug Lot/Batch Number(s): Bevacizumab Lot # M3-RD595
vehicle lot #M3-RD588
GLP Compliance (y/n): Yes.
QA Report (y/n): Yes.

Key Findings:

This study was performed to assess the toxicity of rhuMAb VEGF when administered to cynomolgus monkeys by intravenous injection twice weekly for 4 weeks and to determine the reversibility, persistence, or delayed occurrence of toxic effects after a 4-week recovery period. Three doses of study drug (2.0, 10.0 or 50 mg/kg) or vehicle were administered IV twice weekly. The monkeys were sacrificed at the end of 4 weeks with 2 animals from each of the control and high-dose groups sacrificed after a 4-week recovery period.

The study results demonstrated that all doses of study drug were generally well tolerated. Findings of note are a dose-related trend toward reduced organ to body weight of the uterus and ovaries that did not achieve statistical significance, and a clearly dose-dependent appearance of physeal dysplasia for 4 out of 4 male animals from the middle and high-dose groups. This physeal dysplasia was characterized by a linear cessation of growth line and chondrocyte hyperplasia that did not resolve after the 4-week recovery period.

Other findings that appeared to be related to study drug administration are: Enlarged spleens noted for 1 male and 1 female in the 50 mg/kg dose group and a statistically significant increase in organ to body weight of the pituitary (50%) for high dose females. The biological significance of these two findings is not clear.

Glomerulonephritis was observed histologically in one female from the 2 mg/kg group. Since this group is the lowest dose group, and no other occurrences were noted, this biological significance of this finding is not clear.

Methods:

Species/strain: Cynomolgus monkey

#/sex/group or time point (main study): 6 animals/sex/group for controls, 4 animals per sex/group for low and middle dose, 6 animals/sex/group for high dose (2 animals per group were used for recovery).

Doses: 0, 2, 10 and 50 mg/kg

Route, formulation, volume, and infusion rate:

Study drug was administered by bolus intravenous injection into a saphenous vein twice weekly for at least 4 weeks (on Days 1, 4, 8, 11, 15, 18, 22, 25, and 29). The control group was administered rhuMab VEGF Vehicle by the same method and frequency.

Satellite groups used for toxicokinetics or recovery:

Two animals from the high dose group and two control animals were designated as recovery animals for the 4-week recovery period.

Weight (nonrodents only): 2.2 to 3.6 kg at initiation of study

The following table provided by the sponsor illustrates the study design:

STUDY DESIGN

Animals were stratified by body weight and randomized to groups using computer-generated random numbers according to the following design:

| Group | rhuMab VEGF Dose Level (mg/kg) | Dose Concentration (mg/ml) ^a | Number of Animals ^b | |
|--------------------------|--------------------------------------|---|-----------------------------------|----------------|
| | | | Male | Female |
| 1 (Control) ^c | 0 | 0 | 6 ^d | 6 ^d |
| 2 (Low) | 2 | 0.4 | 4 | 4 |
| 3 (Mid) | 10 | 2 | 4 | 4 |
| 4 (High) | 50 | 10 | 6 ^d | 6 ^d |

a The dose volume was 5 ml/kg.

b Four animals/sex/group were sacrificed during Week 5.

c The control group received rhuMab VEGF Vehicle only.

d Two animals/sex were designated as recovery animals and were dosed with rhuMab VEGF or rhuMab VEGF Vehicle twice weekly for at least 4 weeks, after which dosing was discontinued, and the animals were observed for reversibility, persistence, or delayed occurrence of toxic effects for approximately 4 weeks posttreatment.

Clinical signs:

Animals were checked twice daily. Physical examinations (including rectal body temperatures and respiration rates, blood pressure) were performed twice before initiation of treatment; 1 to 2 hours post-dose on Days 1, 11, and 22; and once on Day 51 (recovery animals).

Body weights:

Body weights were recorded weekly prior to initiation of the study, on Day 1, and weekly thereafter, on day of necropsy.

Food consumption:

Qualitative food consumption observations were made daily and recorded except on days of fasting.

Ophthalmoscopy:

Ophthalmic examinations were performed once before initiation of treatment and once during Week 4. Both eyes examined. Electroretinograms were recorded once before initiation of treatment and once during Week 4.

ECCG:

Electrocardiographic measurements were recorded twice before initiation of treatment; 1 to 2 hours post-dose on Days 1, 11, and 22; and once on Day 51 (recovery animals).

Hematology:

Samples collected twice before initiation of treatment; once during Weeks 1, 2, 3, and 5; and once during Week 8 (recovery animals).

Clinical chemistry:

Samples collected twice before initiation of treatment; once during Weeks 1, 2, 3, and 5; and once during Week 8 (recovery animals).

Urinalysis:

Samples collected twice before initiation of treatment; once during Weeks 1, 2, 3, and 5; and once during Week 8 (recovery animals).

Gross pathology:

The following tissues and organs were harvested at necropsy and preserved: Adrenals, Aorta Brain, Cecum, Cervix, Colon, Duodenum, Epididymides, Esophagus, Eyes, Femur with bone marrow, gallbladder, Heart, Ileum, Kidneys, Liver, Lungs, Ovaries, Pituitary, Prostate, Salivary glands (submandibular), Spleen, Testes, Thymus, Thyroids with parathyroid, Uterus, Injection sites (skin, subcutaneous Tissue), vein (collected from middle, proximal, and distal portions of the intravenous injection sites), Jejunum, Lacrimal glands, Lesions (including tissue masses), Liver, Lungs, Lymph nodes (axillary, mesenteric, and inguinal), Mammary gland, Muscle (thigh), Optic nerve, Ovaries, Pancreas, Rectum, Salivary glands (submandibular), Sciatic nerve, Seminal vesicles, Skin, Spinal cord (cervical, mid-thoracic and lumbar), tongue, Trachea, Ureters, Urethra, Urinary bladder, Vagina, Vitreous fluid.

Organ weights (specify organs weighed if not in histopath table):

Uterus with cervix
Adrenals
Brain
Epididymides
Heart
Kidneys
Liver
Lungs
Ovaries
Pituitary
Prostate
Salivary glands (submandibular)
Spleen
Seminal vesicles
Testes
Thymus
Thyroids with parathyroid

Histopathology: Complete Battery: Yes (X), No ()--explain

Toxicokinetics:

Approximately 1 ml of whole blood was collected from a femoral vein of each animal before the initiation of treatment (Day 1) and on Days 1 (approximately 1 and 8 hours post-dose), 2 (approximately 24 hours post-dose), 4 (pre-dose), 11 (pre-dose and approximately 1 hour post-dose), 14, 29 (pre-dose and approximately 1 and 8 hours post-dose), and 30 (approximately 24 hours post-dose). Blood was collected from the recovery animals on Days 32, 38, 45, and 56. Pre-dose collections were done within approximately 2 hours before dosing.

Other:

Approximately 1 ml of whole blood was collected from a femoral vein of each animal before initiation of treatment (Day 1); within 1 hour pre-dose on Days 15, 22, and 29; and on Day 56 (recovery animals).

Results:

Mortality: All animals survived to the scheduled sacrifice date.

Clinical signs:

- Sporadic observations of liquid, unformed or mucoid feces in all groups except group 3 males and group 4 females are reported but no relationship to dosing of study drug is apparent.
- Sporadic episodes of vomiting were noted with no apparent relationship to the study drug dosing.
- Mild injection site inflammation was noted at terminal sacrifice in treated and control animals. This inflammation was resolved by recovery sacrifice.

Body weights:

- There were no significant changes in body weights that were attributable to administration of rhuMAb VEGF. An approximate 0.2 kg decrease in the mean body weight for all groups at Week 5 was noted. However, the sponsor states that this is due to fasting the animals overnight before the weights were recorded.

Food consumption:

- There were no effects on food consumption that were attributed to administration of rhuMAb VEGF. Low food consumption was noted sporadically in all groups including the control group but did not appear to be related to study drug administration.

Ophthalmoscopy: No visible lesions were reported for any animal in any group.

ECG:

There were no electrocardiographic abnormalities attributed to administration of study drug were reported.

Hematology:

Administration of rhuMAb VEGF was associated with mildly higher red blood cell count, hemoglobin, and hematocrit for males administered 10 or 50 mg/kg/dose. Mean values for these parameters for the affected male groups were approximately 10% to 15% higher than those for the control males. The differences for these parameters appear to be due to mild decreases from baseline levels for the control males. In general, values for these parameters throughout the study for males administered 10 or 50 mg/kg/dose were

similar to their own baseline levels and within the expected ranges for male cynomolgus monkeys. The erythrocyte findings were not accompanied by correlative histopathological findings and were not considered adverse. Reticulocyte counts and bone marrow myeloid-to-erythroid ratios were not affected. The differences were not reversed following 4 weeks of recovery. These findings do not appear to be biologically significant.

Clinical chemistry:

No biologically relevant findings related to study drug administration were noted.

Urinalysis:

No study drug related findings were reported.

Gross pathology:

- Lung adhesions were noted for one male and 2 females but this finding had no relationship to study drug.
- One male in the 10 mg/kg group had a large parathyroid gland
- A high incidence of injection site redness was noted (27 of 32 animals, left side, 14 of 32 animals, right side) for all groups of both sexes but did not show any relationship to study drug administration. This could possibly be attributed to mechanical trauma of the injection itself.
- Lung adhesions were noted for one male and 2 females but this finding had no relationship to study drug.
- One male in the 10 mg/kg group had a large parathyroid gland
- The low incidence of lung and liver adhesions was also noted in the recovery animals. These findings are most likely the result of a pre-existing condition in these monkeys.
- The low incidence of lung and liver adhesions was also noted in the recovery animals. These findings are most likely the result of a pre-existing condition in these monkeys.
- Enlarged spleens noted for 1 male and 1 female in the 50 mg/kg dose group.

Organ weights:

- Mean Salivary gland weight for group 4 males (50 mg/kg) was significantly higher than controls (3.13 gm vs. 1.97 gm). The reduced salivary gland weight was apparent in organ to body weight for the high-dose males only. This finding was not repeated in the female animals.
- Mean spleen weight for group 4 males is significantly greater than controls (16.56 gm vs. 10.53 gm). In organ to body weight the high-dose males showed a 1.6 X increase over the male controls. The mean spleen weight for group 4 females was also greater than female control animals but did not achieve statistical significance. The organ to body weight for the spleen in high-dose females was 1.5 X larger than female controls. This effect did not achieve statistical significance due to higher variability.
- An approximately 50% reduction in mean testis weight was noted for group 4 males compared to control. This reduction did not achieve statistical significance and did not show a clear dose relationship to study drug.

Comment: These animals were among those showing physal dysplasia, indicating that they were immature.

- A statistically significant reduction in thyroid/parathyroid weights of treated males compared to male controls (30-45%). All treatment groups showed the decreased weight with no clear dose relationship. This decrease is also apparent in organ to body weight results. No decrease was noted for any female group.
- A dose-related trend toward reduced organ to body weight of the uterus and ovaries is noted that did not achieve statistical significance. This effect appears to persist through the recovery period.
- The high dose females showed a statistically significant increase in organ to body weight of the pituitary (50%).

Histopathology:

- One of 4 males in each of groups 2 through 4, and 1 female from group 2, showed lymphocytic infiltrate in the left eye. Lymphocytic infiltrate was also noted in the right eye of 2 males from group 2 and 1 female from group 3.
- Lymphocytic infiltrate was noted in the bone marrow from the sternum of one male animal from each of groups 1 through 3 as well as 2 females from group 1 and one female from group 4.
- Physal dysplasia of the femur characterized by a linear cessation of growth line and chondrocyte hyperplasia was reported for 4 out of 4 male animals from groups 3 and 4. This appears to be dose related to the study drug administration and did not recover after 4 weeks without treatment.
- Mineralization in the brain was reported for 2 males (one each from groups 1 and 4) and 1 female from group 4. Lymphocytic infiltration in the brain was reported for 1 female from group 4, and vascular inflammation in the brain was reported for 1 female from group 2. These findings do not appear to be related to study drug administration.
- Evidence of chronic inflammation including fibrosis, parasitism, lymphohistiocytic infiltration, bronchiectasis, presence of foreign material and granulomatous inflammation was reported for multiple animals from all treatment groups. These findings did not show a relationship to study drug administration and are most likely the result of a pre-existing condition in these monkeys.
- Lymphocytic infiltration was observed in 2 males from group 1, 2 males from group 4, 1 female from each of group 1, 3 and 4. Fibrosis of the kidney was observed in one male from group 3 and vascular inflammation was observed in one female from group 2. Glomerulonephritis and chronic inflammation was noted in one of 4 females from group 2 (2 mg/kg).
- Lymphohistiocytic infiltration was observed in the heart of one male from each of groups 3 and 4.
- Histological evidence of subacute inflammation of the liver was noted in 18 of 32 animals across all dose groups and genders. Lymphohistiocytic infiltrate was observed in 2 high-dose males.
- Granulomatous infiltration of the liver was noted in one high-dose animal of each gender. Two male animals (one control and one high dose) showed Kupffer cell hypertrophy. These findings did not show a clear relationship to study drug

administration and no correlation was revealed in results of clinical chemistry parameters.

- No histological findings were reported that correlate with the organ weight changes reported above (male thyroid, spleen, female pituitary or uterus).
- Lymphocytic infiltration was noted for lacrimal glands of both sexes but did not appear to have a relationship to study drug administration.
- Three instances of mineralization of the ovary were noted in one animal from each of the drug treated groups. Zero control showed similar findings.
- Chronic inflammation of the tongue was noted for 3 out of 16 females (mid and high-dose groups), 6 out of 16 males (2 each in control mid- and high dose groups).
- Ductal cysts were noted for both male and female mammary glands with low incidence (2 out of 16 females, 2 out of 16 males) and no apparent relationship to study drug administration.
- Chronic inflammation was noted sporadically for all regions of the gut with no relationship to study drug administration.
- The prostate of 2 out of 4 males in each of groups 2 and 3 were immature as was one from group 4.
- Two instances of chronic inflammation of the vagina, one in the low dose group and one in the high dose group.
- Hypospermia was reported for all male groups (12 out of 16) and immature testis was also reported for all groups (10 out of 16).
- Three instances of ultimobranchial cysts are reported for the 50 mg male recovery group, one female control animal and one 50 mg female animal.

Toxicokinetics:

The sponsor reports that, due to the limited sampling of the non-recovery animals, an adequate compartmental model could not be fit to the data. For this reason, curves based on single dose IV parameters determined in study 96-211-1751 were superimposed on the concentration versus time data from the data derived from the non-recovery animals in the current study. A two-compartment model with central compartment elimination was fit to the data. The values reported for the recovery animals include: weight normalized steady-state volumes of distribution, weight-normalized clearance, initial and terminal half-lives, mean residence time, and total area under the curve. These values are based on compartmental estimates.

The following tables supplied by the sponsor summarize the TK data:

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Groups 2, 3, and 4 - Non-recovery and Recovery Animals

| Parameters ^a | Group 2 - 2 mg/kg IV | | Group 3 - 10 mg/kg IV | | Group 4 - 50 mg/kg IV | |
|--------------------------------------|----------------------|-------------|-----------------------|-------------|-----------------------|-------------|
| | Males | Females | Males | Females | Males | Females |
| n = | 4 | 4 | 4 | 4 | 6 | 6 |
| Weight (kg) | 2.9 ± 0.2 | 2.6 ± 0.1 | 2.9 ± 0.3 | 2.5 ± 0.2 | 3.0 ± 0.2 | 2.6 ± 0.1 |
| T _{max} (days) ^b | Day 29, 1hr | Day 29, 1hr | Day 29, 1hr | Day 29, 1hr | Day 29, 1hr | Day 29, 1hr |
| C _{max} (µg/mL) | 130 ± 5.0 | 140 ± 13 | 840 ± 120 | 820 ± 110 | 3200 ± 490 | 2900 ± 280 |

^a The parameters are reported as mean ± SD, where appropriate. Parameters are the mean of all animals in the group.

^b Median reported.

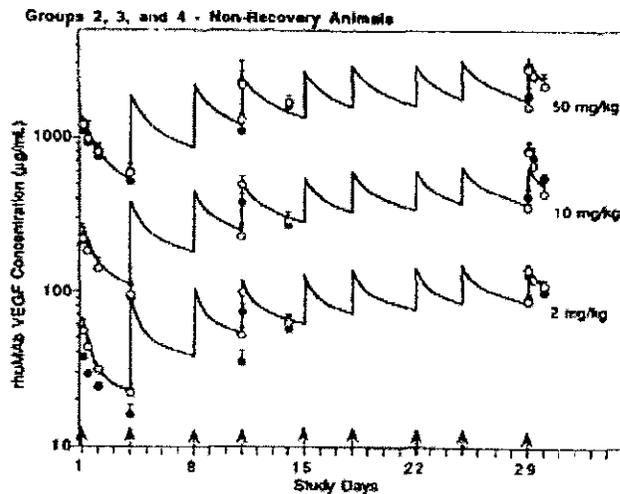


Figure 1. Serum concentration vs. time profiles of rhMAB VEGF determined by ELISA. The arrows represent the administration of an IV dose of rhMAB VEGF. The mean of the non-recovery monkeys (n = 4 per sex) for the 2, 10 and 50 mg/kg group are presented. Curves represent estimates based on single dose IV data. Males - solid symbols; Females - open symbols.

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Group 4 - Recovery Animals:

| Parameters ^a | 50 mg/kg IV ^b | | Males and Females Combined |
|-----------------------------|--------------------------|---------|----------------------------|
| | Males | Females | Mean ± SD |
| n = | 2 | 2 | 4 |
| V _C /W (mL/kg) | 35.5 | 41.5 | 38.5 ± 4.8 |
| V _{SS} /W (mL/kg) | 68.9 | 67.9 | 68.4 ± 5.5 |
| CL _W (mL/kg/day) | 5.08 | 5.07 | 5.07 ± 0.40 |
| AUC (µg·day/mL) | 9910 | 9890 | 9900 ± 760 |
| t _{1/2α1} (hr) | 10.6 | 17.5 | 14.1 ± 4.2 |
| t _{1/2α2} (day) | 8.90 | 9.88 | 9.89 ± 1.2 |
| MRT (day) | 13.6 | 13.5 | 13.6 ± 1.8 |

^a The parameters are reported as mean ± SD, where appropriate.
^b Compartmental parameters are determined from recovery animals in Group 4.

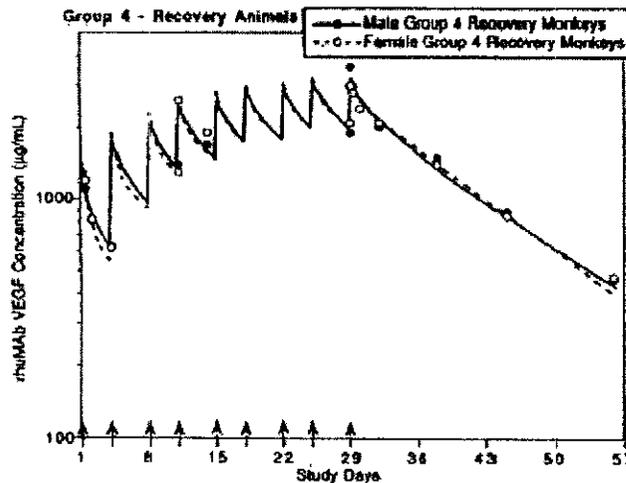


Figure 2. Serum concentration vs. time profiles of recovery animals administered 50 mg/kg IV rhuMab VEGF with estimated curves superimposed on the data. The mean of 2 monkeys per sex is presented.

Anti-VEGF levels were detected in serum samples of control animals. The reason for this and interpretation is unclear. These concentrations were 14-fold less than those of the lowest dose group. Therefore, the sponsor states that the values from the control group were not used in the analysis. They indicate that investigations are ongoing to determine the apparent interference on the rhuMab VEGF ELISA.

Overall, peak concentrations for all groups occurred 1 hour after the last dose on day 29. Two animals in the high dose group showed peak levels 1 hour following the 4th dose on day 11. The peak levels for both animals were comparable to the peak levels for the other animals taken on day 29. A dose-proportional increase in C_{max} was observed among dose groups. The study drug was cleared with an initial half-life of 10.6 and 17.5 hours for male and females, respectively. Terminal half-life of 9.9 and 9.88 days was determined for males and females, respectively. The simulated multiple dosing curves (based on single-dose parameters) indicate that the study drug concentrations are predictable over time.

Other:

Vitreous fluid was collected from the left eye of each animal, and red blood cell counts were done on an aliquot of vitreous fluid. The remaining vitreous fluid was shipped to the Sponsor for additional analyses.

| Group ^a | n = per sex | Males | | Females | |
|------------------------|-------------|--------------------------------|-----------------------------|--------------------------------|-----------------------------|
| | | Vitreous Concentration (ug/ml) | Serum Concentration (ug/ml) | Vitreous Concentration (ug/ml) | Serum Concentration (ug/ml) |
| 2 mg/kg | 4 | 0.15 ± 0.067 | 100 ± 9.0 | 0.12 ± 0.037 | 110 ± 12 |
| 10 mg/kg | 4 | 0.61 ± 0.28 | 560 ± 44 | 0.40 ± 0.047 | 440 ± 90 |
| 50 mg/kg: non-recovery | 4 | 3.6 ± 1.4 | 2200 ± 320 | 2.3 ± 0.74 | 2200 ± 470 |
| 50 mg/kg: recovery | 2 | 0.45 | 460 | 0.41 | 470 |

^a Serum and vitreous fluid collected from non-recovery animals on Day 30; serum and vitreous fluid collected from recovery animals on Days 56 and 58 respectively.

Vitreous levels appear to be proportional to the serum levels with vitreous levels approximately 0.1% of serum levels.

Reviewer Comments:

The monkeys used for this study showed numerous histopathological findings consistent with a pre-existing chronic inflammation of several organ systems. These findings did not show a relationship to administration of study drug and are most likely not of biological significance with respect to toxicities of the study drug.

2.4.2.2. Study Title: 13-week Intravenous Toxicity Study with rhuMab VEGF in Cynomolgus Monkeys with a 4-week Recovery

Testing Facility and Location:

Study Number: 96-182-1751
Date of Study Initiation: 11/7/96
Drug Lot/Batch Number(s): Bevacizumab lot # M3-RD595
Vehicle lot #
GLP Compliance (y/n): Yes.
QA Report (y/n): Yes.

Key Findings:

This study was performed to assess the toxicity of recombinant humanized monoclonal antibody against human vascular endothelial growth factor (rhuMab VEGF, bevacizumab) when administered to cynomolgus monkeys by bolus intravenous injection twice weekly for at least 13 weeks and to determine the reversibility, persistence, or delayed occurrence of toxic effects after 4 weeks post-treatment.

In general, the study drug was well tolerated. No overt toxic effects related to administration of study drug on clinical signs, weight gain, food consumption, ECG, ophthalmic observations, blood pressure or other physical exam parameters were noted.

Anatomical pathology revealed dose-related effects of the study drug. Statistically significant reductions in average absolute ovary and uterus weights are noted for the females in groups 3 and 4 (mid and high dose) (34% and 39%, respectively). The reductions in ovary and uterine weights are maintained in results of organ-to-body-weight comparisons. The organ weights for uterus and ovary were not different from control after the recovery period (n=2), indicating that recovery may be possible.

An apparent dose related absence of corpora lutea in the ovary was noted for 2 of 4 females from group 3 and 4 of 4 females from group 4 and persisted through the recovery period.

These findings for uterus and ovary suggest that bevacizumab may adversely effect fertility in females.

Physeal dysplasia of the femur is reported for all treated male monkeys and all females in the mid- and high-dose groups. Physeal dysplasia is also reported for the humerus in all male animals in groups 3 and 4 as well as 3 of 4 females in group 3, and 4 of 4 females in group 4. The physeal dysplasia did not resolve for either femur or humerus after 4 weeks of recovery.

Methods:

Species/strain: cynomolgus monkeys

#/sex/group or time point (main study): 4 or 6 animals/sex/group

Doses: 0, 2, 10 or 50 mg/kg

Route, formulation, volume, and infusion rate: Drug was administered by IV bolus into the saphenous vein twice per week.

Satellite groups used for toxicokinetics or recovery: 2 animals from each of the control and high dose groups were designated as recovery animals.

Weight (nonrodents only): 2.3 to 3.6 kg at initiation of the study

The study design is summarized in the following table taken from the BLA study report.

| Group | rhuMAb VEGF Dose Level (mg/kg) | Dose Concentration (mg/mL) ^a | Number of Animals ^b | |
|--------------------------|--------------------------------------|---|-----------------------------------|----------------|
| | | | Male | Female |
| 1 (Control) ^c | 0 | 0 | 6 ^d | 6 ^d |
| 2 (Low) | 2 | 0.4 | 4 | 4 |
| 3 (Mid) | 10 | 2 | 4 | 4 |
| 4 (High) | 50 | 10 | 6 ^d | 6 ^d |

^a The dose volume was 5 mL/kg.

^b Four animals/sex/group were sacrificed during Week 14.

^c The control group received rhuMAb VEGF Vehicle only.

^d Two animals/sex were designated as recovery animals and were dosed with rhuMAb VEGF or rhuMAb VEGF Vehicle twice weekly for at least 13 weeks, after which dosing was discontinued, and the animals were observed for reversibility, persistence, or delayed occurrence of toxic effects for approximately 4 weeks posttreatment.

Dosing regimen: The dose preparations were administered by intravenous injection using a minicatheter infusion set into a saphenous vein twice weekly for at least 13 weeks (on Days 1, 4, 8, 11, 15, 18, 22, 25, 29, 32, 36, 39, 43, 46, 50, 53, 57, 60, 64, 67, 71, 74, 78, 81, 85, 88, and 92). The dose preparations were given at a dose volume of 5 ml/kg and a dose rate of approximately 10 ml/minute.

Clinical signs:

The animals were observed twice daily (a.m. and p.m.) for mortality and moribundity. On dosing days, animals were observed pre-dose and approximately 1 hour post-dose, at least twice daily (a.m. and p.m.) on non-dosing days, and during recovery for signs of poor health or abnormal behavior.

Blood pressure measurements were recorded, under ketamine anesthesia, twice before initiation of treatment [Weeks -2 and -1 (Days -13 and -4)]; 1 to 2 hours post-dose on Days 1, 8, 25, 50, and 85; and on Day 114 (recovery animals).

Physical exams were performed twice prior to initiation of dosing, 1-2 hours post dose on days 1, 8, 25, 50, 85 and once on week 17 for the recovery animals.

Body weights:

Individual body weight data were recorded weekly before initiation of treatment, on Day 1, and weekly thereafter.

Food consumption:

Except when animals were fasted, individual qualitative food consumption data were recorded daily by visual inspection, beginning approximately 5 weeks before initiation of treatment.

Ophthalmoscopy:

Ophthalmic exams were performed on anesthetized animals once pre-treatment (week -3) and once during weeks 2, 4, 8 and 13.

ECG:

ECG measurements were taken (under ketamine anesthesia) twice prior to initiation of the study (days -13 and -4) and one to two hours post-dose on days 1, 8, 25, 50, 53, 85 and 114 (recovery animals).

Hematology:

Blood samples were collected from each animal for hematology twice before initiation of treatment (Weeks -2 and -1); once pre-dose during Weeks 2, 4, 8, and 13 (Days 11, 22, 53, and 88, respectively); and once during Week 17 (recovery animals) (except prothrombin time and activated partial thromboplastin time at Weeks -2 and -1). Animals were fasted for 26 to 28 hours before blood sampling.

Clinical chemistry:

Blood samples were collected from each animal twice before initiation of treatment (weeks -2 and -1); once pre-dose during weeks 2, 4, 8, and 13 (Days 11, 22, 53, and 88) for clinical chemistry. Animals were fasted for 26 to 28 hours before blood sampling.

Urinalysis:

Urine samples were collected from each animal twice before initiation of treatment (Weeks -2 and -1); once pre-dose during Weeks 2, 4, 8, and 13 (Days 11, 22, 53, and 88, respectively); and once during Week 17 (recovery animals) for urinalysis.

Gross pathology:

During Weeks 14 (Day 93) and 18 (Day 121, recovery animals), animals were fasted overnight, anesthetized with sodium pentobarbital (administered intravenously), weighed, exsanguinated, and necropsied. Animals were necropsied in random order. The necropsy included a macroscopic examination of the external surface of the body; injection sites; all orifices; eyes; the cranial cavity; the brain and spinal cord; the nasal cavity and paranasal sinuses; and the thoracic, abdominal, and pelvic cavities and viscera.

Organ weights (specify organs weighed if not in histopath table):

Adrenals

Brain

Epididymides

Heart

Kidneys

Liver
Lungs
Ovaries
Pituitary
Prostate
Salivary glands (submandibular)
Spleen
Seminal vesicles
Testes
Thymus
Thyroids with parathyroid
Uterus with cervix

Histopathology: Complete Battery: Yes (X), No ()—explain

Tissues (as appropriate) were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically from each animal. Histopathology slides of the proximal, distal, and shaft regions of the right and left femur with bone marrow; proximal, distal, and shaft regions of the right and left humerus with bone marrow; sternum with bone marrow from all animals; and histopathology slides of kidneys from all animals and the ovaries and uterus from all females were sent to the Sponsor and examined microscopically by the reviewing pathologist (see Protocol Deviations for exception). A directed pathology peer review of the slides and data was performed by the Sponsor's pathologist to assure that all significant findings were described and recorded accurately.

Toxicokinetics:

Samples were taken prior to initiation of treatment, on day1 (1 and 8 hours post-dose), day 2 (24 hours), day 4 (pre-dose), day 11 (pre-dose and 1 hour post-dose), days 14, 53 (pre-dose and 1 hour post-dose), days 91 and 92 (pre-dose and 1 and 8 hours post-dose), and day 93 (24 hours post-dose). Additional samples were taken from recovery animals on days 95, 101, 108, and 119.

Other:

Vitreous fluid samples: Up to 1 ml was collected from the left eye of each animal at sacrifice.

Antibody Analyses:

Approximately 1 ml of whole blood was collected from a femoral vein of each animal before initiation of treatment (Day -6); pre-dose on Days 11, 15, 22, 53, and 92; and on Day 119 (recovery animals). Pre-dose collections were done within approximately 2 hours before dosing. Samples were used for detection of anti-rhu VEGF antibodies.

Results:

Mortality:

All animals survived to the scheduled sacrifice date.

Clinical signs:

No biologically relevant observations related to study drug administration were reported.

Body weights:

No biologically significant effects on body weight were observed.

Food consumption:

Sporadic episodes of low food consumption were noted, but did not show a relationship to administration of the study drug and did not affect body weight.

Ophthalmoscopy:

No treatment related findings were observed.

ECG:

No significant cardiology findings related to study drug administration were observed.

Hematology:

- No biologically significant findings relating to administration of the study drug were observed.
- Statistically significant reduction compared to control in % lymphocytes was noted at week 2 for group 3 and 4 females and weeks 8 and 13 for group 4 females but this finding was not different from baseline and was not consistent over time.
- Statistically significant increase in % neutrophils for groups 2 and 4 females on week 8 and group 2 females on week 13 was noted but no change was noted for group 3. This finding was not sufficiently consistent to indicate a true relationship to study drug administration.

Clinical chemistry:

- No biologically significant findings related to administration of study drug were observed.
- Statistically significant elevation in AST/SGOT was noted for groups 2 and 4 males on weeks 2, 8 and 13. However, this finding was not observed for group 3 (intermediate dose) and values were not higher than the baseline values for these groups. Therefore, biological relevance is questionable.

Urinalysis: No biologically significant findings related to study drug administration were observed.

Gross pathology:

- Lung adhesions were noted in 15 animals across all groups with no apparent relationship to study drug administration.
- Injection site reactions (redness) were noted in a majority of the animals but no relationship to study drug is apparent.

Organ weights:

- Absolute organ weight for kidney in the group 3 females was noted. A trend toward decreasing weights for the kidney in female animals may be present but the changes are not statistically significant and not present in the kidneys from male animals.
- Statistically significant reductions in absolute ovary and uterus weights are noted for the females in groups 3 and 4 (mid and high dose). The reductions in ovary and uterine weights are maintained in results of organ-to-body-weight

comparisons. These findings may be biologically significant and appear to be related to dose of the study drug.

- No statistically significant changes in absolute organ weight are reported for recovery animals. The organ weights for uterus and ovary were not different from control after the recovery period (n=2).

Histopathology:

- Physeal dysplasia of the femur is reported for all treated male monkeys and all females in the mid- and high-dose groups. Physeal dysplasia is also reported for the humerus in all male animals in groups 3 and 4 as well as 3 of 4 females in group 3 and 4 of 4 females in group 4. The severity of the dysplasia was reported as marked to moderately severe. This physeal dysplasia did not resolve for either femur or humerus after 4 weeks of recovery but the severity was rated as less severe (1 male rated as minimal and 1 male rated as marked, one female rated as minimal and one rated as marked).
- Lymphocytic infiltration is noted for the left eye in 1 of 4 female animals in each of groups 2, 3, and 4 and 1 of 4 male animals in groups 2 and 3. Lymphocytic infiltration is also reported in the right eye for 2 of 4 group 3 males.
- Lung histopathology showed lymphohistiocytic infiltration in all animals except 2/4 of the male control animals. Abnormal pigment is also reported for all animals. Evidence of pleural fibrosis is reported for 3/4 animals in groups 1 and 2 males, 2/4 in group 3 males, 1/4 female animals in groups 1 and 2, as well as 2 of 4 females in group 4. Other occasional evidence of lung inflammation is also reported. These findings do not appear to have a relationship to study drug administration and are most likely a result of pre-existing inflammation in these animals.
- Lymphocytic infiltrate is reported in the kidney of 2 of 4 male control animals, 3 of 4 female control animals, 3 of 4 females from group 2, and 1 of 4 female animals from group 4. Tubulointerstitial nephritis is reported for 1 of 4 male animals in the high-dose group (group 4) but no females from the high-dose group showed this finding. Evidence of chronic inflammation of the kidney is reported in 2 of 4 male animals from groups 2 and 3 and 2 of 4 female animals from groups 3 and 4. Evidence of glomerulonephritis is reported in 1/4 males from group 4 (high-dose) and no females from any group. Glomerulosclerosis is reported in one male control animal. These findings do not show a clear relationship to study drug administration.
- Liver histology showed evidence of subacute inflammation in 2 of 4 males and 1 of 4 females from group 2, 3 of 4 males and 1 female from group 4, and 1 control female animal. Kupffer cell pigmentation is reported for some animals from each group except group 3 males. 2 of 8 controls, 3 of 8 from group 2, 1 of 8 from group 3, and 4 of 8 from group 4. Lymphohistiocytic inflammation is reported for one control female animal. Hepatocellular vacuolization is reported for 1 female and 1 male from group 3, and 1 male from group 4. These data do not reflect a clear dose relationship with the study drug and may be indicative of a preexisting condition.

- Evidence of parasitism is reported for skeletal muscle from 2 of 4 males from group 2, 1 of 4 males from each of groups 3 and 4. No females showed this finding. Evidence of skeletal muscle degeneration is reported for one male animal from each of groups 3 and 4. One female from group 3 showed lymphohistiocytic inflammation of skeletal muscle.
- Histopathology of the gallbladder showed lymphocytes infiltration.
- Ultimobranchial cysts were observed for 1 male from each of groups 2 through 4, 3 of 4 females from groups 1 and 2, 2 of 4 females from group 3 and 1 of 4 females from group 4. These findings do not appear to be related to study drug administration.
- Lymphocytic infiltration of the trachea was reported for 2 males from group 2, 1 male from group 3, 2 females from group 1, 2 females from group 2, 3 females from group 3 and 1 female from group 4. These findings do not show a relationship to study drug administration and are further evidence of a pre-existing inflammatory condition.
- Evidence of chronic inflammation was observed in the esophagus for 2 males from group 3, 2 females from groups 2 and 3 and 1 female from group 4. Although no control animals showed this result, there does not appear to be a clear relationship to study drug since frequency is greater in the low and mid-dose groups.
- Evidence of chronic inflammation of the tongue was noted for 2 of 4 male animals from group 2, 1 of 4 female animals from group 1, 2 of 4 female animals from group 3 and 1 of 4 female animals from group 4. One male control animal showed lymphocytic infiltration of the tongue. These findings are indicative of a pre-existing inflammatory condition and are most likely not related to study drug administration.
- Evidence of a pre-existing condition is supported by the observed lymphocytic infiltration in 2 of 4 animals of both sexes across all doses.
- Lymphocytic infiltration is noted for 20 of 32 animals across all dose groups.
- The presence of chronic and subacute inflammation as well as hemorrhage and vascular necrosis at the injection sites was observed in 28 of 32 animals across all dose groups. No study drug relationship is apparent.
- Evidence of chronic inflammation was observed sporadically in the stomach and cecum and colon of animals from all dose groups including controls.
- Chronic inflammation was observed in the urinary bladder in 13 of 32 animals across all dose groups including 3 of 4 male control animals and 2 of 4 female animals.
- Immaturity of the prostate was noted in 2 of 4 high-dose male animals and 1 of 4 males control animals.
- The epididymus of 2 of 4 control male animals and 3 of 4 high-dose males showed hypospermia. Evidence of chronic inflammation was also noted for 1 of 4 males in groups 2 and 3. These findings do not show a relationship to study drug administration.
- Immaturity was observed for 1 of 4 control males and 2 of 4 high-dose males.
- No histologic findings are reported that would account for the dose-dependent reduced uterine weight (See above).

- Ovaries showed mineralization (2 of 4 controls, 3 of 4 from group 3, and 2 of 4 from group 4). Fibrosis was observed in the ovaries of 11 of 16 females across all dose groups.
- An apparent dose related absence of corpora lutea in the ovary was noted for 2 of 4 females from group 3 and 4 of 4 females from group 4 and persisted through the recovery period.

Toxicokinetics:

- Individual peak concentrations and time of peak concentration were calculated based on observed values for all treated animals following dosing on day 92. The serum drug concentration data from the recovery animals in group 4 were analyzed by compartmental methods using a non-linear regression program that accounts for multiple administration of drug.
- A two compartment model with central compartment elimination was fit to the data. An adequate compartmental model could not be fit to each individual animal's data due to limited sampling of the non-recovery animals.

Tables below from the BLA demonstrate the PK calculated parameters:

Groups 2, 3, and 4 - Non-recovery and Recovery Animals

| Parameters | Group 2 - 2 mg/kg IV | | Group 3 - 10 mg/kg IV | | Group 4 - 50 mg/kg IV | |
|-------------------------------|----------------------|-----------|-----------------------|-----------|-----------------------|------------|
| | Males | Females | Males | Females | Males | Females |
| n | 4 | 4 | 4 | 4 | 6 | 8 |
| Weight (kg) | 2.9 ± 0.4 | 2.6 ± 0.3 | 2.8 ± 0.1 | 2.6 ± 0.2 | 2.9 ± 0.2 | 2.6 ± 0.2 |
| T _{max} ^a | 1hr | 1hr | 5hr | 5hr | 1hr | 5hr |
| C _{max} (µg/mL) | 140 ± 22 | 150 ± 5.0 | 660 ± 80 | 810 ± 99 | 3300 ± 750 | 3700 ± 300 |

^a Median time post-dose on Day 92.

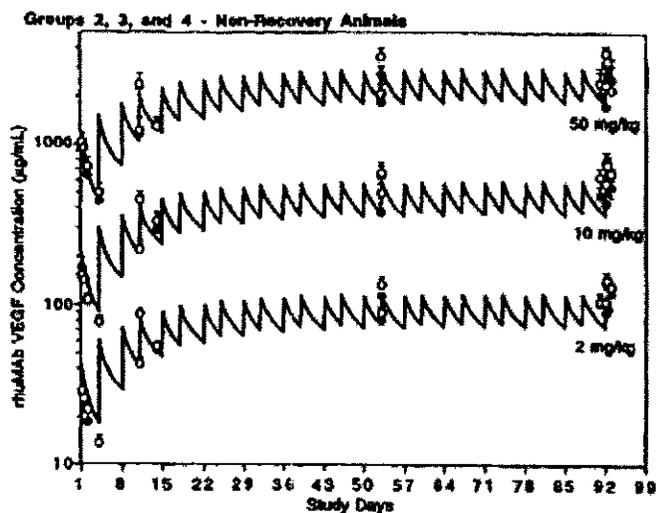


Figure 1. Mean serum rhMAb VEGF concentration vs. time profiles with estimated group curves superimposed on the data (n=4 per sex). Males-solid symbols; Females-open symbols.

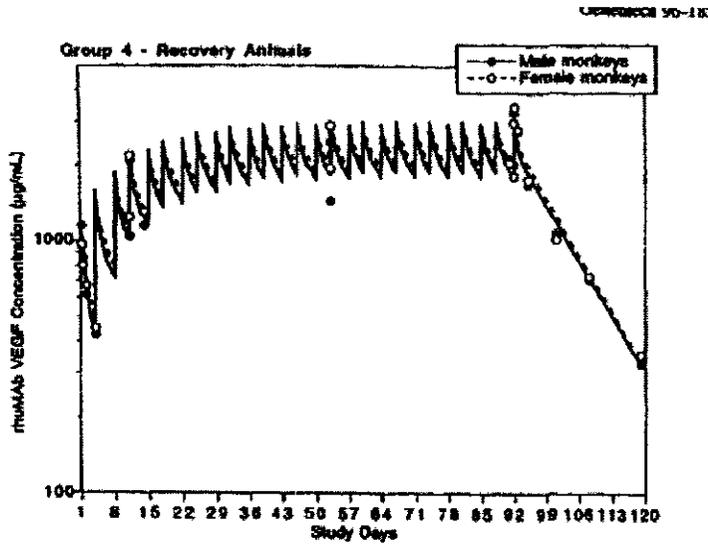


Figure 2. Serum concentration vs. time profiles of recovery animals administered 50 mg/kg IV rhumAb VEGF with estimated curves superimposed on the data. The mean of 2 monkeys per sex is presented.

Group 4 - Recovery Animals

| Parameters ^a | 50 mg/kg IV | | Group Total Mean ± SD |
|------------------------------|-------------|---------|--------------------------|
| | Males | Females | |
| n | 2 | 2 | 4 |
| V _C /W (mL/kg) | 43.1 | 50.8 | 46.9 ± 5.3 |
| V _{ss} /W (mL/kg) | 84.3 | 84.3 | 84.3 ± 2.5 |
| CL/W (mL/kg/day) | 6.48 | 6.03 | 6.25 ± 0.71 |
| AUC (µg·day/mL) ^b | 7821 | 8314 | 8070 ± 860 |
| t _{1/2α1} (hr) | 11.4 | 44.9 | 28.2 ± 34 |
| t _{1/2α2} (day) | 9.6 | 13.1 | 11.4 ± 3.8 |
| MRT (day) | 13.2 | 14.0 | 13.6 ± 1.4 |

^a The parameters are reported as mean where appropriate.

^b First dose AUC calculated.

Other:

Antibody analysis: No detectable anti-bevacizumab antibodies were detected in the serum of any animals throughout the study. One female animal in the control group exhibited anti-bevacizumab antibody starting at day 53. There is no clear reason for this apparent false positive result.

Vitreous analysis: Only animals in the high-dose group had detectable bevacizumab levels in the vitreous. Of the 8 high-dose animals measured, the average levels were 1.8 ± 1.1 µg/ml for the males and 1.7 ± 0.33 µg/ml for the females.

Reviewer Comments:

Macroscopic and microscopic pathology revealed numerous instances of adhesions and lymphocytic infiltration and evidence of chronic inflammation of various organs across all groups. These findings did not show a clear relationship to study drug administration and appear to be results of a pre-existing condition.

Although there appeared to be some recovery for severity of the physal dysplasia in both the femur and humerus, the recovery was not complete within the 4-week period. The potential for recovery of physal dysplasia will be important if bevacizumab is considered for use in pediatric or juvenile populations.

The results of analysis for anti-bevacizumab antibodies indicate that no antibodies were detected at any time point in any animals. However, sampling was performed when significant amounts of study drug were present in the serum making interpretation of the data questionable.

2.4.2.3. Study Title: 26-Week Intravenous Toxicity Study with rhuMab VEGF in Cynomolgus Monkeys with a 12-week Recovery

Testing Facility and Location:

Study Number: 97-194-1751
Date of Study Initiation: 8/22/97
Drug Lot/Batch Number(s): Drug Lot # D9869AX, vehicle lot # M3-RD588
GLP Compliance (y/n): Yes.
QA Report (y/n): Yes.

Key Findings:

This study was performed to assess the toxicity of bevacizumab when administered to cynomolgus monkeys by intravenous injection once or twice weekly for 26 weeks and to determine the reversibility, persistence or delayed effects after a 12 week recovery period.

Physal dysplasia of the femur was reported as severe in 4 of 4 males from group 5, 3 of 4 males from group 4, and 1 of 4 males from group 3. Moderate or mild dysplasia is reported for the one remaining animal in group 4, the remaining 3 in group 3 and 2 of 4 from group 2. This demonstrates a clear dose response relationship to the study drug. Fewer female animals displayed these changes. Severe dysplasia is reported for 1 of 4 from group 5, moderate dysplasia for 2 of 4 females from groups 3 and 4. The difference in toxicity between genders may be explained by differences in ages of the two groups. Severity appeared to be less after 12 weeks of recovery, indicating that some recovery may be possible.

Results of anatomical pathology indicated that treatment with bevacizumab has a probable adverse effect on fertility. Organ weight for the uterus was significantly reduced in the 10 mg/kg, and 50 mg/kg groups compared to control. After 26 weeks of treatment, a dose dependent reduction in ovarian weights (up to approximately 50%) was apparent for all dose groups compared to control. Due to variability, this finding did not reach statistical significance but a clear dose-dependent trend is apparent. Severe reductions in proliferation of the uterine endometrium functionalis layer are reported for females receiving 10 and 50 mg/kg. Follicular arrest is noted for all females in groups 4 (50mg/kg) and 5 (10 mg/kg X2/week). This effect is clearly study drug related. Follicular arrest was not observed after 12 week recovery (n=2) and reduced endometrial proliferation was not observed after the recovery period. However, 1 of 2 recovery female showed absence of corpora lutea indicating incomplete recovery. A reduction of 67% in menstrual cycles was also reported for the 50 mg/kg females. Reduced menstrual cycles were reported for the recovery animals (50 mg/kg, n=2) but, due to individual variations these data are difficult to interpret.

Females in group 4 showed a small elevation in blood pressure that may be study drug related. The changes reported for uterus and ovary in the non-recovery animals are not reported for the recovery animals.

Methods:

Species/strain: Cynomolgus monkeys

#/sex/group or time point (main study): Group 1 (control) consisted of 6 animals/sex, groups 2 (2 mg/kg) and 3 (10 mg/kg) and 5 (10 mg/kg) contained 4 animals/sex, group 4 (50 mg/kg) contained 6 animals/sex. *Doses:* 0, 2 mg/kg weekly, 10 mg/kg weekly, 50 mg/kg weekly, and 10 mg/kg twice weekly

Route, formulation, volume, and infusion rate: The drug was administered by IV infusion in a volume of 5 ml/kg at a rate of 10 ml/min in to the saphenous vein.

Satellite groups used for toxicokinetics or recovery: Two extra animals per sex in groups 1 and 4 were designated as recovery animals.

Age: 4 to 7 years old

Weight (nonrodents only): males: 2.7 to 4.2 kg, females: 2.3 to 2.8 kg

Study design: (Table provided by the sponsor.)

| Group | Dose | Dose | Dose | Dose | Number of Animals ^a | |
|-------------|----------------------------|------------------------|--------------------------|-------------------|--------------------------------|----------------|
| | Frequency (number/week) | Levels (mg/kg/dose) | Concentration (mg/mL) | Volume (mL/kg) | Male | Female |
| 1 (Control) | 1 | 0 | 0 | 5 | 6 ^b | 6 ^b |
| 2 (Low) | 1 | 2 | 0.4 | 5 | 4 | 4 |
| 3 (Mid 1) | 1 | 10 | 2 | 5 | 4 | 4 |
| 4 (High) | 1 | 50 | 10 | 5 | 6 ^b | 6 ^b |
| 5 (Mid 2) | 2 | 10 | 2 | 5 | 4 | 4 |

a Four animals/sex/group were sacrificed after at least 26 weeks of treatment.

b Two animals/sex were designated as recovery animals and were dosed once weekly with rhuMAb VEGF or rhuMAb VEGF Vehicle for at least 26 weeks, after which dosing was discontinued and the animals were observed for reversibility, persistence, or delayed occurrence of toxic effects for approximately 12 weeks posttreatment.

The table above, taken from the BLA, demonstrates the study design. Doses were administered weekly, with the exception of Group 5, in which dosing was twice/week.

Clinical signs:

Animals were observed twice daily for mortality/moribundity. On dosing days, each animal was observed pre-dose and 1 hour post-dose. On non-dosing days and during recovery, animals were observed twice daily for signs of poor health or abnormal behavior. Vaginal swabs were taken daily from all females. Radiographs were taken of the right distal femur to determine the status of growth plate closure. Physical exams were performed under ketamine anesthesia twice prior to study initiation, 1-2 hours post-dose on weeks 4, 13, 19, 26 and 38. Blood pressure was recorded under ketamine anesthesia for each animal, twice prior to study initiation, 1-2 hours post-dosing during weeks 4, 13, 19, 26 and week 38.

Body weights:

On dosing days, each animal was observed pre-dose and 1 hour post-dose. On non-dosing days and during recovery, animals were observed twice daily for signs of poor health or abnormal behavior.

Food consumption:

Food consumption was recorded daily by visual inspection beginning at least 1 week prior to study initiation.

Ophthalmoscopy:

Ophthalmic exams were performed on each animal once prior to initiation of study, and during weeks 4, 13, 19, and 26. Eye exams were not performed during recovery.

EKG:

EKG exams were performed on each animal once prior to initiation of study, and during weeks 4, 13, 19, and 26. Eye exams were not performed during recovery.

Hematology:

Blood and urine samples were taken twice prior to initiation of treatment, and once during weeks 4, 13, 19, 26 and 38. All samples were taken 2 days after the first dose for that given week.

Clinical chemistry:

Blood and urine samples were taken twice prior to initiation of treatment, once during weeks 4, 13, 19, 26 and 38. All samples were taken 2 days after the first dose for that given week.

Urinalysis: See above, Clinical chemistry.

Gross pathology:

Organ weights (specify organs weighed if not in histopath table):

Adrenal, brain, epididymis, heart, kidney, liver/gallbladder, lung, ovary, pituitary, prostate, mandibular salivary gland, spleen, thymus, thyroid/parathyroid, uterus, seminal vesicles and testis.

Histopathology: Complete Battery: Yes (X), No ()--explain

Toxicokinetics:

Blood samples were taken from all animals for determination of study drug levels at the following times:

Day 1: pre-dose, 1 hr, 8 and 24 hours post-dose

Day 4: (prior to dosing for group 5)

Day 8, 22, 43, 85, 134: pre-dose and 1 hour post-dose

Day 183: pre-dose, 1, 8 and 24 hours post-dose.

Recovery animals: on days 186, 192, 204, 210.

Other:

Blood was collected for determination of anti-rhuMAb VEGF antibody formation prior to dosing, and pre-dose on days 8, 15, 22, 85, 183 and day 267.

Results:

Mortality:

All monkeys survived to the scheduled sacrifice date.

Clinical signs:

- Vomiting was noted in all groups in the morning observations: 7 of 12 controls, 4 of 8 from the 2 mg/kg group, 6 of 8 from the 10 mg/kg group, 9 of 12 from the 50 mg/kg. Vomiting was also noted for the recovery animals: 4 of 4 controls, 1 of 4 from the 50 mg/kg group.
- Although not statistically significant, there appears to be a dose-related trend toward a decrease in the number of menstrual cycles in the treated female animals.
- Females in group 4 showed a small elevation in blood pressure that may be study drug related. However, the variation in measurements was wide and the rabbits in that group started the study with a slightly higher average blood pressure than the other treatment groups. This slight elevation was not seen in group 5 (10mg/kg twice per week). On week 26, the blood pressure for the group 4 females was statistically higher than controls at the same time point. No differences were noted for the recovery animals.

Body weights:

A slight decrease in body weight gain was noted for the 50 mg/kg male animals starting at approximately week 14 and continuing through the end of the study. Group 5 males receiving 10 mg/kg showed the decrease in weight gain beginning on week 24. Group 3 males also receiving 10 mg/kg showed the decrease in weight gain on week 27. The decrease in weight gain in the females was apparent on week 27 for all treatment groups. This difference from the control group was small but statistically significant for the male animals (approximately 0.2-0.5 kg) and appears to be dose-related.

Food consumption:

Sporadic vomiting was reported but no clear relationship to the study drug is noted.

Ophthalmoscopy:

No visible lesions were noted for any animal in any group during the study.

EKG:

- A statistically significant lengthening of the Q-T interval was observed for group 5 males at week -2 (0.8 sec vs 0.22 sec.). This was a baseline measurement and did not persist during the study.
- On week 13, a statistically significant increase in Q-T interval was noted for groups 4 and 5 males. However, the values for week 13 are not significantly different from baseline values for the same groups.
- No other changes were noted. These changes were sporadic and inconsistent and were probably not of clinical significance.

Hematology:

Hematology results revealed no changes related to study drug administration.

Clinical chemistry:

- A small, statistically significant, reduction in albumin for groups 4 and 5 males on weeks 19 and 26 that may be study drug related.
- A small, but statistically significant increase in globulins is noted for the group 4 and 5 males at week 26. These changes may be related to study drug but the variation between timepoints is fairly large so there may not be biological significance.

- Bilirubin levels are consistently lower in the treated females than controls but these lower levels are not significantly different than baseline and do not show a clear relationship to study drug administration.

Urinalysis:

No changes related to study drug administration are noted. No other macroscopic findings were noted that were related to study drug administration.

Gross pathology:

Redness at the injection sites is reported but does not show a clear relationship to the study drug administration.

Organ weights:

- Reduced kidney weights are noted for group 3 and 5 males.
- Reduced ovarian weights are noted for all treated females. The decrease in weight does not reach statistical significance, but there appears to be a trend that is dose dependent with the greatest difference from control observed for group 5 (10mg/kg X2/week).
- Significant reduction in uterus weights is noted for groups 3, 4, and 5 compared to control (approximately 50%). Group 2 also shows a reduction in uterus weight that does not reach statistical significance (approximately 40%). These changes appear to be related to study drug administration in a dose dependent manner.
- Reductions in seminal vesicle weight, epididymis and testis weights show a trend that appears to be dose related. However, the reduction in weight does not reach statistical significance. These reductions do not persist through recovery. (n=2)
- The reductions in weight for the uterus and ovary persist through the recovery period. However, at the end of the recovery period, the organ weight reductions do not reach statistical significance. (n=2)

Histopathology:

- Physeal dysplasia of the femur was reported as severe in 4 of 4 males from group 5, 3 of 4 males from group 4, and 1 of 4 males from group 3. Moderate or mild dysplasia is reported for the one remaining animal in group 4, the remaining 3 in group 3 and 2 of 4 from group 2. This demonstrates a clear dose response relationship to the study drug. Fewer female animals displayed these changes. Severe dysplasia is reported for 1 of 4 from group 5, moderate dysplasia for 2 of 4 females from groups 3 and 4. The difference in toxicity between genders may be explained by differences in ages of the two groups.
- Severe reductions in proliferation of the uterine endometrium functionalis layer are reported for females receiving 10 and 50 mg/kg.
- Follicular arrest is noted for all females in groups 4 (50mg/kg) and 5 (10 mg/kg X2/week). This effect is clearly study drug related.
- Incomplete recovery is indicated after the 12-week recovery period. Changes in endometrial proliferation are not reported for the uterus of recovery animals. However, absence of corpora lutea is reported for 1 of 2 high-dose recovery animals.
- Signs of chronic mild inflammation are reported for several organs including eye, kidneys of group 4 and 5 males, heart, skeletal muscle, lung, liver, trachea, esophagus, thyroid, parathyroid, pituitary, stomach, pancreas, duodenum, jejunum, cecum, colon, rectum, ureter, urinary bladder, prostate, vagina. This evidence of inflammation is

reported for control animals as often as treated animals. Thus, the findings may be indicative of pre-existing conditions in these animals.

- Mesangial thickening is reported for 8 of 32 animals but 2 of those animals affected are control animals. The changes are reported as mild to minimal. There is not a clear relationship to study drug administration.
- Evidence of mild to moderate inflammation is reported for the majority of injection sites with no clear relationship to study drug administration.
- Evidence of mild chronic inflammation, as described above for the non-recovery animals, is also present for the recovery animals.

Toxicokinetics:

Pharmacokinetic analysis showed a dose-proportional increase in serum levels of bevacizumab in the dose range used for this study (2 mg/kg to 50 mg/kg). Results suggest that clearance may be slower in males than in females. That finding was not observed in previous studies so the significance is not clear. Clearance, in general, for this study was slightly slower than previous studies for both single and repeated dosing.

Table 1. Groups 2, 3, 4 and 5 - Non-recovery and Recovery Animals (Mean±SD)

| Parameters | Group 2 2 mg/kg IV | | Group 3 10 mg/kg IV | | Group 4 50 mg/kg IV | | Group 5 10 mg/kg q2 IV | |
|--|-----------------------|---------------|------------------------|---------------|------------------------|--------------|---------------------------|--------------|
| | Males | Females | Males | Females | Males | Females | Males | Females |
| n | 4 | 4 | 4 | 4 | 6 | 6 | 4 | 4 |
| Weight* (kg) | 3.1± 0.33 | 2.6± 0.17 | 3.4± 0.71 | 2.8± 0.22 | 3.2± 0.27 | 2.7± 0.22 | 3.3± 0.39 | 2.6± 0.18 |
| T _{max} ^b | 4.5 hr | 1 hr | 1 hr | 8 hr | 8 hr | 1 hr | 1 hr | 1 hr |
| C _{max} ^c (µg/mL) | 119± 8.7 | 95.9± 12.0 | 623± 109 | 492± 44 | 2717± 404 | 2160± 294 | 834± 54.7 | 836± 104 |
| AUC ₀₋₂₄ ^c (x10 ³ µg·day/mL) | 15.9± 0.646 | 14.9± 2.00 | 91.2± 12.0 | 75.7± 9.69 | 325± 71.0 | 268± 17.1 | 119± 6.28 | 122± 20.0 |

- * Mean pre-dose weight.
- ^b Median time post-dose on Day 183.
- ^c Maximum observed concentration post-dose on Day 183.

Table 2. Group 4 - Recovery Animals

| Parameters ^a | 50 mg/kg IV | | Group Total Mean ± SD |
|---------------------------------|-------------|---------|--------------------------|
| | Males | Females | |
| n | 2 | 2 | 4 |
| V _d /W (mL/kg) | 30.5 | 35.7 | 33.1±3.22 |
| V _{ss} /W (mL/kg) | 59.1 | 70.2 | 64.7±9.81 |
| CL/W (mL/kg/day) | 3.29 | 4.67 | 3.98±0.808 |
| AUC ₀₋₂₄ (µg·day/mL) | 15,200 | 10,700 | 13,000±2,600 |
| t _{1/2α1} (hr) | 81.6 | 72.5 | 77.0±30.3 |
| t _{1/2α2} (day) | 23.3 | 17.5 | 20.4±8.67 |
| MRT (day) | 17.9 | 15.1 | 16.5±2.59 |

^a The parameters are reported as mean, where appropriate.

Other:

Antibody analysis: No anti-bevacizumab antibodies were detected in any animals throughout the study with the exception of one vehicle control animals and one group 4 animal that showed positive on week 26.

Reviewer Comments:

2.4.2.4. Study Title: Safety evaluation of rhuMAb VEGF in a dermal rabbit wound model

Testing Facility and Location: Genentech, Inc. 460 Point San Bruno Blvd., South San Francisco, CA 94080
 Study Number: Three studies are combined in this report: 96-407, 96-407 A, 96-407 B
 Date of Study Initiation: 96-407: 11/20/96, 96-407 A: 12/30/96, 96-407 B: 4/1/97
 Drug Lot/Batch Number(s): Study drug lot #: M3-RD595, Placebo lot #: M3-RD588
 GLP Compliance (y/n): No
 QA Report (y/n): No.

Key Findings:

These studies were performed to assess the effects of rhuMAb VEGF in a rabbit model of wound healing. Study 96-407 A was performed to test additional dose levels in the same model as 96-407 and to assess the time required for wound healing. Study 96-407 B was performed to clarify antibody results that were obtained in the previous two studies and to gather information on pharmacokinetics after intravenous bolus administration in male rabbits. Results of studies 96-407 and 96-407A showed that treatment with bevacizumab was associated with a decrease in wound healing capacity at all doses. Although the sponsor states that results of Study 96-407A suggest that wound healing rate can recover after terminating treatment, those data are not included in the study report. Therefore, conclusions regarding recovery of wound healing capacity cannot be drawn.

Methods:

Species/strain: New Zealand white rabbits purchased from _____
#/sex/group or time point (main study): Study 96-407
 Study 96-407B: two males/group
 Study 96-407A

Doses: 96-407 1) vehicle, 2) 50 mg/kg bevacizumab, 3) 35 mg/kg methylprednisolone
 96-407A 1) vehicle, 2) 2 mg/kg bevacizumab, 3) 10 mg/kg bevacizumab
 96-407B 1) 10 vehicle, 2) 10 mg/kg bevacizumab

Route, formulation, volume, and infusion rate: For all three studies, study drug was administered IV except methylprednisolone, which was administered IM. For study 96-407, the 50 mg/kg dose and vehicle were each administered in a volume of 5 ml/kg. For 96-407A and -B, all doses were administered in volumes of 1 ml/kg.

Weight (nonrodents only): 2.0-2.5 kg

Study design: (Tables taken from BLA)
 96-407

| Group | Dose | Study Days animals dosed |
|-------|-----------------------------|--------------------------|
| 1 | rhuMAb VEGF vehicle | 1, 3, 5, 7, 10 |
| 2 | 50 mg/kg rhuMAb VEGF | 1, 3, 5, 7, 10 |
| 3 | 35 mg/kg methylprednisolone | -2, 1, 3, 5, 7, 11 |

Animals were sacrificed on day 12. One animal was found dead on day 12.

96-407A

| Group | Dose | Study Days animals dosed |
|-------|----------------------|--------------------------|
| 1 | rhUMAb VEGF vehicle | 1, 4, 8, 11 |
| 2 | 2 mg/kg rhUMAb VEGF | 1, 4, 8, 11 |
| 3 | 10 mg/kg rhUMAb VEGF | 1, 4, 8, 11 |

Animals were sacrificed when all wounds in all dose groups were healed. There is no data to support the claim that wounds had healed. It is also not clear when these rabbits were sacrificed.

Blood samples were taken for detection of anti-bevacizumab antibodies prior to dosing and 5 minutes post-dose on days 1 and 8, and on days 17 and 30.

96-407B

| Group | Dose | Study Days animals dosed |
|-------|----------------------|--------------------------|
| 1 | rhUMAb VEGF vehicle | 1, 4, 8, 11 |
| 2 | 10 mg/kg rhUMAb VEGF | 1, 4, 8, 11 |

Blood samples were collected out to day 50. Samples were collected on day 1 pre-dose, 5 minutes, 1 and 8 hours post-dose, day 2, day 4 pre-dose and 5 minutes post-dose, day 8 pre-dose and 5 minutes post-dose, day 11 pre-dose, 5 minutes 1 and 8 hours post-dose, and on days 12, 15, 22, 29, 36, 43 and 50. There is not mention of the disposition of the rabbits from this study. No necropsy or histopathology data are reported.

The model wounds were produced by a circular 8 mm punch biopsy instrument creating two partial thickness wounds in the skin of the inside of the right ear of each animal.

Clinical signs: Animals were observed daily for general appearance.

Body weights: Body weights were recorded each time each animal was dosed.

Food consumption: The presence of feces was noted as evidence that the animals were eating.

Antibody analyses:

Samples were collected for antibody detection as described above under study design. For study 96-407B, anti-bevacizumab antibodies were detected in both animals receiving study drug at day 11.

Gross pathology: For study 96-407, the right ear of each animal was removed and fixed in buffered formalin. For study 96-407A, bone samples from the femurs and humeri were taken. Ears were not examined histologically.

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

Histopathology: Complete Battery: Yes (), No (X)—explain These studies address only wound healing capabilities after bevacizumab treatment. Bone was also taken for 96-407A only because bone lesions were seen in the repeat-dose toxicology studies.

Toxicokinetics:

Samples were taken for toxicokinetic analysis as described above under study design. Calculated TK parameters are listed in the following Table, provided by the sponsor:

Individual and Mean Pharmacokinetic Parameters of rhuMAb VEGF following Intravenous Administration of 10 mg/kg

| Parameter | Rabbit 2-3 | Rabbit 2-4 | Mean |
|------------------------------------|------------|------------|------|
| Dose (mg/kg) | 10 | 10 | 9.3 |
| C _{max} on Day 11 (µg/mL) | 479 | 441 | 460 |
| T _{max} on Day 11 (min) | 5 | 5 | 5 |
| V _C (mL/kg) | 37.2 | 42.7 | 39.9 |
| V _{SS} (mL/kg) | 59.1 | 67.0 | 62.9 |
| CL (mL/day/kg) | 8.10 | 8.15 | 8.13 |
| t _{1/2} initial (hr) | 4.14 | 7.50 | 5.82 |
| t _{1/2} terminal (day) | 5.18 | 5.87 | 5.52 |
| MRT (day) | 7.29 | 8.20 | 7.74 |
| AUC ₀₋₁₁ (µg·day/mL) | 1230 | 1230 | 1230 |

Ear wound measurements: The diameter of each wound gap was measured daily. For each animal, the diameter of two wounds was averaged and wound area was calculated from the average diameter. Rate of wound healing was calculated as the change in wound gap area from day 1.

Results

Results for study 96-407 showed that the rate of wound healing in animals receiving methylprednisolone was significantly reduced than that of control animals. By day 12, the control animal wounds were 78% closed but the wounds of the methylprednisolone treated animals were 38% closed. The area of the wounds for the group treated with 50 mg/kg bevacizumab had reached 46% closure by day 12.

Study 96-407A was conducted to study additional doses of bevacizumab and to evaluate whether the wound healing would return to normal rate after termination of bevacizumab dosing. Area of wound healing on day 12 was 69% for vehicle control, 47% for 2 mg/kg group and 36% for the 10 mg/kg group. In comparing data from day 12, it appears that, although bevacizumab does inhibit wound healing, there is no clear dose relationship. All treated animals showed reduced wound healing capacity regardless of dose. No data could be located in this report for recovery as stated in the study objectives.

At sacrifice on day 12, 8 out of 12 control animals showed complete healing of wounds. For the 50 mg/kg group, 2 out of 12 (17%) had completely healed wounds. The methylprednisolone treated animals had 50% complete wound closure.

Microscopic changes were not observed on histological examination of bone samples taken from rabbits from 96-407A.

Reviewer Comments:

Most of the blood samples taken for anti-bevacizumab antibody detection were taken at time points when the study drug was still present in the plasma. Therefore, results of those analyses are not reliable.

As stated above, one objective for this study was to determine if the wound healing capacity for animals treated with bevacizumab would return to normal after dosing was stopped. The sponsor reports in their summary, that the rate of wound healing does return to normal after cessation of bevacizumab dosing but no data to support this claim could be located.

No physal dysplasia was noted upon microscopic examination of bone samples collected from animals in study #96-407A. This may be due to the fact that the animals were mature and did not have open growth plates.

2.4.2.5. Study Title: Effect of rhuMab VEGF on ovarian function in rabbits.

Testing Facility and Location: Genentech, Inc., South San Francisco, CA

Study Number: 97-166-1751, 97-166A-1751

Date of Study Initiation: 6/6/97

Drug Lot/Batch Number(s): bevacizumab lot # M3-RD595, control antibody (rhuMab E25) lot # H-28-056

GLP Compliance (y/n): No.

QA Report (y/n): No

Key Findings:

In toxicology study 96-182-1751, ovarian and uterine weights were reduced in cynomolgus monkeys administered 10 or 50 mg/kg rhuMab VEGF twice weekly for 13 weeks. Follicular maturation arrest and absence of corpora lutea were also noted. These studies were carried out to further investigate the effect of VEGF inhibition on ovarian function due to the clinical relevance for fertility.

A preliminary study was conducted to determine if human chorionic gonadotropin (hCG) would cause ovulation in rabbits. The sponsor states that no differences were observed between saline and hCG-treated animals in this study. Therefore, no findings are discussed for study 97-166. The purpose of study 97-166A was to determine if treatment with bevacizumab had any effect on hCG-induced responses. Three groups were assigned with 8 animals per group: group 1 received bevacizumab vehicle, group 2 received 50 mg/kg control antibody (E25)

Methods:

Species/strain: Female New Zealand white rabbits

#/sex/group or time point (main study): 8 female rabbits per group

Doses: See Table provided by the sponsor, below.

Route, formulation, volume, and infusion rate: Dose solutions were administered IV in a volume of 5 ml/kg. All rabbits received hCG on day 1. The test articles (rhuMab VEGF vehicle, E25 or rhuMab VEGF) were administered on days -3, 1, 4 and 7. On day 9, 4 rabbits from each group were euthanized. Remainder of the rabbits survived an additional 31 days followed by restimulation with hCG on Day 40. The recovery rabbits were sacrificed on day 48.

Age: 26 – 28 weeks at arrival

Study design:

| Group | No. of Animals | Test Material | Dose Volume (mL/kg) |
|-------|----------------|---------------------------------|---------------------|
| 1 | 8 | rhuMAb VEGF Vehicle | 5 |
| 2 | 8 | 50 mg/kg antibody control (E25) | 5 |
| 3 | 8 | 50 mg/kg rhuMAb VEGF | 5 |

Weight (nonrodents only): 3.5-4.5 kg

Body weights:

Body weights recorded twice weekly throughout the study.

Hormone levels:

Blood was drawn for from all animals detection of progesterone and estradiol levels on days -4, -1, 2, 5 and 8. Blood was collected from the recovery animals on days 39, 44 and 47 (corresponding to days -1, 5 and 8 after the second hCG administration.

Gross pathology:

Ovaries were examined at necropsy for fluid-filled cysts, raised white (corpora lutea) or black (corpora hemorrhagica) nodules.

Organ weights: Ovaries and uteri were weighed at necropsy.

Histopathology: Complete Battery: Yes (), No (X)--explain

This was a research study limited to analysis of hormone levels in response to administration of hCG in rabbits treated with rhuMAb VEGF.

Other:

Results:

Mortality: All animals survived to scheduled sacrifice.

Body weights:

No changes in body weight attributable to study drug administration were noted.

Hormone levels:

The ELISA used to detect estradiol levels was not sensitive enough to determine estradiol levels prior to or after hCG administration. Progesterone levels could be detected only after serum samples were concentrated. Serum progesterone levels prior to hCG administration were approximately 0.5 ng/ml. On days 5 and 8, progesterone levels of animals treated with rhuMAb VEGF were significantly lower than controls. Graphic representation included in the BLA (actual numbers not provided) suggest that the reduction in progesterone levels for animals treated with rhuMAb VEGF approached 50% on day 5 and began to recover slowly to about a 25% reduction on day 8. The recovery animals showed a trend toward persistence of the reduced progesterone levels in response to hCG stimulation but the differences were not statistically significant. The line listings for the data were not included so statistics could not be confirmed.

Gross pathology: No macroscopic findings were reported.

Organ weights:

Ovarian and uertine weights were reduced in animals treated with rhuMAb VEGF compared to controls. The ovaries from bevacizumab treated animals had reduced surface follicles, corpora lutea and corpora hemorrhagica compared to controls. The

ovaries and uteri of the recovery animals appeared similar for all groups at necropsy but reduced weights for the bevacizumab treated animals persisted..

Histopathology:

Although surface follicles and corpora lutea were reduced, pre-ovulatory follicles were observed near the surface of the ovary in study drug treated rabbits indicating arrested development at that stage. The recovery animals showed no histological changes that could account for the reduced organ weights.

Reviewer Comments: This non-GLP study was performed as an exploratory research study to investigate the effect of bevacizumab treatment on ovarian function. The results indicate that treatment with the study drug inhibits ovulation. There appears to be a trend toward recovery but full recovery is not documents in this study. Independent assessment of group differences could not be made because actual data from the study was provided in this document.

2.4.2.6. Study Title: Hemolytic potential and blood compatibility testing with rhuMAB VEGF

Testing Facility and Location: /

Study Number: 96-221-1751
 Date of Study Initiation: 9/19/96
 Drug Lot/Batch Number(s): Bevacizumab Lot No. M3-RD595, vehicle Lot No. M3-RD588
 GLP Compliance (y/n): Yes.
 QA Report (y/n): Yes.

Key Findings:

This study was performed as an early exploratory study to assess the hemolytic potential of rhuMAB VEGF for cynomolgus monkey and human whole blood and the compatibility of rhuMAB VEGF for cynomolgus monkey and human serum and plasma. Hemolytic potential and blood compatibility tests were performed on rhuMAB VEGF (Lot No. M3-RD595) at a concentration of 10 mg/mL. After equal dilution with whole blood, serum, or plasma, the final test material concentration was 5 mg/mL. Identical testing was performed on rhuMAB VEGF Vehicle (Lot No. M3-RD588).

The hemolytic potential was evaluated by measuring the concentration of hemoglobin in the supernatant plasma of a mixture of equal volumes of rhuMAB VEGF or rhuMAB VEGF Vehicle and cynomolgus monkey or human whole blood that had been incubated for 40 minutes at 38°C.

Compatibility with serum, plasma, or both was determined by the absence of precipitation or coagulation in a mixture of equal volumes of rhuMAB VEGF or rhuMAB VEGF Vehicle and cynomolgus monkey or human serum or plasma that had been incubated for 25 minutes at room temperature (22.1°C).

Results indicated that rhuMAB VEGF (at a final concentration of 5 mg/mL) and rhuMAB VEGF Vehicle (at a dilution equivalent to a final concentration of 5-mg/mL rhuMAB VEGF) do not cause hemolysis of cynomolgus monkey or human erythrocytes and are compatible with cynomolgus monkey and human serum and plasma.

2.4.2.7. Study Title: Effects of rhuMAB VEGF on physal dysplasia in young rabbits

Testing Facility and Location: Genentech, Inc.
Study Number: 98-075-1751
Date of Study Initiation: 4/7/98
Drug Lot/Batch Number(s): Bevacizumab lot #M3-RD595, vehicle lot #M3-RD588,
rhuMAB E-25 (IgG1 isotype control) lot #E98624.
GLP Compliance (y/n): No.
QA Report (y/n): No.

Key Findings: This study was performed to establish a reproducible dose-response relationship between rhuMAB VEGF (bevacizumab) and expected physal dysplasia in young growing rabbits (42 days old). Physal dysplasia was observed in cynomolgus monkeys (studies 96-181-1751, 96-182-1751). In addition, reduced wound healing capacity was noted in a rabbit model of primary wound healing. This study was performed in an effort to bridge the two studies to determine if equivalent doses could cause both adverse effects. The results showed that, at doses up to 75 mg/kg in the rabbit, bevacizumab treatment resulted in thickening of the femur and tibia growth plates with hypertrophied and greater numbers of chondrocytes. However, the effects on the bone growth plates was much less severe than those seen in the cynomolgus monkey model treated with similar dose ranges. In considering those data, it should be noted that rabbit has approximately 8 fold lower affinity for bevacizumab than primates. Of note is that the doses used in this study (up to 75 mg/kg) in rabbits are over 30X greater than those that resulted in reduced wound healing capacity in the same species (2mg/kg).

Methods:

Species/strain: Female New Zealand White rabbits

#/sex/group or time point (main study): 3 females per group

Doses: Five dose groups, each containing 3 animals. See Table, below provided by the sponsor.

Route, formulation, volume, and infusion rate: The dose was administered IV through the marginal ear vein in a volume of 7.5 ml/kg over 2 minutes.

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Study Design:

| Group | Rabbit # | Administered |
|-------|----------|--|
| 1 | 1-1 | rhuMAb VEGF Vehicle |
| | 1-2 | rhuMAb VEGF Vehicle |
| | 1-3 | rhuMAb VEGF Vehicle |
| 2 | 2-4 | 10 mg/kg rhuMAb VEGF |
| | 2-5 | 10 mg/kg rhuMAb VEGF |
| | 2-6 | 10 mg/kg rhuMAb VEGF |
| 3 | 3-7 | 50 mg/kg rhuMAb VEGF |
| | 3-8 | 50 mg/kg rhuMAb VEGF |
| | 3-9 | 50 mg/kg rhuMAb VEGF |
| 4 | 4-10 | 75 mg/kg rhuMAb VEGF |
| | 4-11 | 75 mg/kg rhuMAb VEGF |
| | 4-12 | 75 mg/kg rhuMAb VEGF |
| 5 | 5-13 | 75 mg/kg rhuMAb E-25 (isotype control) |
| | 5-14 | 75 mg/kg rhuMAb E-25 (isotype control) |
| | 5-15 | 75 mg/kg rhuMAb E-25 (isotype control) |

Animals were dosed on days 1, 4, 7, and 10, then sacrificed on day 14.

Age: young, 42 days old

Weight (nonrodents only): 1.27-1.46

Clinical signs:

Animals were observed daily for general appearance.

Body weights:

Body weights were recorded on dosing days.

Gross pathology: At sacrifice, the right femur and right humerus were collected and fixed in formalin.

Organ weights (specify organs weighed if not in histopath table): Not done.

Histopathology: Complete Battery: Yes (), No (X)--explain

Only femur, tibia and humerus growth plates were evaluated histologically. This study was performed as an exploratory study to investigate specifically the effect of bevacizumab on bone growth.

Results:

Mortality:

All animals survived to scheduled sacrifice.

Clinical signs:

None reported.

Body weights:

No apparent body weight effects attributable to the study drug administration were observed.

Gross pathology:

Right femurs and tibias were collected at necropsy.

Organ weights:

Not Done.

Histopathology:

Histopathology was performed on distal femoral and proximal tibial physis. Examination showed both proliferating and hypertrophied chondrocytes contributing to thickened growth plates of the animals treated with bevacizumab compared to control groups. No inhibition of vascular invasion of the growth plates was not observed in doses up to 75 mg/kg. In monkeys, this effect was noted at doses as low as 5 mg/kg. Although doses of 2 mg/kg resulted in reduced wound healing capacity in rabbits, doses up to 75 mg/kg resulted in only partial inhibition of long bone growth plates.

Reviewer Comments: The study shows less adverse effects on the long bone growth plates in the rabbit compared to the results at lower doses in the monkey. However, the doses cannot be directly compared between species because bevacizumab is known to have approximately 8 fold lower affinity in rabbits than primates. In addition, no data were provided on serum levels of bevacizumab during this study. It is of interest that the doses used for this study were much larger than those that resulted in reduced wound healing capacity in the same species.

2.4.2.8. Study Title: Study to determine the deposition of rhuMAb VEGF in the kidney

| | |
|--------------------------------|---|
| Testing Facility and Location: | Genentech, Inc. |
| Study Number: | 99-537-1751 |
| Date of Study Initiation: | 1/10/00 |
| Drug Lot/Batch Number(s): | bevacizumab lot #M3-RD-595, vehicle lot #M8-RD560 |
| GLP Compliance (y/n): | No. |
| QA Report (y/n): | No. |

Key Findings: This exploratory study was performed to assess the potential for deposition of rhuMAb VEGF in the kidney. Results from the clinical studies indicate that treatment of individuals with impaired renal function with bevacizumab may exacerbate the condition. Two rabbits per group were given doses up to 100 mg/kg, IV on days 1 and 3 of this 5 day study. No changes attributable to study drug administration were noted for any of the limited parameters examined (including kidney histopathology). See reviewer's comments, below.

Methods:

Species/strain:

New Zealand white rabbits. The rabbits used for this study were transferred from another study (#99-476). The animals had previously been administered surrogate antibody. Based upon the knowledge of the serum half-life of MHM23 in rabbits, no MHM23

would have been present in the serum of these animals at initiation of dosing with rhuMab VEGF, and the animals having been exposed to MHM23 would not affect the current study.

#/sex/group or time point (main study): 8 females, 2/group

Doses: 0, 2, 10 and 100 mg/kg

Route, formulation, volume, and infusion rate: See study design table provided by the sponsor, below.

Satellite groups used for toxicokinetics or recovery: N/A

STUDY DESIGN

| Group # | Administered | Dosing regimen | # of animals |
|---------|--------------------------|--|--------------|
| 1 | rhuMab VEGF Vehicle | Single IV bolus injection on Days 1 and 3 | 2 |
| 2 | 2 mg/kg rhuMab VEGF | Single IV bolus injection on Days 1 and 3 | 2 |
| 3 | 10 mg/kg rhuMab VEGF | Single IV bolus injection on Days 1 and 3 | 2 |
| 4 | 100 mg/kg rhuMab VEGF | 15 minute IV infusion on Days 1 and 3 | 2 |

Animals were sacrificed on day 5.

Weight (nonrodents only): 3.28-3.44

Clinical signs:

Observations were made daily for general appearance.

Body weights:

Body weights were recorded in the days the rabbits were dosed.

Food consumption:

Food consumption was not measured, but the presence of feces was noted daily as an indication that the rabbits were eating.

Clinical chemistry:

Blood samples were taken from all animals prior to dosing on day 1 and prior to sacrifice on day 5.

Urinalysis:

Urine was collected from all animals at necropsy on day 5.

Gross pathology:

Necropsy was performed n day 5. No gross observations were recorded. Only the kidney was harvested.

Organ weights (specify organs weighed if not in histopath table): None done.

Histopathology: Complete Battery: Yes (), No (X)—explain

Only kidney was examined. This study was performed as an exploratory study to address potential for kidney toxicity specifically.

Toxicokinetics: N/A

Results:

Mortality:

All animals survived to scheduled sacrifice.

Clinical signs:

o clinical signs attributable to study drug administration were noted.

Body weights:

No alterations in body weight gain attributable to study drug administration were noted.

Food consumption:

No effects of the study drug on food consumption were noted.

Clinical chemistry:

No trends attributable to study drug administration were noted for clinical chemistry. No statistical analysis was possible because only 2 animals per group were studied.

Urinalysis:

No urinalysis results are provided with this study report.

Gross pathology:

No gross pathology examination

Organ weights:

No organ weights were taken

Histopathology:

No findings related to study drug administration were noted. Only kidney was examined.

Reviewer Comments:

This study was intended to explore the potential for administration of bevacizumab to contribute to reduced kidney function by deposition of antibody in the kidney. No antibody deposition was noted by immunohistochemical analysis. However, study duration (5 days) was possibly too short for such deposition to be observed and animal group size was too small to draw firm conclusions.

2.4.2.9. Study Title: The effects of anti-VEGF in New Zealand white rabbits with cisplatin-induced mild renal injury.

| | |
|--------------------------------|---|
| Testing Facility and Location: | Genentech, Inc. |
| Study Number: | 01-220-1751 |
| Date of Study Initiation: | 5/3/02 |
| Drug Lot/Batch Number(s): | Bevacizumab lot #L9838AX, vehicle lot #L9861A |
| GLP Compliance (y/n): | No. |
| QA Report (y/n): | |

Key Findings: The purpose of this study was to determine the effects of rhuMAb VEGF in a cisplatin-induced mild renal injury model in New Zealand White (NZW) rabbits. Cisplatin, a known nephrotoxin, is used clinically to treat many types of cancer and is expected to be used in combination with bevacizumab in treatment of colorectal cancer. Therefore, this study was

carried out to explore the potential for bevacizumab treatment to exacerbate kidney damage when used in combination with cisplatin.

Although mild renal damage was detected in groups receiving cisplatin, no differences were detected in groups receiving bevacizumab in addition to cisplatin compared to those receiving cisplatin alone. These data suggest that administration of rhuMAb VEGF does not exacerbate the renal damage induced by treatment with cisplatin or alter coagulation parameters in NZW rabbits.

Methods:

Species/strain:

New Zealand white rabbits, male (normal rabbit serum chemistry control data were used as historical control for this study and compiled from previous studies 01-220-1751 and 01-193-1751. All rabbits in the historical control group were male, NZW rabbits purchased from _____ (body weight 2.5-3.5 kg). The control data represent results from 52 naïve animals.

#/sex/group or time point (main study):

8 rabbits were purchased from _____ 23 were transferred from study #01-145-1751 and 01-133-1751

Doses: See study design table provided by the sponsor, below.

Route, formulation, volume, and infusion rate:

Study drug was administered IV on days 1, 3, 5, 8, 10, and 12.

Study Design

| Group | Rabbit No. | Treatment | Dosing Regimen | Dose Volume |
|-------|----------------------------|---------------------|--|---------------------------------|
| 1 | 1, 2, 3, 4, 5, 6, 7 | Saline | Saline on Study Days 1, 3, 5, 8, 10, 12 | 1 mL/kg for saline |
| | | rhuMAb VEGF Vehicle | rhuMAb VEGF Vehicle on Study Days 8, 10, 12 | 2 mL/kg for rhuMAb VEGF Vehicle |
| 2 | 9, 10, 11, 12, 13, 14, 15 | Cisplatin | 1 mg/kg cisplatin on Study Days 1, 3, 5, 8, 10, 12 | 1 mL/kg for cisplatin |
| | | rhuMAb VEGF Vehicle | rhuMAb VEGF Vehicle on Study Days 8, 10, 12 | 2 mL/kg for rhuMAb VEGF Vehicle |
| 3 | 17, 18, 19, 20, 21, 22, 23 | Cisplatin | 1 mg/kg cisplatin on Study Days 1, 3, 5, 8, 10, 12 | 1 mL/kg for cisplatin |
| | | rhuMAb VEGF | 50 mg/kg rhuMAb VEGF on Study Days 8, 10, 12 | 2 mL/kg for rhuMAb VEGF |
| 4 | 25, 26, 27, 28, 29, 30, 31 | Saline | Saline on Study Days 1, 3, 5, 8, 10, 12 | 1 mL/kg for saline |
| | | rhuMAb VEGF | 50 mg/kg rhuMAb VEGF on Study Days 8, 10, 12 | 2 mL/kg for rhuMAb VEGF |

Cisplatin or saline was administered into the marginal ear vein at a dose volume of 1 mL/kg on days 1, 3, 5, 8, 10, and 12. rhuMAb VEGF or rhuMAb VEGF Vehicle was administered into the marginal ear vein at a dose volume of 2 mL/kg on days 8, 10, and 12.

Animals were sacrificed on day 14.

Satellite groups used for toxicokinetics or recovery: N/A

Weight (nonrodents only): 2.1-2.9 kg at initiation of dosing.

Clinical signs:

Animals were observed for general appearance every other day during the dosing period.

Body weights:

Body weights were recorded twice (on two separate days) prior to study initiation, and on Days 1, 3, 5, 6, 8, 10, 12, and 13. Food supplements were given to animals dosed with cisplatin to prevent treatment-induced weight decrease.

Hematology:

Blood samples for hematology were collected prior to study initiation and at necropsy.

Clinical chemistry:

Baseline samples were collected twice, on 2 separate days, prior to study initiation, and on days 6, and 13.

Urinalysis:

Urine was collected from 2 animals per group. Samples were collected twice, 4 days apart prior to study initiation and on days 6, and 13.

Gross pathology:

Samples (cross-sections) of right and left kidney were collected at necropsy.

Organ weights (specify organs weighed if not in histopath table): Organ weights were not recorded.

Histopathology: Complete Battery: Yes (), No (X)—explain:

Histopathologic examination was performed only on samples of kidney tissue. This study was designed to address kidney function issues only.

Toxicokinetics: N/A

Results:

Mortality:

All animals survived to scheduled sacrifice.

Clinical signs:

All animals appeared healthy throughout the study duration.

Body weights:

- The mean weight of the control group continued to increase throughout the study.
- Cisplatin treatment alone reduced the average weight gain from day 0 to day 13 in comparison to the control group (gains of 0.38 kg and 0.11 kg, respectively).
- Combined treatment with cisplatin and rhuMAb VEGF resulted in a loss of body weight (-0.02 kg) over the study period.
- Treatment with rhuMAb VEGF alone did not result in a significant change in mean body weight gain on day 13 compared to control.

Hematology:

- A trend toward reduced WBC was noted in groups treated with cisplatin. However, no difference between was noted between the cisplatin alone group and

the cisplatin plus bevacizumab group. No difference was noted between the bevacizumab alone group and control.

- WBC counts were reduced from baseline at day 13 for all groups but no difference between groups were noted.

Clinical chemistry:

- BUN values were significantly elevated on day 13 in cisplatin treated groups compared to control. Treatment with bevacizumab alone did not alter BUN values compared to control. No differences were noted between groups treated with cisplatin or cisplatin plus bevacizumab.
- Creatinine levels increased significantly by day 13 in cisplatin treated groups (2-fold increase over baseline and historical control) but cisplatin plus bevacizumab did not result in any difference from cisplatin alone. Bevacizumab alone did not result in values different from control.

Urinalysis:

- Due to highly variable results, no trend in urinary protein levels attributable to study drug could be determined.
- Urinary specific gravity values were significantly lower than baseline and historical control values by day 6 for cisplatin treated animals. By day 13 the cisplatin plus bevacizumab showed significantly lower specific gravity than cisplatin alone as well as control. The group receiving bevacizumab alone showed no effect of study drug compared to baseline or historical control.

Gross pathology: No remarkable findings are reported.

Organ weights: Not reported.

Histopathology:

Histological analysis was performed on kidney tissue only.

- Cisplatin treated animals showed dilated medullary tubules and scattered interstitial inflammatory changes. No additional pathology was observed in animals treated with cisplatin plus bevacizumab.

Reviewer Comments: The changes in serum chemistry, hematology and kidney histology noted in this study are expected in response to cisplatin treatment, as this agent is known to cause renal damage. For the most part, bevacizumab did not appear to exacerbate the cisplatin effects. The added reduction in urinary specific gravity reported in the group receiving cisplatin plus bevacizumab was very small and not likely to be of significant biological relevance in the context of this study. However, the potential for exacerbation of kidney affects with the combination of cisplatin and bevacizumab cannot be ruled out. Use of this drug combination in humans should include monitoring for enhanced renal damage.

2.4.2.10. Study Title: Effect of rhuMAb VEGF on thrombus formation in a rabbit venous thrombosis model

Testing Facility and Location: Genentech, Inc.

Study Number: 01-125-1751
 Date of Study Initiation: 3/12/01
 Drug Lot/Batch Number(s): Bevacizumab Lot # L9838A, vehicle Lot #L9856A
 GLP Compliance (y/n): No.
 QA Report (y/n): No.

Key Findings:

Several thrombotic episodes were observed in Phase 2 clinical trials of rhuMAb VEGF use in treatment of cancer patients. The majority of the thromboses occurred in the venous system. This study was performed to investigate the effect of rhuMAb VEGF in a rabbit model of venous thrombosis.

The objective of the study was to determine the effect of rhuMAb VEGF on clot formation in a rabbit jugular vein at the site of stenosis and damage, and to measure whole blood and plasma *ex vivo* for signs of hypercoagulability or for the presence of a systemic prothrombotic state.

Rabbits were given 75 mg/kg rhuMAb VEGF or rhuMAb VEGF Vehicle intravenously each day for 8 consecutive days. Following the final dose, a thrombus was allowed to form in the jugular vein by application of a flow-reducing stricture immediately proximal to the site of clamp-induced damage. The presence or absence of occlusion was noted, as well as time to occlusion and weight of the excised clot. Cuticle bleeding time was measured. Assays of coagulation and fibrinolysis were then performed *ex vivo*.

Study results indicate that rabbits treated with rhuMAb VEGF do not develop a prothrombotic state in this venous thrombosis model nor is such a state demonstrable by the biomarkers measured.

Methods:

Species/strain: Adult, male New Zealand white rabbits from _____

#/sex/group or time point (main study): 2 groups, 7 rabbits per group

Doses: 75 mg/kg of bevacizumab or vehicle

Route, formulation, volume, and infusion rate: IV, Study drug was administered by slow infusion into the marginal ear vein at a volume of 3.0 ml/kg.

Satellite groups used for toxicokinetics or recovery: Not Done.

Weight (nonrodents only): 3.8-4.2 kg

Study Design

| Group No. | No./Sex | Treatment |
|-----------|---------|-------------------------|
| 1 | 7/M | 0 (rhuMAb VEGF Vehicle) |
| 2 | 7/M | 75 mg/kg rhuMAb VEGF |

On day eight, surgery was performed for placement of a flow probe around the jugular vein. Three occluders were placed around the vein and branches to reduce flow to less than 5 ml/min. Using an atraumatic forcep, damage was induced to the internal and external jugular veins. Time to complete occlusion of the vessel was measured.

Following occlusion (or at 5 hours), the animals were sacrificed. The jugular vein was excised and the clot removed and weighed.

Clinical signs:

Rabbits were observed daily for signs of discomfort or adverse drug reactions and general appearance.

Body weights:

Body weights were recorded prior to study initiation and after the final dose prior to surgery.

Food consumption:

Food consumption was monitored qualitatively on a daily basis.

Ophthalmoscopy: Not done.

ECG: Not done.

Hematology:

Samples were collected prior to initiation of treatment, after the final dose on day 8, and at 2 and 4 hours after damage to the jugular vein. Parameters measured include: prothrombin time, activated partial thromboplastin time, whole blood recalcification time, activated clotting time, platelet aggregation to adenosine diphosphate, cuticle bleeding time and d-dimer concentrations. Complete blood cell counts were measured. Other hematology parameters included WBC, RBC, Hgb, HCT, MCV, MCH, MCHC, RDW, PLT, MPV.

Clinical chemistry: Not done

Urinalysis: Not done.

Gross pathology:

Limited to venous thrombosis only.

Organ weights (specify organs weighed if not in histopath table): Not done.

Histopathology: Complete Battery: Yes (), No (X)—explain

This study was exploratory in nature and addressed the issues of clot formation only. Immunohistochemical assay for von Willebrand Factor/Factor VIII complex was performed on sections of the jugular vein adjacent to the site of clot formation and on sections from the contralateral undamaged vessel.

Toxicokinetics: Not done

Results:

Mortality:

All animals survived to scheduled sacrifice.

Clinical signs:

No clinical signs attributable to study drug administration were noted.

Body weights:

No effect of treatment on body weight was noted.

Food consumption:

Food consumption was not effected by study drug administration.

Hematology:

- One animal from the vehicle group failed to fully occlude during the allowed 5 hour period.
- Average clot weight was 41 ± 29 mg for the vehicle group and 22 ± 13 mg.
- No difference in time to occlusion was noted between groups.
- No differences in hematology parameters were noted between groups.
- In both groups, a decrease in WBC levels was noted over the duration of the study. (41% for vehicle and 32% for bevacizumab)
- No differences between groups in cuticle bleeding time were noted.
- No differences between groups in any of the clotting time parameters were noted between groups.

Histopathology:

Immunohistochemical staining for von Willebrand Factor VIII was limited to the area of clot formation in the jugular vein. The intensity of staining was qualitatively not different between groups. No other histology was performed.

Reviewer Comments:

Treatment with bevacizumab in this study had no apparent effects on thrombus formation or clotting time in this rabbit model. The duration of this study was short and, it cannot be ruled out that treatment for longer duration may have an effect on thrombus formation. It is not known how predictive this model is for humans undergoing anti-tumor therapy.

2.4.2.11. Study Title Development of a full-thickness linear incision skin wound model in rabbits.

A. Dose range finding study of rhuMAb VEGF in a full- thickness linear incision skin wound model in rabbits.

B. Use of a full-thickness linear incision skin wound model in rabbits to assess that activity of rhuMAb VEGF preparations.

| | |
|--------------------------------|---|
| Testing Facility and Location: | Genentech, Inc. South San Francisco, CA 94080 |
| Study Number: | 97-373-1751, 97-373-A-1751, 97-373-B-1751 |
| Date of Study Initiation: | 12/12/97 |
| Drug Lot/Batch Number(s): | Bevacizumab lot Nos. M3-RD595 and 29154-04-A, Vehicle lot No. M3-RD588 |
| GLP Compliance (y/n): | No. |
| QA Report (y/n): | No. |

Key Findings:

The purpose of study 97-373 was to develop a full-thickness linear incision skin wound model in New Zealand white rabbits and to use the model in assessment of the capacity of bevacizumab to inhibit wound healing in this model. The purpose of study 97-373-A was to determine the dose of rhuMAb 1751 that would result in a significant effect on wound healing in the rabbit skin wound model. That dose would then be compared to rhuMAb VEGF 1754. The purpose of study 97-373-B was to compare two generations of rhuMAb VEGF (1751 and 1754)

by comparison of the capacity to inhibit wound healing. 1754 is a variant of rhuMAB VEGF with increased affinity for VEGF.

Results of this series of studies demonstrated a dose-related reduction in wound healing capacity after treatment with rhuMAB VEGF in this rabbit skin wound model at doses of 0.5 and 2.0 mg/kg. In addition, the studies demonstrated that rhuMAB VEGF has approximately twice the activity on wound healing than for drug variant 1754 than variant 1751 (as indicated by measurement of healed wound tensile strength) in spite of the longer calculated serum clearance for variant 1754 of approximately twice that of variant 1751.

2.4.3.12. Study Title: Dose response of 2nd generation rhuMAB VEGF in a full-thickness linear incision skin wound model in rabbits.

| | |
|--------------------------------|---|
| Testing Facility and Location: | Genentech, Inc. South San Francisco, CA 94080 |
| Study Number: | 99-009-1751 |
| Date of Study Initiation: | 1/26/99 |
| Drug Lot/Batch Number(s): | RhuMAB VEGF 1751 lot # M3-RD595, rhuMAB VEGF 1754 lot # 29154-04-A, vehicle lot # M3-RD588. |
| GLP Compliance (y/n): | No. |
| QA Report (y/n): | No. |

Key Findings:

The purpose of this study was to determine a dose response of 1754 rhuMAB VEGF in a full-thickness linear incision skin wound model in rabbits. The activity of 1754 rhuMAB VEGF was demonstrated in 97-373-B (See above). Results of that study showed that drug variant 1754 had approximately twice the inhibitory activity on wound healing compared to drug variant 1751. No differences in tensile strength of fully healed wounds were noted between the two groups receiving the lowest doses of 0.25 and 0.5 mg/kg in that study. In the current study, doses of drug variant 1754 included 0.5, 1.0 and 2.0 mg/kg were administered to groups of 42 day old female New Zealand white rabbits.

A clear dose response was achieved between the higher doses of drug variant 1754 used in this study. The group receiving 2.0 mg/kg had such fragile wound healing that no tensile strength could be determined. Significantly different wound strength values were obtained in comparing groups of animals receiving equivalent doses in mg/kg of the two variants. In comparison between wounds from animals treated drug variant 1751 and drug variant 1754, no difference was noted between 0.5 mg/kg of 1751 and 0.25 mg/kg of 1754. These are consistent with the conclusion that drug variant 1754 has twice the wound inhibition capacity of drug variant 1751.

2.4.3.13. Study Title: Model development to assess the anti-angiogenic effects of 1st generation rhuMAB VEGF (GN1751) administered by intravenous injection to cynomolgus monkeys.

Testing Facility and Location: \

Study Number: 99-158-1754
 Date of Study Initiation: 5/4/99
 Drug Lot/Batch Number(s): GN1751 lot # M3-TOX5, R-30793
 Vehicle lot # M3-TOX3
 GLP Compliance (y/n): No. (In general compliance.)
 QA Report (y/n): No.

Table
 Age and Weight of Animals Used in Study 99-158-1754

| Group | Animal ID | Test Material | Age (years) | Weight (kg) |
|-------|-----------|-----------------------|-------------|-------------|
| 1 | F10000M | rhuMab Vehicle | 3 | 3 |
| 3 | F10034M | 2.0 mg/kg rhuMab VEGF | 3 | 2.8 |

Each animal received four doses of the test or control article via intravenous injection into a saphenous vein. Doses were administered on Days -2, 1, 3 and 5 (relative to surgery), with the site of administration alternating between the left and right saphenous veins. The animals were sedated prior to dose administration on Days 1, 3 and 5 in conjunction with surgery (Day 1) and wound site observations.

On Day 1, each animal received 6 surgically-induced wounds. Each wound site was a 2-cm-length cutaneous incision (approximately 4 mm full thickness) made in a diagonal craniocaudal direction in the intrascapular region. The incisions were sutured and protected using standard techniques.

The animals were monitored for clinical signs including body weight, food consumption and general appearance and behavior as well as wound site inspection. On day 7, the animals were euthanized and the 6 wound sites were excised and examined histologically for estimation of blood vessel density.

Key Findings:

This study was performed to develop a model for assessment of the anti-angiogenic effects of 1st Generation rhuMab VEGF (GN1751) when administered by intravenous injection to cynomolgus monkeys prior to and following surgically induced wounds.

Intravenous administration of GN1751 at a dose level of 2.0 mg/kg was apparently well tolerated by the one cynomolgus monkey that received this material. No test-article-related effects on clinical signs or changes in the gross appearance of the wound sites were observed. Vessel length density was lower in the test-article-treated animal compared to the single control animal. There was a decrease in the force required for wound site separation in the test-article-treated animal as compared to the control animal. From these data it was concluded that development of vasculature at the wound site, and the associated wound healing, was reduced following treatment with the study drug. The data from the two monkeys used in this study was used as an indicator that this wound healing model for assessing the anti-angiogenic effects of rhuMab VEGF is appropriate.

2.4.3.14. Study Title: A study of the anti-angiogenic effects of 1st and 2nd generation rhuMab VEGF (GN1751 and GN1754) administered by intravenous injection to cynomolgus monkeys.

Testing Facility and Location:

Study Number: 99-159-1754
 Date of Study Initiation: 5/27/99
 Drug Lot/Batch Number(s): GN1751 lot # M3-TOX5, R-30793
 GN1754 lot # M3-TOX4, R-30792
 Vehicle lot # M3-TOX3
 GLP Compliance (y/n): No. (In general compliance.)
 QA Report (y/n): No.

Study design:

A total of 16 male cynomolgus monkeys, experimentally naïve and weighing 2.2 to 4.2 kg and ranging in age from 2.9 to 6.7 years at the outset of the study, were assigned to 6 treatment groups. Each animal received four doses of a test or control article via intravenous injection into a saphenous vein. Doses were administered on Days -2, 1, 3 and 5 (relative to surgery), with the site of administration alternating between the left and right saphenous veins. The study was performed as described above for study 99-158-1754.

The animals were observed twice daily for changes in general appearance and behavior, including daily qualitative assessment of food consumption, beginning 1 day prior to the first dose (Study Day -3) and continuing through Day 7. Body weights were measured one day prior to the first dose administration to calculate absolute dosing volumes. Incision sites were observed immediately following surgery on Day 1, and on Days 3 and 5. The incision sites were evaluated for changes in general appearance (i.e., reddening, swelling) and suture integrity.

The table below, provided by the sponsor, illustrates the study design:

| Group No. | Number of Animals | Test Material | Dose Level (mg/kg) | Dose Vol. (mL/kg) | Dose Solution Conc. (mg/mL) |
|-----------|-------------------|---------------|--------------------|-------------------|-----------------------------|
| 1 | 2 | Vehicle | 0 (control) | 1.0 | - |
| 2 | 3 | GN1751 | 0.5 | 1.0 | 0.5 |
| 3 | 2 | GN1751 | 2.0 | 1.0 | 2.0 |
| 4 | 3 | GN1754 | 0.25 | 1.0 | 0.25 |
| 5 | 3 | GN1754 | 0.5 | 1.0 | 0.5 |
| 6 | 3 | GN1754 | 1.0 | 1.0 | 1.0 |

Key Findings:

The purpose of the study was to assess and compare the anti-angiogenic effects of GN1751 and GN1754 when administered by intravenous injection to cynomolgus monkeys prior to and following surgical formation of linear incisions in the skin.

Both drug variations, at the doses used, appeared to be well tolerated. No study drug effects on clinical observations were noted. The two drug treatments resulted in differences in extent of wound healing (as indicated by tensitometric analysis). Dose-dependent reductions in wound healing were not demonstrated by either drug variant compared to vehicle control. Histological examination of the tissues did not indicate study drug-related differences. The sponsor indicates that interpretation of the data is limited by inter- and intra-animal variation and the small number of animals used. No clear conclusions were drawn.

2.4.2.15 Study Title: The immunohistochemical investigation of the cross-reactivity of rhuMAb VEGF with Cynomolgus monkey and rabbit tissues

Testing Facility and Location: /

Study Number: 96-327-1751
 Date of Study Initiation: 10/29/96
 Drug Lot/Batch Number(s): bevacizumab lot #M3-RD595
 Biotinylated bevacizumab lot # 25020-3
 Negative control antibody (rhuMAb E25) lot # 25020-8, lot #25019-73, lot # 25020-83
 GLP Compliance (y/n): Yes.
 QA Report (y/n): Yes.

Key Findings:

The purpose of this study was to assess the binding capacity of rhuMAb VEGF, the recombinant humanized monoclonal antibody against vascular endothelial growth factor (AVASTIN) to tissues from rabbit and Cynomolgus monkeys. These are the two main species used in toxicology studies to support approval of AVASTIN. The goal was to identify tissue binding capacity of the test article. The results of this study are intended to compliment those from study #96 326-1751. In this study, the AVASTIN molecule was biotinylated and tested against various tissues from Cynomolgus monkeys and New Zealand white rabbits using the immunohistochemical technique. The negative control antibody used was rhuMAb E25 also conjugated to E25 is a humanized antibody with constant region structure similar to that of AVASTIN, with specificity for human IgE.

The positive control was rat skin injected subcutaneously *in vivo* with _____ loaded with VEGF. Normal rat skin without the _____ was used as an internal negative control. Study design is in compliance with appropriate guidance. The test article was tested at 2 dilutions (10 and 400µg/ml) on tissues from 3 cynomolgus monkeys and rabbits. To test the effects of biotinylation on binding capacity of the test article, additional rabbit tissues were stained with a non-biotinylated version of AVASTIN using the indirect immunohistochemistry technique. The tissues examined include: kidney, liver, lung, ovary, placenta, retina, skin, spleen, uterus for the rabbit; for the monkey: adrenal, bladder, bone marrow, blood cells cerebral cortex, cerebellum, colon, duodenum, breast, fallopian tube, heart, kidney, liver, lung, lymph node, ovary, pancreas, prostate pituitary retina, skin, spleen, spinal cord, striated muscle, testis, thymus, thyroid, ureter, endometrium and cervix.

Results:

The biotinylated AVASTIN molecule, supplied by the sponsor was compared to binding characteristics with the non-biotinylated version on rabbit tissues. Testing results confirmed that the biotinylation process did not affect the binding capabilities of AVASTIN. The rat positive-control tissue showed appropriate specific staining. No cross-reactivity was observed in any tissues analysed in this study. All results were either clearly negative or judged to be non-specific.

2.4.2.16 Study Title: The immunohistochemical investigation of the cross-reactivity of rhuMAb VEGF with human tissues

Testing Facility and Location: /

Study Number: 96-326-1751

Date of Study Initiation: 10/2/96

Drug Lot/Batch Number(s): biotinylated bevacizumab lot # 25020-3
 Negative control antibody lot # 25020-8, 25019-73, 25020-83

GLP Compliance (y/n): Yes.

QA Report (y/n): Yes.

Key Findings:

Biotinylated bevacizumab was used as primary antibody for immunohistochemical staining to determine the staining patterns of the study drug in 36 human tissues. Two concentrations of bevacizumab were used on tissues from 1-4 donors. The results showed appropriate staining of the positive control rat tissues and only non-specific staining for the negative control (rhuMAbE25). No specific staining was observed with the biotinylated study drug. These results are consistent with available biochemical and pharmacological data regarding bevacizumab binding indicating that this drug binds only circulating VEGF.

Methods:

A panel of _____ harvested from 1 to 4 donors, _____ were obtained from Genentech

The study design is in compliance with appropriate regulatory guidance. The positive control was rat skin injected subcutaneously *in vivo* with _____ loaded with VEGF. Normal rat skin without the _____ was used as an internal negative control. Each tissue was stained with either biotinylated bevacizumab or biotinylated E25 (control antibody) at two concentrations (10 and 400 µg/ml).

The effects of biotinylation on binding capacity of the test article was tested by staining tissues with a non-biotinylated version of AVASTIN using the indirect immunohistochemistry technique as described for study 96-327, above. The results confirmed that biotinylation did not alter binding capacity of bevacizumab in these studies.

Results:

Of the tissues tested, no specific staining was observed. Non-specific staining was seen associated with blood vessels and endothelium in the brain. The sponsor states that this non-specific staining is due to the presence of lipofuscin pigment.

Reviewer Comments:

The results of this study are in agreement with the expected outcome for bevacizumab binding. This monoclonal antibody is reported to bind only circulating VEGF.

Genotoxicity

No genotoxicity studies were performed.

2.4.3. Carcinogenicity

No carcinogenicity studies were performed.

2.4.4.1. Study Title

2.5. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

2.5.1. Fertility and Early Embryonic Development

No studies of this type were performed.

2.5.2. Embryo-Fetal Development

2.5.2.1. Study Title

Intravenous Dose-Range Developmental Toxicity Study of rhuMab VEGF in Rabbits

Testing Facility and Location:

Study Number: 01-223-1751
Date of Study Initiation: 6/29/01
Drug Lot/Batch Number(s): Study Drug Lot # L9847A; placebo Lot # K9818A
GLP Compliance (y/n): Yes.
QA Report (y/n): Yes.

Key Findings:

This study was performed as a preliminary dose ranging study to determine the appropriate dosing for adequate design of a more detailed toxicity study addressing the potential for bevacizumab treatment of pregnant dams to affect embryo-fetal development. Thus, the extent of the analyses is limited. The major findings for this study are that the study drug does cross the placenta and effects are observed at the doses used in this model. Bevacizumab was detected in fetal serum and amniotic fluid. The effects observed in this study that appear to be

study drug dependent are: an increase in fetal resorptions and one gross fetal deformity (downward flexed forepaw) in the 100 mg/kg dose group.

Methods:

Species/strain: New Zealand white rabbits, 5-5.5 months old.

Number/sex/group: A total of 35 timed pregnant rabbits were used. The animals were assigned to 7 groups, 5/group.

Doses: 0, 30, 100 mg/kg

Route, formulation, volume, and infusion rate: The study drug was infused IV at a rate of 2 ml/min. High dose: 100 mg/kg.

Satellite groups used for toxicokinetics or recovery: None.

Study design:

Unique study design or methodology (if any): See the table below provided by the sponsor.

2.7.1. Dose Administration

| Dose Group | Dose (mg/kg/dose) | Concentration (mg/ml) | Volume (ml/kg) | Injection Rate (ml/min) | Number of Rabbits | Day of Arrival (DCI) | Days of Dosing (DCIs) | Assigned Rabbit Numbers |
|------------|-------------------|-----------------------|----------------|-------------------------|-------------------|----------------------|-----------------------|-------------------------|
| I | 0 (Placebo) | 0 | 4 | 2 | 5 | 1 | 6, 9, 12, 15, 18 | 6701-6705 |
| II | 10 | 2.5 | 4 | 2 | 5 | 3 | 6, 9, 12 | 6706-6710 |
| III | 30 | 7.5 | 4 | 2 | 5 | 2 | 6, 9, 12 | 6711-6715 |
| IV | 100 | 25.0 | 4 | 2 | 5 | 2 | 6, 9, 12 | 6716-6720 |
| V | 10 | 2.5 | 4 | 2 | 5 | 1 | 12, 15, 18 | 6721-6725 |
| VI | 30 | 7.5 | 4 | 2 | 5 | 1 | 12, 15, 18 | 6726-6730 |
| VII | 100 | 25.0 | 4 | 2 | 5 | 1 | 12, 15, 18 | 6731-6735 |

The test article was considered 100% active/pure for the purpose of dose calculations.

This study was performed on two groups of rabbits that arrived on different dates. See table from the BLA, above. Study drug was administered during the period of organogenesis on days 6, 9, 12, 15 and 18 (Group I, Days 6, 9 and 12 (Groups II, III, IV) or days 12, 15, and 18 ((Groups V, VI and VII). The sponsor reports that the doses used were based on previous experimental evidence taking into consideration the difference in pharmacokinetics between rabbit and human as well as the fact that antibodies have been found to develop between eight and eleven days after initiation of dosing. For these reasons, abbreviated dosing was incorporated into this study.

Parameters and endpoints evaluated:

In-life observations (Dams):

Clinical signs:

Animals were observed twice daily for general appearance and viability. They were examined pre-dose and 1 hour post-dose for effects of the test article, abortions, premature deliveries and deaths on dosing days and once daily for non-dosing days.

Body weight:

Body weights were recorded on gestational day 0, on gestational day 5 (on arrival at the testing facility) and daily through day 29.

Food consumption:

Food consumption was monitored qualitatively on a daily basis.

Blood collection:

Two ml of blood was collected from each pregnant doe on day 5 of presumed gestation and prior to sacrifice on DG 29. Blood was collected and pooled from fetuses on the day of C-section (DG 29).

Toxicokinetics:

Blood was collected from pregnant does and fetuses as described above. Samples of amniotic fluid was also collected from each uterine horn at the time of C-section.

Terminal and necroscopic evaluations: C-section data: At necropsy, thoracic, abdominal and pelvic viscera were examined for gross lesions. Any gross lesions were preserved for possible future evaluation. All other tissues were discarded. The number of corpora lutea was determined. The uterus of each rabbit was excised and examined for pregnancy, number and distribution of implant sites, early and late resorptions, and live and dead fetuses.

Offspring:

Live fetuses were defined as a fetus that responded to stimuli. Non-responding fetuses were designated as dead. The difference between late resorption and dead term fetus was determined by the amount of autolysis observed. Fetuses were weighed and examined for gross external deformities. Live fetuses were euthanized by IP injection of sodium pentobarbital. All fetuses were examined internally to identify sex.

Results:

In-life observations (Dams):

Mortality: All animals survived to scheduled sacrifice.

Clinical signs:

No clinical findings related to the study drug were observed. Scant feces was noted for 1 out of 5 animals in the placebo group and 2 out of 5 animals in group VIIc (100mg/kg). Liquid feces was noted for 1 out of 5 animals in group VIc (30mg/kg).

Body weight:

Body weight gain was slightly reduced for group VIIc (100 mg/kg on days 12,15,18). This correlated with slightly reduced food consumption for this group. All treated animals appear to have a slightly slower rate of weight gain than the placebo group. However, at sacrifice, the final body weights were not significantly different from placebo for any treated groupgroup.

Food consumption:

No significant difference in food consumption between groups was noted. Food consumption for group VII was slightly reduced (stated above) compared to placebo but this difference did not reach statistical significance.

Toxicokinetics:

In pregnant female rabbits, concentrations of rhuMAb VEGF were detected in maternal serum, fetal serum, and amniotic fluid of most animals at Day 29 of

gestation (11 or 17 days after the last dose) following rhuMAb VEGF administration on either Days 6, 9, and 12 or Days 12, 15, and 18 of gestation.

Antibodies against rhuMAb VEGF were detected in some samples on Day 29 of gestation. Due to assay interference by the presence of study drug, the data should be interpreted with caution.

Terminal and necroscopic evaluations: C-section data:

Fetal resorptions (early): 5 early and 5 late resorptions were noted for 2 dams in group IIb (10 mg/kg). One late resorption in one dam for group IIIb (30 mg/kg). One early resorption for one dam was noted for group VIa (30 mg/kg). One late resorption for one dam in group Va (10 mg/kg). 4 late resorptions for 2 dams for group VIIa (100 mg/kg).

| Group number | I | II | III | IV | V | VI | VII |
|-------------------------------|---|-----------|---------|----|---------|---------|----------|
| % Resorbed conceptuses/litter | 0 | 20.2±39.4 | 2.0±4.5 | 0 | 2.2±5.0 | 2.2±5.0 | 8.2±13.1 |

No resorptions were noted for the placebo group. All placentae appeared grossly normal.

The intermediate lobe of the lung was absent for one placebo doe and one doe from group Va (10 mg/kg). This finding is apparently not unusual for this species (sponsor information).

Offspring: One gross malformation was observed in the offspring from all groups. The left forepaw was flexed downward: one fetus, group VIIc (100 mg/kg). No microscopic examinations were performed for this study.

Reviewer Comments:

This study was intended as a preliminary study to determine appropriate dosing for a more in depth embryo-fetal developmental toxicity study. Thus, the extent of the analyses is limited. The major findings for this study are that the study drug does cross the placenta and effects are observed at the doses used in this model.

2.5.2.2. Study Title Intravenous Developmental Toxicity Study of rhuMab VEGF in Rabbits

Testing Facility and Location:

Study Number: 02-029-1751
 Date of Study Initiation: 3/22/02
 Drug Lot/Batch Number(s): Study drug Lot number: N9806AX; Placebo lot number: L9856A
 GLP Compliance (y/n): Yes.
 QA Report (y/n): Yes.

Key Findings:

This study was performed to assess the effects of bevacizumab on embryo-fetal development when administered IV to pregnant rabbits during the period of organogenesis. No clinical signs of toxicity were noted for the pregnant dams with the exception of an early (DG 6 and 7) significant reduction in mean body weight gain for the low dose group and a mean body weight loss for the middle and high-dose groups. This occurred after the first dose of the test article but all remaining body weight gains and body weights were comparable among the Placebo, 10, 30 and 100 mg/kg dose groups.

In contrast, clear evidence of a dose related teratogenic effect was identified upon examination of the 73 litters and over 600 fetuses. Multiple skeletal deformities were noted in the fetuses at all dose groups with increased incidence for the high dose group. A clear NOAEL for developmental toxicity was not defined in this study. From the data generated in this study the best estimate of NOAEL is less than 10 mg/kg/dose. The data indicate that bevacizumab can have a teratogenic effect on developing fetuses.

Methods:

Species/strain: New Zealand white rabbits

Number/sex/group: 103 timed pregnant rabbits were assigned to 4 groups (20/group). See study design, below.

Doses: 10, 30 and 100 mg/kg/day

Route, formulation, volume, and infusion rate:

Study drug was administered via catheter into the marginal ear vein as a slow bolus at a constant rate of 2 ml/min.

Satellite groups used for toxicokinetics or recovery: Rabbits assigned to part A and Part B were used for TK.

Study design:

| Dose Group | Dose ^a (mg/kg/day) | Concentration (mg/mL) | Volume (mL/kg) | Injection Rate (mL/min) | Number of Rabbits | Assigned Rabbit Numbers | | |
|------------|-------------------------------|-----------------------|----------------|-------------------------|---|------------------------------------|-----------|-----------|
| | | | | | | Main Study | Part A | Part B |
| I | 0 (Placebo) | 0 | 4 | 2 | 20 ^b | 9401-9420 | NA | NA |
| II | 10 | 2.5 | 4 | 2 | 20 ^b + 5 ^c + 5 ^d | 9421-9440 | 9481-9485 | 9486-9490 |
| III | 30 | 7.5 | 4 | 2 | 20 ^b | 9441-9460 | NA | NA |
| IV | 100 | 25 | 4 | 2 | 20 ^b + 5 ^c + 5 ^d | 9461-9478, 889 ^e , 9480 | 9491-9495 | 9496-9500 |

NA - Not Applicable

- a. The test article was considered 100% active/pure for the purpose of dose calculations.
- b. Main study rabbits (Dosed on DGs 6, 9, 12, 15 and 18).
- c. Satellite rabbits assigned to Part A (Dosed on DG 18).
- d. Satellite rabbits assigned to Part B (Dosed on DGs 6, 9, 12, 15 and 18).
- e. Doe 9479 lost body weight between DGs 0 and 5 and was replaced with doe 889.

The test article or placebo was administered to the pregnant rabbits once daily during the period of organogenesis on DGs 6, 9, 12, 15 and 18 (all rabbits assigned to the main study and satellite rabbits assigned to Part B) or DG 18 only (satellite rabbits assigned to Part A). Doses were adjusted for the body weight collected on the day of dosing and given at approximately the same time each day during the dosing interval.

Parameters and endpoints evaluated:

In-life observations (Dams):

Clinical signs:

Animals were observed daily for general appearance, mortality and moribundity and signs of abortion.

Body weight:

Body weights were recorded on DG 0, the day of arrival at the Testing Facility, DG 5, and daily from DGs 6 to 29.

Food consumption:

Food consumption was recorded daily throughout the study.

Toxicokinetics:

On DG 5 and before euthanasia on DG 29, approximately 2 ml of blood was collected from the medial auricular artery of all rabbits assigned to the main study.

On DG 5, before and approximately 5 minutes after dosing on DG 18, on DG 19 (approximately 24 hours after dosing on DG 18), DG 20 (approximately 48 hours after dosing on DG 18), and before euthanasia on DG 21, approximately 2 ml of blood was collected from the medial auricular artery of all rabbits assigned to Part A.

On DG 5, before and approximately 5 minutes after dosing on DGs 6, 9, and 18, and on DG 15 (before dosing), DG 21 and DG 29 (before euthanasia), approximately 2 ml of blood was collected from the medial auricular artery of all rabbits assigned to Part B.

On DG 21 (all rabbits assigned to Part A) and on DG 29 (all rabbits assigned to the Main study and Part B), fetal blood samples (two samples per litter at approximately 2 ml per uterine horn, when possible) were obtained at euthanasia. Fetal blood samples were collected via the inferior vena cava following euthanasia. The blood was pooled per uterine horn and pooled per litter.

On DG 21 (all rabbits assigned to Part A) and on DG 29 (all rabbits assigned to the Main study and Part B), amniotic fluid (at least 2.0 ml) surrounding each fetus was collected and pooled per uterine horn per rabbit.

Terminal and necroscopic evaluations: C-section data:

All rabbits were euthanized either on DG 21 (rabbits assigned to Part A) or DG 29 (rabbits assigned to the main study or Part B) gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Uteri of apparently nonpregnant does were examined while being pressed between glass plates to confirm the absence of implantation sites. The number of corpora lutea in each ovary was recorded. (Data are not located in the BLA.)The uterus of each rabbit was excised and examined for pregnancy, number and distribution of implantation sites, early and late resorptions and live and dead fetuses.

Caesarean-sectioning observations for rabbits assigned to Part A and Part B were not performed.

Offspring:

Each fetus was weighed, examined for gross alterations, sex identified and organs evaluated by dissection. The brain was examined *in situ*.

Results:

In-life observations (Dams):

Mortality:

All animals survived to scheduled sacrifice.

Clinical signs:

No clinical observations related to study drug administration were noted.

Body weight:

Significantly reduced mean body weight gain ($p \leq 0.05$) and significant mean body weight loss ($p \leq 0.01$) occurred in the 30 and 100 mg/kg dose groups, respectively on DGs 6 to 7 (after the first dose of the test article). All remaining body weight gains and body weights were comparable among the 0 (Placebo), 10, 30 and 100 mg/kg dose groups.

Food consumption:

No effect on food consumption related to study drug administration was noted.

Toxicokinetics:

Following single and multiple doses, rhuMAb VEGF concentrations in maternal serum and amniotic fluid increased linearly with dose, while concentrations in fetal serum increased but with more variability.

Antibody titers to bevacizumab were detected in maternal serum in 9 of 73 does (12%). Anti-bevacizumab antibodies were also detected in fetal serum from 9 of 71 does (13%) and in amniotic fluid from 7 of 71 does (10%). Titers were determined from sample collected on DG29 at C-section and sacrifice.

Terminal and necroscopic evaluations: C-section data:

On gross examination, two does showed absence of the intermediate lobe of the lung. One doe from the high dose group had a cyst on the left horn of the uterus filled with clear fluid.

- An dose related increase in late resorptions was observed (3 for the control group vs. 14 for the 100 mg/kg group, 42 and 60%, respectively).
- All placentae appeared to be grossly normal.
- No apparent differences were observed for implantations, # of live fetuses, or gender of fetuses.

Offspring:

- Fetal body weights (total, male and female) were significantly reduced by approximately 17% ($p \leq 0.01$) in the 10, 30 and 100 mg/kg dose groups, as compared to the Placebo group. Values for total and male body weights in the 30 and 100 mg/kg dose groups and in the 100 mg/kg dose group for female fetal body weights were outside the ranges

observed historically at the testing facility. This finding showed a clear relationship to study drug administration.

- The number of late resorptions was increased in the 100 mg/kg dose group. One dead fetus was found in the 100 mg/kg group.
- The number of litters containing fetuses with any type of malformation was increased for the 30 and 100 mg/kg groups (42.1 % for placebo, 76.5% for the 30 mg/kg group and 95.0% for the 100 mg/kg group). The total number of fetal alterations per litter was increased for the 100 mg/kg group (9.1% for placebo, 14.8% for the 30 mg/kg group and 61.2% for the 100 mg/kg).
- Fetal abnormalities included: reduced metacarpal ossification sites for all dose groups compared to placebo control, reduced number of ossification sites for caudal vertebrae and fore and hindlimb phalanges, corneal opacity.
- Meningocele reported in 14 fetuses (9.2%) from 6 litters in the 100 mg/kg group.
- Thin skin over the fontanelle was observed in one fetus in the 30 mg/kg dose group and 18 fetuses (11.8%) from seven litters in the 100 mg/kg dose group.
- Four of the fetuses in the 100 mg/kg dose group had additional gross external alterations.
- Downward flexed forepaw was observed in 3 of fetuses (2%).
- Malformations of the hindlimb (laterally rotated) were seen in 10 fetuses (6.5%).
- Skeletal abnormalities: Irregular ossification of the skull was seen in 3 fetuses (2.1%) from 3 litters for the 10 mg/kg group and one fetus (0.6%) from one litter for the 100 mg/kg group. Abnormal shape of the posterior fontanel was observed for 15 litters (75%) and in 28 fetuses (18.4%) from dams receiving 100 mg/kg. Abnormally large size of the posterior fontanelle was observed in 8 litters (40.0%) and 33 fetuses (21.7%) from the 100 mg/kg group. A hole was reported in the parietal bone of a small number of fetuses from all dose groups 1 from the 10 mg/kg group, 5 from the 30 mg/kg group and 2 from the 100 mg/kg group. Incompletely ossified pre-maxilla was observed in 7 fetuses (4.6%) from 3 (15%) of litters.
- Rib malformations were noted in all dose groups: flat ribs (3 litters and 5 fetuses from the 100 mg/kg group), wavy ribs (9 litters and 26 fetuses from the 100 mg/kg group, 3 litters and 5 fetuses from the 30 mg/kg group), thickened ribs (3 litters and 3 fetuses from the 10 mg/kg group, 2 litters and 2 fetuses from the 30 mg/kg group and 9 litters and 17 fetuses from the 100 mg/kg group), incompletely ossified ribs (3 litters and 6 fetuses from the 10 mg/kg group, 4 litters and 5 fetuses from the 30 mg/kg group and 6 litters and 12 fetuses from the 100 mg/kg group), bent ribs (1litter and 1 fetus from the 30 mg/kg group and 4 litters and 6 fetuses from the 100 mg/kg group). No rib deformities were reported for the placebo group.
- Multiple other skeletal deformities were observed in the treated groups at low incidence including incomplete formation of cervical, thoracic and lumbar vertebrae, irregularly shaped scapula, asymmetric sternal centra, and absent hindlimb phalanges.
- Irregularly shaped tibias were noted for the high dose group only (5 litters and 11 fetuses).

Reviewer Comments:

A clear NOAEL for developmental toxicity was not clearly defined since fetal deformities were observed at all doses. From the data generated in this study the best estimate of NOAEL is less than 10 mg/kg/dose. Although some of the deformities noted do not show a clear dose relationship, the data clearly indicate that bevacizumab can be teratogenic.

2.5.3. Prenatal and Postnatal Development

No. Studies of this type were performed.

2.5.4. Juvenile Animal Toxicity Studies

NO studies of this type were performed.

2.6. LOCAL TOLERANCE

No studies of this type were performed.

2.7. OTHER TOXICITY STUDIES

2.7.1. Study Title

Testing Facility and Location:

Study Number:

Date of Study Initiation:

Drug Lot/Batch Number(s):

GLP Compliance (y/n):

QA Report (y/n):

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Key Findings:

Methods:

Dosing:

Observations and Times:

Results:

Reviewer Comments:

2.8. CONCLUSIONS AND RECOMMENDATIONS

2.8.1. Pharmacology

Pharmacology, pharmacodynamics and pharmacokinetics were reviewed by Dr. Anita O'Conner and are not included in this review.

2.8.2. Toxicology

Conclusions and recommendations:

Repeat-dose toxicity: The data provided from the three repeat-dose toxicology studies indicated that, in the intended patient population under the proposed conditions of use, Avastin should be reasonable safe. The drug appeared to be well tolerated at all doses used (up to 50 mg/kg administered weekly for up to 26 weeks). No apparent study drug related hematology or clinical chemistry or physical exam findings were reported with the exception of a small rise in blood pressure for female monkeys after 26 weeks of treatment at 50 mg/kg/wk. The biological significance is not clear since the male animals did not show this finding. However, careful monitoring of blood pressure for human use is recommended.

Anatomical pathology analyses revealed several findings that indicate toxic effects on general growth and skeletal development, fertility, and wound healing capacity. In general, the toxic effects revealed in these studies appear to be predictable extensions of the pharmacodynamic activity of Avastin.

Physeal dysplasia of the femur and humerus was reported as severe in 4 of 4 males from group 5, 3 of 4 males from group 4, and 1 of 4 males from group 3. Moderate or mild dysplasia is reported for the one remaining animal in group 4, the remaining 3 in group 3 and 2 of 4 from group 2. This physeal dysplasia was characterized by a linear cessation of growth line and chondrocyte hyperplasia.

These data demonstrate a clear dose response relationship to the study drug. Fewer female animals displayed these changes. Severe dysplasia is reported for 1 of 4 from group 5, moderate dysplasia for 2 of 4 females from groups 3 and 4. The difference in toxicity between genders may be explained by differences in ages of the two groups. The dysplasia did not resolve after 4-weeks of recovery. Severity appeared to be less after 12 weeks of recovery, indicating that some recovery may be possible.

[.]

No specific studies assessing the effect of Avastin on fertility were performed. However, results of anatomical pathology from the repeat-dose toxicity studies indicate that treatment with Avastin may have an adverse effect on fertility. In all studies, a dose dependent reduction in uterine and ovarian organ weight was identified the 10 mg/kg, and 50 mg/kg groups compared to control. After 26 weeks of treatment, the dose dependent reduction in uterine ovarian weights (up to approximately 50%) was apparent for all dose groups compared to control. This reduction in organ weight was still apparent after as long as 12 weeks of recovery (n=2) although, due to

variability and the small number of recovery animals, this finding did not reach statistical significance.

The reductions in ovarian and uterine weights correlated with notable reductions in proliferation of the uterine endometrium functionalis for females receiving 10 and 50 mg/kg for 26 weeks. Follicular arrest is noted for all females in groups 4 (50 mg/kg) and 5 (10 mg/kg X2/week) treated for 26 weeks. Examination of the ovaries after 13 or 26 weeks of treatment revealed an absence of corpora lutea for the 30 mg/kg groups as well as 100% of the animals receiving 50 mg/kg. This effect is clearly study drug related. After 4 weeks of recovery absence of corpora lutea persisted for 50 mg/kg group (n=2) but, after 12 weeks, 50% of the 50 mg/kg animals (n=2) showed this finding. Follicular arrest and the reduced endometrial proliferation were not observed after 12 week recovery (n=2) suggesting that at least partial recovery is possible. However, the persistence of the absence of corpora lutea in 1 of 2 recovery females that received 26 weeks of treatment at 50 mg/kg/wk indicates that recovery is not complete within this time period. A reduction of 67% in menstrual cycles was also reported for the 50 mg/kg females. Reduced menstrual cycles were reported for the recovery animals (50 mg/kg, n=2) but, due to individual variations and the small number of recovery animals, these data are difficult to interpret. (See Appendix for detailed tabulation of the histopathological data relating to fertility.)

Other findings that may be study drug-related are: enlarged spleens noted for 1 male and 1 female in the 50 mg/kg dose group and a statistically significant increase in organ to body weight of the pituitary (50%) for high dose females after 4 weeks of treatment twice weekly. The biological significance of these findings is not clear since they did not occur in other studies with longer exposure.

Glomerulonephritis was observed histologically in one female from the low dose group after 4 weeks of dosing and one male control animals after 13 weeks of dosing. Since these findings occurred in the lowest dose and control groups, and no other occurrences were noted in the three repeat-dose studies, this finding does not appear to be related to administration of study drug and, for the purposes of these studies does not appear to be of biological significance. In all studies there was a high occurrence of signs of chronic inflammation in multiple organ systems suggesting the presence of a pre-existing condition. However, the presence of a pre-existing inflammatory condition could possibly confound interpretation of results by masking a study drug related low level toxicity.

The NOAEL for repeat dosing of Avastin identified in these studies was 2 mg/kg.

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Embryo-fetal development toxicity:

Two studies were performed addressing the potential for treatment with Avastin during pregnancy to cause fetal developmental toxicities when administered IV to pregnant rabbits during the period of organogenesis. No clinical signs of toxicity were noted for the pregnant dams with the exception of an early (DG 6 and 7) significant reduction in mean body weight gain for the low dose group and a mean body weight loss for the middle and high-dose groups. This occurred after the first dose of the test article and was temporary. All remaining body weight gains and body weights were comparable among the Placebo, 10, 30 and 100 mg/kg dose groups.

In contrast, clear evidence of a dose related teratogenic effect was identified upon examination of the 73 litters and over 600 fetuses. Multiple skeletal deformities were noted in the fetuses at all dose groups with increased incidence for the high dose group.

A clear NOAEL for developmental toxicity was not defined in this study. From the data generated in this study the best estimate of NOAEL is less than 10 mg/kg/dose. The data indicate that bevacizumab can have a teratogenic effect on developing fetuses.

Reduced wound healing:

The toxicity package included several non-GLP studies addressing the potential for Avastin to reduce wound healing capacity. Two rabbit models of skin wound healing were developed: a full-thickness skin incision model and a rabbit dermal wound model of incomplete skin lesion. Results of both studies demonstrated a dose-dependent reduction in wound healing capacity in rabbits treated with Avastin at doses of 2, 10 and 50 mg/kg. Reductions in wound healing capacity were noted for all doses examined.

2.8.2. Labeling

The major toxicities revealed in the non-clinical studies relate to four sections of the package insert: Impairment of fertility, Pregnancy Category, Pediatric Use and Precautions due to reduced wound healing.

Impairment of fertility

The original suggested language for the package insert acknowledged the potential for Avastin treatment to impair fertility. No specific toxicity study was performed addressing the potential for impairment of fertility by Avastin treatment. However, numerous findings from the repeat-dose toxicity studies indicate that impairment of fertility is likely. The language describing the data indicating this potential was not located in the fertility section and no clear indication of the doses that resulted in those changes were included. In subsequent versions, the description of the toxicities that indicate impairment in fertility was consolidated in appropriate section and the sponsor clarified the language in this section according to the CFR by adding details regarding the treatment regimens and doses that resulted in the apparent toxicities. The major negotiation regarding the language of this section related to the potential for recovery. The data from the repeat-dose toxicology studies showed a potential for recovery, but complete recovery was not achieved in any study. A detailed review integrating the findings taken from the study reports of studies 96-182-1751 and 97-194-1751 was prepared and discussed with the sponsor. The sponsor agreed that, in light of those data, full recovery was not achieved. Final language was agreed upon and is provided below.

Carcinogenesis, Mutagenesis, Impairment of Fertility

No carcinogenicity data are available for AVASTIN in animals or humans.

AVASTIN may impair fertility. Dose-related decreases in ovarian and uterine weights, endometrial proliferation, number of menstrual cycles, and arrested follicular development or

absent corpora lutea were observed in female cynomolgus monkeys treated with 10 or 50 mg/kg of AVASTIN for 13 or 26 weeks. Following a 4- or 12-week recovery period, which examined only the high-dose group, trends suggestive of reversibility were noted in the two females for each regimen that were assigned to recover. After the 12-week recovery period, follicular maturation arrest was no longer observed, but ovarian weights were still moderately decreased. Reduced endometrial proliferation was no longer observed at the 12-week recovery time point, but uterine weight decreases were still notable, corpora lutea were absent in 1 out of 2 animals, and the number of menstrual cycles remained reduced (67%).

Embryo-fetal development and teratogenicity:

This section of the package insert should clearly state that treatment with Avastin during pregnancy can result in serious fetal deformities. No NOAEL was identified as toxic effects were observed at all doses. The original language did not contain information about the dosing that resulted in the toxicity or its relationship to the recommended human dose. Relating the doses in the rabbit to human doses is difficult as Avastin has approximately 8-fold lower affinity for rabbit VEGF than human. The final language agreed upon with the most accurate information regarding dose relationships between the doses used in the rabbit studies and the recommended human dose is provided below.

Pregnancy Category C

AVASTIN has been shown to be teratogenic in rabbits when administered in doses that are two-fold greater than the recommended human dose on a mg/kg basis. Observed effects included decreases in maternal and fetal body weights, an increased number of fetal resorptions, and an increased incidence of specific gross and skeletal fetal alterations. Adverse fetal outcomes were observed at all doses tested.

Angiogenesis is critical to fetal development and the inhibition of angiogenesis following administration of AVASTIN is likely to result in adverse effects on pregnancy. There are no adequate and well-controlled studies in pregnant women. AVASTIN should be used during pregnancy or in any woman not employing adequate contraception only if the potential benefit justifies the potential risk to the fetus. All patients should be counseled regarding the potential risk of AVASTIN to the developing fetus prior to initiation of therapy. If the patient becomes pregnant while receiving AVASTIN, she should be apprised of the potential hazard to the fetus and/or the potential risk of loss of pregnancy. Patients who discontinue AVASTIN should also be

counseled concerning the prolonged exposure following discontinuation of therapy (half-life of approximately 20 days) and the possible effects of AVASTIN on fetal development.

Pediatric populations:

One of the major consistent toxic effects of Avastin administration revealed in the repeat-dose toxicity studies was physal dysplasia of long bones (humerus and femur) in monkeys with open growth plates. This raises concern for the potential use of Avastin in pediatric populations. Information was added under the Pediatric Use section describing the dose-related occurrence of physal dysplasia and an indication of the relationship of the toxic dose to the recommended human dose. The 4 –week toxicology study is quoted because that serves as the worst case scenario since it was the shortest exposure. The lack of complete recovery is also indicated.

Pediatric Use

The safety and effectiveness of AVASTIN in pediatric patients has not been studied. However, physal dysplasia was observed in juvenile cynomolgus monkeys with open growth plates treated for four weeks with doses that were less than the recommended human dose based on mg/kg and exposure. The incidence and severity of physal dysplasia were dose-related and were at least partially reversible upon cessation of treatment.

Wound healing:

The effect of reduced wound healing capacity after treatment with Avastin was investigated in several non-GLP studies in response to adverse events in the clinical studies. These studies did not include complete pathology reports but sufficient data was included to confirm that a reduction of wound healing capacity resulted from Avastin treatment at all doses tested in two rabbit wound healing models. The language of the package insert was modified in appropriate section to provide appropriate warnings and precautions that should be taken for human use. The final language is provided below.

WARNINGS

**Gastrointestinal Perforations/Wound Healing Complications
(See DOSAGE AND ADMINISTRATION: Dose Modifications)**

Gastrointestinal perforation and wound dehiscence, complicated by intra-abdominal abscesses, occurred at an increased incidence in patients receiving AVASTIN as compared to controls. AVASTIN has also been shown to impair wound healing in pre-clinical animal models.

Surgery

AVASTIN therapy should not be initiated for at least 28 days following major surgery. The surgical incision should be fully healed prior to initiation of AVASTIN. Because of the potential for impaired wound healing, AVASTIN should be suspended prior to elective surgery. The appropriate interval between the last dose of AVASTIN and elective surgery is unknown; however, the half-life of AVASTIN is estimated to be 20 days (see **CLINICAL PHARMACOLOGY: Pharmacokinetics**) and the interval chosen should take into consideration the half-life of the drug. (See **WARNINGS: Gastrointestinal Perforations/Wound Healing Complications**.)

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2.9. Appendices / Attachments

Review of fertility data for Avastin BLA

Background:

The data reflecting a potential effect of Avastin treatment on fertility are taken from two repeat-dose toxicology studies. Study #96-182-1751 is a 13-week treatment with a 4-week recovery period in cynomolgus monkeys. Study #97-194-1751 is a 26-week treatment with a 12 week recovery period in cynomolgus monkeys. For both studies the doses used were 2mg/kg for the low dose, 10 mg/kg for the mid-dose and 50 mg/kg for the high dose (all doses administered IV weekly). For the 26-week treatment study, they added an additional group that received 10 mg/kg, twice weekly.

For both studies, there were 4 animals/sex/group. The control groups and high-dose groups had two additional animals designated as recovery animals-so those groups in both studies had a total of 6 animals to start the study. The low and mid dose groups did not have recovery animals. So no data on recovery is available for those dose groups for either study.

ORGAN WEIGHTS-OVARY AND UTERUS

At sacrifice at the end of treatment for each study, 4 animals/sex/group were euthanized, leaving 4 females per group at what the sponsor refers to as terminal sacrifice.

For both studies, at terminal sacrifice, absolute organ weight for ovary and uterus was reduced in a dose dependent manner.

- After 13 weeks of treatment statistically significant reductions were observed for the uterus in both the mid and high dose groups. After 26 weeks of treatment, uterus weights reductions were statistically significant in mid, high and the twice per week dose groups.
- After 13 weeks of treatment, ovarian weight reductions were statistically significant in the mid and high dose groups. After 26 weeks of treatment, data shows a dose dependent reduction in ovarian weights for all dose groups, but did not reach statistical significance. However, ignoring the SD, the numbers show a nearly 50% drop in weight between the control and high-dose group. At sacrifice of the recovery animals (2 controls and 2 high-dose animals for each study), the weight differences were still apparent but less pronounced and not considered by the sponsor to be statistically significant. This was true for both studies.

MACROSCOPIC FINDINGS-OVARY AND UTERUS

Macroscopic findings at terminal sacrifice for the 13-week study included only and ovarian cyst in one control animal. No uterus findings are reported. No macroscopic findings are reported for ovary or uterus at terminal sacrifice for the 26-week study except small ovary for one animals in the low dose group.

MICROSCOPIC FINDINGS

| Organ | Study | Sacrifice | Findings | Number Of animals | Dose group |
|--------|-------|-----------|--|--|---|
| Uterus | 13/4 | Terminal | Hemorrhage | 2/4 | Control |
| Ovary | 13/4 | Terminal | Mineralization Fibrosis Corpora lutea absent | 2/4 2/4 3/4 each 2/4 4/4 | Control Control All other dose group 10 mg/kg group 50 mg/kg group |
| Uterus | 13/4 | Recovery | Not remarkable Pigment | 2/2 1/2 1/2 | Control 50 mg/kg group 50 mg/kg group |
| Ovary | 13/4 | Recovery | CORPORA LUTEA ABSENT | 2/2 | 50 mg/kg group |
| Uterus | 26/12 | Terminal | Amyloid Thickness, functionalis layer, endometrium | 3/4 4/4 2/4 1/4 slight 1/4 moderately severe 2/4 severe 1/4 moderate 1/4 moderately severe 2/4 severe 1/4 slight 2/4 moderate 1/4 moderately severe 1/4 minimal 3/4 moderate 1/4 minimal 2/4 slight 1/4 moderate | 10 mg/kg group 50 mg/kg group 10 mg/kg X2 Control Control Control 2 mg/kg group 2 mg/kg group 2 mg/kg group 10 mg/kg group 10 mg/kg group 10 mg/kg group 50 mg/kg group 50 mg/kg group 10 mg/kg X2/wk 10 mg/kg X2/wk 10 mg/kg X2/wk |
| Ovary | 26/12 | Terminal | Mineralization | 1/4 moderate 2/4 minimal 1/4 moderate | 2 mg/kg group 10 mg/kg group 50 mg/kg group |

| | | | | | |
|--------|-------|----------|--|---|--|
| | | | Amyloid Maturation arrest, follicular | 1/4 moderately severe 1/4 severe 1/4 slight 4/4 present 4/4 present | 50 mg/kg group 50 mg/kg group 10 mg/kg group 50 mg/kg group 10 mg/kg X2/wk |
| Uterus | 26/12 | Recovery | THICKNESS ENDOMETRIUM AMYLOID | NO FINDINGS Absent | ALL GROUPS |
| Ovary | 26/12 | Recovery | Follicular arrest Corpora lutea absent Amyloid Mineralization | Absent 1/2 Absent Absent | 50 MG/KG GROUP All groups |

Ratings for severity were summarized only for the 26 week study: 1= minimal
2=slight
3=moderate
4=moderately severe
5=severe

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Structure and formulation information:

The following figures containing amino acid sequences of bevacizumab , and clinical formulation were provided by the sponsor.

Figure 1
Primary Sequence of Bevacizumab

The residues underlined are part of the complementarity-determining regions of the antibody (Presta et al. 1997).

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ON ORIGINAL

Figure 2
Primary Sequence of Bevacizumab

⌈

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residues underlined are part of the complementarity-determining regions of the antibody (Ito et al. 1997).

Table 1
Composition of Drug Product

| Components, Drug Product | Specification | Code | Component Function | Amount per Batch ^a | Nominal Amount per 100 mg Vial | Nominal Amount per 400 mg Vial | Nominal Amount per 1 Vial |
|---|-------------------|--------|--------------------|-------------------------------|--------------------------------|--------------------------------|---------------------------|
| Bevacizumab | Section 3.2.S.4.1 | — | Active ingredient | — | 100 mg | 400 mg | 100 mg |
| <u>α,α-Trehalose dihydrate</u> | — | G20251 | | — | 340 mg | 960 mg | 100 mg |
| <u>Sodium phosphate, monobasic, monohydrate</u> | — | G20045 | | — | 23.2 mg | 92.8 mg | 100 mg |
| <u>Sodium phosphate, dibasic, anhydrous</u> | — | G20035 | | — | 4.8 mg | 19.2 mg | 100 mg |
| Sterile Water for Injection | USP, Ph. Eur. | G9025 | | — | qs to 4 mL | qs to 16 mL | 16 mL |
| Polysorbate 20 | USP, Ph. Eur. | G20091 | | — | 1.6 mg | 6.4 mg | 100 mg |

^a Batch size may vary.

^b The specifications for α,α-trehalose dihydrate and sodium phosphate (dibasic, anhydrous) are included in Section 3.2.S.2.3.1.

^c Refer to Section 3.2.P.2.1.2.

Signatures:

Reviewer Signature *[Handwritten Signature]* /S/

Supervisor Signature *[Handwritten Signature]* /S/ Concurrence Yes No