

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

202799Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Omontys (peginesatide)

Date: February 27, 2012

To: File for NDA 202799

From: John K. Leighton, PhD, DABT

Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review of Drs. Ringgold and Gehrke and labeling and secondary memorandum provided by Dr. Saber. I concur with Dr Saber's conclusion that Omontys may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
02/27/2012

MEMORANDUM

Date: February 27, 2012
From: Haleh Saber, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
NDA: 202799
Drug: OMONTYS[®] (peginesatide)
Indication: For treatment of anemia due to chronic kidney disease (CKD) in adult patients on dialysis

OMONTYS[®] (peginesatide) is an erythropoiesis-stimulating agent (ESA). Peginesatide is a synthetic, pegylated dimeric peptide. The two identical peptide chains are covalently attached through a linker derived from iminodiacetic and β -alanine. The amino acid sequence of peginesatide is not related to that of erythropoietin (EPO), however, peginesatide binds to and activates the recombinant human erythropoietin receptor with high specificity. Peginesatide showed activities similar to EPO and approved ESAs, Aranesp and Epogen/Procrit. Therefore, the pharmacologic class assigned to peginesatide is “erythropoiesis-stimulating agent”, to be consistent with the label for Aranesp and Epogen/Procrit. The pharmacologic class is described in INDICATIONS AND USAGE in the HIGHLIGHTS section of the label.

Pharmacology, safety pharmacology, pharmacokinetic/ADME (absorption, distribution, metabolism and excretion), and toxicology studies were conducted in *in vitro* systems and/or in animal species. Peginesatide was administered subcutaneously or intravenously to animals in toxicology studies, consistent with the intended route of administration in patients. Drug-related toxicities were similar after subcutaneous or intravenous administration and after single- or repeat-dose administration. Only repeat-dose general toxicology studies were reviewed for this NDA. Toxicities were mostly related to pharmacology of the drug and were consistent with those observed with marketed ESAs. Adverse effects in animals included: increased RBCs, hemoglobin, and hematocrit, enlarged spleens, and increased hematopoiesis/hypercellularity and hyperplasia in the bone marrow. Increased congestion was seen in multiple organs. Cardiac toxicity (thrombosis, stromal proliferation of the atrio-ventricular valve, and myocardial degeneration) was evident in rats after ≥ 3 months of dosing. There were no adverse cardiac conduction findings, based on the results of the hERG study and the ECG parameters assessed in the monkey in the toxicology study. Hemo-concentration was speculated to be the cause of cardiac toxicity and multi-organ congestion. Renal toxicities were mostly evident in the rat and included tubular degeneration, dilated tubules with cytoplasmic vacuolation, and congestion/inflammation.

Peginesatide was not genotoxic in the ICH battery of genotoxicity assays or carcinogenic in the rat and in Tg.rasH2 transgenic mice.

When administered intravenously during the period of organogenesis, peginesatide was teratogenic to rats and rabbits or caused embryo-fetal lethality.

Peginesatide may reduce male and female fertility. Administration of the drug to male and female rats in a dedicated fertility study, resulted in reduced weight of seminal vesicles and prostate, and decreased viable fetuses in females. The effects in females may be the result of pre- and post-implantation losses. There was no apparent drug-related effect on estrous cycles or number of corpora lutea. Increased morphological abnormalities of the sperm was reported in the pharmacology/toxicology review. Upon further examination of the data, there are no drug-related morphological abnormalities in the sperm. Reduced sperm count was also observed in males and reported in the pharmacology/toxicology review; however, the Applicant provided data indicating that values are within the historical range.

The Applicant proposed a Category C for pregnancy; their justification included the following:

- “The embryofetal effects in the rat and rabbit reproductive toxicity studies are indirect and associated with profound maternal polycythemia (increases in hemoglobin [Hgb] up to \approx 3-5 g/dL over normal controls), which would adversely impact placental blood flow.
- Negligible fetal placental transfer of peginesatide in the rat further supports that peginesatide-related embryofetal findings are not direct drug effect.
- Because peginesatide injection is not dosed to achieve polycythemic Hgb effects in dialysis patients but rather Hgb levels well below normocythemic physiologic levels (even at the highest doses), the embryofetal effects, which were associated with sustained maternal polycythemia, are not clinically relevant.”

DHOT accepts the Category C. This is also consistent with the labels for the marketed ESAs, Aranesp and Epogen/Procrit.

The nonclinical studies were reviewed by Dr. Kimberly Ringgold and Dr. Brenda Gehrke. The nonclinical findings are summarized in the “Executive Summary” of the NDA review and reflected in the product label.

Recommendation: I concur with Drs. Ringgold and Gehrke that from a nonclinical perspective, OMONTYS may be approved for the proposed indication. No additional nonclinical studies are needed to support approval of OMONTYS for the proposed indication.

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/s/

HALEH SABER
02/27/2012

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 202799
Submission date: 5/23/2011
Received date: 5/27/2011
Product: Peginesatide; Omontys®
Indication: Treatment of anemia due to chronic kidney disease in adult patients on dialysis
Applicant: Affymax, Inc
Review Division: DHOT (for DHP)
Reviewers: Kimberly Ringgold, PhD
Brenda Gehrke, PhD
Supervisor/Team Leader: Haleh Saber, PhD
Division Director: John Leighton, PhD, DABT (DHOT)
Ann Farrell, MD (DHP)
Project Managers: Trinh Scott
Ebla Ali-Ibrahim

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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability

The nonclinical studies submitted to this NDA provide sufficient information to support the use of peginesatide for the treatment of anemia due to chronic kidney disease in adult patients on dialysis.

1.1.2 Additional Non Clinical Recommendations

None

1.1.3 Labeling

The data referenced in the labeling is contained in this review.

There is no recommended top human dose of peginesatide due to titration of the drug dose to a desired hemoglobin level. For the animal: human exposure comparisons, the AUC in humans at a dose of 0.35 mg/kg was used. This dose is considered the maximum (covering 95%) human dose based on data from the Phase 3 trial in patients on dialysis. This dose was also used by the Applicant on Nov 3, 2011, for the carcinogenicity study for the animal: human exposure comparisons. The dose of 0.35 mg/kg is an achievable dose in patients. Where animal AUC data were unavailable, dose to dose comparisons (based on body surface area) were made using a dose of 0.35 mg/kg in patients.

1.2 Discussion of Nonclinical Findings

Pharmacology

Peginesatide is an erythropoiesis-stimulating agent. The amino acid sequence of peginesatide is not related to that of erythropoietin (EPO), however, it binds to and activates the recombinant human erythropoietin receptor with high specificity. Further, peginesatide dose-dependently stimulated the proliferation of UT-7/EPO cells (an EPO-responsive human leukemia cell line) and inhibited apoptosis of UT-7/EPO cells. Peginesatide protects cells from apoptosis through the suppression of caspase activation, the maintenance of the expression of the anti-apoptotic protein BCL-X_L, and activating JAK2 phosphorylation. Peginesatide can also stimulate erythroid progenitors from primary human CD34+ cells to form erythroid colonies. The activity of peginesatide was supported by *in vivo* rat studies, where peginesatide increased reticulocytes, red blood cells, hemoglobin, and hematocrit levels in Sprague-Dawley rats. The Applicant has also shown that variants of peginesatide (b) (4) displayed similar erythropoietic activity to peginesatide.

Peginesatide had no effects on the central nervous system in mice and the respiratory system in guinea pigs. Peginesatide administration to rats caused decreases in serum

Na⁺ and Cl⁻ concentrations as well as Cl⁻ excretion at the highest dose tested. No effects were observed on urinary volume and pH. Although cardiovascular safety studies in the anesthetized dog show that peginesatide did not cause any effects, the Applicant also showed that peginesatide has no activity in dogs. Therefore, the effects of peginesatide on the cardiovascular system can not be determined based on the dog study. Electrocardiography evaluations were incorporated into the chronic study in monkeys. There were no peginesatide-related effects on heart rate, PR, QRS, RR, and QT interval. Increases in QTc were noted in the mid-dose (0.2 mg/kg) at 3, 6, and 9 months for males and females and in the high-dose (20 mg/kg) female group at 9 months. These effects were not dose-dependent. While peginesatide does not affect the conduction in the heart, it causes cardiovascular toxicities as shown by histopathology findings in the general toxicology studies.

Pharmacokinetics

The pharmacokinetics (PK) of peginesatide was studied in multiple species including the rat and monkey, the non-clinical species tested for chronic toxicity. The PK was characterized by a long half-life (~15 – 31 hours), small volume of distribution, and slow clearance. Peginesatide exposure was lower and the rate of absorption was slower with subcutaneous administration compared to intravenous administration. The subcutaneous bioavailability was 26%. Tissues with high concentrations of peginesatide included the liver, renal cortex, kidney, pituitary gland, lymph nodes, renal medulla, spleen, and choroid plexus. Peginesatide remains mostly unchanged in plasma, urine, and feces following intravenous or subcutaneous administration in rats. Peginesatide is not significantly metabolized by liver or renal S9 fractions. This is expected because peginesatide is a peptide. Renal excretion is the main route of elimination of peginesatide. Peginesatide does not bind to albumin or lipoproteins. Twenty-four to 48 hours after administration of radiolabeled peginesatide, 11% - 13% of radioactivity was detected in the milk of lactating rats as compared to the plasma levels of radioactivity.

General Toxicity

The toxicological profile of peginesatide was consistent for erythropoietin-stimulating agents. Nonclinical findings in the rat and monkey show that peginesatide treatment caused notable changes in red blood cell hematology parameters (red blood cells, hemoglobin, and hematocrit) and morphology, enlarged spleens, and increased hematopoiesis/hypercellularity and hyperplasia in the bone marrow. Increased congestion and periarteritis were also noted in rat and monkey studies, which can also be attributed to the exaggerated pharmacology of peginesatide. Other target organ toxicities included:

Heart: Changes included thrombosis, stromal proliferation of the atrio-ventricular valve, and myocardial degeneration in mid- and high-dose rats dosed intravenously for 6 months. Rats dosed subcutaneously for 3 months also showed these effects. Cardiac toxicity was the cause of deaths in some animals. No major findings were observed in the intravenous or subcutaneous studies in monkeys. Electrocardiography exams in the monkey showed no peginesatide-related effects on heart rate, PR, QRS, RR, and QT interval. Increases in QTc were noted in the 0.2 mg/kg dose group at 3, 6, and 9

months for males and females and in the 20 mg/kg female group at 9 months, however, these increases were not dose-dependent.

Kidney: Nephropathy characterized by clusters of degenerating cortical tubular cells, dilated tubules with cytoplasmic vacuolation, and lumens filled with proteinaceous fluid in rats treated with a single intravenous dose of peginesatide. Congestion and periarteritis were noted in rats dosed intravenously and subcutaneously. Renal toxicity, e.g. hyaline droplets, casts, and tubular degeneration likely contributed to mortalities in rats receiving repeat doses of peginesatide intravenously. There was also an increased incidence and severity of tubular regeneration, casts, chronic interstitial inflammation and pigmentation in the surviving rats of these studies. Congestion and chronic inflammation were observed in monkeys dosed intravenously and subcutaneously, respectively.

Brain: Hemorrhage, congestion, and mononuclear cell infiltrates were noted in the intravenous study in the monkey. Vacuolation of the choroid plexus and lymphohistiocytic infiltrates were seen in the subcutaneous study in the monkey.

Genotoxicity

Peginesatide was not mutagenic in the *in vitro* reverse mutation assay (Ames test). Peginesatide was not clastogenic when tested *in vitro* in the CHO cell for chromosomal aberrations or *in vivo* in mouse bone marrow for micronucleus formation.

Carcinogenicity

Peginesatide was not carcinogenic in the rat and in Tg.rasH2 transgenic mice.

Reproductive and Developmental Toxicity

Peginesatide was teratogenic or caused embryofetal lethality in the rat at doses of ≥ 1 mg/kg and at doses of ≥ 0.25 mg/kg in the rabbit. The dose of 1 mg/kg in rats results in exposures (AUC) comparable to those estimated in humans after IV administration at the highest human dose. Reduced fetal weight and reduced ossification were also seen in a separate embryofetal developmental study in rats at a lower dose of 0.25 mg/kg. The adverse findings in rabbits included an increased incidence of premature delivery observed at ≥ 0.05 mg/kg (approximately 5% of the dose of 0.35 mg/kg in patients based on body surface area). Thus, administration of peginesatide during pregnancy may pose a risk to the human fetus. Peginesatide should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

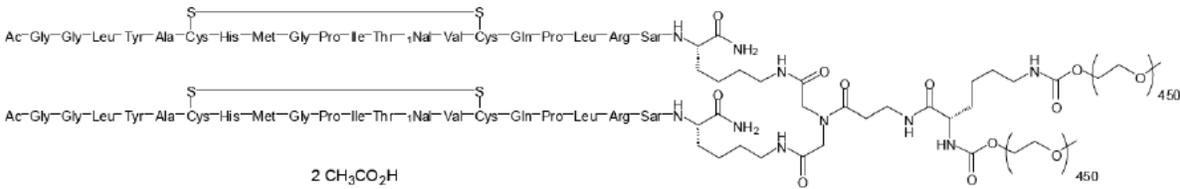
When administered intravenously to male and female rats at weekly intervals prior to and during mating, fertility was reduced at ≥ 0.1 mg/kg and was most evident at toxic doses ≥ 1.0 mg/kg of peginesatide. Adverse effects in males included reduced weight of seminal vesicles and prostate, increased morphological abnormalities of the sperm, and reduced sperm count. Decreased viable fetuses at ≥ 0.1 mg/kg in females appeared to be due to pre- and post-implantation losses.

Special Toxicity

Increased concentrations of the impurity, (b) (4) did not alter the toxicological profile of peginesatide. Peginesatide is compatible with human blood, plasma, and serum. Peginesatide was not antigenic and did not result in dermal sensitization in the guinea pig.

2 Drug Information

2.1 Peginesatide

2.1.1 CAS Registry Number:	1185870-58-9
2.1.2 Generic Name:	Peginesatide
2.1.3 Code Name:	AF37702 injection
2.1.4 Chemical Name:	N ² , N ⁶ -Bis-(methoxypolyethyleneglycol 20000-oxycarbonyl)-Lysyl-N,N-bis-†Acetyl-Glycyl-L-Leucyl-L-Tyrosyl-L-Alanyl-L-Cysteiny-L-Histidyl-L-Methionyl-L-Glycyl-L-Prolyl-L-Isoleucyl-L-Threonyl-L-1-Naphthylalanyl-LValyl-L-Cysteiny-L-Glutaminy-L-Prolyl-Leucyl-L-Arginy-L-Sarcosyl-N ⁶ -(1-oxyethyl-2-yl)-L-Lysinamide, cyclic (6→15)-disulfide↓-β-Alaninamide acetate (salt)
2.1.5 Molecular Formula/Molecular Weight	C ₂₀₃₁ H ₃₉₅₀ N ₆₂ O ₉₅₈ S ₆ . (b) (4) (d) (4)
2.1.6 Structure	 <p>2 CH₃CO₂H</p>
2.1.7 Pharmacologic class:	Erythropoiesis stimulating agent (ESA)

2.2 Relevant IND/s, NDA/s, and DMF/s

IND 63,257

2.3 Clinical Formulation

2.3.1 Drug Formulation

Single Dose Vial (SDV): 2 mg/0.5gm/mL to 6 mg/0.5mL AF37702 Injection
 Pre-filled Syringe (PFS): 1 mg/0.5gm/mL to 6 mg/0.5mL AF37702 Injection
 Multiple Dose Vial (MDV): 10 mg/mL & 20 mg/mL AF37702 Injection

Other excipients include: sorbitol (47 mg), (L-) methionine (1.5 mg), glacial acetic acid (0.6 mg) Phenol (5 mg), sodium hydroxide (adjusted to pH 5.4), and water (b) (4)

2.3.2 Comments on Novel Excipients: none

2.3.3 Comments on Impurities/Degradants of Concern: none

2.4 Proposed Clinical Population and Dosing Regimen

Patients with adult dialysis patients with anemia associated with chronic renal failure. Peginesatide will be administered either intravenously (IV) or subcutaneously (SC) as a single monthly injection according to the following:

- Initial treatment: 0.04 to 0.08 mg/kg body weight administered once monthly
- Conversion from another ESA: dose once monthly based on total weekly Epoetin or darbepoetin alfa dose at time of conversion

2.5 Regulatory Background

Affymax met with FDA on a number of occasions during which discussions on nonclinical, clinical, and chemistry, manufacturing and controls (CMC) aspects of the development of AF37702 Injection occurred. These include Pre-IND (4 March 2005), End-of-Phase 2 (EOP2, 1 February 2007 and 23 February 2007), Pre-NDA (21 October 2010), and clinical, statistical, and CMC advice meetings. A special protocol assessment (SPA) was used for carcinogenicity studies.

3 Studies Submitted

3.1 Studies Reviewed

Study Title	Study No.
Primary Pharmacodynamics	
<i>In vitro Pharmacology</i>	
Determination of the IC50 of AF37702 (Hematide™) for the Human Erythropoietin Receptor Using a Radioligand Competition Binding Assay	BIOL-EPO-04-004
Erythropoietin Receptor-Specific Responsiveness of Engineered Reporter Cells to AF37702 (Hematide™)	BIOL-EPO-04-006
Response of UT-7/EPO Cells to AF37702 (Hematide™): Proliferation and Rescue From Apoptosis	BIOL-EPO-04-008

Signal Transduction in Response to AF37702 (Hematide™)	BIOL-EPO-04-007
Erythroid Colony Formation in Response to AF37702 (Hematide™)	BIOL-EPO-04-009
<i>In vivo Pharmacology</i>	
Erythropoietic Activity Analysis of AF37702 Following Repeated Intravenous or Subcutaneous Injection in Male Sprague-Dawley Rats	AF03-29A
The Effect of the Erythropoiesis Stimulating Agent Hematide™, AF37702, on the Correction of Anemia in Rats with Experimental Renal Failure Induced by Five-Sixth Nephrectomy	AF04-001
Evaluation of Erythropoiesis Stimulating Agents AF37702 (b) (4) in Normocythemic Rats Following Single Intravenous Administration at 10 mg/kg	AF05-009O
Safety Pharmacology	
Neuropharmacological Profile (NPP) of AF37702 in Mice	AF03-38
Effects of AF37702 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	AF07-032
Cardiovascular (Hemodynamic) Evaluation of AF37702 in Anesthetized Dogs	AF03-37
Safety Pharmacology Studies of AF37702: Effects on the Cardiovascular System in Anesthetized Dogs	BA06089
Pulmonary Assessment of AF37702 in the Anesthetized Guinea Pig	AF03-39
Determination of Electrolyte Concentrations and Volume Diuresis after Intravenous Administration of AF37702 in Rats	AF03-40
Pharmacokinetics	
<i>Absorption</i>	
Single-Dose Intravenous Pharmacokinetic Analysis of AF37702 in Male Sprague-Dawley Rats (non-GLP)	AF03-49
Single-Dose Intravenous and Subcutaneous Pharmacokinetic Analysis of AF37702 in Male Sprague-Dawley Rats (non-GLP)	AF03-28A
Single-Dose Intravenous Pharmacokinetic Analysis of Three AF37702 Dose Levels in Male Non-Naïve Cynomolgus Monkeys (non-GLP)	AF03-48
<i>Distribution</i>	
Quantitative Whole-Body Autoradioluminography in Sprague-Dawley Rats Following Intravenous and Subcutaneous Administration of 14C-AF37702 and Intravenous Administration of (b) (4)	AF05-017
Binding of AF37702 to Serum Proteins and Lipoproteins	AF09-010
Feto-Placental Transfer of Radioactivity in Rats After Single Intravenous Administration of [14C]AF37702	09-808-020:AF37702-10417
<i>Metabolism</i>	
<i>In Vitro</i> Metabolism of [14C]AF37702 by Hepatic and Renal Microsomes and Hepatic and Renal S9 Fractions From Humans, Rats, and Monkeys (non-GLP)	B117-702-032:AF37702-10425
Preliminary Metabolite Profiles in Plasma, Urine, and Feces Following a Single Intravenous or Subcutaneous Dose of [14C]-AF37702 to Male and Female Sprague-Dawley Rats (non-GLP)	AF08-024
<i>Excretion</i>	
Excretion Mass Balance and Pharmacokinetics of Radioactivity Following a Single Intravenous or Subcutaneous Dose of [14C]AF37702 to Male and Female Sprague-	AF08-022

Dawley Rats (non-GLP)	
Transfer of the Radioactivity Into the Breast Milk of Lactating Rats After Single Intravenous Administration of [14C]AF37702	09-808-021:AF37702-10469
General Toxicology	
Single Dose Toxicity	
Single Dose Intravenous Toxicity Study of AF37702 in Rats Followed by a 14-Day Recovery	AF03-33
Single Dose Intravenous Toxicity Study of AF37702 in Cynomolgus Monkeys Followed by a 14-Day Recovery	AF03-34
Repeat-dose Toxicity	
A 3-Month Toxicity Study of AF37702 With Intravenous Administration Every Three-Weeks in Mice	AF06-018
Four-Week Intravenous Toxicity Study of AF37702 in Rats Followed by a Six-Week Recovery	AF03-35
A 6-Month Intravenous Toxicity Study of AF37702 in Rats, Including a 3-Month Interim Sacrifice, Followed by a Six-Week Recovery	AF04-009
A 3-Month Subcutaneous Toxicity Study of AF37702 in Rats Followed by a Six-Week Recovery	AF04-011
Four-Week Intravenous Toxicity Study of AF37702 in Cynomolgus Monkeys Followed by a Twelve-Week Recovery	AF03-36
A 9-Month Intravenous Toxicity Study of AF37702 in Cynomolgus Monkeys, Including 3-Month and 6-Month Interim Sacrifices, Followed by a Fourteen-Week Recovery	AF04-010
A 4-Week Subcutaneous Toxicity Study of AF37702 in Cynomolgus Monkeys	AF05-011
Genotoxicity	
Bacterial Reverse Mutation Assay	AF03-46
<i>In Vitro</i> Mammalian Chromosome Aberration test	AF03-47
Mammalian Erythrocyte Micronucleus Test	AF04-019
Carcinogenicity	
A Two Year Carcinogenicity Study of AF37702 in Rats Following Intravenous Administration Every Three-Weeks	AF06-013
26 Week Carcinogenicity Study of AF37702 with Intravenous Administration Every Three-Weeks in Tg.rash2 Mice	AF08-004
Reproductive and Developmental Toxicity	
Study for Effects of AF37702 on Fertility and General Reproductive Performance in Rats (SEG I)	AF05-020
Study for Effects of AF37702 on Embryo-Fetal Development in Rats (SEG II)	AF04-017
Effects of AF37702 on Embryo-Fetal Development in Rats–Supplemental Study	BA06123
Study for Effects of AF37702 on Embryo-Fetal Development in Rabbits (SEG II)	AF05-003
Effects of AF37702 on Embryo-Fetal Development in Rabbits–Supplemental Study	BA06124
Intravenous Developmental and Perinatal/Postnatal Reproduction Toxicity Study of AF37702 in Rats, Including a Postnatal Behavioral/Functional Evaluation	AF08-006
Special Toxicity Studies	
<i>Antigenicity</i>	
Antigenicity Study of AF37702 in Guinea Pigs: Systemic Anaphylaxis	AF03-41
AF37702: Guinea Pig Sensitization–Maximization Test (Magnusson-Kligman)	AF03-42
<i>Impurity</i>	

A Four-Week Intravenous Toxicity Study of AF37702 Containing (b) (4) in Rats Followed by a Six-Week Recovery	AF08-012
<i>Other Toxicity Studies</i>	
Evaluation of AF37702 to Induce Hemolysis in Human Blood	AF03-43
Evaluation of AF37702 to Induce Flocculation in Human Plasma and Serum	AF03-44

Studies Not Reviewed

Study Title	Study No.
Primary Pharmacodynamics	
<i>In vivo Pharmacology</i>	
AF37702 Erythropoietic Activity in Normocythemic Mice Following a Single Bolus Intravenous Injection	AF03-51B
A 3-Month Murine Hematology Study of AF37702 With Intravenous administration Every Three-Weeks for a Total of Five Injections Followed by an Eight-Week Recovery Period	AF08-026
AF37702 Erythropoietic Activity in Normocythemic Male Sprague-Dawley Rats Following Single-Dose Intravenous Administration	AF03-28B
Effect of a Single Intravenous Administration of AF37702 on Erythropoiesis in Normal Rats	AF37702-00037
Erythropoiesis Study of AF37702 Following Intravenous Administration Once Every Three Weeks for a Total of Eighteen Injections in Male Sprague-Dawley Rats	AF08-003C
Effect of Repetitive Intravenous and Subcutaneous Administrations of AF37702 on Erythropoiesis in Normal Rats	AF37702-00039
Single Dose Intravenous Pharmacokinetic and Erythropoietic Analysis of AF37702 in New Zealand White Rabbits	AF04-014
Single Intravenous Dose Pharmacokinetic and Erythropoietic Analysis of AF37702 (1 mg/kg) in Male Beagle Dogs	AF03-52
Single-Dose Intravenous Pharmacokinetic and Erythropoietic Analysis of 1.35 mg AF37702/kg in Male Non-Naïve Cynomolgus Monkeys	AF03-31
Single-Dose Intravenous Pharmacokinetic Analysis of Three AF37702 Dose Levels in Male Non-Naïve Cynomolgus Monkeys	AF03-48
Effect of a Single Intravenous Administration of AF37702 on Erythropoiesis in Cynomolgus Monkeys	AF37702-00041
Erythropoiesis-Stimulating Effects of a Single Intravenous Administration of AF37702 in 5/6 Nephrectomized Rats	AF37702-00038
AF09-008: Evaluation of the Erythropoietic Activity of AF37702 at Doses of 0.1, 1, and 10 mg/kg in Rats with Experimental Renal Failure Induced by 5/6 Nephrectomy	AF09-008
Evaluation of the Erythropoietic Activity of 10 mg/kg AF37702 Following Single Administration in Normocythemic Male Rats using 2, 10 or 20 mg/mL Formulation Strengths	AF09-007A
Evaluation of AF37702, Formulated in Acetate Buffer and Phosphate Buffer, in Male Sprague-Dawley Rats Following Single Bolus Intravenous Injections	AF05-009B
Evaluation of Erythropoiesis Stimulating Agents AF37702, (b) (4) in Normocythemic Rats Following Single Intravenous Administration at 1 mg/kg	AF06-003C
Secondary Pharmacodynamics	
AF37702 Leadprofiling Receptor Assay: Specificity Screening of AF37702	AF03-50

(Hematide™) Against a Panel of 66 Radioligand Binding Assays	
The Evaluation of AF37702 to Induce Proliferation (or Not) of Human Tumor and TF-1 Erythroleukemia Cells	AF05-019
Pharmacokinetics	
<i>Absorption</i>	
28-Day Toxicity and Pharmacokinetic Study of AF37702 With Intravenous Administration Every Three-Weeks in CByB6F1 Hybrid Mice (GLP)	AF07-013
Linearity in the Plasma Concentrations of AF37702 in Rats After Single Subcutaneous and Intravenous Administration of AF37702 (non-GLP)	09-808-028:AF37702-10421
Pharmacokinetics of Radioactivity After Single Intravenous Administration of [14C]AF37702 to Male Rats (non-GLP)	09-808-016:AF37702-10516
Excretion Mass Balance and Pharmacokinetics of Radioactivity Following a Single Intravenous or Subcutaneous Dose of [14C]AF37702 to Male and Female Sprague Dawley Rats (non-GLP)	AF08-022
Single Dose Intravenous Pharmacokinetic and Erythropoietic Analysis of AF37702 in New Zealand White Rabbits (non-GLP)	AF04-014
Single Intravenous Dose Pharmacokinetic and Erythropoietic Analysis of AF37702 (1 mg/kg) in Male Beagle Dogs (non-GLP)	AF03-52
Single-Dose Intravenous Pharmacokinetic and Erythropoietic Analysis of 1.35 mg AF37702/kg in Male Non-Naïve Cynomolgus Monkeys (non-GLP)	AF03-31
Dose Proportionality in the Plasma Concentrations of AF37702 in Monkeys After Single Subcutaneous and Intravenous Administration of AF37702 (non-GLP)	09-808-029:AF37702-10422
Plasma Concentrations and Urinary and Fecal Excretion of Radioactivity in Monkeys After Single Intravenous Administration of [14C]AF37702 (non-GLP)	09-808-015:AF37702-10410
<i>Distribution</i>	
Tissue Distribution of 14C-AF37702 in Male Sprague Dawley Rats Following a Single Intravenous Dose Using Quantitative Whole-Body Autoradioluminography	AF04-002
Concentrations of the Radioactivity in the Tissues of Albino and Pigmented Rats After Single Intravenous Administration of [14C]AF37702	09-808-017:AF37702-10481
Concentrations of the Radioactivity in the Tissues of Rats After Single Subcutaneous Administration of [14C]AF37702	09-808-033:AF37702-11552
A Tissue Distribution Study of [14C]AF37702 Following Intravenous Administration in Male Cynomolgus Monkeys Using Quantitative Whole-Body Autoradiography (QWBA) and Microautoradiography (MARG)	AF09-001
<i>In Vitro</i> Partitioning of [14C]AF37702 Into Blood Cells in Rats, Monkeys, and Humans	09-808-019:AF37702-10401
Intravenous Developmental and Perinatal/Postnatal Reproduction Toxicity Study of AF37702 in Rats, Including a Postnatal Behavioral/Functional Evaluation (GLP)	AF08-006
<i>Metabolism</i>	
Metabolite Profiles in the Plasma After Single Intravenous Administration of [14C]AF37702 to Rats (non-GLP)	09-808-022:AF37702-10482
Metabolite Profiles in the Urine and Feces After Single Intravenous Administration of [14C]AF37702 to Rats (non-GLP)	09-808-024:AF37702-10485
Metabolite Profiles in the Tissues After Single Intravenous Administration of [14C]AF37702 to Rats	09-808-035:AF37702-11558
Metabolite Profiles in the Plasma After Single Intravenous Administration of [14C]AF37702 to Monkeys (non-GLP)	09-808-023:AF37702-10483
Metabolite Profiles in the Urine and Feces After Single Intravenous Administration of [14C]AF37702 to Monkeys (non-GLP)	09-808-025:AF37702-10484

Metabolite Profiles in the Maternal and Fetal Plasma After Single Intravenous Administration of [14C]AF37702 to Pregnant Rats (non-GLP)	09-808-026:AF37702-10475
Metabolite Profiles in the Plasma and Milk After Single Intravenous Administration of [14C]AF37702 to Lactating Rats (non-GLP)	09-808-027:AF37702-10476
<i>In Vitro</i> Evaluation of Two Lots of AF37702 as Inhibitors of Human Cytochrome P450 Enzymes (GLP)	AF08-013
<i>In Vitro</i> Evaluation of Two Lots of AF37702 as Inducers of Human Cytochrome P450 Enzymes Expressed in Cultured Human Hepatocytes (GLP)	AF08-014
<i>Drug interactions</i>	
Inhibitory Effects of AF37702 (b) (4) (b) (4) on Cytochrome P450 Activities (non-GLP)	270-2002:AF-37702-00024
Evaluation of CYP3A Induction by AF37702 (b) (4) (b) (4) in Human Hepatocytes (non-GLP)	A957-702-004:AF37702-00025
<i>Excretion</i>	
Urinary, Fecal and Expiratory Excretion of the Radioactivity in Rats After Single Intravenous Administration of [14C]AF37702 (non-GLP)	09-808-018:AF37702-10418
Plasma Concentrations and Urinary and Fecal Excretion of Radioactivity in Monkeys After Single Intravenous Administration of [14C]AF37702 (non-GLP)	09-808-015:AF37702-10410
<i>Other Pharmacokinetic Studies</i>	
Single-Dose Intravenous Pharmacokinetic Analysis of AF37702 in Male Sprague-Dawley Rats with Experimental Renal Failure Induced by Five-Sixth Nephrectomy (non-GLP)	AF04-020
Single Dose Intravenous Pharmacokinetic Analysis of (b) (4) (b) (4) in Male Sprague Dawley Rats (non-GLP)	AF08-002C
Genotoxicity	
Salmonella/Escherichia coli Spot Test Mutagenicity Assay	AF03-45
Carcinogenicity	
28-Day Toxicity and Pharmacokinetic Study of AF37702 With Intravenous Administration Every Three-Weeks in CByB6F1 Hybrid Mice	
Reproductive and Developmental Toxicity	
Intravenous Dosage-Range Developmental and Perinatal/Postnatal Reproductive Toxicity Study of AF37702 in Rats	AF07-027
Dose-Range Finding Study for Effects of AF37702 at Doses of 0, 1, 10 and 50 mg/kg From Gestation Day 6 to Day 20 on the Embryo-Fetal Development in Sprague Dawley Rats	AF04-012
Local Tolerance	
A 14-Day Single Dose Subcutaneous Local Tolerance Study of AF37702 in Male Sprague-Dawley Rats	AF05-006
Special Toxicology Studies	
An Antibody Induction Study of AF37702 in Male Cynomolgus Monkeys	AF05-007
A Single Dose Subcutaneous Systemic and Local Tolerance Study of AF37702 Containing Linear Alkylbenzene Sulfonate (LAS) in Male Sprague-Dawley Rats	AF05-021
Antibody Detection Direct ELISA	
Antibody Detection Direct ELISA - Positive Control Antibodies	
Antibody Detection Direct ELISA - Definition of Positives (Signal Cut Point)	
Antibody Detection Direct ELISA - Antibody Specificity Retest	

Antibody Detection Direct ELISA - Assay Validation	
Antibody Detection Direct ELISA - Assay Sensitivity	
Antibody Detection Direct ELISA - Drug Assay Interference	
Neutralizing Antibody Assays	
Anti-EPO Antibody Radioimmunoprecipitation Assay	

3.3 Previous Reviews Referenced

Non-clinical reviews under IND 63,257

4 Pharmacology

4.1 Primary Pharmacology

Study title: Determination of the IC₅₀ of AF37702 (Hematide™) for the human erythropoietin receptor using a radioligand competition binding assay

Study no.: BIOL-EPO-04-004

Study report location: eCTD 4.2.1.1

The binding of AF37702 (Hematide™) to recombinant human erythropoietin receptor was assessed by determining the IC₅₀ of AF37702 in a radioligand competition binding assay. In this assay, recombinant human erythropoietin receptor extracellular domain that had been fused to the Fc portion of human IgG was combined with a serial dilution of AF37702, (b) (4) of AF37702), or a control agonist (Aranesp or erythropoietin), and a constant amount of ¹²⁵I radiolabeled recombinant human erythropoietin (¹²⁵I-EPO). This was allowed to equilibrate overnight at 4°C, and bound ¹²⁵I-EPO was quantitated the next day. A total of 3 replicate IC₅₀ determinations were conducted for all compounds. Results indicate that AF37702 binds to recombinant human erythropoietin receptor with a mean IC₅₀ value of 36.97 pM.

Table 1: IC₅₀ values for AF37702, (b) (4) Aranesp, and erythropoietin

Assay	IC ₅₀ (pM)			
	AF37702	(b) (4)	Aranesp	Erythropoietin
Replicate 1	34.79	7.219	12.44	7.634
Replicate 2	41.58	7.113	12.45	6.612
Replicate 3	34.54	5.225	12.45	6.091
Mean	36.97	6.52	12.45	6.78

Study title: Erythropoietin receptor-specific responsiveness of engineered reporter cells to AF37702 (Hematide™)

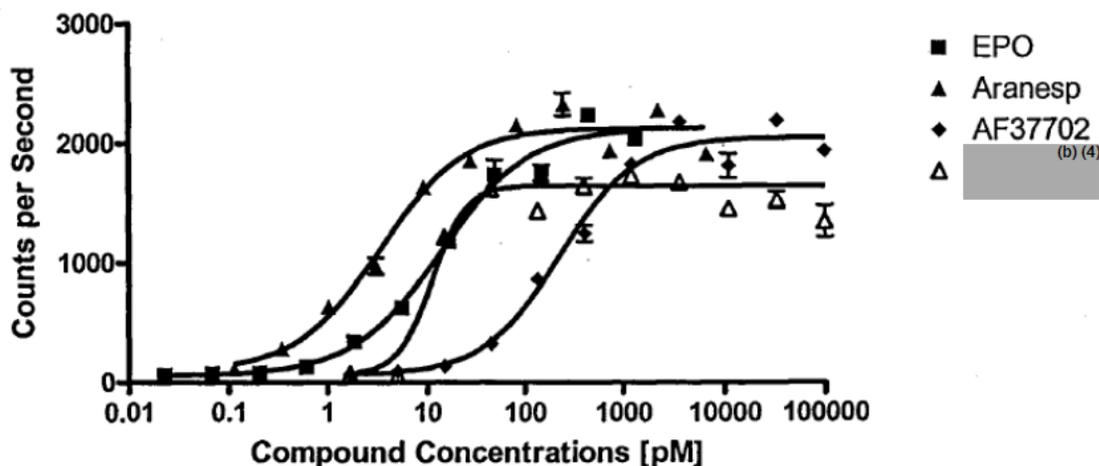
Study no.: BIOL-EPO-04-006

Study report location: eCTD 4.2.1.1

The functional activity and specificity of AF37702 was evaluated using engineered reporter cells developed from an IL-3 dependent murine pre-B cell line (Ba/F3) transfected with DNA encoding a chimeric human erythropoietin receptor (HuEPOr), human granulocyte colony stimulating factor receptor (HuG-CSFr), or human thrombopoietin receptor (HuTPOr). Cell proliferation assays were conducted with AF37702, (b) (4), erythropoietin (EPO), and Aranesp. G-CSF was used as a positive control in the HuG-CSFr cell assay and TPO was used as a positive control for the HuTPOr cell assay.

AF37702 ($EC_{50}=280$ pM), (b) (4) ($EC_{50}=10$ pM), EPO ($EC_{50}=14$ pM), and Aranesp ($EC_{50}=4.4$ pM) produced a dose-dependent activation of the HuEPOr reporter cells (see Figure 1 below). None of the compounds stimulated the proliferation of HuG-CSFr or HuTPOr reporter cells. These results suggest that AF37702 has activity at the human erythropoietin receptor, but does not have activity at the human granulocyte colony stimulating factor receptor or the human thrombopoietin receptor.

Figure 1: Dose response curves of Ba/F3 HuEPOr/HuG-CSFr fos-luc reporter cells incubated with EPO, Aranesp, AF37702, or (b) (4) (Excerpted from Applicant's submission)



Study title: Response of UT-7/EPO cells to AF37702 (Hematide™): Proliferation and rescue from apoptosis

Study no.: BIOL-EPO-04-008

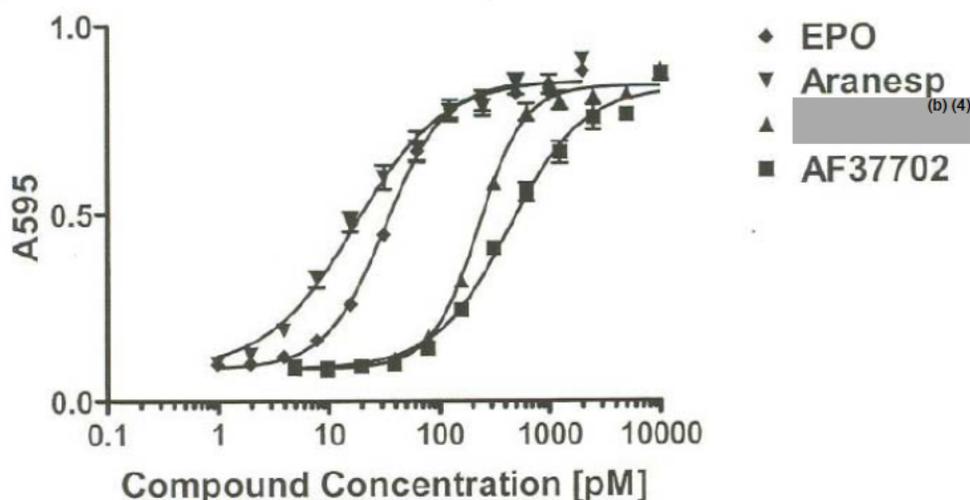
Study report location: eCTD 4.2.1.1

This study was conducted to assess the ability of AF37702 to stimulate the proliferation of UT-7/EPO cells, an EPO-responsive human leukemia cell line, and to study the ability of AF37702 to rescue UT-7/EPO cells from apoptosis. Tissue culture experiments in

96-well plates were conducted to assess the proliferation of UT-7/EPO cells in response to AF37702, (b) (4) of AF37702), and control compounds erythropoietin (EPO) and Aranesp. To assess the activity of AF37702 in rescuing UT-7/EPO cells from apoptosis, Annexin V-FITC and propidium iodide (PI) staining of cells was conducted on cells cultured in the following culture conditions: 1) 120 mL of cells with 3 nM EPO, 2) 120 mL of cells with 3 nM Aranesp, 3) 120 mL of cells with 50 nM AF37702, and 4) 400 mL of cells in basic growth medium without EPO (starved cells). Following 0, 24, 48, 72 and 96 hours of culture, 1×10^6 cell aliquots were collected from each condition for Annexin V staining, and $15\text{--}20 \times 10^6$ cell aliquots were collected from each condition for caspase activity measurements. Caspase-3 and caspase-8 activity were measured using Colorimetric assay kits.

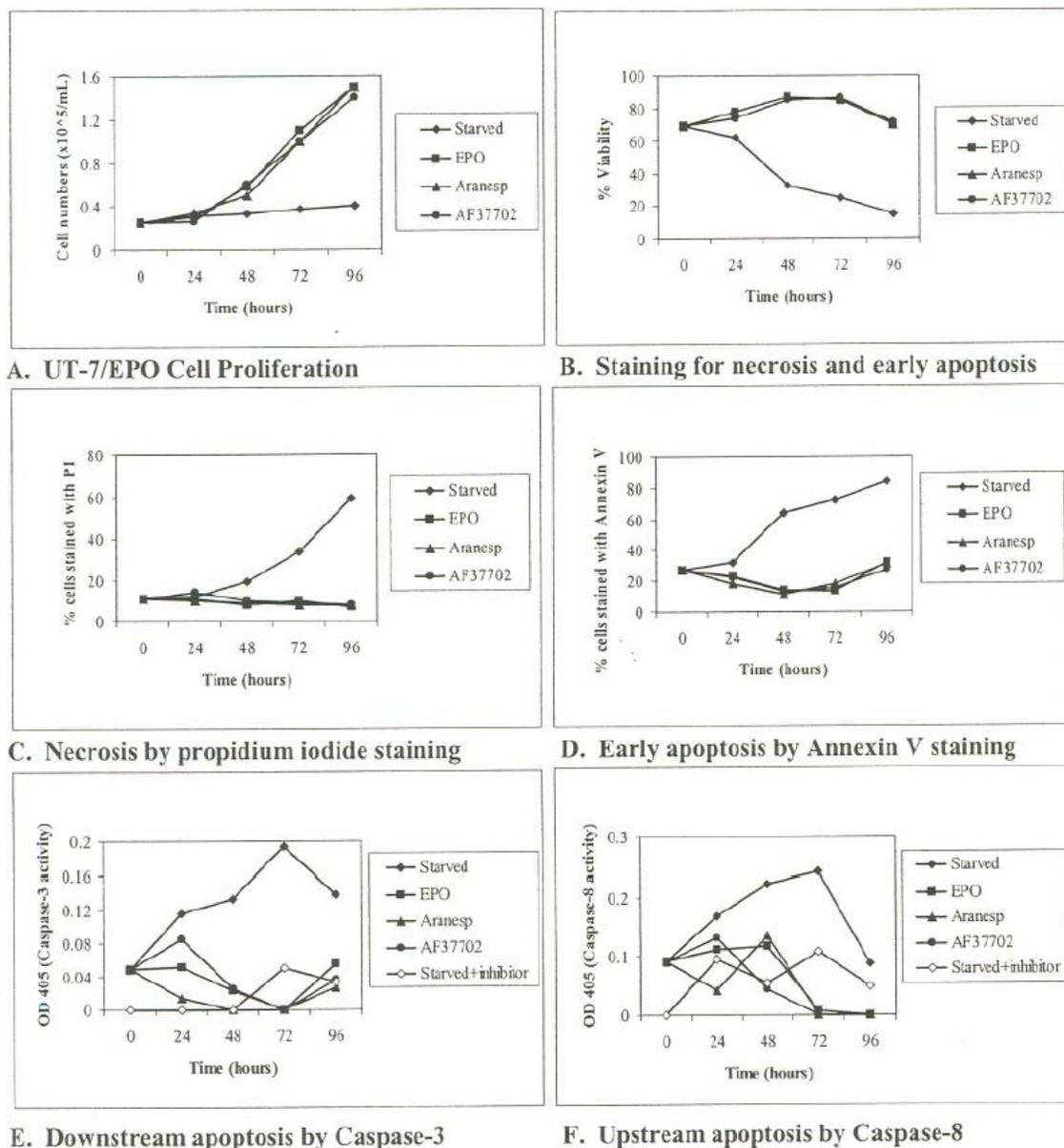
AF37702 dose-dependently stimulated the proliferation of UT-7/EPO cells with an EC_{50} value of 457 pM, and a maximal response similar to that of EPO and Aranesp. The EC_{50} values for EPO, Aranesp, and (b) (4) were 36 pM, 17 pM, and 258 pM respectively.

Figure 2: Proliferation of UT-7/EPO cells
(Excerpted from Applicant's submission's submission)



Induction of apoptosis was observed in the starved UT-7/EPO cells cultured without EPO, resulting in reduced cell growth and viability, increasing numbers of cells stained positive for Annexin V, and increased activity of caspase-3 and caspase-8. In contrast, UT-7/EPO cells cultured with AF37702, EPO, or Aranesp proliferated with a high viability and low death rate (% cells stained with PI). The number of AF37702-, EPO-, or Aranesp-cultured cells stained positive for Annexin V remained low compared to starved cells and caspase activity did not increase. Cell proliferation and viability, staining for Annexin V and PI, and caspase-3 and caspase-8 activity are shown in the figure below. These results indicate that AF37702 inhibits apoptosis of UT-7/EPO cells.

Figure 3: Inhibition of UT-7/EPO cell apoptosis by AF37702, EPO and Aranesp (Excerpted from Applicant's submission)



Study title: Signal transduction in response to AF37702 (Hematide™)

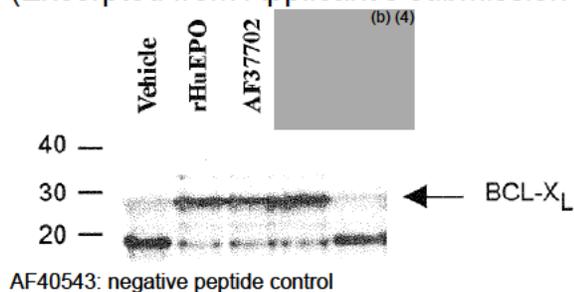
Study no.: BIOL-EPO-04-007

Study report location: eCTD 4.2.1.1

The signal transduction response to AF37702 was characterized and compared to the response to erythropoietin, the natural ligand for the human erythropoietin receptor, through multiple *in vitro* assays and techniques with UT-7/EPO cells including immunoprecipitation, western blotting, PARP cleavage FACS assay, DNA fragmentation FACS assay, and BCL-X_L FACS assay. The PARP cleavage FACS, DNA fragmentation FACS, and BCL-X_L FACS assays were conducted with medium containing AF37702, (b) (4) of AF37702), recombinant human erythropoietin

X_L FACS assay. AF37702 and (b) (4) maintained the expression level of BCL-X_L (see Figure 5 below). The activity of AF37702 was similar to the activity of rHuEPO for these assays, and these results suggest that AF37702 protects cells from apoptosis through the suppression of caspase activation and maintenance of the expression of the anti-apoptotic protein BCL-X_L.

Figure 5: BCL- X_L expression in AF37702-treated cells
(Excerpted from Applicant's submission's submission)



Study title: Erythroid colony formation in response to AF37702 (Hematide™)

Study no.: BIOL-EPO-04-009

Study report location: eCTD 4.2.1.1

The ability of AF37702 and (b) (4) of AF37702) to stimulate proliferation and differentiation of primitive erythroid progenitors was assessed in an erythroid (BFU-E) colony assay using primary human CD34+ cells. A methylcellulose based medium supplemented with recombinant human stem cell factor (rhSCF), recombinant human granulocyte macrophage colony stimulating factor (rhGM-CSF), and recombinant human interleukin 3 (rhIL-3) was used in the colony assay. Human CD34+ cells were incubated with EPO, Aranesp, AF37702, or (b) (4) for 14 days. The number of BFU-E colonies per 1000 cells was calculated.

The controls EPO and Aranesp stimulated dose dependent formation of erythroid colonies with EC₉₀ values of approximately 180 pM and 400 pM respectively. AF37702 and (b) (4) also stimulated the formation of erythroid colonies from human CD34+ cells with EC₉₀ values of 3 to 4 nM (3000 to 4000 pM). These results indicate that AF37702 can stimulate erythroid progenitors from primary human CD34+ cells to form erythroid colonies.

Study title: Erythropoietic activity analysis of AF37702 following repeated intravenous or subcutaneous injection in male Sprague-Dawley rats

Study no.: AF03-29A

Study report location: eCTD 4.2.1.1

The erythropoietic activity of AF37702 was assessed following repeated intravenous or subcutaneous injections in normocythemic male Sprague-Dawley rats. AF37702 (0.135 or 1.35 mg/kg) was administered intravenously or subcutaneously once weekly or once

every two weeks for 6 weeks. Vehicle was administered intravenously or subcutaneously once weekly. Hematology parameters (reticulocytes, hemoglobin, hematocrit, RBC, MCV, MCHC, MCH, and WBC) were measured on Days 15, 29, 43, 50, and 64 for the once weekly groups and on Days 15, 29, 43, and 57 for the once every two weeks groups. Anti-AF37702 antibody assessment was performed on Day 43 for all dose groups, on Day 78 for the intravenous dose groups, and on Day 85 for the subcutaneous dose groups.

Repeated intravenous or subcutaneous administration of AF37702 resulted in hematologic changes including increases and decreases in reticulocytes, increases in hemoglobin, hematocrit, and RBC, and decreases in MCV, MCHC, and MCH. Reticulocytes were significantly increased compared to vehicle controls at the high dose of 1.35 mg/kg following weekly intravenous or subcutaneous dosing, with the largest increase observed at Day 15. Blood sampling was conducted 14 days following an AF37702 injection in the once every two weeks groups, so drug was not on board at the time of sampling. Reticulocytes were significantly decreased in the once every two weeks groups compared to vehicle controls due to functional iron deficiency following massive reticulocytosis. Hemoglobin and RBC levels were significantly increased at both 0.135 and 1.35 mg/kg AF37702 for intravenous and subcutaneous administration for both dosing schedules. These increases were dose-dependent for once every two weeks administration, but were not dose-dependent for once weekly administration most likely due to maximization of the erythropoietin response. The changes in reticulocytes, hemoglobin, and RBC are shown in the tables below. No anti-AF37702 antibodies were detected in any of the rats administered AF37702 intravenously or subcutaneously.

Table 2: Mean percent reticulocyte levels in once weekly groups
(Excerpted from Applicant's submission)

Day	IV			SC		
	0	0.135	1.35	0	0.135	1.35
15	3.85 ± 0.87	3.93 ± 0.50	12.20 ± 1.54*	3.70 ± 0.34	4.88 ± 1.01	10.13 ± 0.97*
29	3.20 ± 0.39	3.20 ± 0.24	6.73 ± 1.64*	2.63 ± 0.30	3.53 ± 0.75	6.40 ± 1.12*
43	2.33 ± 0.36	2.28 ± 0.39	6.60 ± 1.30*	2.65 ± 0.24	2.98 ± 0.43	6.45 ± 1.33*

*Significant Difference from Vehicle Control, $P < 0.05$.

Table 3: Mean percent reticulocyte levels in once every 2 weeks groups
(Excerpted from Applicant's submission)

Day	IV			SC		
	0	0.135	1.35	0	0.135	1.35
15	3.85 ± 0.87	1.12 ± 0.83*	1.50 ± 0.22*	3.70 ± 0.34	1.48 ± 0.46*	1.10 ± 0.18*
29	3.20 ± 0.39	0.70 ± 0.20*	1.33 ± 0.15*	2.63 ± 0.30	0.65 ± 0.31*	0.95 ± 0.19*
43	2.33 ± 0.36	0.60 ± 0.00*	1.30 ± 0.29*	2.65 ± 0.24	0.40 ± 0.20*	1.07 ± 0.32*

*Significant Difference from Vehicle Control, $P < 0.05$.

Table 4: Mean hemoglobin levels in once weekly groups
(Excerpted from Applicant's submission)

Day	IV			SC		
	0	0.135	1.35	0	0.135	1.35
15	15.65 ± 0.54	18.13 ± 0.97*	19.08 ± 0.81*	14.58 ± 0.30	17.38 ± 0.72*	18.13 ± 0.66*
29	15.20 ± 0.54	20.43 ± 1.04*	20.70 ± 1.61*	15.30 ± 1.01	19.80 ± 0.94*	19.13 ± 0.57*
43	16.00 ± 0.29	21.90 ± 0.98*	22.20 ± 0.85*	15.40 ± 0.47	19.88 ± 0.90*	20.70 ± 0.77*
50	15.70 ± 0.35	20.35 ± 1.47*	22.30 ± 1.33*	15.30 ± 0.48	20.10 ± 1.14*	21.20 ± 0.85*
64	15.23 ± 0.90	15.85 ± 0.97	16.18 ± 1.24	13.77 ± 1.62	16.53 ± 0.49*	14.83 ± 0.93

*Significant Difference from Vehicle Control, $P < 0.05$.

Table 5: Mean hemoglobin levels in once every 2 weeks groups
(Excerpted from Applicant's submission)

Day	IV			SC		
	0	0.135	1.35	0	0.135	1.35
15	15.65 ± 0.54	15.65 ± 0.93	17.30 ± 0.76*	14.58 ± 0.30	14.98 ± 0.61	17.30 ± 1.16*
29	15.20 ± 0.54	16.77 ± 0.15*	18.78 ± 0.38*	15.30 ± 1.01	16.95 ± 0.70	19.10 ± 1.47*
43	16.00 ± 0.29	17.70 ± 0.10*	20.43 ± 1.07*	15.40 ± 0.47	17.83 ± 1.02*	20.17 ± 1.21*
57	15.73 ± 0.68	15.23 ± 0.86	16.60 ± 1.15	15.73 ± 0.21	15.75 ± 0.52	16.83 ± 1.63

*Significant Difference from Vehicle Control, $P < 0.05$.

Table 6: Mean RBC levels (106/ μ L) in once weekly groups
(Excerpted from Applicant's submission)

Day	IV			SC		
	0	0.135	1.35	0	0.135	1.35
15	7.64 ± 0.38	9.38 ± 0.27*	10.70 ± 0.25*	7.30 ± 0.14	9.05 ± 0.18*	10.33 ± 0.45*
29	7.80 ± 0.50	11.49 ± 0.21*	13.42 ± 0.75*	8.03 ± 0.65	11.49 ± 0.20*	13.08 ± 0.37*
43	8.14 ± 0.41	12.83 ± 0.28*	14.90 ± 0.91*	8.06 ± 0.31	12.17 ± 0.46*	14.95 ± 0.51*
50	8.22 ± 0.47	12.17 ± 0.45*	15.60 ± 1.34*	8.34 ± 0.24	12.40 ± 1.10*	15.28 ± 0.69*
64	7.99 ± 0.53	9.74 ± 0.24	11.23 ± 1.60*	7.38 ± 0.64	10.10 ± 0.56*	11.23 ± 1.46*

*Significant Difference from Vehicle Control, $P < 0.05$.

Table 7: Mean RBC levels (106/ μ L) in once every 2 weeks groups
(Excerpted from Applicant's submission)

Day	IV			SC		
	0	0.135	1.35	0	0.135	1.35
15	7.64 ± 0.38	7.94 ± 0.47	9.68 ± 0.86*	7.30 ± 0.14	7.40 ± 0.36	9.21 ± 0.32*
29	7.80 ± 0.50	9.24 ± 0.34*	11.58 ± 1.01*	8.03 ± 0.65	8.98 ± 0.45	11.02 ± 0.49*
43	8.14 ± 0.41	9.74 ± 0.26*	12.87 ± 1.01*	8.06 ± 0.31	9.78 ± 0.61*	12.11 ± 0.44*
57	8.10 ± 0.47	8.54 ± 0.37	10.46 ± 1.01*	8.51 ± 0.15	8.89 ± 0.30	10.33 ± 0.61*

*Significant Difference from Vehicle Control, $P < 0.05$.

Study title: The effect of the erythropoiesis stimulating agent Hematide™, AF37702, on the correction of anemia in rats with experimental renal failure induced by five-sixth nephrectomy**Study no.:** AF04-001**Study report location:** eCTD 4.2.1.1

The erythropoietic activity of AF37702 was evaluated following a single-bolus intravenous administration (0, 0.1, 1, and 10 mg/kg) in a rodent model of anemia. Male Sprague-Dawley rats were nephrectomized using the 5/6 nephrectomy procedure involving the complete removal of one kidney and 2/3 of the other kidney to induce renal failure and anemia. Twenty-five days later, rats were assigned to one of four dosing groups, and were administered a single intravenous bolus dose (1 mL/kg) of vehicle or AF37702 (0.1, 1, or 10 mg/kg). The day of dosing was designated Day 0. Hematologic parameters (reticulocytes, hemoglobin, hematocrit, RBC, MCV, MCHC, MCH, and WBC) were measured on Days 3, 5, 9, 15, 19, 23, 29, 33, 38, and 44.

Treatment with AF37702 dose-dependently increased both reticulocyte count and hemoglobin levels compared to vehicle-controls (see Figures 6 and 7 below). The maximum increase in reticulocyte count was observed on Day 5. Hematocrit and RBC levels were also significantly increased by AF37702 treatment at all doses compared to vehicle-controls.

Figure 6: Reticulocyte counts in nephrectomized rats following a single intravenous administration of vehicle or AF37702

(Excerpted from Applicant's submission)

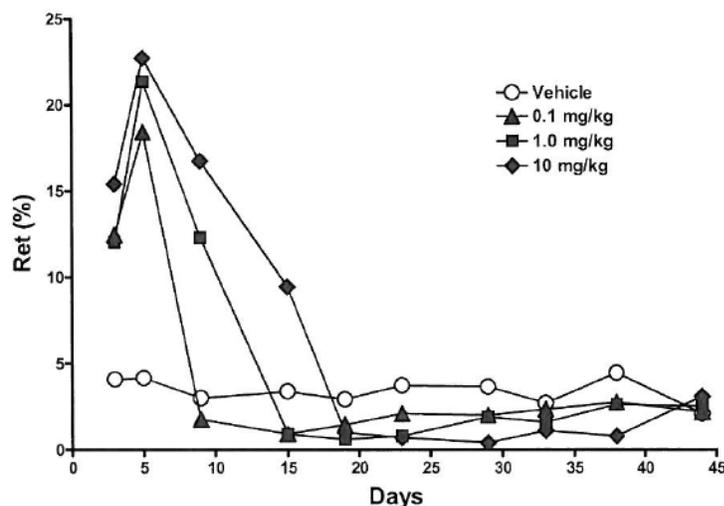
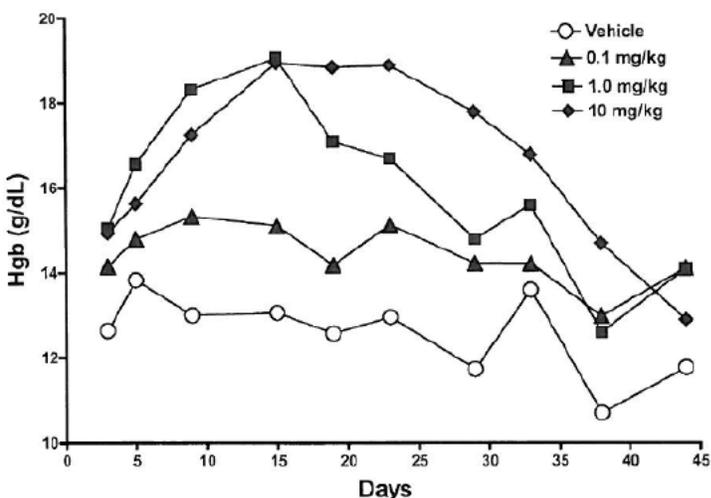


Figure 7: Hemoglobin levels in nephrectomized rats following a single intravenous administration of vehicle or AF37702
(Excerpted from Applicant's submission's submission)



Study title: Evaluation of erythropoiesis stimulating agents AF37702, (b) (4)

in normocythemic rats

following single intravenous administration at 10 mg/kg

Study no.: AF05-0090

Study report location: eCTD 4.2.1.1

The erythropoietic activity of three AF37702 variants was evaluated and compared with the activity of AF37702 following intravenous administration at 10 mg/kg in normal rats. The variants tested were (b) (4)

Male Sprague-Dawley rats were administered a single intravenous bolus dose (1 mL/kg) of vehicle, (b) (4) or AF37702 on Day 0. Hematologic parameters (reticulocytes, hemoglobin, hematocrit, RBC, MCV, MCHC, MCH, WBC, and absolute neutrophils) were measured on Days 0, 5, 9, 14, 20, 23, 28, 34, and 43.

The (b) (4) forms of AF37702 (b) (4) were comparable in erythropoietic activity to AF37702 at 10 mg/kg. The (b) (4) showed *in vivo* erythropoietic activity, but was less active than AF37702, which is expected given the shorter circulating half-life of (b) (4). The means for percent reticulocytes, hemoglobin, and RBC levels for each compound are presented in the tables below.

Table 8: Mean percent reticulocytes following single intravenous administration of vehicle or 10 mg/kg (b) (4), or AF37702

(Excerpted from Applicant's submission)

Day	Control	(b) (4)			AF37702
0	6.5 ± 0.7	6.8 ± 1.2	6.4 ± 1.0	6.4 ± 1.4	6.8 ± 0.6
5	6.1 ± 0.8*	20.2 ± 1.0#	20.6 ± 1.9#	22.9 ± 2.0#	19.3 ± 1.5#
9	5.4 ± 0.8*	5.7 ± 1.1*	16.6 ± 0.6#	16.3 ± 2.4#	15.2 ± 1.3#
14	5.0 ± 0.6	1.4 ± 0.1*#	3.7 ± 0.3*#	7.3 ± 0.9*#	5.4 ± 0.5
20	4.1 ± 0.1*	1.6 ± 0.5*#	0.7 ± 0.1#	0.9 ± 0.3#	0.7 ± 0.1#
23	3.5 ± 0.5*	2.1 ± 0.7*#	0.7 ± 0.2#	0.3 ± 0.1#	0.5 ± 0.1#
28	3.2 ± 0.7*	3.0 ± 0.6*	2.0 ± 0.3#	0.6 ± 0.2#	1.5 ± 0.6#
34	3.1 ± 0.6	3.8 ± 0.6*	2.8 ± 0.4	1.8 ± 1.1	2.4 ± 0.6
43	2.6 ± 0.6	3.5 ± 0.3	3.8 ± 0.9	3.6 ± 1.1	2.9 ± 0.5

* Denotes significant difference from AF37702, $P < 0.05$. # Denotes significant difference from controls, $P < 0.05$.

Table 9: Mean hemoglobin levels following single intravenous administration of vehicle or 10 mg/kg (b) (4), or AF37702

(Excerpted from Applicant's submission)

Day	Control	(b) (4)			AF37702
0	13.3 ± 0.5	13.6 ± 0.4	13.5 ± 0.3	13.5 ± 0.8	13.6 ± 0.8
5	14.1 ± 0.4*	16.2 ± 0.4#	16.2 ± 0.3#	16.2 ± 0.8#	16.2 ± 0.5#
9	14.7 ± 0.6*	17.1 ± 0.8#	17.2 ± 0.3#	17.8 ± 1.1#	17.5 ± 0.3#
14	14.9 ± 0.4*	17.1 ± 0.6#	18.2 ± 0.1#	19.2 ± 0.3#	18.3 ± 0.3#
20	15.0 ± 0.4*	15.9 ± 0.7*	16.7 ± 0.6#	18.4 ± 1.3#	17.3 ± 0.1#
23	15.4 ± 0.3*	15.6 ± 0.5*	16.2 ± 0.5	17.7 ± 0.9#	16.8 ± 0.4#
28	15.7 ± 0.6	15.4 ± 0.2	15.5 ± 0.5	16.6 ± 1.0	15.8 ± 0.4
34	15.5 ± 0.7	15.4 ± 0.1	15.1 ± 0.4	15.2 ± 1.1	15.5 ± 0.4
43	15.5 ± 0.6*	15.3 ± 0.2*	14.4 ± 0.2#	14.0 ± 0.6#	14.3 ± 0.6#

* Denotes significant difference from AF37702, $P < 0.05$. # Denotes significant difference from controls, $P < 0.05$.

Table 10: Mean RBC levels following single intravenous administration of vehicle or 10 mg/kg (b) (4) or AF37702

(Excerpted from Applicant's submission)

Day	Control	(b) (4)			AF37702
0	6.62 ± 0.29	6.60 ± 0.17	6.55 ± 0.29	6.64 ± 0.25	6.60 ± 0.49
5	7.00 ± 0.32*	7.69 ± 0.24#	7.84 ± 0.25#	7.77 ± 0.27#	8.01 ± 0.48#
9	7.15 ± 0.39*	8.38 ± 0.39#	8.44 ± 0.23#	8.77 ± 0.29#	8.70 ± 0.19#
14	7.54 ± 0.33*	8.84 ± 0.40#	10.06 ± 0.46#	10.73 ± 0.25#	10.21 ± 0.12#
20	7.57 ± 0.28*	8.25 ± 0.44*	9.19 ± 0.37*#	10.32 ± 0.57#	9.99 ± 0.33#
23	7.80 ± 0.31*	8.02 ± 0.49*	8.87 ± 0.32#	9.95 ± 0.50#	9.55 ± 0.46#
28	8.05 ± 0.39*	8.00 ± 0.19*	8.66 ± 0.20	9.48 ± 0.47#	9.30 ± 0.47#
34	8.12 ± 0.52*	8.16 ± 0.33*	8.42 ± 0.28	8.93 ± 0.70	9.17 ± 0.46#
43	8.45 ± 0.41	8.37 ± 0.39	8.22 ± 0.14	8.37 ± 0.48	8.64 ± 0.65

* Denotes significant difference from AF37702, $P < 0.05$. # Denotes significant difference from controls, $P < 0.05$.

Similar results were observed with (b) (4) at a lower dose of 1 mg/kg in Study AF06-0003C.

4.2 Secondary Pharmacology

Studies not reviewed.

4.3 Safety Pharmacology

Neurological effects:

Study title: Neuropharmacological Profile (NPP) of AF37702 in Mice

Study no.: (Affymax) AF03-38

Study report location: eCTD 4.2.1.3.1

Conducting laboratory and location:

(b) (4)

Date of study initiation: 29 March 2004

GLP compliance: Statement included and signed

QA statement: Statement included and signed

Drug, lot #, and % purity: AF37702, 12AB1, 99.9 %

Key Findings:

- No neurological effects on CD-1 mice were observed

Methods:

Species: Crl:CD-1^o (ICR) BR mice

Route: IV, bolus

Vehicle: 10 mM acetate in 0.9% sodium chloride for

injection
 Procedure: The test article or vehicle dosing preparations were administered once to each mouse intravenously via a tail vein. Each animal received 10 ml/kg as a bolus dose.

Group	Number of Animals Male	Treatment	Dose (mg/kg)	Volume (ml/kg)	Concentration (mg/ml)
1	10	Vehicle	0	10	0
2	10	AF37702	1	10	0.1
3	10	AF37702	10	10	1
4	10	AF37702	100	10	10

Results:

(Excerpted from Applicant's submission)

Group Number (n=10)	Intravenous Treatment (10 ml/kg)	Dose (mg/kg)	Mean Body Temperature ^a (°C)	Signs Observed
1	Vehicle for AF37702	0	38.4±0.20	0-24 hours: no signs
2	AF37702	1	38.3±0.22	0-24 hours: no signs
3	AF37702	10	38.9±0.08	0-24 hours: no signs
4	AF37702	100	38.6±0.12	0-24 hours: no signs

^aData are presented as the Mean±SEM

Summary:

Intravenous administration of AF37702 did not produce any neuropharmacological signs at doses of 1, 10, and 100 mg/kg in CD-1 mice and had no effect on body temperatures.

Renal effects:**Study title: Determination of Electrolyte Concentrations and Volume Diuresis after Intravenous Administration of AF37702 in Rats**

Study no.: (Affymax) AF03-40

Study report location: eCTD 4.2.1.3.1

Conducting laboratory and location:

(b) (4)

Date of study initiation: 23 Mar 2004

GLP compliance: Statement included and signed

QA statement: Statement included and signed

Drug, lot #, and % purity: AF37702, 12AB1, 99.9 %

Key Findings:

- A dose of 50 mg/kg AF37702 resulted in decreased Na⁺ and Cl⁻ excretion

Methods:

Three groups of ten male Sprague Dawley rats were intravenously (i.v.) administered AF37702 at 0.5, 5 or 50 mg/kg. An additional group of ten male rats was administered the vehicle at 5 ml/kg, IV. The vehicle was 10 mM acetate in 0.9% sodium chloride for injection. The fasted rats were weighed and orally hydrated with 0.9% saline at 25 ml/kg immediately following the test article or vehicle i.v. administration. The rats were immediately force urinated by massage, placed in individual metabolism cages and urine collected over 4 hours (+5 minutes). Urine volumes and pH were recorded and urinary electrolytes (Na⁺, K⁺ and Cl⁻) were analyzed.

Group	Number of Animals Male	Treatment	Dose (mg/kg)	Volume (ml/kg)	Concentration (mg/ml)
1	10	Vehicle	0	5	0
2	10	AF37702	0.5	5	0.1
3	10	AF37702	5	5	1
4	10	AF37702	50	5	10

Results:

Urinary volume and pH were unaffected by AF37702 at any dose. Na⁺ and Cl⁻ were unaffected at doses ≤ 5 mg/kg. At 50 mg/kg, decreases were noted in Na⁺ and Cl⁻ as well as Cl⁻ excretion.

(Excerpted from Applicant's submission)

Urine Electrolyte Concentration, pH and Volume Output

Group Number (n=10)	Intravenous Treatment (5 ml/kg)	Dose (mg/kg)	Urine Volume (ml)	% Volume Diuresis	pH	Electrolyte Concentrations			Electrolyte Excreted/ 100 Grams Body Weights		
						Na ⁺ (mEq/L)	K ⁺ (mEq/L)	Cl ⁻ (mEq/L)	Na ⁺ (μEq)	K ⁺ (μEq)	Cl ⁻ (μEq)
1	Vehicle	0	4.4±0.33	-	6.57±0.11	156±7.5	38±2.5	164±6.3	342±22.5	84±7.3	357±15.9
2	AF37702	0.5	4.8±0.37	9.1	6.70±0.09	146±4.8	47±2.9	144±4.0	357±25.4	112.6*±8.3	349.8±23.2
3	AF37702	5	4.4±0.39	-	6.62±0.06	140±8.2	48±2.3	147±7.8	307±11.7	106±7.2	324±15.9
4	AF37702	50	4.8±0.31	9.1	6.64±0.07	122*±2.8	44±3.5	113*±5.0	297±21.3	105±7.4	273*±17.7

* = Statistically significant (p<0.05) change relative to the vehicle group - ANOVA/Tukey HSD Multiple Comparison Test

Summary:

Under the conditions tested, intravenous administration of AF37702 to rats did not produce any renal effects at doses up to 5 mg/kg. However, electrolyte excretion was reduced at the 50 mg/kg dose. The excretion was statistically significant (↓24% compared to controls).

Pulmonary effects:**Study title: Pulmonary Assessment of AF37702 in the Anesthetized Guinea Pig**

Study no.: (Affymax) AF03-39; 1082GA33.001 (b) (4)
 Study report location: eCTD 4.2.1.3.1
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 7 April 2004
 GLP compliance: Statement included and signed
 QA statement: Statement included and signed
 Drug, lot #, and % purity: AF37702, 12AB1, 99.9 %

Key Findings:

- Intravenous administration of AF37702 did not produce any pulmonary effects at doses of 1, 10, and 100 mg/kg in the guinea pig

Methods:

Species: Guinea pig (Hartley)
 Route: IV via jugular catheter
 Vehicle: 10 mM acetate in 0.9% sodium chloride for injection
 Procedure: The test article or vehicle dosing preparations were administered once to each animal
 Parameters evaluated: Airway resistance (cmH₂O/mL/sec); dynamic lung compliance (mL/cmH₂O), respiratory rate

(breaths/min), tidal volume (mL), minute volume (mL/min)

Group	Number of Animals	Treatment	Dose (mg/kg)	Volume (ml/kg)	Concentration (mg/ml)
	Male				
1	4	Vehicle	0	5	0
2	4	AF37702	0.5	5	0.1
3	4	AF37702	5	5	1.0
4	4	AF37702	50	5	10

Results:

There were no statistically or biologically relevant differences between any of the treatment groups with respect to respiratory rate, lung compliance, airway resistance, tidal volume or minute volume.

Cardiovascular effects:

Study title: Safety Pharmacology Studies of AF37702: Effects on the Cardiovascular System in Anesthetized Dogs

Study no.: BA06089
 Study report location: eCTD 4.2.1.3.1
 Conducting laboratory and location: (b) (4)

Date of study initiation: 10 April 2006
 GLP compliance: Statement included and signed
 QA statement: Statement included and signed
 Drug, lot #, and % purity: AF37702, 12AD2, 99.1 %

Key Findings:

- AF37702 did not produce any cardiovascular effects at doses of 0.2, 2, and 20 mg/kg in the anesthetized dog

Methods:

Species: Beagle dog
 Route: IV, bolus via right femoral vein
 Vehicle: Saline containing 10 mmol/L acetic acid
 Procedure: The vehicle and AF37702 were administered intravenously by bolus injection for 1 minute at 35-minute intervals

Parameters evaluated: Blood pressure, heart rate and electrocardiographic (ECG)

Treatment	Substance	Dosage level (mg/kg)	Dosage volume (mL/kg)	Concentration (mg/mL)	Number of animals [Animal Nos.]
A	Vehicle ^{a)}	0	2	0	4 [101-104]
B	AF37702	0.2	2	0.1	
C	AF37702	2	2	1	
D	AF37702	20	2	10	

^{a)} Saline containing 10 mmol/L acetic acid

Results:

There were no statistically significant differences in QTc interval, heart rate, mean blood pressure, and PR interval at any time point between the vehicle and AF37702 treatments.

Study title: Cardiovascular (Hemodynamic) Evaluation of AF37702 in Anesthetized Dogs

Study no.: AF03-37 (Affymax); 0247DA33.001 (b) (4)
 Study report location: eCTD 4.2.1.3.1
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 31 March 2004
 GLP compliance: Statement included and signed
 QA statement: Statement included and signed
 Drug, lot #, and % purity: AF37702, 12AD2, 99.1 %

Key Findings:

- AF37702 did not produce any cardiovascular effects at doses of 0.2, 2, and 20 mg/kg in the anesthetized dog

Methods:

Species: Beagle dog
 Route: IV, bolus via a vena cava catheter
 Vehicle: 10 mM acetate in 0.9% sodium chloride for injection
 Procedure: The vehicle and AF37702 were administered 4 times on one day
 Parameters evaluated: Arterial blood pressure, heart rate, left ventricular pressure (LVP), left ventricular end diastolic pressure (LVEDP), +dP/dt, cardiac output (CO),

and lead II ECG (gross analysis)

Group	Number of Animals	Treatment	Dose
	Male		
1	4	AF37702	0, 0.2, 2 and 20 mg/kg

^aEach dose was administered 30 minutes (minimum) following the preceding treatment.

Results:

There were no statistically significant differences in blood pressure, cardiac output, heart rate, LVP, left ventricular end diastolic pressure, or +dP/dt at any time point between the vehicle and AF37702 treatments. Transient changes in cardiac output and left ventricular end diastolic pressure were observed, however, these changes were not dose-dependent and similar to effects of the vehicle.

Study title: Effects of AF37702 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells

Study no.: AF07-32 (Affymax); 070926.BSJ (b) (4)
 Study report location: eCTD 4.2.1.3.1
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 10 Oct 2007
 GLP compliance: Statement included and signed.
 Exception: Positive control formulation was not analyzed for stability, homogeneity, or concentration (potency was demonstrated via comparison to historical controls)
 QA statement: Statement included and signed
 Drug, lot #, and % purity: AF37702, SF353/6AF1, 99.1 %
 Vehicle: HEPES buffered physiological saline
 Positive control: Terfenadine

Key Findings:

- AF37702 had minimal inhibitory effect on hERG potassium current.

Methods:

Human embryonic kidney (HEK293) cells stably expressing the hERG potassium channel were exposed to AF37702 at the concentration of 1 and 5 μ M. Each concentration was tested in at least three cells ($n \geq 3$). The highest concentration tested (5 μ M) represented a concentration 50times higher than the expected C_{max} value. 5 μ M also represents the approximate upper limit of solubility of AF37702 in HB-PS. The positive control was applied in two cells ($n = 2$). Vehicle control solution was applied in three cells ($n = 3$). hERG-mediated potassium current

was achieved by patch-clamp method. The incubation time was not provided in this submission

Results:

An inhibitory effect on hERG potassium current amplitude of 6.5% was observed at 1 μ M.

4.4 Overall Discussion and Conclusions

The pharmacologic activity of peginesatide (AF37702) was compared to the activity of [REDACTED]^{(b) (4)} of peginesatide, and known erythropoiesis-stimulating agents erythropoietin and Aranesp in *in vitro* studies. In a radioligand competition binding assay for the recombinant human erythropoietin receptor, peginesatide demonstrated binding to recombinant human erythropoietin receptor with a mean IC_{50} value of 36.97 pM, which was slightly less than the binding of erythropoietin (IC_{50} =6.78 pM) and Aranesp (IC_{50} =12.45 pM) to the receptor. Peginesatide produced a dose-dependent activation of reporter cells transfected with DNA encoding a chimeric human erythropoietin receptor (EC_{50} =280 pM), but did not demonstrate activity at reporter cells transfected with the human granulocyte colony stimulating factor receptor or the human thrombopoietin receptor. Peginesatide also dose-dependently stimulated the proliferation of UT-7/EPO cells, an erythropoietin-responsive human leukemia cell line, with an EC_{50} value of 457 pM, and a maximal response similar to that of erythropoietin and Aranesp. In an erythroid (BFU-E) colony assay using primary human CD34+ cells, peginesatide stimulated the formation of erythroid colonies from human CD34+ cells with EC_{90} value of 4 nM (4000 pM). Although, peginesatide was less potent than [REDACTED]^{(b) (4)} erythropoietin, and Aranesp in these assays, the results indicate that peginesatide demonstrates similar pharmacologic activity at the erythropoietin receptor as erythropoietin and Aranesp.

The signal transduction response to peginesatide was characterized and compared to the response to erythropoietin, the natural ligand for the human erythropoietin receptor. Peginesatide produced similar tyrosine phosphorylation of JAK2, STAT5A, STAT5B, and MAPK as erythropoietin in UT-7/EPO cells, indicating that peginesatide has similar stimulating activity for the recombinant human erythropoietin receptor as erythropoietin in the erythropoietin-responsive UT-7/EPO leukemic cell line. Additionally, the ability of peginesatide to rescue UT-7/EPO cells from apoptosis was evaluated through multiple *in vitro* assays. Annexin V-FITC and propidium iodide staining and caspase activity measurements were conducted on cells cultured in peginesatide, erythropoietin, Aranesp, or in basic growth medium without erythropoietin (starved cells). While induction of apoptosis was observed in the starved UT-7/EPO cells, peginesatide was shown to inhibit apoptosis of UT-7/EPO cells with increased cell proliferation and viability, and lower levels of apoptotic indexes (Annexin V staining and caspase-3 and caspase-8 activity) compared to starved cells. Peginesatide prevented caspase-3 activation (EC_{50} =64.6 pM) in the PARP cleavage FACS assay, and prevented DNA fragmentation (EC_{50} =40.6 pM) in the DNA fragmentation FACS assay. In the BCL-X_L

FACS assay, peginesatide maintained the expression level of BCL-X_L, an index for the activation of the PI 3-kinase/AKT/Protein kinase B signaling pathway. The activity of peginesatide was similar to the activity of erythropoietin for these assays, and these results suggest that peginesatide protects cells from apoptosis through the suppression of caspase activation and maintenance of the expression of the anti-apoptotic protein BCL-X_L.

The erythropoietic activity of peginesatide was evaluated *in vivo* in mice, rats, rabbits, dogs, and monkeys. In a rat study, repeated intravenous or subcutaneous administration of peginesatide (0.135 or 1.35 mg/kg; 0.81 or 8.1 mg/m²) once weekly or once every two weeks for 6 weeks resulted in hematologic changes including increases in reticulocytes at the high dose, and increases in hemoglobin and RBC at both doses. In a rat model of renal failure and anemia, treatment with a single intravenous bolus dose of peginesatide (0.1, 1, or 10 mg/kg; 0.6, 6, or 60 mg/m²) 25 days after a 5/6 nephrectomy procedure dose-dependently increased both reticulocyte count and hemoglobin levels compared to vehicle-controls. Based on these results, peginesatide has erythropoietic activity *in vivo* in both normal and anemic animals.

The *in vivo* erythropoietic activity of three peginesatide variants was evaluated and compared with the activity of peginesatide following intravenous administration at 1 or 10 mg/kg (6 or 60 mg/m²) in normal rats. The (b) (4) (b) (4) forms of peginesatide (b) (4) were comparable in erythropoietic activity to peginesatide at both doses. (b) (4) (b) (4) showed *in vivo* erythropoietic activity, but was less active than peginesatide, which is expected given the shorter circulating half-life of (b) (4)

Peginesatide had no effects on the central nervous system in mice and the respiratory system in guinea pigs. Peginesatide administration to rats caused decreases in Na⁺ and Cl⁻ concentrations as well as Cl⁻ excretion at the highest dose tested. No effects were observed on urinary volume and pH. Although cardiovascular safety studies in the anesthetized dog show that peginesatide did not cause any effects, the Applicant also showed that peginesatide has no activity in dogs. Therefore, the effects of AF37702 on the cardiovascular system can not be determined based on the study in the dog. Electrocardiography evaluations, however, were incorporated into the chronic study in the monkey. There were no peginesatide-related effects on heart rate, PR, QRS, RR, and QT interval. Increases in QTc were noted in the 0.2 mg/kg at 3, 6, and 9 months for males and females and in the 20 mg/kg female group at 9 months. These effects were not dose-dependent.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 Absorption

Study title: Single dose intravenous and subcutaneous pharmacokinetic analysis of AF37702 in male Sprague-Dawley rats

Study no.: AF03-28A

Study report location: eCTD 4.2.2.2

Study was reviewed under IND 63257 by Dr. Ke Zhang, in the Division of Gastroenterology Products. Review was slightly modified to fit this NDA review.:

To characterize the pharmacokinetics of AF37702 in rats, AF37702 was given by single intravenous or subcutaneous administration at 1.35 mg/kg. Blood samples were collected at predose, 1, 4, 8 (intravenous only), 12 (subcutaneous only), 24, 48, 72, 96, 120, 144, 168, 196, 216, and 240 hours after dosing. Plasma samples were analyzed for AF37702 using a competition ELISA with a lower limit of quantification of 31.5 ng/mL.

The results indicated that following intravenous administration, AF37702 was eliminated from plasma with half-life of 21.6 hours. The plasma clearance and volume of distribution were low (clearance = 1.49 ml/h/kg and volume of distribution, V_{ss} = 49.9 ml/kg). Following subcutaneous administration, the peak plasma level (T_{max}) was reached within 48 hours and AF37702 was slowly eliminated from plasma with a half-life of 18 hours. The subcutaneous bioavailability was 26%.

Table 11: Summary of pharmacokinetics parameters
(Excerpted from Applicant's submission's submission)

Route	C_{max} (ng/mL)	t_{max} ^a (h)	AUC(0-t) (ng•h/mL)	AUC(inf) (ng•h/mL)	$t_{1/2}$ (h)	CL (mL/h/kg)	V_{ss} (mL/kg)
IV	46073	1	911518	913166	21.6	1.49	49.9
SC	3699	42	269989	270141	18	5.05	26.41

Study title: Single-dose intravenous pharmacokinetic analysis of AF37702 in male Sprague-Dawley rats

Study no.: AF03-49

Study report location: eCTD 4.2.2.2

Study was reviewed under IND 63257 by Dr. Ke Zhang, in the Division of Gastroenterology Products. Review was slightly modified to fit this NDA review.:

To characterize the pharmacokinetics of AF37702 in rats, AF37702 was given to rats by single intravenous administration at 0.138, 0.69, 1.38, 6.9, and 13.8 mg/kg. Blood samples were collected at predose, 0.083, 0.5, 1, 4, 10, 24, 72, and 96 hours after dosing. Plasma samples were analyzed for AF37702 using a competition ELISA.

The results indicated that following intravenous administration, AF37702 was eliminated from plasma in a biexponential fashion with a rapid initial phase followed by a prolonged terminal phase. The terminal half-life was ranging from 21.5 to 30.7 hours. There were dose proportional increases of C_{max} and AUC values. Clearance was small ranging from 1.3 to 2.3 mL/h/kg. Volume of distribution was also small, ranging from 44 to 66 mL/kg.

Table 12: Mean plasma pharmacokinetic parameters of AF37702 in male Sprague-Dawley rats (*Excerpted from Applicant's submission*)

Mean plasma pharmacokinetic parameters of AF37702 in male Sprague-Dawley rats (n=5)								
Dose (mg/kg)	C_{max} (ng/mL)	t_{max} ^a (h)	AUC(0-t) (ng•h/mL)	AUC(inf) (ng•h/mL)	$t_{1/2}$ (h)	CL (mL/h•kg)	V_{ss} (mL/kg)	MRT (h)
0.138	2685	0.083	53392	59998	23.1	2.34	65.6	28.6
0.69	13143	0.083	356641	373049	21.5	1.91	56.1	30.5
1.38	36990	0.083	883867	924883	22.2	1.54	42.7	27.7
6.9	194571	0.083	5092503	5552466	27.5	1.30	44.1	34.3
13.8	324727	0.083	8871897	9921825	30.7	1.44	56.8	39.3

^a: median

Study title: Single-dose intravenous pharmacokinetic analysis of AF37702 in male *Cynomolugus* monkeys

Study no.: AF03-48

Study report location: eCTD 4.2.2.2

Study was reviewed under IND 63257 by Dr. Ke Zhang, in the Division of Gastroenterology Products. Review was slightly modified to fit this NDA review:

To characterize the pharmacokinetics and erythropoietic activity of AF37702 in monkeys, AF37702 was given by single intravenous administration at 0.019, 0.095, and 0.475 mg/kg. Blood samples were collected at predose, 0.25, 2, 6, 12, 24, 48, 72, 120, 144 and 168 hours after dosing. Plasma samples were analyzed for AF37702 using a competition ELISA. Additional blood samples (1 mL) were collected pre-dose and at 4, 7, 10, 13, 16, 19, 22, 25, 28, and 31 days after dosing for hematology assessment.

The results indicated that following intravenous administration, AF37702 was eliminated from plasma with a short initial phase followed by a dominant, prolonged terminal phase. Plasma clearance was dose-dependent with values of 1.74, 1.0 and 0.6 mL/h/kg for the low, mid and high dose, respectively. Similarly, the half-life was 2 times longer

(29.9 hours) following the high dose than that (14.6 hours) observed following the low dose. There were dose-proportional increases of C_{max} but the increases of AUC values were greater than dose-proportional. Saturation of receptor was evident at concentrations ≥ 0.095 mg/kg.

Table 13: Mean plasma pharmacokinetic parameters of AF37702 in male Cynomolgus monkeys (*Excerpted from Applicant's submission*)

Dose (mg/kg)	C_{max} (ng/mL)	t_{max}^a (h)	AUC(0-t) (ng•h/mL)	AUC(inf) (ng•h/mL)	$t_{1/2}$ (h)	CL (mL/h•kg)	V _{ss} (mL/kg)	MRT (h)
0.019	516.2 (21.2)	0.25 (0.25 –0.25)	10648 (1651)	11107 (1825)	14.6 (4.3)	1.74 (0.29)	31.3 (4.02)	18.5 (4.90)
0.095	3290 (700.5)	0.25 (0.25 –0.25)	98142 (25392)	98793 (25467)	16.4 (1.7)	1.00 (0.23)	28.8 (4.86)	29.0 (2.0)
0.475	15395 (1747)	6.0 (0.25 –6.0)	822535 (237122)	851758 (267124)	29.9 (9.4)	0.60 (0.21)	27.4 (2.45)	48.3 (12.2)

Data were mean (SD); ^a: median value

5.2 Distribution

Study title: Quantitative whole-body autoradioluminography in Sprague-Dawley rats following intravenous and subcutaneous administration of ^{14}C -AF37702 and intravenous administration of (b) (4)

Study no.: AF05-017

Study report location: eCTD 4.2.2.3

Tissue concentration and related distribution of drug-derived radioactivity was determined after single intravenous and single subcutaneous administration of ^{14}C -AF37702 (17 mg/kg) to male Sprague-Dawley rats using quantitative whole-body autoradiography. Additionally, the tissue concentration and related distribution of drug-derived radioactivity was determined after single intravenous administration of (b) (4) (17 mg/kg). (b) (4) Rats were euthanized at 72, 120, 168, 336, and 672 hours after intravenous dosing of ^{14}C -AF37702, at 1, 4, 8, 24, 72, 120, 168, 240, 336, and 672 hours after subcutaneous dosing of ^{14}C -AF37702, and at 1, 4, 8, 24, 72, 120, 168, 336, and 672 hours after intravenous dosing of (b) (4), with 1 rat/time point. Whole blood was collected via cardiac puncture and carcasses were processed for whole-body autoradiographic analysis. Concentrations of drug-derived radioactivity were determined in blood, plasma, tissues, and bodily fluids to evaluate tissue distribution and elimination of the test compounds.

^{14}C -AF37702-derived radioactivity was widely distributed following either intravenous or subcutaneous administration with the majority of tissues reaching C_{max} at 72 hours after dosing (see Tables 14 and 15 below). Concentrations steadily declined, but elimination of radioactivity was not complete at 672 hours after dosing with either route. Tissues

with high concentrations of radioactivity included the liver, renal cortex, kidney, pituitary gland, lymph nodes, renal medulla, spleen, and choroid plexus. High concentrations of radioactivity observed in the urine and kidney suggest that renal excretion of drug-derived radioactivity was the primary route of elimination. Following a single subcutaneous dose of ^{14}C -AF37702, concentrations of radioactivity associated with the dose site were high throughout the experiment, suggesting a potential depot effect resulting in slow absorption of the administered dose over time. Blood and plasma concentrations of ^{14}C -AF37702-derived radioactivity were highest at 72 hours following intravenous dosing (see Table 16, which was the first time point for this route of administration, and at 24 hours following subcutaneous administration (see Table 17).

Table 14: Concentrations of radioactivity in tissues and fluids in rats following a single intravenous dose of ^{14}C -AF37702 (*Excerpted from Applicant's submission*)

Tissue Type	Tissue	Tissue Concentration ($\mu\text{g equiv/g}$)		
		Animal Number/Time Point		
		1-72 72 h	1-336 336 h	1-672 672 h
Vascular/Lymphatic	Aorta	7.790	5.791	2.045
	Blood	16.117	1.034	0.388
	Bone marrow	18.245	8.859	9.152
	Lymph nodes	29.302	35.369	10.194
	Spleen	21.003	29.004	25.139
Excretory/Metabolic	Bile	BLQ	BLQ	BLQ
	Kidney	35.597	7.474	4.476
	Liver	45.674	17.080	10.091
	Renal cortex	38.339	7.059	3.791
	Renal medulla	22.424	6.896	7.481
	Urinary bladder	19.089	9.591	1.218
	Urine	14.578	1.876	0.619
Central Nervous System	Cerebellum	0.604	BLQ	BLQ
	Cerebrum	0.427	BLQ	BLQ
	Choroid plexus	18.715	12.834	6.421
	Medulla	0.347	BLQ	BLQ
	Pineal gland	NR	14.100	NR
	Spinal cord	0.348	BLQ	BLQ
Endocrine	Adrenal gland	19.626	11.140	10.829
	Pituitary gland	33.767	16.039	20.562
	Thymus	5.307	2.425	1.765
	Thyroid	17.926	12.367	9.153

Tissue Type	Tissue	Tissue Concentration ($\mu\text{g equiv/g}$)		
		Animal Number/Time Point		
		1-72 72 h	1-336 336 h	1-672 672 h
Secretory	Exorbital lacrimal gland	10.171	6.745	5.565
	Harderian gland	11.254	7.432	4.461
	Intraorbital lacrimal gland	9.616	4.449	2.790
	Pancreas	15.210	10.810	4.779
	Parotid gland	10.096	7.488	5.748
	Salivary gland	13.830	11.810	7.416
Fatty	Fat (abdominal)	5.201	1.710	0.651
	Fat (brown)	9.500	8.264	5.393
Dermal	Nonpigmented skin	7.802	3.825	1.359
Reproductive	Bulboglndular muscle	10.347	5.645	3.710
	Epididymis	5.108	3.342	1.372
	Preputial gland	12.486	NR	NR
	Prostate	5.683	4.012	1.060
	Seminal vesicle	1.764	1.006	0.518
	Testes	9.138	9.571	6.767
Skeletal/Muscular	Bone	0.680	0.461	0.251
	Diaphragm	4.783	1.860	0.667
	Incisor pulp	19.306	5.971	2.063
	Muscle	1.465	0.694	0.317
	Myocardium	8.930	6.274	3.067
Respiratory Tract	Lung	13.461	2.599	1.949
	Nasal turbinates	NR	2.162	0.945
	Trachea	6.467	2.392	0.996

Tissue Type	Tissue	Tissue Concentration (µg equiv/g)		
		Animal Number/Time Point		
		1-72 72 h	1-336 336 h	1-672 672 h
Alimentary Canal	Buccal mucosa	BLQ	BLQ	BLQ
	Cecum contents	NR	0.421	0.299
	Cecum	NR	5.793	2.478
	Esophageal contents	1.137	0.204	0.271
	Esophagus	3.331	0.623	0.233
	Gastric mucosa	6.160	2.734	1.989
	Large intestinal contents	7.503	0.819	0.504
	Large intestine	9.656	3.880	1.195
	Small intestinal contents	2.243	0.309	0.213
	Small intestine	5.239	3.578	1.685
	Stomach contents	0.251	0.341	0.161
Stomach	4.810	1.293	0.631	
Ocular	Ciliary body/processes	8.058	4.678	BLQ
	Cornea	BLQ	BLQ	BLQ
	Eye (whole)	2.269	0.372	0.160
	Lens	BLQ	BLQ	BLQ
	Iris	1.596	BLQ	BLQ
	Retina/choroid	11.876	4.323	BLQ

BLQ: Below the limit of quantitation (0.159 µg equiv/g).

NR: Not represented during quantitative analysis.

Table 15: Concentrations of radioactivity in tissues and fluids in rats following a single subcutaneous dose of ¹⁴C-AF37702 (Excerpted from Applicant's submission)

Tissue Type	Tissue	Tissue Concentration (µg equiv/g)							
		Animal Number/Time Point							
		2-1 1 h	2-4 4 h	2-8 8 h	2-24 24 h	2-72 72 h	2-168 168 h	2-336 336 h	2-672 672 h
Vascular/Lymphatic	Aorta	BLQ	BLQ	BLQ	BLQ	3.273	3.025	2.188	1.237
	Blood	0.283	1.126	3.303	14.016	7.383	1.944	0.579	0.698
	Bone marrow	0.209	0.772	1.529	5.256	5.729	5.256	3.873	3.573
	Lymph nodes	BLQ	0.314	0.301	17.381	23.299	9.920	10.298	9.965
	Spleen	0.179	0.584	1.210	5.001	6.353	6.799	13.344	12.822
Excretory/Metabolic	Bile	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Kidney	2.784	11.563	21.490	29.273	18.535	7.937	3.556	3.636
	Liver	0.968	4.055	8.591	16.140	19.088	12.498	4.938	4.178
	Renal cortex	2.952	12.425	23.635	30.826	22.675	8.928	3.521	3.468
	Renal medulla	1.101	5.440	7.464	13.872	10.214	4.632	3.883	4.402
	Urinary bladder	BLQ	1.712	BLQ	2.176	17.837	4.011	2.312	1.041
Urine	33.631	48.288	87.203	50.981	14.121	3.017	1.497	1.586	
Central Nervous System	Cerebellum	BLQ	BLQ	BLQ	0.337	0.241	BLQ	BLQ	BLQ
	Cerebrum	BLQ	BLQ	BLQ	0.285	0.236	BLQ	BLQ	BLQ
	Choroid plexus	BLQ	0.255	0.257	2.974	4.405	3.656	2.997	4.116
	Medulla	BLQ	BLQ	BLQ	0.235	BLQ	BLQ	BLQ	BLQ
	Pineal gland	NR	0.363	BLQ	0.448	4.790	3.637	2.986	4.521
	Spinal cord	BLQ	BLQ	BLQ	0.214	0.181	BLQ	BLQ	BLQ
Endocrine	Adrenal gland	BLQ	0.431	1.139	4.232	4.383	3.817	2.698	2.833
	Pituitary gland	BLQ	0.359	1.017	4.653	6.658	5.564	8.667	5.743
	Thymus	BLQ	0.173	0.329	1.286	1.663	1.668	0.874	0.707
	Thyroid	BLQ	0.377	0.594	3.704	9.357	6.216	3.665	3.347

		Tissue Concentration (µg equiv/g)							
		Animal Number/Time Point							
Tissue Type	Tissue	2-1 1 h	2-4 4 h	2-8 8 h	2-24 24 h	2-72 72 h	2-168 168 h	2-336 336 h	2-672 672 h
Secretory	Exorbital lacrimal gland	BLQ	0.202	0.378	1.793	3.193	3.239	2.102	2.966
	Harderian gland	BLQ	0.164	0.313	1.389	3.653	2.931	1.891	2.329
	Intraorbital lacrimal gland	BLQ	0.252	0.337	1.703	3.091	1.923	1.323	2.028
	Pancreas	BLQ	0.370	0.879	3.387	4.447	4.441	2.785	2.443
	Parotid gland	BLQ	0.249	0.561	2.700	3.491	1.151	2.117	2.157
	Salivary gland	BLQ	0.305	0.613	3.075	4.817	4.663	2.857	3.302
Fatty	Fat (abdominal)	BLQ	BLQ	BLQ	0.531	0.949	1.149	BLQ	0.164
	Fat (brown)	BLQ	0.292	0.719	4.896	7.109	4.146	2.245	3.048
Dermal	Nonpigmented skin	BLQ	0.258	0.451	1.927	2.882	2.541	1.704	0.903
Reproductive	Bulboglandular muscle	NR	0.325	NR	2.440	3.748	2.716	1.535	1.404
	Epididymis	BLQ	0.180	0.320	1.549	2.360	1.361	0.835	0.846
	Preputial gland	BLQ	0.165	0.472	1.879	1.502	2.695	0.911	0.527
	Prostate	0.164	BLQ	0.298	1.326	2.059	1.697	0.818	0.561
	Seminal vesicle	BLQ	BLQ	0.188	0.280	0.541	1.161	0.642	0.402
	Testes	BLQ	BLQ	0.417	1.703	1.943	3.362	2.459	2.996
Skeletal/Muscular	Bone	BLQ	BLQ	BLQ	0.364	0.472	0.192	0.291	0.228
	Diaphragm	BLQ	0.218	0.488	1.762	1.389	1.099	0.548	0.453
	Incisor pulp	BLQ	0.397	1.084	4.969	5.892	5.060	3.234	1.653
	Muscle	BLQ	BLQ	BLQ	0.398	0.578	0.416	0.260	0.212
	Myocardium	BLQ	0.379	0.910	3.774	3.033	2.118	1.197	1.094
Respiratory Tract	Lung	0.230	1.063	2.329	8.892	5.232	2.153	0.839	0.864
	Nasal turbinates	BLQ	BLQ	0.188	1.141	1.227	0.982	0.588	0.471
	Trachea	BLQ	NR	NR	3.087	4.159	2.058	1.256	0.822

		Tissue Concentration (µg equiv/g)							
		Animal Number/Time Point							
Tissue Type	Tissue	2-1 1 h	2-4 4 h	2-8 8 h	2-24 24 h	2-72 72 h	2-168 168 h	2-336 336 h	2-672 672 h
Alimentary Canal	Buccal mucosa	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Cecum contents	BLQ	1.863	1.051	1.896	1.312	0.841	0.282	0.211
	Cecum	BLQ	0.448	0.975	4.162	4.461	3.692	2.620	1.728
	Esophageal contents	BLQ	0.174	BLQ	0.928	0.851	0.533	BLQ	BLQ
	Esophagus	BLQ	0.282	0.354	2.657	2.126	1.486	0.570	0.308
	Gastric mucosa	BLQ	0.353	0.657	2.351	2.602	1.843	0.988	0.920
	Large intestinal contents	BLQ	BLQ	1.293	2.957	2.533	1.337	0.437	NR
	Large intestine	BLQ	0.322	1.429	3.057	3.071	3.318	1.812	1.240
	Small intestinal contents	1.200	1.394	1.103	1.995	1.072	0.752	0.245	0.274
	Small intestine	BLQ	0.328	0.980	2.160	3.139	1.714	1.193	0.871
	Stomach contents	0.482	0.563	BLQ	0.285	BLQ	0.199	BLQ	BLQ
	Stomach	BLQ	0.300	0.478	2.179	2.458	0.766	0.677	0.538
Ocular	Ciliary body/processes	BLQ	BLQ	0.522	1.140	3.451	3.419	BLQ	BLQ
	Cornea	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Eye (whole)	BLQ	BLQ	BLQ	0.241	0.403	0.308	BLQ	BLQ
	Lens	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Iris	BLQ	BLQ	0.298	2.739	1.325	BLQ	BLQ	BLQ
	Retina/choroid	BLQ	BLQ	0.901	3.138	6.110	2.085	1.508	BLQ
Dose site	Subcutaneous dose site	780.418	769.309	430.862	1007.910	791.743	505.398	386.727	222.659

BLQ: Below the limit of quantitation (0.159 µg equiv/g)

NR: Not represented during quantitative analysis

Table 16: Concentration of radioactivity in blood and plasma in rats following a single intravenous dose of ¹⁴C-AF37702 (Excerpted from Applicant's submission)

Time (h)	Animal Number	Concentration (µg equiv/g)			
		QWBA-Derived Blood	LSC-Derived		
			Blood	Plasma	B/P Ratio
72	1-72	16.117	16.624	32.904	0.505
120	1-120	ND	6.060	13.479	0.450
168	1-168	ND	3.003	7.246	0.414
336	1-336	1.034	0.980	2.266	0.432
672	1-672	0.388	0.492	0.855	0.575

ND: Not determined

BLQ: Below the limit of quantitation (QWBA blood = 0.110 µg equiv/g; LSC blood = 0.008 µg equiv/g; and LSC plasma = 0.005 µg equiv/g).

Table 17: Concentration of radioactivity in blood and plasma in rats following a single subcutaneous dose of ^{14}C -AF37702 (*Excerpted from Applicant's submission*)

Time (h)	Animal Number	Concentration ($\mu\text{g equiv/g}$)			
		QWBA-Derived	LSC-Derived		
			Blood	Blood	Plasma
1	2-1	0.283	0.311	0.509	0.611
4	2-4	1.126	1.304	2.513	0.519
8	2-8	3.303	3.339	5.986	0.558
24	2-24	14.016	15.594	28.842	0.541
72	2-72	7.383	7.281	14.860	0.490
120	2-120	ND	3.850	8.345	0.461
168	2-168	1.944	2.022	4.153	0.487
240	2-240	ND	1.372	3.401	0.403
336	2-336	0.579	0.632	1.356	0.466
672	2-672	0.698	0.810	1.532	0.529

ND: Not determined

BLQ: Below the limit of quantitation (QWBA blood = 0.110 $\mu\text{g equiv/g}$; LSC blood = 0.008 $\mu\text{g equiv/g}$; and LSC plasma = 0.005 $\mu\text{g equiv/g}$).

Following a single intravenous dose of (b) (4) drug-derived radioactivity was extensively distributed with C_{max} observed in the majority of organs and blood and plasma at 1 hour after dosing. Tissues with high concentrations of radioactivity included the liver, renal cortex, kidney, renal medulla, aorta, trachea, and spleen. High concentrations of radioactivity observed in the urine and kidney suggest that renal excretion of drug-derived radioactivity was the primary route of elimination.

Study title: Binding of AF37702 to serum proteins and lipoproteins

Study no.: AF09-010

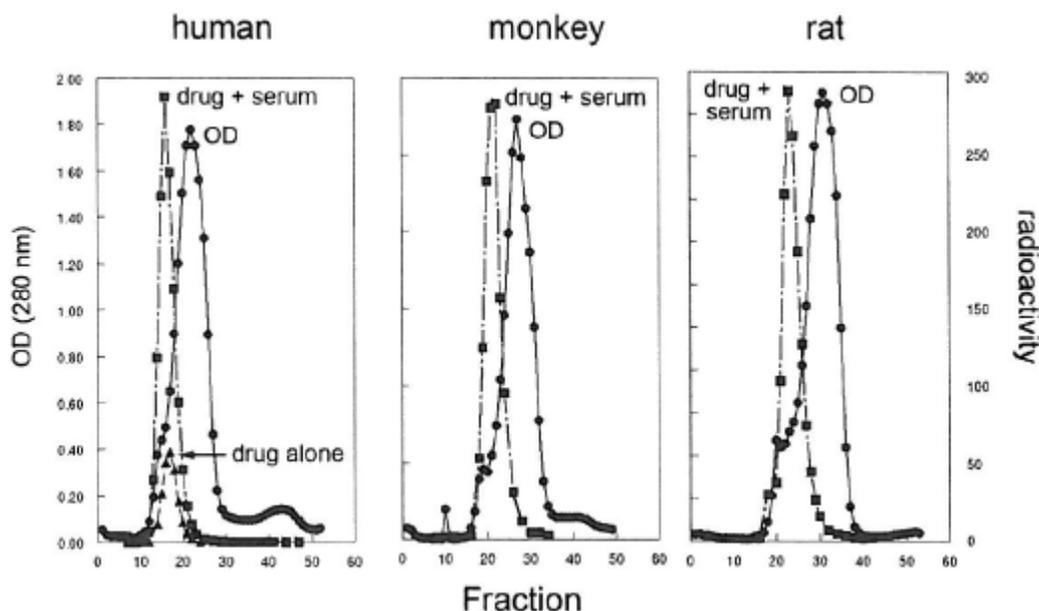
Study report location: eCTD 4.2.2.3

The binding of AF37702 to proteins and lipoproteins in sera from rats, monkeys, and humans was assessed. Because of the high molecular weight of AF37702, conventional protein binding methods such as equilibrium dialysis and ultrafiltration were not feasible. Column (gel-exclusion) chromatography was conducted to determine whether AF37702 binds to albumin. Samples contained 250 μl portions of human, monkey, or rat serum, each containing 1 μg of radioactive AF37702. Total drug concentration on the column was 4 $\mu\text{g/mL}$ radioactive AF37702. A separate elution analysis was conducted using AF37702 alone. The radioactivity and optical density was determined at 280 nm. Ultracentrifugation was conducted using both analytical and bulk flotation methods to determine if AF37702 binds to very low-density lipoproteins (VLDL; <1.006 g/mL), low-density lipoproteins (LDL; 1.006-1.063 g/mL), or high-density lipoproteins (HDL; 1.063-1.21 g/mL). For analytical ultracentrifugation, samples contained 250 μl portions of human, monkey, or rat serum, each containing 1 μg of radioactive AF37702. For bulk flotation ultracentrifugation, samples contained 3.9 mL portions of human, monkey, or rat serum, each containing 2 μg of radioactive AF37702. Separate elution analyses were conducted using AF37702 alone for both ultracentrifugation methods. The total concentration of radioactive AF37702 on the

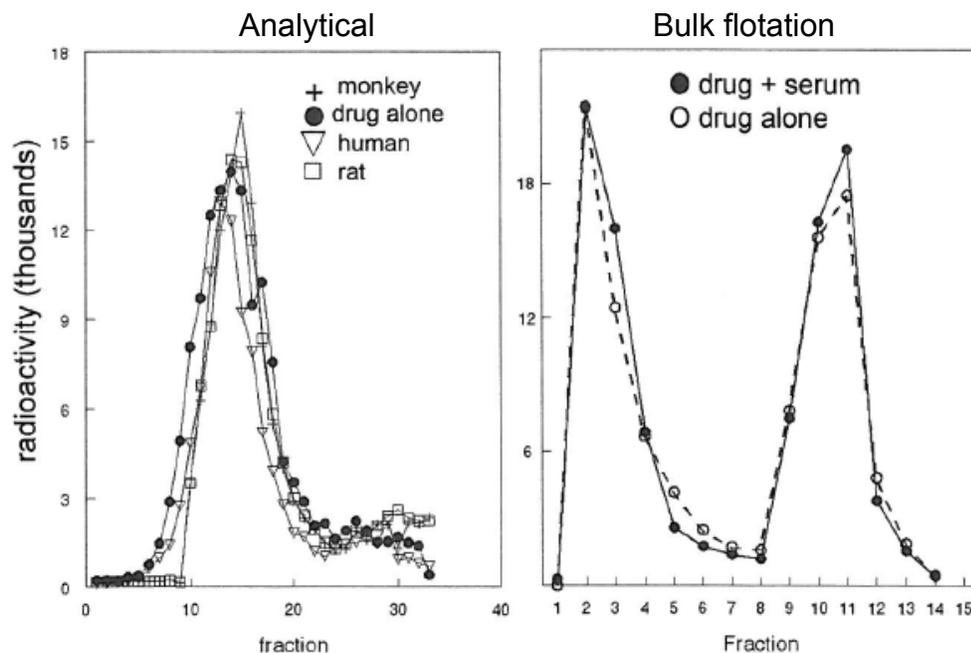
column was 4 $\mu\text{g/mL}$ for analytical ultracentrifugation and 5 $\mu\text{g/mL}$ for bulk flotation ultracentrifugation. Radioactivity present in each fraction was determined.

Column chromatography results indicated that the radioactive drug (centered at fraction 22) was eluted before albumin (centered at fraction 24) and that the elution profile of drug alone was similar to the profile with drug and serum. Since the presence of serum did not alter the elution profile of the radioactive AF37702, there is no binding to albumin.

Figure 8: Column chromatography
(Excerpted from Applicant's submission)



In the analytical ultracentrifugation experiment, radioactivity associated with AF37702 appeared in the central fraction that corresponds to low-density lipoproteins (fractions 12-18). These results rule out binding of the drug to very low-density or high-density lipoproteins, but cannot rule out binding to low-density lipoproteins. In the bulk flotation ultracentrifugation procedure, all lipoproteins rise to the top of the gradient because of their high lipid content, while other proteins will be found within the lower portion of the gradient. The distribution of radioactivity in the flotation experiment showed no significant level of radioactivity at the top of the gradient (fraction 1), which rules out affinity of AF37702 for all lipoproteins. Therefore, the data indicate that AF37702 does not bind to albumin (based on column chromatography results) or lipoproteins (based on ultracentrifugation results).

Figure 9: Ultracentrifugation (*Excerpted from Applicant's submission*)

Study title: Feto-placental transfer of radioactivity in rats after single intravenous administration of [¹⁴C]AF37702

Study no.: 09-808-020

Study report location: eCTD 4.2.2.3

The transfer of AF37702 into the placentas, fetuses, and amniotic fluid of pregnant rats was evaluated following a single intravenous administration of [¹⁴C]AF37702 at a dose of 5 mg/kg on gestational day 18. The pregnant rats were euthanized and samples including maternal blood, fetal blood, amniotic fluid, placentas, and fetuses were collected at 0.5, 24, and 48 hours after drug administration with 3 pregnant rats per time point. Radioactivity was measured with a liquid scintillation counter by the direct method for maternal plasma, amniotic fluid, and fetal plasma and by the combustion method for the homogenates of the placenta and fetus.

The concentrations of radioactivity in the maternal plasma, placenta, fetal plasma, fetus, and amniotic fluid are presented in the table below. In the maternal plasma, the concentration of radioactivity was 116.672 µg equiv./mL at 0.5 hours after administration and decreased at 24 and 48 hours. In the placenta, the concentration of radioactivity was the highest (21.202 µg equiv./mL) at 24 hours after administration. The concentrations of radioactivity were low in the fetal plasma and were below the lower limit of quantitation at all time points. Based on these results, fetal exposure to AF37702-related radioactivity following intravenous administration was limited with the transfer of AF37702 restricted by the placental barrier.

Table 18: Concentrations of the radioactivity in the tissues of pregnant rats after single intravenous administration of [¹⁴C]AF37702 (5 mg/kg)
(Excerpted from Applicant's submission)

Tissue	Concentration (µg equiv./g or mL)		
	0.5 h	24 h	48 h
Maternal plasma	116.672 ± 12.718	44.485 ± 4.372	16.321 ± 0.261
Placenta	15.341 ± 1.722	21.202 ± 3.644	17.543 ± 0.936
Fetal plasma	0.029 ± 0.006	0.013 ± 0.022	0.031 ± 0.002
Fetus	LOQ	LOQ	LOQ
Amniotic fluid	LOQ	LOQ	LOQ

Each value represents the mean ± SD for three animals.

LOQ: Below the lower limit of quantitation

5.3 Metabolism

Study title: *In vitro* metabolism of [¹⁴C]AF37702 by hepatic and renal microsomes and hepatic and renal S9 fractions from humans, rats, and monkeys

Study no.: B117-702-032

Study report location: eCTD 4.2.2.4

The *in vitro* metabolism of [¹⁴C]AF37702 was examined using hepatic and renal microsomes and hepatic and renal S9 fractions from humans, male rats, and monkeys. The ^{(b) (4)} content was also evaluated. [¹⁴C]AF37702 (10 µg/mL) was incubated with hepatic and renal microsomes and hepatic and renal S9 fractions in the presence of an NADPH-generating system at 37°C for 1 and 2 hours. For each of the microsomes and S9 fractions, zero-time incubations served as controls. Measurement of the radioactivity and HPLC analysis was conducted on the aliquots of the analytical samples.

Results show that the initial percentage of ^{(b) (4)} to the total radioactivity in the 0-hour incubation mixture was approximately 10%. The ratio of ^{(b) (4)} to the total radioactivity was slightly increased in a time-dependent manner by incubation with hepatic microsomes from each species and human renal microsomes. The maximal increase of 4% was observed over 2 hours with human hepatic microsomes. No significant metabolic peak other than ^{(b) (4)} was observed with hepatic and renal microsomes or S9 fractions from humans, rats, and monkeys, however, one small peak was observed with the rat renal S9 fractions. Although the rate of degradation of [¹⁴C]AF37702 to ^{(b) (4)} with hepatic and renal microsomes was slightly increased in humans and other species, these data suggest that AF37702 is not metabolized by liver or renal S9 enzymes.

Study title: Preliminary metabolite profiles in plasma, urine, and feces following a single intravenous or subcutaneous dose of [¹⁴C]AF37702 to male and female Sprague-Dawley rats

Study no.: AF08-024

Study report location: eCTD 4.2.2.4

This study was conducted to identify and profile metabolites of AF37702 in plasma, urine, and feces from Sprague-Dawley rats. The plasma, urine, and fecal samples were obtained from a rat mass balance/excretion study (Study # AF08-022, reviewed below), in which rats were administered a single intravenous or subcutaneous dose of [¹⁴C]AF37702 (5 mg/kg). Urine was collected at pre-dose, 0-6 hours, 6-24 hours, and at 24 hour intervals up to 336 hours after drug administration and feces were collected pre-dose and at 24 hour intervals up to 336 hours after drug administration. Plasma samples were collected at 0.25, 1, 4, 8, 24, 48, 72, 120, 168, 240, and 336 hours after drug administration (3 rats/group/time point) in separate groups of rats. After radio analysis for Study AF08-022, remaining plasma, urine, and fecal samples were used for metabolite profiling and identification in this study. Samples of the same type were pooled for analysis. HPLC (reverse phase and size exclusion)/tandem MS coupled with a radio flow-through detector was used to search for (b) (4) metabolites, metabolite profiling, and identification.

The radio-chromatogram of the 0-24 hour pooled plasma from male rats administered a single intravenous dose of [¹⁴C]AF37702 showed one metabolite peak at retention time of 21.87 minutes (P1) and AF37702 at retention time of 23.73 minutes. AF37702 and P1 accounted for 90.06% and 9.94% of the total radioactivity, respectively. Only AF37702 was observed in the radio-chromatogram of pooled plasma from male rats following subcutaneous administration of [¹⁴C]AF37702. Very low radioactivity was detected in the pooled urine samples. For intravenous administration AF37702 was the dominant peak and a small peak was detected at the P1 region, while only AF37702 was detected in the subcutaneous administration samples. AF37702 was also detected in the pooled feces from rats administered intravenous [¹⁴C]AF37702, but there were no distinguishable peaks in the pooled feces samples from rats administered subcutaneous [¹⁴C]AF37702. Reverse phase and size exclusion HPLC methods showed that P1 had a similar retention time as the reference standard (b) (4) therefore, P1 was identified as (b) (4)

AF37702 remained mostly unchanged in plasma, urine and feces following intravenous or subcutaneous administration in rats. (b) (4) a (b) (4) derivative of AF37702 was detected following intravenous administration only.

5.4 Excretion

Study title: Excretion mass balance and pharmacokinetics of radioactivity following a single intravenous or subcutaneous dose of [¹⁴C]AF37702 to male and female Sprague-Dawley rats

Study no.: AF08-022

Study report location: eCTD 4.2.2.5

An excretion mass balance study was conducted to determine the rate and extent of excretion of total radioactivity in urine and feces following administration of a single

intravenous or subcutaneous dose of [¹⁴C]AF37702 (5 mg/kg) to Sprague Dawley rats. Groups of male and female rats (n=4) were administered a single intravenous dose of [¹⁴C]AF37702 and another group of male rats (n=4) was administered a single subcutaneous dose of [¹⁴C]AF37702. Urine was collected at pre-dose, 0-6 hours, 6-24 hours, and at 24 hour intervals up to 336 hours after drug administration. Feces were collected pre-dose and at 24 hour intervals up to 336 hours after drug administration. Additionally, a cage rinse was performed at each 24 hour interval after feces collections and a cage wash and wipe were conducted after the last feces collection at 336 hours. Carcasses and the subcutaneous injection site from rats in the subcutaneous administration group were also collected. The pharmacokinetics of plasma total radioactivity were evaluated following a single intravenous or subcutaneous administration of [¹⁴C]AF37702 in separate groups of male rats (n=9). Plasma samples were collected at 0.25, 1, 4, 8, 24, 48, 72, 120, 168, 240, and 336 hours after drug administration (3 rats/group/time point). Excretion radioactivity in expired air was also evaluated following intravenous dosing in a separate group of male rats (n=4). Rats were individually housed in sealed glass metabolism chambers and expired [¹⁴CO₂] gas was trapped in a solution of Carbo-Sorb®. The [¹⁴CO₂] scrubber liquids were collected from the system at 0-24 and 24-48 hours after dosing. Urine, feces, cage residue (cage rinses, washes, and wipes), plasma, expired air, carcasses, and subcutaneous injection sites were analyzed for radioactivity by liquid scintillation counting.

The target dose of [¹⁴C]AF37702 was 5 mg/kg, however, the actual dose administered was 4.1 to 4.4 mg/kg. The cumulative recovery of radioactivity from the urine, feces, cage residue, carcass and subcutaneous injection site following a single intravenous or subcutaneous dose of [¹⁴C]AF37702 is listed in the table below. Urinary excretion was the predominant route of excretion of [¹⁴C]AF37702-derived radioactivity following intravenous or subcutaneous administration. [¹⁴C]AF37702-derived radioactivity was also excreted in the feces. The excretion rate was very slow and reached a steady state in 5 to 7 days, with approximately 1% of the dose recovered in urine or feces in each 24 hour interval thereafter. The majority of the [¹⁴C]AF37702-derived radioactivity (58.0-65.5%) was in the carcass at 336 hours after the administration of [¹⁴C]AF37702. Following subcutaneous administration of [¹⁴C]AF37702, 8.9% of the [¹⁴C]AF37702-derived radioactivity was recovered in the injection site. Following intravenous administration in a separate group of rats, the mean total radioactivity recovery from the expired air was 0.1% of the dose.

Table 19: Mean cumulative recovery of radioactivity following a single intravenous or subcutaneous dose of [¹⁴C]AF37702

Sample	% of administered dose (0-336 hours)		
	Intravenous		Subcutaneous
	Males	Females	Males
Urine	30.9	29.4	17.8
Feces	11.4	9.9	4.6
Carcass	59.4	58.0	65.5
Injection site	NA	NA	8.9
Cage residue	3.2	2.9	2.4
Total	104.9	100.2	99.2

NA= Not applicable

The radioactivity concentrations in plasma were quantifiable through 336 hours after a single intravenous or subcutaneous administration of [¹⁴C]AF37702. The pharmacokinetic parameters for radioactivity in the plasma are shown in the table below. Following intravenous administration, the C_{max} (110.794 $\mu\text{g equiv./mL}$) was observed at the first time point of 0.25 hours and concentrations decreased with a terminal half-life of 34.9 hours. The estimated plasma clearance was 1.5 mL/h/kg. The rate of absorption was slower and the exposure was lower for subcutaneous administration of [¹⁴C]AF37702 with an observed C_{max} of 9.64 $\mu\text{g equiv./mL}$ at 24 hours after administration and a terminal half-life of 63.5 hours. The apparent bioavailability based on the comparison of the $AUC_{(inf)}$ following subcutaneous and intravenous doses was 35%.

Table 20: Pharmacokinetic parameters for radioactivity in plasma of male rats following a single intravenous or subcutaneous administration of [¹⁴C]AF37702

Pharmacokinetic parameters	Intravenous	Subcutaneous
C_{max} ($\mu\text{g equiv./mL}$)	110.494	9.64
T_{max} (hours)	0.25	24
$AUC_{(last)}$ (h. $\mu\text{g equiv./mL}$)	3288	1078
$AUC_{(inf)}$ (h. $\mu\text{g equiv./mL}$)	3307	1167
$t_{1/2}$ (hours)	34.9	63.5

Study title: Transfer of the radioactivity into the breast milk of lactating rats after single intravenous administration of [¹⁴C]AF37702

Study no.: 09-808-021

Study report location: eCTD 4.2.2.5

The transfer of AF37702 into the breast milk of lactating female Sprague-Dawley rats was assessed following intravenous administration of [¹⁴C]AF37702 (5 mg/kg) on the 14th day after parturition. During the experiment each lactating rat (n=3) was housed with 5 suckling pups. Blood and milk samples were collected from the lactating rats at 0.5, 24, and 48 hours after the administration of [¹⁴C]AF37702. At approximately 0.5 hours before sample collection the lactating rats were separated from the suckling rats,

and at approximately 0.25 hours before sample collection oxytocin (1.0 I.U./kg) was injected subcutaneously to the lactating rat. The radioactivity in the plasma and milk samples was measured with a liquid scintillation counter using the direct method.

The concentrations of radioactivity in the plasma and milk of the lactating rats following a single intravenous administration of [^{14}C]AF37702 (5 mg/kg) are presented in the table below. These results indicated that low levels (11% – 13% at 24-48 hours post-dose) of radioactivity were transferred to the breast milk of lactating rats.

Table 21: Concentrations of radioactivity in the plasma and milk of lactating rats following a single intravenous administration of [^{14}C]AF37702 (5 mg/kg)
(*Excerpted from Applicant's submission*)

Time after dosage (h)	Concentration ($\mu\text{g equiv./mL}$)	
	Plasma	Milk
0.5	129.250 \pm 2.934	0.026 \pm 0.046
24	54.964 \pm 3.007	6.039 \pm 1.354
48	27.438 \pm 2.349	3.597 \pm 0.348

Each value represents the mean \pm SD for three animals.

5.5 Discussion and Conclusions

The pharmacokinetics of peginesatide was studied in multiple species including the rat and monkey, the non-clinical species tested for toxicity. Absorption studies were reviewed under IND 63257 by Dr. Ke Zhang. Following intravenous administration of peginesatide in rats (0.138, 0.69, 1.38, 6.9, or 13.8 mg/kg; 0.828, 4.14, 8.28, 41.4, or 82.8 mg/m²) or monkeys (0.019, 0.095, or 0.475 mg/kg; 0.228, 1.14, or 5.7 mg/m²) the half-life ranged from 14.6 to 30.7 hours. In an absorption study conducted in rats following a single intravenous or subcutaneous dose of peginesatide (1.35 mg/kg; 8.1 mg/m²), exposure was lower and the rate of absorption was slower with subcutaneous administration (C_{max} = 3699 ng/mL; t_{max} = 42 hours; $\text{AUC}_{(\text{inf})}$ = 270,141 ng·h/mL) than intravenous administration (C_{max} = 46,073 ng/mL; t_{max} = 1 hour; $\text{AUC}_{(\text{inf})}$ = 913,166 ng·h/mL). The subcutaneous bioavailability was 26%.

In an *in vitro* binding assay assessing the binding of peginesatide to proteins and lipoproteins in serum from rats, monkeys, and humans, data indicated that peginesatide does not bind to albumin or lipoproteins. A distribution study in rats following a single intravenous or subcutaneous dose of [^{14}C] peginesatide (17 mg/kg; 102 mg/m²) showed that peginesatide was widely distributed with C_{max} occurring at 72 hours after dosing in the majority of tissues. Tissues with high concentrations of peginesatide included the liver, renal cortex, kidney, pituitary gland, lymph nodes, renal medulla, spleen, and choroid plexus. High concentrations of peginesatide in the urine and kidney suggest that renal excretion is the primary route of elimination for the drug and are consistent with findings in the excretion study. An excretion study in rats following a single

intravenous or subcutaneous dose of [^{14}C] peginesatide (5 mg/kg; 30 mg/m²) showed that urinary excretion was the predominant route of excretion of peginesatide following either intravenous (29-31%) or subcutaneous (18%) administration, with some excretion in the feces (5-11%). In the plasma, the rate of absorption was slower and the exposure was lower for subcutaneous administration (C_{max} = 9.64 $\mu\text{g equiv./mL}$; t_{max} = 24 hours; $t_{1/2}$ = 63.5 hours) than for intravenous administration (C_{max} = 110.794 $\mu\text{g equiv./mL}$; t_{max} = 0.25 hours; $t_{1/2}$ = 34.9 hours) of [^{14}C] peginesatide. The apparent bioavailability based on the comparison of the $\text{AUC}_{(\text{Inf})}$ following subcutaneous and intravenous doses was 35%. These plasma findings are consistent with results from the absorption study and the pharmacokinetic results in humans.

A metabolism study conducted with the samples from the excretion study in rats showed that peginesatide remained mostly unchanged in plasma, urine and feces following intravenous or subcutaneous administration in rats. (b) (4)

(b) (4) derivative of peginesatide was detected following intravenous administration only. In an *in vitro* metabolism study of [^{14}C] peginesatide using hepatic and renal microsomes and hepatic and renal S9 fractions from humans, male rats, and monkeys, no significant metabolic peak other than (b) (4) was observed. These data indicate that peginesatide is not metabolized by liver or renal S9 enzymes. This finding is expected since peginesatide is a peptide and is expected to undergo proteolytic degradation. Based on the results of the non-clinical metabolism studies, an *in vivo* metabolism study in humans was not conducted.

The transfer of peginesatide-related radioactivity into the placentas, fetuses, and amniotic fluid of pregnant rats was evaluated in a distribution study following a single intravenous administration of [^{14}C] peginesatide at a dose of 5 mg/kg (30 mg/m²) on gestational day 18. The concentrations of radioactivity were low in the fetal plasma and were below the lower limit of quantitation at all time points. Based on these results, fetal exposure to peginesatide-related radioactivity following intravenous administration was very limited with the transfer of radioactive material/substances restricted by the placental barrier. In an excretion study assessing the transfer of peginesatide-related radioactivity into the breast milk of lactating female rats following intravenous administration of [^{14}C] peginesatide (5 mg/kg; 30 mg/m²) on the 14th day after parturition, low levels of peginesatide-related radioactivity were transferred to the breast milk of lactating rats (up to 13% 48 hours post-dose).

6 General Toxicology

6.1 Single-dose Toxicity

Single-dose toxicity studies (AF03-33 & AF03-34) were reviewed under IND 63257 by Ke Zhang, PhD. in the Division of Gastroenterology Products. Review was slightly modified to fit this NDA review:

Methods: Acute intravenous dose toxicity studies were conducted with AF37702 in rats (Study AF03-33) and monkeys (Study AF03-34). The dosing information was summarized by Dr. Zhang in a table along with the results in the result section. AF37702 was given to rats and monkeys by intravenous injection at 0, 0.1, 1, 10, and 50 mg/kg. The vehicle used was 10 mM Acetate and 0.9% NaCl for injection. Rats and monkeys were observed for mortality and clinical signs of toxicity daily for 14 days. Following parameters for both rats and monkeys were conducted: body weights, food consumption, hematology, clinical chemistry, organ weight, gross pathology, and histopathology on the heart, liver, kidneys, lungs, and spleen were conducted.

Results: The results are summarized in the following table.

Study #/Animal	Dosage (mg/kg)	Mortality	Clinical Signs of Toxicity
Rats 5/sex/group	iv. 0, 0.1, 1, 10, and 50	None	Mid (~18%) and high (~28%) dose males had less body weight gain during study as compared to the control. Other treatment related changes were exaggerated pharmacological activity (erythropoiesis) at all doses. Increased extramedullary hematopoiesis was also noted mainly in mid and high dose groups. Histopathological examination revealed minimal to mild nephropathy in all treatment groups. The nephropathy was characterized by clusters of degenerating cortical tubular cells, dilated tubules with cytoplasmic vacuolation, and lumens filled with proteinaceous fluid.
Monkeys 1/sex/group	iv. 0, 0.1, 1, 10, and 50	None	The treatment related changes were exaggerated pharmacological activity (erythropoiesis) at all doses. Histopathological examination revealed higher proportion of erythroid cells in femoral bone marrow.

Study Summary:

In conclusion, there were no treatment related clinical signs of toxicity in either rats or monkeys. In rats, mid (~18%) and high (~28%) dose males had less body weight gain during study as compared to the control. The treatment related changes were exaggerated pharmacological activity (erythropoiesis) at all doses in both studies. Histopathological examination revealed minimal to mild nephropathy in all treatment groups in rats. The nephropathy was characterized by clusters of degenerating cortical tubular cells, dilated tubules with cytoplasmic vacuolation, and lumens filled with proteinaceous fluid.

6.2 Repeat-dose Toxicity

Intravenous Administration

Title: A 6-month intravenous toxicity study of AF37702 in Rats, including a 3-month sacrifice, followed by a 6-week recovery period.

Study reviewed under IND 63257 by David Bailey, PhD. in the Division of Medical Imaging and Hematology Products. Review was slightly modified to fit this NDA review:

Study Number: AF04-009

Laboratory: (b) (4)

Applicant: Affymax, Inc, Palo Alto, CA

In-Life Dates: August 25, 2004 – April 15, 2005

Test Material: AF37702, Lot #12AB1, 98.7%

GLP: Statement included and signed

QA: Statement included and signed

Key Findings:

- 3 deaths and 40 deaths in the 1 and 10 mg/kg groups, respectively.
- Increases in RBC, HGB, HCT along with enlarged spleens, hypercellularity of femoral bone marrow, increased erythropoiesis in sternal bone marrow and extramedullary hematopoiesis in the spleen are consistent with the pharmacology of AF37702
- Target organ toxicities were noted in the heart, liver, kidney, lung and thymus

Design: To assess shorter term toxicity, an interim sacrifice at 3 months was conducted. To evaluate the potential reversibility of possible drug effects, some animals were carried for an additional 6-week drug free recovery period. The vehicle was 10 mM acetate and 0.9% NaCl for injection and the drug was administered once every 3 weeks at the dose levels shown in the table below:

Group	Dose of AF37702 (mg/kg)	Number Animals Assigned			Day of Deaths	Total Deaths, M/F (% mortality)
		M/F				
		3 Month	6 Month	6 Wk Recovery		
Toxicology						
1	0 (Vehicle)	10/10	20/20	5/5	31	1/0 (2/0)
2	0.1	10/10	20/20	5/5	0	0/0
3	1.0	10/10	20/20	5/5	8, 116, 180	2/1 (5/2)
4	10.0	10/10	20/20	5/5	57-190	19/21(54/60)

Group	Dose of AF37702 (mg/kg)	Number Animals Assigned			Day of Deaths	Total Deaths, M/F (% mortality)
		M/F				
		3 Month	6 Month	6 Wk Recovery		
Toxicokinetics						
5	0.1		9/9			0/0
6	1.0		9/9			0/0
7	10.0		9/9		Omitted	9/9 (100)

The parameters observed and intervals, in Groups 1-4 (toxicology) included:

Clinical signs: Daily

Food intake and Body weights: Weekly

Ophthalmology, Pretest and prior to termination

Clinical pathology: just prior to termination for animals scheduled at 3 and 6 months and end of recovery period),

Gross necropsy, organ weights, and histopathology: at necropsy

Results and Discussion

Toxicology Phase (Groups 1-4)

All animals survived the duration of the study in the low-dose group at 0.1 mg/kg. One male was sacrificed in a moribund condition in the control group after a cage accident. There were 3 deaths and 40 deaths in the mid- and high-dose groups, respectively.

No AF37702 effects on body weight, food intake, and ophthalmoscopy were observed.

The primary effects observed were consistent with the pharmacological action of known erythropoiesis-stimulating agents and were observed at the first bleeding on Day 20 in the mid- and high-dose groups. By Day 90 (after 5th dose) and thereafter during treatment (to Day 196), all groups receiving AF37702 had significantly elevated RBC, HGB and HCT.

Hematology:

Hematological values for male rats after 13 weeks dosing:

Peginesatide (mg/kg)		RBC	HGB	HCT
0	Control	8.8	15.8	49.8
0.1	% change from controls	↑9**	↑18**	↑21**
1.0		↑32**	↑36**	↑44**
10		↑65**	↑47**	↑58**

*: statistically significant compared to controls $P \leq 0.05$; **: statistically significant compared to controls $P \leq 0.01$

Hematological values for female rats after 13 weeks dosing:

Peginesatide (mg/kg)		RBC	HGB	HCT
0	Control	8.1	15.3	46.1
0.1	% change from controls	↑7	↑20**	↑24**
1.0		↑31**	↑40**	↑50**
10		↑72**	↑54**	↑69**

Hematological values for male rats after 26 weeks dosing:

Peginesatide (mg/kg)		RBC	HGB	HCT
0	Control	9.2	17.2	52.2
0.1	% change from controls	↑4**	↑9**	↑7**
1.0		↑40**	↑35**	↑38**
10		↑51**	↑31**	↑40**

*: statistically significant compared to controls $P \leq 0.05$; **: statistically significant compared to controls $P \leq 0.01$

Hematological values for female rats after 26 weeks dosing:

Peginesatide (mg/kg)		RBC	HGB	HCT	PLT
0	Control	9.2	17.2	52.2	
0.1	% change from controls	-	↑8*	↑6	↓12
1.0		↑42**	↑47**	↑48**	↓30**
10		↑80**	↑56**	↑65**	↓35**

*: statistically significant compared to controls $P \leq 0.05$; **: statistically significant compared to controls $P \leq 0.01$

During the recovery period, hematology values returned to comparable control values for the low-dose group receiving 0.1 mg/kg. For the mid-dose group, values were reduced during the recovery period but were still elevated compared to control values. For the high-dose group, none of the animals assigned to the recovery period survived to enter the period.

Serum Chemistry:

Clinical Chemistry values for male rats after 13 weeks of dosing:

Peginesatide (mg/kg)		CREA	TRIG	PHOS	ALP	GLU
0	Control	0.4	36	7.4	76	137
0.1	% change from controls	↑25**	↑70*	↑6	↓17**	↓15**
1.0		↑50**	↑143**	↑6*	↓26**	↓29**
10		↑50**	↑209**	↑10*	↓31**	↓32**

*: statistically significant compared to controls $P \leq 0.05$; **: statistically significant compared to controls $P \leq 0.01$

Clinical Chemistry values for male rats after 26 weeks of dosing:

Peginesatide (mg/kg)		CREA	TRIG	PHOS	ALP	GLU
0	Control	0.4	23	8	84	124
0.1	% change from controls	-	↑67	↑9	↓3	↓19**
1.0		↑25*	↑153**	↑14**	↓36**	↓36**
10		↑25*	↑211**	↑15**	↓43**	↓47**

*: statistically significant compared to controls $P \leq 0.05$; **: statistically significant compared to controls $P \leq 0.01$

Clinical Chemistry values for female rats after 13 weeks of dosing:

Peginesatide (mg/kg)		CREA	TRIG	PHOS	ALP	AST	GLU	K	FE
0	Control	0.5	23	8	73	86	117	6.7	359
0.1	% change from controls	↑20*	↑23*	↑6	↓18	-	↓20**	↓12*	↓54**
1.0		↑20*	↑85**	↑6*	↓34**	↑21	↓27**	↓22**	↓88**
10		↑40**	↑146**	↑10*	↓47**	↑123**	↓35**	↓64**	NA

*: statistically significant compared to controls $P \leq 0.05$; **: statistically significant compared to controls $P \leq 0.01$

Clinical Chemistry values for female rats after 26 weeks of dosing:

Peginesatide (mg/kg)		CREA	TRIG	ALP	AST	GLU	K	FE
0	Control	0.5	24	61	75	131	6.5	432
0.1	% change from controls	-	↑25	↓15*	13	↓13*	-	↓66**
1.0		↑20**	↑113**	↓36**	19	↓35**	↓23**	↓89**
10		↑20*	↑300**	↓41**	155**	↓40**	↓31**	↓97**

*: statistically significant compared to controls $P \leq 0.05$; **: statistically significant compared to controls $P \leq 0.01$

Necropsy Findings:

At necropsy at the end of the dosing period, the most frequent finding was increased spleen weight which occurred in a few animals of the low dose group and in all animals in the mid- and high-dose group. Other organs exhibiting increased weights in the mid and high-dose groups were liver, heart, kidney, lung and thymus. At the end of the recovery period, all organ weights were comparable between control and low-dose animals. In the mid-dose group, spleen and heart weights were still elevated above control values.

Histopathology:

Histopathologically, cardiac changes were common in rats at the end of the treatment period, including thrombosis, stromal proliferation of the valves, and myocardial degeneration in mid- and high-dose rats. At the end of the recovery period, myocardial degeneration and fibrosis were observed in the mid-dose group, and not in the control or low-dose animals. No animals assigned to the high-dose recovery group survived to be assessed for recovery of toxicities.

There was an increase in nephropathy, which was considered the cause of death, with hyaline droplets, casts and tubular regeneration in animals that died or were sacrificed moribund. At terminal sacrifice, there was an increase in incidence and severity of tubular regeneration, casts, chronic interstitial inflammation and pigmentation in mid- and high-dose group rats.

Toxicokinetic Phase: (Groups 5-7)

Methods: Whole blood was taken for drug level determination from surviving animals on Days 1, 85 and 190 at the following intervals: predose and at 1, 4, 8, 24, 48, 72, 120, and 168 hrs post-dose. After final blood drawing, all surviving animals in Groups 5-6 were euthanized and discarded without necropsy. Pharmacokinetic parameters were determined from 3 samples for male and female animals as shown in the table below (*Excerpted from Applicant's submission*):

Dose (mg/kg)	Sex	T _{max} (h)	C _{max} (µg/mL)	AUC(0,24) (µg-h/mL)	T _{1/2} (h)	CL/F (mL/h/kg)	V _z /F (mL/kg)
Day 1							
0.1	M	1.0	2.10	57.1	15.5	1.75	38.5
	F	1.0	2.26	57.0	16.4	1.75	39.8
1.0	M	1.0	21.6	811	19.9	1.23	37.6
	F	1.0	22.7	685	20.1	1.46	42.5
10	M	4.0	268	11324	31.3	0.88	38.1
	F	1.0	356	13769	40.6	0.73	39.4
Day 85							
0.1	M	1.0	2.62	69.7	17.9	1.44	35.0
	F	8.0	1.80	62.4	18.7	1.60	43.9
1.0	M	4.0	30.1	1151	19.0	0.89	26.4
	F	1.0	25.6	989	20.0	1.01	35.3
10	M	1.0	314	14826	29.7	0.68	30.1
	F	1.0	311	15143	30.4	0.66	29.0
Day 190							
0.1	M	1.0	1.94	56.1	13.9	1.79	39.0
	F	4.0	2.66	62.3	14.2	1.61	40.3
1.0	M	1.0	43.9	1314	17.0	0.76	18.1
	F	1.0	43.3	1285	17.0	0.78	21.2
10	M	No survival in PK groups					
	F	No survival in PK groups					

Increases greater than proportional to dose were observed at all dose levels and all intervals in the range of 0.1 to 10 mg/kg for AUC and C_{max} suggesting a deviation from linear kinetics over that dose range

For doses of 0.1 to 10 mg/kg, C_{max} values of 1.8 to 356 µg/mL were achieved in the 0.5 to 2.0 hour range, with corresponding AUC of 62 and 13769 µg-h/mL. C_{max} and AUC values were consistently close for males and females at all sampling periods.

Plasma concentrations decreased with a half-life of 15.5 hr (0.1 mg/kg) to 40.6 hr (10 mg/kg) for the first dose. For the values at Days 85 and 190, the range of half-life values did not show a pattern of change with dose levels or repeated dosing.

In general, after the initial Day 1 collection, exposure was observed to increase over time between Day 85 and 190 indicating an accumulation of drug. In addition, the increase in AUC was more than dose-proportional (indicating drug accumulation is likely due to saturation of the receptor).

Study title: A 9-month intravenous toxicity study of AF37702 in Cynomolgus Monkeys, including 3-month and 6-month interim sacrifices, followed by a 4-week recovery period

Study no.: AF04-010 (b) (4):0472SA33.001

Study report location: eCTD 4.2.3.2

Conducting laboratory and location:

(b) (4)

Date of study initiation: 10 August 2004

GLP compliance: Statement included and signed

QA statement: Statement included and signed

Drug, lot #, and % purity: AF37702, 12AB1, 99.9 %

Key Study Findings

- Mortalities: 3 animals at 20 mg/kg dose found dead with multi-organ congestion noted microscopically. No clinical signs noted leading up to death
- Dilated/dark retinal venules, focal retinal hemorrhage, and lens opacities at 20 mg/kg
- ↑ RBCs and indices (HGB, HCT, & RET); ↑ PT and APTT
- 3 monkeys in the 20 mg/kg group developed antibodies to AF37702
- Multi-organ congestion (bone marrow, brain, GI, kidney, liver, lung, spleen)
- Hypercellularity observed in bone marrow

Methods

This study was designed to determine the toxicity and TK of AF37702 when given to Cynomolgus monkeys via IV, bolus injection, once every three weeks for 3, 6, and 9 months. The Applicant also assessed the ability of AF37702 to induce antibodies. The animals were assigned according to the table below.

(Excerpted from Applicant's submission)

Group	Dose Level (mg/kg/day)	Concentration (mg/ml)	Dose Volume (ml/kg)	Number of Animals	
				Male	Female
1. Control (vehicle)	0	0	5	16	16
2. Low-dose	0.2	0.04	5	8	8
3. Mid-dose	2.0	0.4	5	8	8
4. High-dose	20	4.0	5	16	16

Observations and Results**Mortality**

Animal #	Dose (mg/kg)	Sex	Day of Death	Observations	
				Reason	General (include pathology)
42	20	M	79	Found dead	No observed clinical signs, multi-organ congestion (kidney, lungs, lymph nodes, stomach, bone marrow, brain, GI, liver, salivary gland, seminal vesicle, spleen, thymus,
85	20	F	104	Found dead	No observed clinical signs, multi-organ congestion (kidney, heart, lymph nodes, stomach, bone marrow, brain, GI, liver, salivary gland, ovary, spleen, thymus, uterus, adrenals
90	20	F	207	Found dead	No observed clinical signs, blood-filled fluid in brain with corresponding hemorrhage, multi-organ congestion (kidney, heart, lymph nodes, stomach, bone marrow, brain, GI, liver, ovary, spleen, thymus, uterus, adrenals, lung, pancreas

Clinical observations

Unremarkable

Bodyweight

Unremarkable

Food consumption

Unremarkable

Ophthalmoscopy

	Male				Female			
	Control	% change			Control	% change		
Dose (mg/kg)	0	0.2	2.0	20	0	0.2	2.0	20
No. animals (T/R)	8/8	8	8	8/8	16	8	8	8/8
Dilated and dark retinal venules	-	-	-	-	-	-	-	2/0
Focal retinal hemorrhage	-	-	-	1/0	-	-	-	-
Lens opacities	-	-	-	1/0	-	-	-	1/0

Electrocardiography

There were no AF37702-related effects on heart rate, PR, QRS, RR, and QT interval. Increases in QTc were noted in the 0.2 mg/kg at 3, 6, and 9 months for males and females and in the 20 mg/kg female group at 9 months.

(Excerpted from Applicant's submission)

The Effects of Vehicle and AF37702 on Heart Rate and ECG Intervals – Males - Mean Values

Group	Treatment	n	Interval	HR	PR	QRS	RR	QT (sec)	QTc (sec) ^a	QTc % Change ^b
1	Vehicle control	16	Baseline	209 ± 12.7	0.083 ± 0.002	0.041 ± 0.001	0.305 ± 0.020	0.169 ± 0.008	0.230 ± 0.006	-
		16	3 month	253* ± 5.4	0.082 ± 0.001	0.041 ± 0.000	0.239* ± 0.006	0.156 ± 0.006	0.222 ± 0.006	-3.5
		12	6 month	256* ± 5.7	0.094* ± 0.003	0.042 ± 0.001	0.235* ± 0.005	0.161 ± 0.004	0.228 ± 0.004	-0.9
		8	9 month	255* ± 4.4	0.095* ± 0.001	0.041 ± 0.001	0.235* ± 0.004	0.161 ± 0.004	0.227 ± 0.003	-1.3
2	AF37702 0.2 mg/kg/day	8	Baseline	205 ± 20.4	0.081 ± 0.003	0.040 ± 0.000	0.319 ± 0.039	0.157 ± 0.007	0.217 ± 0.005	-
		8	3 month	234 ± 7.6	0.084 ± 0.001	0.041 ± 0.000	0.258* ± 0.008	0.154 ± 0.004	0.218 ± 0.003	0.5
		8	6 month	246* ± 7.8	0.092* ± 0.003	0.043* ± 0.001	0.245* ± 0.008	0.156 ± 0.006	0.221 ± 0.007	1.8
		4	9 month	260* ± 14.0	0.083 ± 0.003	0.042 ± 0.001	0.232* ± 0.012	0.157 ± 0.009	0.224 ± 0.009	3.2
3	AF37702 2.0 mg/kg/day	8	Baseline	221 ± 11.0	0.081 ± 0.003	0.041 ± 0.000	0.276 ± 0.015	0.166 ± 0.002	0.229 ± 0.002	-
		8	3 month	222 ± 13.6	0.084 ± 0.002	0.040 ± 0.000	0.278 ± 0.019	0.161 ± 0.003	0.224 ± 0.002	-2.2
		8	6 month	225 ± 16.5	0.090 ± 0.003	0.043 ± 0.001	0.277 ± 0.020	0.165 ± 0.004	0.227 ± 0.003	-0.9
		4	9 month	204 ± 17.2	0.093* ± 0.003	0.040 ± 0.000	0.300 ± 0.024	0.167 ± 0.007	0.228 ± 0.006	-0.4
4	AF37702 20 mg/kg/day	16	Baseline	221 ± 10.7	0.086 ± 0.002	0.040 ± 0.000	0.284 ± 0.017	0.158 ± 0.004	0.221 ± 0.003	-
		16	3 month	231 ± 5.5	0.083 ± 0.002	0.042 ± 0.001	0.262 ± 0.007	0.159 ± 0.003	0.223 ± 0.003	0.9
		12	6 month	232 ± 4.9	0.089 ± 0.002	0.044* ± 0.001	0.259 ± 0.006	0.165 ± 0.004	0.229 ± 0.003	3.6
		8	9 month	223 ± 11.0	0.096* ± 0.003	0.041 ± 0.001	0.275 ± 0.017	0.180 ± 0.005	0.243 ± 0.004	10.0

Data presented as Mean ± SEM

^aCalculated using Van de Water formula

^bCalculated from 15 min post-dose of vehicle treatment

* Statistically significant change compared to the predose value - Two-way repeated measures ANOVA followed by a Bonferroni Multiple Comparison Test (SigmaStat, v2.03)

(Excerpted from Applicant's submission)

The Effects of Vehicle and AF37702 on Heart Rate and ECG Intervals – Females - Mean Values

Group	Treatment	n	Interval	HR	PR	QRS	RR	QT (sec)	QTc (sec) ^a	QTc % Change ^b
1	Vehicle control	16	Baseline	222 ± 14.7	0.083 ± 0.001	0.041 ± 0.001	0.291 ± 0.021	0.162 ± 0.004	0.223 ± 0.003	-
		16	3 month	244 ± 5.9	0.087 ± 0.003	0.042 ± 0.001	0.247* ± 0.006	0.163 ± 0.004	0.228 ± 0.004	2.2
		12	6 month	249 ± 6.3	0.085 ± 0.002	0.042 ± 0.001	0.242* ± 0.006	0.168 ± 0.003	0.234 ± 0.002	4.9
		8	9 month	248 ± 7.5	0.092 ± 0.003	0.044* ± 0.002	0.243* ± 0.007	0.164 ± 0.004	0.230 ± 0.004	3.1
2	AF37702 0.2 mg/kg/day	8	Baseline	249 ± 19.3	0.081 ± 0.005	0.040 ± 0.000	0.254 ± 0.031	0.144** ± 0.007	0.209 ± 0.005	-
		8	3 month	249 ± 10.2	0.096* ± 0.003	0.041 ± 0.000	0.244 ± 0.012	0.160 ± 0.002	0.226* ± 0.001	8.1
		8	6 month	259 ± 4.7	0.090 ± 0.007	0.041 ± 0.001	0.232 ± 0.005	0.160 ± 0.004	0.227* ± 0.004	8.6
		4	9 month	255 ± 11.4	0.089 ± 0.003	0.043 ± 0.003	0.237 ± 0.011	0.165* ± 0.008	0.231* ± 0.008	10.5
3	AF37702 2.0 mg/kg/day	8	Baseline	242 ± 6.9	0.085 ± 0.003	0.040 ± 0.000	0.249 ± 0.007	0.161 ± 0.005	0.226 ± 0.005	-
		8	3 month	241 ± 11.6	0.089 ± 0.004	0.041 ± 0.001	0.254 ± 0.016	0.166 ± 0.003	0.231 ± 0.003	2.2
		8	6 month	234 ± 10.8	0.093 ± 0.002	0.041 ± 0.001	0.260 ± 0.013	0.177 ± 0.006	0.242 ± 0.006	7.1
		4	9 month	243 ± 13.5	0.086 ± 0.003	0.043 ± 0.003	0.248 ± 0.013	0.170 ± 0.005	0.235 ± 0.005	4.0
4	AF37702 20 mg/kg/day	16	Baseline	241 ± 8.1	0.083 ± 0.002	0.040 ± 0.000	0.254 ± 0.011	0.161 ± 0.005	0.225 ± .0004	-
		16	3 month	253 ± 5.6	0.086 ± 0.003	0.043* ± 0.001	0.238 ± 0.007	0.166 ± 0.003	0.233 ± 0.003	3.6
		11 ^c	6 month	238 ± 6.4	0.094 ± 0.003	0.042 ± 0.001	0.254 ± 0.007	0.172 ± 0.005	0.237 ± 0.004	5.3
		6 ^d	9 month	238 ± 7.3	0.099* ± 0.004	0.042 ± 0.001	0.253 ± 0.008	0.175 ± 0.003	0.240* ± 0.003	6.7

Data presented as Mean ± SEM

^aCalculated using Van de Water formula

^bCalculated from 15 min post-dose of vehicle treatment

^cOne animal dead

^dTwo animals dead

* Statistically significant change compared to the predose value - Two-way repeated measures ANOVA followed by a Bonferroni Multiple Comparison Test (SigmaStat, v2.03)

** Statistically significant change compared to the vehicle control group - Two-way repeated measures ANOVA followed by a Bonferroni Multiple Comparison Test (SigmaStat, v2.03)

Hematology

	Male				Female			
	Control	% change			Control	% change		
Dose (mg/kg)	0	0.2	2.0	20	0	0.2	2.0	20
RBC								
3 month	5.67	↑20	↑56	↑68	5.44	↑21	↑66	↑88
6 month	5.51	↑15	↑52	↑63	5.36	↑19	↑60	↑79
9 month	5.58	↑11	↑47	↑65	5.41	↑13	↑55	↑84
Recovery	5.14	NA	NA	-	5.23	NA	NA	-
HGB								
3 month	13.9	↑22	↑56	↑68	13.3	↑25	↑69	↑66
6 month	13.6	↑16	↑61	↑74	13.2	↑23	↑71	↑75
9 month	13.7	↑13	↑60	↑68	13.2	↑20	↑64	↑73
Recovery	12.7	NA	NA	-	13.1	NA	NA	-
HCT								
3 month	44.5	↑28	↑66	↑76	43	↑27	↑73	↑80
6 month	43.5	↑20	↑65	↑80	42.3	↑27	↑73	↑85
9 month	43.5	↑16	↑63	↑77	42.1	↑51	↑89	↑89
Recovery	39.1	NA	NA	-	40.7	NA	NA	-
RET (abs)								
3 month	107	↑86	↑110	↑181	90.3	↑162	↑176	↑246
6 month	111	↑124	↑62	↑168	88.8	↑84	↑153	↑184
9 month	82	↑182	↑133	↑271	105.5	↑42	↑131	↑169
Recovery	77.1	NA	NA	-	67.71	NA	NA	↓25

NA: not applicable

Coagulation

	Male				Female			
	Control	% change			Control	% change		
Dose (mg/kg)	0	0.2	2.0	20	0	0.2	2.0	20
PT								
3 month	10.1	↑20	↑56	↑68	10.3	↑21	↑66	↑88
6 month	10.2	↑15	↑52	↑63	10.1	↑19	↑60	↑79
9 month	10.3	↑11	↑47	↑65	9.9	↑13	↑55	↑84
Recovery	9.9	NA	NA	-	9.6	NA	NA	-
APTT								
3 month	18.1	NA	NA	↑188	17.8	NA	NA	↑104
6 month	16.8	-	↑28	↑46	16.2	-	↑59	↑59
9 month	20.2	-	-	↑18	16.0	-	↑33	↑49
Recovery	17.7	NA	NA	-	16.6	NA	NA	-

NA: not applicable

Clinical chemistry

Blood samples were obtained at pre-dose, day 5, weeks 13, 26, 39, and 43 (recovery)

	Male				Female			
	Control	% change			Control	% change		
Dose (mg/kg)	0	0.2	2.0	20	0	0.2	2.0	20
No. animals (T/R)	6/2	4/0	4/0	6/2	6/2	4/0	4/0	6/2
AST								
Week 13	32	-	-	↑19	28	-	-	↑25
Week 27	39	-	-	↑26	29	↑14	-	↑23
	33	-	↑15		27	↑15	-	↑24

	Male				Female			
	Control	% change			Control	% change		
Dose (mg/kg)	0	0.2	2.0	20	0	0.2	2.0	20
No. animals (T/R)	6/2	4/0	4/0	6/2	6/2	4/0	4/0	6/2
Week 39	38	NA	NA	↑52	27	NA	NA	-
Week 43				-				

T: Terminal necropsy; R: Recovery necropsy; NA: not applicable

Thyroid function

Blood samples were obtained at pre-dose, day 5, weeks 13, 26, 39, and 43 (recovery). No drug-related changes observed.

Troponin

Blood samples were obtained at pre-dose, day 5, weeks 13, 26, 39, and 43 (recovery). No drug-related changes were observed.

Urinalysis

Urine samples were obtained at pre-dose, day 5, weeks 13, 26, 39, and 43 (recovery). No drug-related changes observed.

AF37702 Antibody Detection Analysis (Study 05-509)

(Excerpted from Applicant's submission)

Three of the 64 monkeys treated intravenously with AF37702 once every third week over 274 days developed antibodies to AF37702. All 3 positive monkeys were from the high dose (20 mg/kg) group.

Parallel toxicokinetic analysis of these animals, however, has shown that levels of AF37702 were present at Day 190 and Day 274 (averages of 410 and 250 ng/mL, respectively) in 20 mg/kg dosing Group 4 that have known potential to interfere with the antibody-detection ELISA. The later Day 342/344 and Day 372 samples were the only tested post-dose time points from high-dose animals likely to be completely free of potentially interfering concentrations of AF37702. It is therefore possible that some of the Group 4 animals, especially those sacrificed before the later time points produced AF37702-specific antibodies that were undetectable due to the lack of available samples with sufficiently low levels of AF37702.

Erythropoietin Antibody Detection Analysis (Study 05-510)

(Excerpted from Applicant's submission)

Samples from Cynomolgus monkeys dosed intravenously with AF37702 for up to nine months were tested for EPO-specific antibodies. The samples chosen for testing were from all nine study animals that had scored above the Signal Cut Point at any time point

in an initial ELISA screening test for AF37702-reactive antibodies. No EPO-specific antibodies were detectable in any of the tested samples.

Gross Pathology

Macroscopic findings - Terminal		Male				Female			
Dose (mg/kg)		0	0.2	2.0	20	0	0.2	2.0	20
3-month sacrifice									
Injection site	Red discoloration	-	-	-	1	-	-	-	-
6-month sacrifice									
Spleen	Dark	-	-	-	1	-	-	-	7
Injection site	Red discoloration	1	1	1	4		1		
Liver	Dark	-	-	-	-	-	1	3	4
Stomach	Black foci	-	-	-	-	-	-	-	1
	Mucosa reddened	-	-	-	-	--	-	-	1
	Dark areas in pyloric region	-	-	-	-	-	-	-	1
9-month sacrifice									
Injection site	Red discoloration	-	-	-	3				
Stomach	Dark red focus	-	-	-	1				
	Mucosa dark red	-	-	-	1				
Ovaries	Right-clear cyst	-	-	-	-	-	-	-	1
Uterus	Dark red	-	-	-	-	-	-	-	1
Recovery sacrifice									
Heart	Epicardium: white discoloration	-	-	-	1	-	-	-	-
Lung	Dark red discoloration	-	-	-	1	-	-	-	-
	White foci	-	-	-	1	-	-	-	-
Testis	Blood clot on surface (left)	-	-	-	1	-	-	-	-
Injection site	Red discoloration (right)	-	-	-	-	-	-	-	1
	Thickened	-	-	-	-	-	-	-	1

Organ Weights

Organ samples were taken at scheduled necropsy

	Male				Female			
	Control	% change			Control	% change		
Dose (mg/kg)	0	0.2	2.0	20	0	0.2	2.0	20
No. animals (T/R)	6/2	4/0	4/0	6/2	6/2	4/0	4/0	6/2
Spleen								
Week 39	0.78	-	-	↓13	1.053	↓24	↓28	↓60
Week 43	1.145	NA	NA	-	0.811	NA	NA	↑56
Thyroid								
Week 39	0.081	↓17	↓35	↓35	-	-	-	-
Week 43	0.007	NA	NA	↓40				

T: Terminal necropsy; R: Recovery necropsy; NA: not applicable

Histopathology

Adequate Battery (yes)

Peer Review (yes)

Histological Findings: Tables excerpted from Applicant's submission

3-month sacrifice

Group/Sex	1M	4M	1F	4F
	N=4	N=3	N=4	N=4
Tissue/Finding				
Bone Marrow, Sternum/Hypercellularity	0	1	0	3
Average Severity	0.0	0.3	0.0	0.8
Bone Marrow, Sternum/Congestion	0	3	0	4
Average Severity	0.0	2.0	0.0	1.8
Brain/Infiltration, Mononuclear Cell	0	3	0	4
Average Severity	0.0	1.3	0.0	1.0
Brain/Congestion	0	2	1	4
Average Severity	0.0	1.0	0.3	1.8
Cecum/Congestion	0	0	0	0
Average Severity	0.0	0.0	0.0	0.0
Colon/Congestion, Mucosa	0	1	0	0
Average Severity	0.0	0.7	0.0	0.0
Duodenum/Congestion, Mucosa	0	3	0	3
Average Severity	0.0	2.0	0.0	1.5
Ileum/Congestion, Mucosa	0	1	0	0
Average Severity	0.0	0.7	0.0	0.0
Jejunum/Congestion, Mucosa	0	1	0	0
Average Severity	0.0	0.7	0.0	0.0
Rectum/Congestion	0	0	0	0
Average Severity	0.0	0.0	0.0	0.0
Liver/Congestion	0	3	0	2
Average Severity	0.0	2.0	0.0	0.8
Lung/Congestion	1	3	0	2
Average Severity	0.3	1.7	0.0	0.5
Kidney/Congestion	1	3	0	4
Average Severity	0.3	2.0	0.0	1.5
Kidney/Deposition, Glomerulus	0	0	0	0
Average Severity	0.0	0.0	0.0	0.0
Spleen/Congestion, Red Pulp	0	2	0	4
Average Severity	0.0	1.3	0.0	1.5

*Average severity based upon a grading scale where: 0 = normal, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. N = Number of animals, M= Male, F=Female, Average severity is calculated based on total number of incidence, severity, and total number of tissues examined.

6-month sacrifice

Tissue/Finding	Group/Sex							
	1M N=4	2M N=4	3M N=4	4M N=4	1F N=4	2F N=4	3F N=4	4F N=4
Bone Marrow, Sternum/Hypercellularity	0	0	3	4	0	0	4	4
Average Severity	0.0	0.0	1.3	1.8	0.0	0.0	1.5	1.5
Bone Marrow, Sternum/Congestion	0	0	2	4	0	0	4	4
Average Severity	0.0	0.0	1.0	1.5	0.0	0.0	1.5	1.3
Brain/Infiltration, Mononuclear Cell	0	0	0	4	0	0	2	4
Average Severity	0.0	0.0	0.0	1.8	0.0	0.0	0.8	1.5
Brain/Congestion	0	1	4	4	0	1	4	4
Average Severity	0.0	0.3	1.8	2.0	0.0	0.3	2.0	2.0
Cecum/Congestion, Mucosa	0	0	0	4	0	0	1	4
Average Severity	0.0	0.0	0.0	2.0	0.0	0.0	0.3	1.8
Colon/Congestion, Mucosa	0	0	3	4	0	0	2	0
Average Severity	0.0	0.0	0.8	2.0	0.0	0.0	0.8	0.0
Duodenum/Congestion, Mucosa	0	0	0	4	0	0	2	3
Average Severity	0.0	0.0	0.0	2.0	0.0	0.0	0.8	1.5
Ileum/Congestion, Mucosa	0	0	0	3	0	0	0	1
Average Severity	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.5
Jejunum/Congestion, Mucosa	0	0	0	3	0	0	0	0
Average Severity	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.0
Rectum/Congestion, Mucosa	0	0	1	0	0	0	1	2
Average Severity	0.0	0.0	0.3	0.0	0.0	0.0	0.3	0.7
Liver/Congestion	0	0	4	4	0	1	4	4
Average Severity	0.0	0.0	1.5	1.3	0.0	0.3	2.0	2.0
Lung/Congestion	0	0	2	4	0	0	3	4
Average Severity	0.0	0.0	0.5	2.0	0.0	0.0	1.0	1.8
Kidney/Congestion	0	0	4	4	1	2	4	4
Average Severity	0.0	0.0	1.5	2.0	0.3	0.5	2.0	2.0
Kidney/Deposition, Glomerulus	0	0	0	2	0	0	2	3
Average Severity	0.0	0.0	0.0	0.5	0.0	0.0	0.5	0.8
Spleen/Congestion, Red Pulp	0	0	2	4	0	0	2	4
Average Severity	0.0	0.0	0.8	1.8	0.0	0.0	0.8	1.3

*Average severity based upon a grading scale where: 0 = normal, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. N = Number of animals, M= Male, F=Female, Average severity is calculated based on total number of incidence, severity, and total number of tissues examined.

9-month sacrifice

Tissue/Finding	Group/Sex							
	1M N=4	2M N=4	3M N=4	4M N=4	1F N=4	2F N=4	3F N=4	4F N=3
Bone Marrow, Sternum/Hypercellularity	0	0	4	4	0	0	4	3
Average Severity	0.0	0.0	1.5	2.0	0.0	0.0	1.0	2.0
Bone Marrow, Sternum/Congestion	0	0	3	4	0	0	3	2
Average Severity	0.0	0.0	0.8	1.8	0.0	0.0	0.8	1.3
Brain/Infiltration, Mononuclear Cell	0	0	3	4	1	1	3	3
Average Severity	0.0	0.0	0.8	2.0	0.3	0.3	0.8	1.7
Brain/Congestion	0	0	4	4	0	1	4	3
Average Severity	0.0	0.0	1.8	2.0	0.0	0.3	1.8	2.0
Cecum/Congestion, Mucosa	0	0	2	3	0	0	3	2
Average Severity	0.0	0.0	0.8	0.8	0.0	0.0	0.8	1.0
Colon/Congestion, Mucosa	0	0	2	3	0	0	0	2
Average Severity	0.0	0.0	0.5	1.0	0.0	0.0	0.0	0.7
Duodenum/Congestion, Mucosa	0	0	2	3	1	0	2	2
Average Severity	0.0	0.0	0.5	0.8	0.3	0.0	0.5	1.0
Ileum/Congestion, Mucosa	0	0	1	2	1	0	3	2
Average Severity	0.0	0.0	0.3	0.5	0.3	0.0	0.8	0.7
Jejunum/Congestion, Mucosa	0	0	2	1	0	0	2	2
Average Severity	0.0	0.0	0.5	0.3	0.0	0.0	0.5	0.7
Rectum/Congestion, Mucosa	0	0	1	2	0	0	0	0
Average Severity	0.0	0.0	0.3	1.3	0.0	0.0	0.0	0.0
Liver/Congestion	0	0	4	4	0	0	3	3
Average Severity	0.0	0.0	1.5	1.8	0.0	0.0	1.3	2.0
Lung/Congestion	0	0	1	4	0	0	2	3
Average Severity	0.0	0.0	0.3	1.8	0.0	0.0	0.8	2.0
Kidney/Congestion	1	0	4	4	0	1	4	3
Average Severity	0.3	0.0	2.0	2.0	0.0	0.3	2.0	2.0
Kidney/Deposition, Glomerulus	0	0	4	4	0	0	4	1
Average Severity	0.0	0.0	1.0	2.0	0.0	0.0	1.0	0.7
Spleen/Congestion, Red Pulp	0	0	3	4	0	0	2	2
Average Severity	0.0	0.0	1.0	1.3	0.0	0.0	0.5	0.7

*Average severity based upon a grading scale where: 0 = normal, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. N = Number of animals, M= Male, F=Female, Average severity is calculated based on total number of incidence, severity, and total number of tissues examined.

Recovery sacrifice

Tissue/Finding	Group/Sex			
	1M N=4	4M N=4	1F N=4	4F N=3
Bone Marrow, Sternum/Hypercellularity	0	0	0	1
Average Severity	0.0	0.0	0.0	0.3
Brain/Infiltration, Mononuclear Cell	0	4	0	3
Average Severity	0.0	2.0	0.0	2.0
Lung/Congestion	1	1	0	0
Average Severity	0.5	0.5	0.0	0.0
Kidney/Congestion	0	1	0	0
Average Severity	0.0	0.3	0.0	0.0
Kidney/Dilation, Glomerulus	0	4	0	2
Average Severity	0.0	1.0	0.0	0.7
Kidney/Interstitialium, Fibrosis	1	4	0	2
Average Severity	0.3	1.0	0.0	1.0

*Average severity based upon a grading scale where: 0 = normal, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. N = Number of animals, M= Male, F=Female, Average severity is calculated based on total number of incidence, severity, and total number of tissues examined.

Toxicokinetics**Methods**

Blood samples collection: on day 1, 106, 190, and 274 at predose, 1, 4, 8, 24, 48, 72, 120, and 168 hours after dosing.

Results

Toxicokinetic Parameters of AF37702 in Plasma of Male Monkeys						
Dose (mg/kg)	Month	AUC _(0-120hr)	AUC/dose	C _{max}	C _{max} /dose	t _{max}
0.2	0	212	1060	5.51	28	1
	3	231	1155	5.98	30	1
	6	222	1110	5.43	27	1
	9	229	1145	5.19	26	2.5
2	0	3926	1963	73.2	37	1
	3	3266	1633	70.1	35	2.5
	6	4406	2203	85.1	43	1
	9	2789	1395	58	29	2.5
20	0	47266	2363	675.2	34	1
	3	48227	2411	757.4	38	2.5
	6	74506	3725	1389.7	69	1
	9	41256	2063	691.5	35	2.5
Toxicokinetic Parameters of AF37702 in Plasma of Female Monkeys						
Dose (mg/kg)	Week	AUC _(0-120hr)	AUC/dose	C _{max}	C _{max} /dose	t _{max}
0.2	0	239	1195	6.94	35	1
	3	234	1170	6.1	31	1
	6	209	1045	5.14	26	1
	9	196	980	3.16	16	1
2	0	3803	1902	80.7	40	2.5
	3	3112	1556	59.1	30	2.5
	6	5085	2543	116.1	58	6
	9	2616	1308	45.5	23	4
20	0	46576	2329	677.8	34	1
	3	46498	2325	670.1	34	2.5
	6	87202	4360	1715.8	86	14
	9	42993	2150	714.7	36	8

Study Summary:

AF37702 was administered to male and female monkeys via IV, bolus injection, once every three weeks for 3, 6, and 9 months at doses of 0, 0.2, 2, and 20 mg/kg, followed by a 4-week recovery period. AF377002 exposure generally increased with dose. C_{max} and AUC values were also similar between all time points at the 0.2 and 2 mg/kg doses, indicating no drug accumulation. In the 20 mg/kg group, however, C_{max} and AUC values at 6 months were higher than at the 3 and 9 month time points. Concentrations were similar between males and females.

Three animals in the 20 mg/kg group (1 male and 2 female) were found dead at days 79, 104, and 207. Multi-organ congestion was noted in all three animals and one female showed brain hemorrhage. There were no clinical signs that lead to death.

In surviving animals, ophthalmoscopy reports show dilated and dark retinal venules, focal retinal hemorrhage, and lens opacities in the 20 mg/kg groups. Increased in RBCs and indices, enlarged spleens, and hypercellularity in the bone marrow were likely due to the pharmacology of AF37702. Multi-organ congestion was the primary toxicity and was noted in the bone marrow, brain, GI, kidney, liver, lung, and spleen. Antibody analyses show that 3 monkeys in the 20mg/kg group developed AF37702 antibodies. Most effects were generally reversible.

Subcutaneous Administration

Title: A 3-Month Subcutaneous Toxicity Study of AF37702 in Rats Followed by a Six-Week Recovery

Study Number: AF04-011

Laboratory: (b) (4)

In-Life Dates: September 17, 2005 – February 11, 2005

Test Material: AF37702, Lot #12AB1A, 98.7%

GLP: Statement included and signed

QA: Statement included and signed

Design: This study was designed to evaluate the potential toxicity and toxicokinetics of AF37702 after once every three weeks subcutaneous dosing to Sprague-Dawley rats for a total of 5 doses. The vehicle was 10 mM acetate in 0.9% sodium chloride for injection. The dose levels are shown in the Applicant's tables below:

Toxicology Groups

Group	Dose Level (mg/kg)	Concentration (mg/ml)	Dose Volume (ml/kg)	Number of Animals*	
				Male	Female
1. Vehicle-Control	0	0	5	15	15
2. Low-dose	0.1	0.02	5	15	15
3. Mid-dose	1.0	0.2	5	15	15
4. High-dose	10	2.0	5	15	15

* On Day 90, ten animals/sex/group were euthanized and necropsied. The remaining five animals/sex/group remained on test following the final dose, untreated, until they were euthanized following a six-week recovery.

Toxicokinetic Groups

Group	Dose Level (mg/kg)	Concentration (mg/ml)	Dose Volume (ml/kg)	Number of Animals**	
				Male	Female
5. Low-dose	0.1	0.02	5	9	9
6. Mid-dose	1.0	0.2	5	9	9
7. High-dose	10	2.0	5	9	9

**The toxicokinetic groups, in this report, were only evaluated for toxicokinetics.

Results and Summary:

Male and female rats were administered AF37702 at 0.1, 1.0 or 10 mg/kg subcutaneously (dorsal thoracic region) once every three weeks (Days 1, 22, 43, 64 and 85) followed by a six-week recovery period. AF37702 exposure increased with dose between all dose groups following subcutaneous administration. C_{max} and AUC values were dose-proportional between all doses on both days. Slight drug accumulation was observed in the 0.1 and 1 mg/kg males groups and in all female groups (see table from Applicant's submission):

Plasma pharmacokinetic parameters of AF37702 following single and multiple SC administration of AF37702 in rats.

Day	Sex	Dose (mg/kg)	C _{max} (µg/mL)	t _{max} (h)	AUC _{0-t} (µg•h/mL)	AUC _{0-inf} (µg•h/mL)	t _{1/2} (h)	CL/F (mL/h•kg)	AI ^a C _{max}	AI ^b AUC
1	Male	0.1	0.274	24	12.8	ND	ND	ND	NA	NA
		1	2.924	48	177.8	184.1	18.9	5.43	NA	NA
		10	50.879	24	3391	3403	15.6	2.94	NA	NA
	Female	0.1	0.279	24	13.6	ND	ND	ND	NA	NA
		1	5.079	24	293.4	298.1	16.1	3.35	NA	NA
		10	54.329	48	3907	4245	26.6	2.36	NA	NA
85	Male	0.1	0.819	24	27.9	30.5	16.6	3.28	2.99	2.18
		1	3.807	48	223.9	245.1	26.1	4.08	1.30	1.26
		10	33.003	48	2070.7	2459.5	37.1	4.07	0.65	0.61
	Female	0.1	1.131	24	50.6	52.6	22.6	1.91	4.05	3.71
		1	8.875	48	559.6	589.7	22.0	1.70	1.75	1.91
		10	63.191	48	4851.4	5622.3	33.0	1.78	1.16	1.24

^a C_{max} accumulation index = C_{max} (Day85) / C_{max} (Day 1)

^b AUC accumulation index = AUC(0-t, Day85) / AUC(0-t, Day 1)

ND: not determined, insufficient data points on terminal phase

NA: not applicable

Two animals in the 1 mg/kg male group were euthanized during this study. One animal was sacrificed on day 50 due to a moribund condition with clinical signs decreased activity, hunched posture, thin body condition, cold to touch, decreased skin turgor and red staining in cage and urogenital area. The other animal was sacrificed on

day 119 due to an abrasion on its eye. No other clinical signs were noted in the surviving animals. Except for the one animal described above, AF37702 had no effects on ophthalmoscopy. There were no drug-related effects body weights, and food consumption. No animals developed antibodies to AF37702. Statistically significant increases in RBC, HGB, and HCT were observed in all AF37702 treatment groups. Enlarged spleens and increased erythropoiesis in bone marrow are likely due to the pharmacology of AF37702. Other major target organs of AF37702 were the heart, kidney, and pancreas. In the heart, toxicities included minimal to mild stromal proliferation of the atrio-ventricular valve and in the interstitium of the myocardium in the 10 mg/kg group. In the kidney, minimal to moderate congestion (≥ 1 mg/kg) and minimal to mild periarteritis (≥ 10 mg/kg) were observed. In the pancreas, minimal to moderate periarteritis was observed in the 10 mg/kg group. All changes appear to be reversible. An increased incidence and severity of increased congestion, erythropoiesis, and myelopoietic infiltration in the liver were also noted.

Title: A 4-Week Subcutaneous Toxicity Study of AF37702 in Cynomolgus Monkeys

Study Number: AF05-011

Laboratory: (b) (4)

In-Life Dates: April 8, 2005 – May 25, 2005

Test Material: AF37702, Lot #031114A, 98.7%

GLP: Statement included and signed

QA: Statement included and signed

Design: This study was designed to evaluate the potential toxicity and toxicokinetics of AF37702 to Cynomolgus monkeys after once weekly subcutaneous dosing for 5 consecutive weeks. The vehicle was 10 mM acetate in 0.9% sodium chloride for injection. The dose levels are shown in the Applicant's table below:

Study Design

Group Number	Number of Animals		Test Article	Dosage Level (mg/kg)	Dosage Volume (mL/kg)	Dosing Regimen	Necropsy Day
	Males	Females					
1	3	3	Vehicle	0	1	SC on Days 1, 8, 15, 22, and 29	Day 35
2	3	3	AF37702	0.5			
3	3	3		5			
4	3	3		50			

Results and Summary:

AF37702 had no effects on mortality, ophthalmic parameters, body weights, food consumption, urinalysis, clinical chemistry, and ECG. Erythemas and swelling were noted at the injection sites in AF37702 treated animals. Increases in RBCs and indices (HGB, HCT, MCV) along with hypercellularity in the bone marrow (sternum) were

expected given the pharmacology of AF37702. Levels of APTT were elevated at doses ≥ 5 mg/kg. Three animals in the 50 mg/kg group showed mild cytoplasmic vacuolation of the choroid plexus (brain).

AF37702 exposure increased with dose between all dose groups following subcutaneous administration. C_{max} and AUC values were greater than dose-proportional between 0.5 and 5 mg/kg; whereas, C_{max} and AUC values were dose-proportional between 5 and 50 mg/kg. Slight drug accumulation was observed in the 5 and 50 mg/kg groups (see table from Applicant) upon repeated dosing:

Mean (SD) plasma pharmacokinetic parameters of AF37702 following a single or multiple SC administration (once weekly) in monkeys (n=3/sex/group).

	Dose (mg/kg)	C_{max} ($\mu\text{g/mL}$)	t_{max}^a (h)	$AUC_{(0-1)}$ ($\mu\text{g}\cdot\text{h/mL}$)	$t_{1/2}$ (h)	AI^b AUC
Day 1						
Male	0.5	5.30 (0.89)	48 (8-48)	511 (68.2)	37.9 (9.8)	NA
	5	168.1 (33.3)	120 (48-120)	17784 (944)	ND	NA
	50	1707 (496.3)	48 (48-96)	151783 (25593)	62.4 ^c	NA
Female	0.5	3.49 (0.64)	48 (24-72)	365 (54.3)	ND	NA
	5	148.4 (48.3)	96 (72-120)	15100 (5087)	ND	NA
	50	1225 (278.9)	72 (48-72)	125293 (20430)	ND	NA
Day 29						
Male	0.5	5.44 (0.50)	8 (8-24)	440 (63.5)	50.3 ^d	0.86
	5	209.5 (71.1)	48 (8-72)	20928 (8582)	63.9 ^d	1.18
	50	2107 (358.7)	72 (72-72)	238032 (45090)	ND	1.57
Female	0.5	3.10 (0.54)	24 (8-48)	262 (65.5)	ND	0.72
	5	234.8 (73.9)	72 (72-72)	22776 (7274)	28.7 ^c	1.51
	50	2348 (692.6)	120 (24-120)	249554 (76277)	ND	1.99

^a: median(range); ^b: AUC accumulation index = $AUC(0-168\text{h, Day1}) / AUC(0-144\text{h, Day 29})$

^c: n=1; ^d: n=2

ND: not determined, insufficient data point on terminal phase; NA: not applicable

6.3 Discussion and Conclusion

The toxicological profile of peginesatide was consistent with its pharmacology. Nonclinical findings in the rat and monkey show that peginesatide treatment caused notable changes in red blood cell hematology parameters (red blood cells, hemoglobin, and hematocrit) and morphology, enlarged spleens, and increased hematopoiesis/hypercellularity and hyperplasia in the bone marrow. Increased congestion and periarteritis were also noted in studies in the rat and monkey, which can also be attributed to the exaggerated pharmacology of peginesatide. Other target organ toxicities were seen in the heart, liver, kidney, and brain. Toxicities were comparable between the intravenous and subcutaneous routes of administration.

7 Genetic Toxicology

Genotoxicity reviews were completed by Ke Zhang, Ph.D. in the Division of Gastroenterology Products on April 13, 2007. Reviews were slightly modified to fit this NDA review.

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: AF37702 bacterial reverse mutation assay

Study report No: AF03-46

Testing Laboratory: (b) (4)

Date of study initiation: February 27, 2004

Date of study report: November 18, 2004

GLP Compliance: Statements included and signed

QA-report: Statements included and signed

Drug Batch No.: 12AB1

Key Finding: AF37702 was negative for mutagenicity in this Ames Test.

Methods:

To examine the potential mutagenic effects of AF37702, the reverse mutation assay (Ames test) was conducted using the direct plate incorporation method.

Strain/species/cell line: Four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and one strain of *E. Coli* (WP2uvrA).

Basis of concentration selection: The highest concentration of 5000 µg/plate was used based on ICH S2.

Metabolic activation system: Metabolic activation, S-9 mix, was from rat liver.

Negative control: dimethyl sulfoxide.

Positive controls: Positive controls (sodium azide, 2- nitrofluorene, 2-aminoanthracene, 9-aminoacridine, and methyl methanesulfonate) were tested.

Test conditions: The reverse mutation assay (Ames test) was conducted using the direct plate incorporation method.

Concentrations used in defining study: The following concentrations were tested: 2.5, 7.5, 25, 75, 200, 600, 1800, and 5000 µg/plate with and without S-9.

Counting method: The condition of the bacterial background lawn was evaluated macroscopically and microscopically using a dissecting microscope.

Cytotoxic endpoints: The condition of the bacterial background lawn was evaluated for evidence of cytotoxicity.

Genetic toxicity endpoints/results: Number of revertant colonies.

Statistical methods: Number of revertant colonies was averaged for each concentration.

Criteria for positive results: The results were considered positive if the test substance induced a 2-fold increase in the mean revertant colonies as compared to the control and this increase should have shown a concentration response to increasing concentrations of the test article.

Results:

Study validation: The positive controls significantly increased the colonies compared to the solvent controls.

Study outcome: AF37702 did not significantly increase the colonies as compared to the solvent control in the presence and absence of S-9 mix. The results were reproducible. The results were summarized in the following tables (*Excerpted from Applicant's submission*):

Test Article Id : AF37702, Lot# 12AB1
 Study Number : AA87VA.503.BTL Experiment No : B1

Average Revertants Per Plate \pm Standard Deviation

Liver Microsomes: None

Dose ($\mu\text{g}/\text{plate}$)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	16 \pm 1	163 \pm 1	14 \pm 4	5 \pm 1	16 \pm 3
2.5	17 \pm 1	183 \pm 22	20 \pm 1	5 \pm 1	16 \pm 4
7.5	15 \pm 1	176 \pm 8	19 \pm 3	6 \pm 1	15 \pm 1
25	19 \pm 4	165 \pm 9	18 \pm 0	6 \pm 2	18 \pm 2
75	14 \pm 1	182 \pm 16	24 \pm 2	6 \pm 1	20 \pm 2
200	18 \pm 1	189 \pm 5	20 \pm 6	7 \pm 4	16 \pm 3
600	16 \pm 5	171 \pm 1	18 \pm 1	8 \pm 4	21 \pm 3
1800	18 \pm 6	178 \pm 18	21 \pm 6	6 \pm 2	20 \pm 1
5000	15 \pm 5	199 \pm 18	23 \pm 1	4 \pm 0	22 \pm 4
Positive	157 \pm 27	587 \pm 22	298 \pm 35	721 \pm 49	136 \pm 5

Liver Microsomes: Rat liver S9

Dose ($\mu\text{g}/\text{plate}$)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	18 \pm 1	205 \pm 0	17 \pm 6	7 \pm 2	14 \pm 3
2.5	23 \pm 3	225 \pm 34	14 \pm 0	6 \pm 1	18 \pm 0
7.5	22 \pm 2	229 \pm 5	12 \pm 1	8 \pm 4	20 \pm 0
25	29 \pm 1	221 \pm 15	17 \pm 1	9 \pm 2	19 \pm 6
75	20 \pm 1	245 \pm 16	15 \pm 1	5 \pm 0	15 \pm 7
200	17 \pm 2	240 \pm 11	14 \pm 1	7 \pm 0	17 \pm 2
600	27 \pm 3	253 \pm 7	15 \pm 1	4 \pm 1	16 \pm 1
1800	22 \pm 0	242 \pm 1	16 \pm 4	5 \pm 3	15 \pm 1
5000	28 \pm 2	247 \pm 16	23 \pm 0	8 \pm 2	18 \pm 3
Positive	939 \pm 208	1598 \pm 420	118 \pm 26	135 \pm 12	648 \pm 112

Vehicle = Vehicle Control

Positive = Positive Control (50 μL plating aliquot)

Plating aliquot: 100 μL

Test Article Id : AF37702, Lot# 12AB1
 Study Number : AA87VA.503.BTL Experiment No : B2

Average Revertants Per Plate \pm Standard Deviation

Liver Microsomes: None

Dose ($\mu\text{g}/\text{plate}$)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	18 \pm 3	136 \pm 13	20 \pm 1	5 \pm 3	20 \pm 3
75	16 \pm 3	123 \pm 15	18 \pm 3	5 \pm 2	19 \pm 6
200	23 \pm 6	149 \pm 6	21 \pm 6	8 \pm 4	21 \pm 2
600	22 \pm 3	131 \pm 19	17 \pm 3	6 \pm 1	20 \pm 3
1800	18 \pm 5	141 \pm 7	21 \pm 7	6 \pm 2	21 \pm 5
5000	18 \pm 1	130 \pm 4	19 \pm 1	8 \pm 2	18 \pm 1
Positive	101 \pm 7	594 \pm 8	204 \pm 24	406 \pm 48	72 \pm 5

Liver Microsomes: Rat liver S9

Dose ($\mu\text{g}/\text{plate}$)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	33 \pm 3	140 \pm 4	11 \pm 4	8 \pm 2	24 \pm 5
75	29 \pm 2	173 \pm 13	14 \pm 2	5 \pm 1	24 \pm 4
200	31 \pm 4	165 \pm 14	13 \pm 5	3 \pm 0	20 \pm 2
600	29 \pm 3	144 \pm 15	15 \pm 2	5 \pm 2	23 \pm 4
1800	32 \pm 3	171 \pm 15	17 \pm 3	6 \pm 0	20 \pm 3
5000	34 \pm 5	161 \pm 26	21 \pm 3	5 \pm 3	20 \pm 2
Positive	1363 \pm 27	1381 \pm 143	122 \pm 7	140 \pm 46	704 \pm 71

Vehicle = Vehicle Control

Positive = Positive Control (50 μL plating aliquot)

Plating aliquot: 100 μL

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Study title: *In vitro* chromosomal aberration test in Chinese hamster ovary cell (CHO)

Study report No: AF03-47

Testing Laboratory: (b) (4)

Date of study initiation: February 26, 2004

Date of study report: November 30, 2004

GLP Compliance: Statements included and signed

QA-report: Statements included and signed

Drug Batch No.: 12AB1

Key Finding: AF37702 was negative for clastogenicity in this chromosome aberration assay

Methods: To examine the potential induction of chromosomal aberrations by AF37702, the *in vitro* chromosomal aberration test was conducted in Chinese ovary cells.

Strain/species/cell line: Chinese ovary cells

Basis of concentration selection: The highest concentration of 5000 µg/ml was used per ICH S2.

Metabolic activation system: Metabolic activation, Aroclor 1254-induced rat liver S-9

Negative control: Water

Positive controls: Mitomycin and cyclophosphamide

Exposure conditions: The cultures were incubated with or without metabolic activation at 37°C. In all groups, cells were arrested in metaphase using colcemid ~2-3 hours before harvest.

Concentrations used in defining study: 625, 1250, 2500, 5000 µg/ml with and without S-9

Treatment condition	Treatment Time	Recovery Time	AF37702 Concentrations (µg/mL)
Non-activated (-S9)	4	16	625, 1250, 2500, 5000
	20	0	
Activated (+S9)	4	16	

Statistical methods: Number of cells with aberration was averaged for each concentration.

Criteria for positive results: The results should be considered positive if the test substance induced a significant increase in the number of cells with chromosomal aberrations at one or more concentrations as compared to the control and this increase should be a concentration response to increasing concentrations of the test article.

Results:

AF37702 did not significantly increase the frequency of the chromosomal aberration in the presence and absence of S-9. However, the positive controls significantly increased it. The results were summarized in the following table (*Excerpted from Applicant's submission*):

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
Water	-S9	4	13.3	200	200	0.000	±0.000	3.0	0.0
AF37702, Lot # 12AB1									
1250	-S9	4	12.7	200	200	0.000	±0.000	5.0	0.0
2500	-S9	4	12.8	200	200	0.000	±0.000	4.0	0.0
5000	-S9	4	8.9	200	200	0.000	±0.000	5.5	0.0
MMC, 0.2	-S9	4	10.9	200	100	0.270	±0.548	2.0	22.0**
Water	+S9	4	12.3	200	200	0.005	±0.071	10.0	0.5
AF37702, Lot # 12AB1									
1250	+S9	4	11.9	200	200	0.000	±0.000	8.5	0.0
2500	+S9	4	13.5	200	200	0.005	±0.071	9.0	0.5
5000	+S9	4	14.6	200	200	0.010	±0.100	9.5	1.0
CP, 10	+S9	4	9.2	200	200	0.225	±0.562	4.5	16.5**
Water	-S9	20	15.4	200	200	0.000	±0.000	3.5	0.0
AF37702, Lot # 12AB1									
1250	-S9	20	12.9	200	200	0.000	±0.000	4.5	0.0
2500	-S9	20	13.0	200	200	0.015	±0.122	5.5	1.5
5000	-S9	20	12.4	200	200	0.010	±0.100	5.5	1.0
MMC, 0.1	-S9	20	9.6	200	100	0.230	±0.468	2.5	21.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using Fisher's exact test.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: AF37702: mammalian erythrocyte micronucleus test

Study report No: AF04-19

Testing Laboratory: (b) (4)

Date of study initiation: November 23, 2004

Date of study report: March 4, 2005

GLP Compliance: Statements included and signed

QA-report: Statements included and signed

Drug Batch No.: 12AB1

Key Finding: AF37702 was negative in this *in vivo* micronucleus assay

Methods:

To examine the potential genotoxic effects of AF37702, a micronucleus test was conducted using mouse bone marrow cells. AF37702 (500, 1000, or 2000 mg/kg) was administered to mice by intraperitoneal injection in two divided doses (3 hours apart).

Strain/species/cell line: ICR mice

Basis of dose selection: The highest dose tested was 2000 mg/kg per ICH S2.

Vehicle control: Sterile water for injection

Positive control: Cyclophosphamide

Exposure conditions: Mice were sacrificed 24 and 48 hours after dosing and bone marrow was collected.

Doses used: 500, 1000, or 2000 mg/kg.

Counting method: Slides were prepared and examined for presence of micronucleated polychromatic erythrocytes.

Cytotoxic endpoints: Proportion of reticulocytes to total erythrocytes was determined as an indicator of bone marrow toxicity.

Genetic toxicity endpoints/results: Frequency of micronucleated reticulocytes.

Statistical methods: Frequency of micronucleated reticulocytes was analyzed.

Criteria for positive results: The result was considered positive if a significant increase in the micronucleated reticulocytes was observed dose-dependently.

Results:

Study validation: The positive controls significantly increased the frequency of micronucleated reticulocytes.

Study outcome: No deaths occurred in this study. Piloerection was noted at doses of 1000 and 2000 mg/kg. AF37702 did not significantly increase the frequency of micronucleated reticulocytes at the doses tested. The results were summarized in the following table (*Excerpted from Applicant's submission*):

**Summary of Bone Marrow Micronucleus Analysis
Following Administration of AF37702, Lot # 12AB1 in ICR Mice**

Treatment (20 mL/kg x 2)	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes	
						Number per 1000 PCEs (Mean +/- SD)	Number per PCEs Scored ¹
Water	M	24	5	0.476 ± 0.04	---	0.3 ± 0.27	3 / 10000
	F	24	5	0.478 ± 0.04	---	0.5 ± 0.35	5 / 10000
AF37702 500 mg/kg	M	24	5	0.450 ± 0.04	-5	0.5 ± 0.35	5 / 10000
	F	24	5	0.440 ± 0.02	-8	0.4 ± 0.42	4 / 10000
1000 mg/kg	M	24	5	0.437 ± 0.08	-8	0.7 ± 0.27	7 / 10000
	F	24	5	0.414 ± 0.05	-13	0.3 ± 0.27	3 / 10000
2000 mg/kg	M	24	5	0.452 ± 0.09	-5	0.6 ± 0.22	6 / 10000
	F	24	5	0.262 ± 0.08	-45	0.7 ± 0.57	7 / 10000
Cyclophosphamide** 50 mg/kg	M	24	5	0.344 ± 0.02	-28	24.1 ± 7.78	*241 / 10000
	F	24	5	0.325 ± 0.03	-32	25.5 ± 7.58	*255 / 10000
Water	M	48	5	0.493 ± 0.03	---	0.7 ± 0.27	7 / 10000
	F	48	5	0.490 ± 0.03	---	0.7 ± 0.45	7 / 10000
AF37702 2000 mg/kg	M	48	5	0.483 ± 0.04	-2	0.4 ± 0.22	4 / 10000
	F	48	5	0.418 ± 0.02	-15	0.7 ± 0.45	7 / 10000

¹Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables)

**CP administered only once at a volume of 20 mL/kg

7.4 Discussions and Conclusions

Peginesatide was negative in the standard battery of genotoxicity assays. Peginesatide was not mutagenic in the *in vitro* reverse mutation (Ames) assay, and was not clastogenic in an *in vitro* chromosomal aberration assay in CHO cells or in an *in vivo* micronucleus assay in mouse bone marrow.

8 Carcinogenicity

8.1 Rat Carcinogenicity Study

Study title: A Two Year Carcinogenicity Study of AF37702 in Rats Following Intravenous Administration Every Three-Weeks.

Study no.: AF06-013 (Affymax), 0474RA33.001
(b) (4)

Study report location: eCTD 4.2.3.4.2

Conducting laboratory and location: (b) (4)

Date of study initiation: 10/13/2006

GLP compliance: Statement included and signed

QA statement: Statement included and signed

Drug, lot #, and % purity: AF37702, Lot PLI012-06, 99.3%

ECAC concurrence: Yes (Exec. CAC meeting of 9/27/2006)

Key Study Findings

Neoplastic findings:

- At the doses tested, no dose-related increases in the incidence of neoplastic lesions compared to the controls were observed

Non-neoplastic findings:

- Statistically significant increase in mortality in 0.1 and 0.25 mg/kg male groups compared to controls; the increase was not dose-dependent
- Increased incidences of dark pink extremities observed amongst AF37702 treatment groups
- Increased red blood cells correlated with increase hemoglobin and hematocrit levels observed
- Enlarged spleen likely due to increased hematopoiesis seen microscopically
- Hyperostosis and hyperplasia observed in sternal bone marrow and femoral bone, respectively

Methods

Doses: Saline control, vehicle control, 0.01, 0.1, 0.25 mg/kg AF37702
 Frequency of dosing: Every 3 weeks
 Dose volume: 5 mL/kg
 Route of administration: Intravenous injection (lateral tail vein)
 Formulation/Vehicle: 20 mM phosphate and 0.003% Tween 20 in 4.7% sorbitol, pH range 5.90-6.01
 Basis of dose selection: General toxicology studies and Exec. CAC recommendations
 Species/Strain: SD rats
 #/Sex/Group: 53/sex/group
 Age: 6 – 7 weeks
 Animal housing: Individual
 Paradigm for dietary restriction: Ad lib food and water
 Dual control employed: Yes
 Interim sacrifice: No
 Satellite groups: TK rats: 3/sex/control; 9/sex/dose for AF37702 treated animals

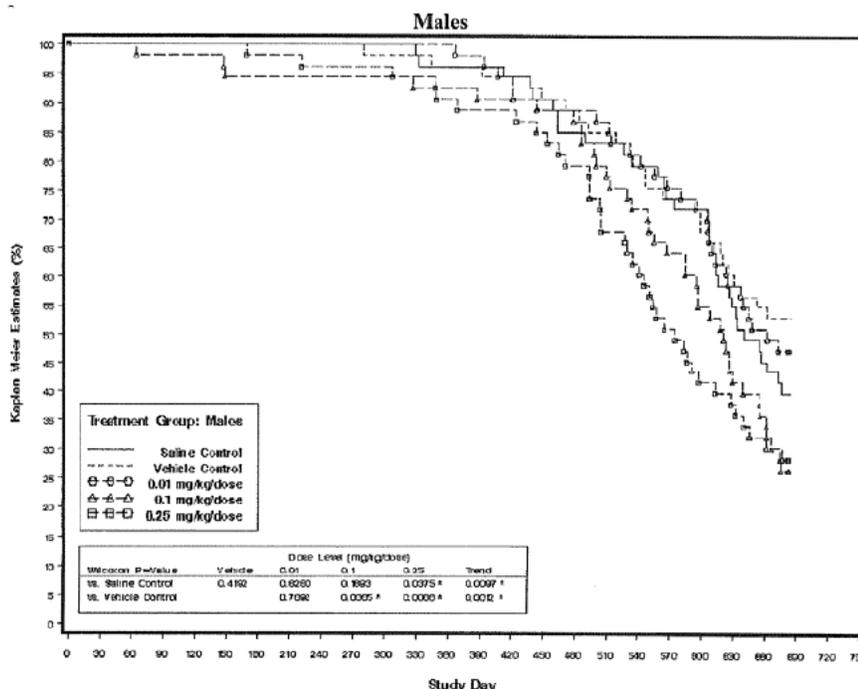
Observations and Results**Mortality**

Overall survival was higher in females than males. No difference in survival observed in females compared to controls. Although there was a statistically significant dose-response relationship between the high-dose (HD) group and the vehicle control group in survivals in male rats, this relationship was not dose-dependent. Table below shows percent survival and survival curve amongst males only.

Week	Adjusted % Survival				
	Male				
	Saline control	Vehicle control	0.01 mg/kg	0.1 mg/kg	0.25 mg/kg
1	100	100	100	100	100
10	100	100	100	98	100
21	100	100	100	96	100
25	100	100	100	94	98
32	100	100	100	94	96
40	100	98	100	94	96
44	100	98	100	94	94
48	96	98	100	92	94
53	96	96	98	92	89
56	96	94	96	91	89
60	96	94	91	91	89
64	91	91	89	89	85
67	85	89	89	89	79
72	83	85	87	79	63
76	79	81	81	72	64
80	77	75	77	66	53

Week	Adjusted % Survival				
	Male				
	Saline control	Vehicle control	0.01 mg/kg	0.1 mg/kg	0.25 mg/kg
84	72	74	74	60	45
88	58	64	62	51	40
92	49	57	53	40	32
97	40	53	47	26	28

Excerpted from Applicant's submission



* - statistically significant at the 0.05 significance level

Clinical Signs

Sex	No. of animals affected									
	Males					Females				
Dose (mg/kg)	SC	VC	0.01	0.1	0.25	SC	VC	0.01	0.1	0.25
Dark pink extremities										
Period (Days):										
-183 – 364	-	3	1	50	52	-	-	-	53	51
-365 – 546	-	1	-	1	48	1	-	-	2	48
-547 – 682 (males)	-	1	-	13	31	NA	NA	NA	NA	NA
-546 – 709 (females)	NA	NA	NA	NA	NA	1	-	-	11	45

SC: saline control; VC: vehicle control, NA: not applicable due to different days of sacrifice; -: not observed

Summary: dark pink extremities were seen as early as day 202 for males and 211 for females. No significant difference in palpable masses amongst all groups.

Body Weights

Unremarkable

Food Consumption

Unremarkable

Hematology

	Males				Females			
	VC	% change			VC	% change		
Dose (mg/kg)	0	0.01	0.1	0.25	0	0.01	0.1	0.25
Reticulocytes (abs)	238	-	-	↑165	331	-	↓28	↓72
RBC	8.2	-	↑11	↑20	7.1	-	↑13	↑21
HGB	15	-	↑18	↑30	13.9	-	↑16	↑27
HCT	46.7	-	↑18	↑32	43.9	-	↑14	↑24

VC: vehicle control; -: no change

Summary: Increased red blood cells and corresponding hemoglobin and hematocrit levels were observed in males and females. A large increase in reticulocytes was observed in males only, while decreases were observed in females. The Applicant suggests that this discrepancy may have been due to the difference in collection with male rat sample collection occurring 8-10 days after the final dosing and female rat sample collection occurring 12-15 days after final dosing (samples for both sexes were taken prior to sacrifice). However, considering the rapid maturation of reticulocytes into erythrocytes and with AF37702 $t_{1/2}$ values between 0.25 and 4 hr (see toxicokinetic section below), the cause for the different values in males and females cannot be determined without additional information.

Gross Pathology

		No. of animals affected/%affected									
		Males					Females				
Dose (mg/kg)		SC	VC	0.01	0.1	0.25	SC	VC	0.01	0.1	0.25
Early death											
No. of animals		32	25	28	39	38	35	28	23	25	31
Adrenals	Enlarged	-	-	-	-	-	-	1/4	-	-	2/6
Heart	Enlarged	5/16	-	2/4	4/10	12/32	-	-	1/4	-	2/6
Spleen	Enlarged	1/3	1/4	1/4	13/33	22/58	3/9	1/4	2/9	5/20	12/39
Terminal sacrifice											
No. of animals		21	28	25	14	15	18	25	30	28	22
Adrenals	Enlarged	-	-	-	-	-	-	-	-	1/4	3/14
Spleen	Enlarged	-	-	1/4	1/7	11/73	-	1/4	2/6	2/7	7/32

SC: saline control; VC: vehicle control; -: no change

Summary: Enlarged adrenals and hearts did not have histopathology correlates. Organ weight data was not provided. Enlarged spleens were likely due to increased hematopoiesis observed microscopically (see histopathology section).

Histopathology

Peer Review: Yes, statement included and signed.

Neoplastic findings:

Sex		No. of animals affected									
		Males					Females				
Dose (mg/kg)		SC	VC	0.01	0.1	0.25	SC	VC	0.01	0.1	0.25
No. of animals		53	50	51	52	49	50	51	50	51	53
Lymphoma	Hemolymphoreticular	-	1	1	1	3	1	-	-	2	3
	Rectum	-	-	1	-	-	-	-	-	-	-
	Total	-	1	2	1	3	1	-	-	2	3
Adenoma	Pituitary	20	4*	11*	12*	9*	17	25	27	21	21

SC: saline control; VC: vehicle control; -: no change; *: statistically significant when compared to vehicle control, but not saline control

Statistical Analysis: There were no drug-related tumor findings. See the statistical review by Dr. Min.

Non-neoplastic:

Sex			No. of animals affected										
			Males					Females					
Dose (mg/kg)			SC	VC	0.01	0.1	0.25	SC	VC	0.01	0.1	0.25	
No. of animals			53	53	53	53	53	53	53	53	53	53	
Tissue	Findings	Severity											
Bone, femur	Hyperostosis	Minimal	-	-	1	1	-	13	21	15	25	24	
		Mild	1	-	-	-	-	1	4	1	6	9	
		Moderate	-	-	-	-	-	-	-	-	-	1	-
		Total	1	-	1	1	-	14	25	16	32	33	
Bone marrow, sternum	Hyperplasia	Minimal	7	5	7	10	20	12	11	6	7	18	
		Mild	3	1	2	3	11	10	6	9	10	11	
		Moderate	-	-	-	-	-	-	1	-	-	2	2
		Total	10	6	9	13	31	22	18	15	19	31	
	Megakaryocytosis	Minimal	-	-	-	-	-	5	5	3	9	13	
		Mild	-	-	-	-	-	2	2	-	-	6	
		Total	-	-	-	-	-	7	7	3	9	19	
Spleen	Hematopoiesis increased	Minimal	12	13	14	12	15	24	22	20	11	11	
		Mild	6	4	3	5	10	8	9	11	3	7	
		Moderate	-	-	-	1	6	5	8	3	4	6	
		Marked	-	-	-	-	-	-	-	1	2	1	
		Total	18	17	17	18	31	37	39	35	20	35	

SC: saline control; VC: vehicle control; -: no change

Summary: Increased hematopoiesis in the spleen is expected from AF037702, which activates the EPO receptor. The increase in megakaryocytes in the bone marrow is small and without correlative thrombocytopenia. Hyperplasia and hyperostosis were noted in the sternal bone marrow and femoral bone of both sexes, respectively.

Toxicokinetics

Blood samples were collected from non-fasted animals on days 43 and 190 via retro-orbital puncture under CO₂ anesthesia at predose, 0.25, 1, 4, 8, 24, 48, 72, 120 hr postdose. Plasma samples were analyzed for AF37702 by (b) (4). Pharmacokinetic parameters are listed below.

Day	Sex	Dose (mg/kg)	C _{max} (µg/mL)	t _{1/2} (h)	AUC _{0-∞} (µg·h/mL)	AUC _{0-∞} /dose	C _{max} / dose
43	M	0.01	0.16	0.25	4	357	16
		0.1	2.07	0.25	48	476	21
		0.25	4.93	4	160	641	20
	F	0.01	0.20	0.25	2*	225	20
		0.1	1.47	4	48	485	15
		0.25	3.98	1	139	556	16
109	M	0.01	0.12	4	3	284	12
		0.1	2.55	0.25	60	605	25
		0.25	5.67	4	187	746	23
	F	0.01	0.13	0.25	2*	230	13
		0.1	1.98	0.25	63	625	20
		0.25	4.69	0.25	164	656	19

*The value of AUC_{0-24h} is displayed for 0.01 mg/kg group females as after 24 h the concentration became less than the lower limit of quantitation.

Summary: C_{max} was dose-proportional while AUC values were greater than dose proportional indicating a reduced clearance at higher doses. The mean predicted human IV and SC AUC values of AF37702 were 1264.6 µg·h/mL and 332.7 µg·h/mL, respectively. Rat to human AUC values are shown below.

Species	Route	Dose (mg/kg)	AUC (µg·h/mL)	Rat:Human Ratio	
Human	IV	0.35	1265	NA	
Rat	IV	Day 43	0.01	3	0.002
			0.1	48	0.038
			0.25	150	0.119
		Day 109	0.01	3	0.002
			0.1	62	0.049
			0.25	176	0.139

*Summary of individual study findings:*Adequacy of carcinogenicity study and Appropriateness of test models:

The test model was appropriate. The Applicant followed ECAC recommendations; PD effects were seen in the animals and were severe/substantial at the HD (0.5 mg/kg). No changes in body weights were observed.

Evaluation of Tumor Findings:

There are no drug-related tumors.

The findings in the pituitary are statistically significant but are not likely due to AF37702 for the following reasons: The number of pituitary adenomas was higher in the saline controls (20) compared to the incidence for the AF37702-treated groups and there was a lack of dose response amongst AF37702-treated groups. Pituitary adenomas are common tumors and are reported to occur in 0.77 – 70% of Sprague Dawley rats based on historical control data published by (b) (4) in March 2001. The factors above suggest that the pituitary adenomas are not related to AF37702 exposure.

The lymphoma found were all lymphocytic and systemic except for the lymphoma found in the rectum of the LD (0.1 mg/kg) male rat. Lymphoid neoplasms are reported to occur at a 1.1% incidence rate in Harlan Sprague Dawley female rats (for this study, <1 per group would be expected, and a total of 2 for all groups combined).

8.2 Tg.rasH2 Mouse Carcinogenicity Study**Study title: 26 Week Carcinogenicity Study of AF37702 with Intravenous Administration Every Three Weeks in Tg.rasH2 Mice**

Study no.:	AF08-004 (Affymax), AB46YG.7V8R.BTL (b) (4)
Study report location:	eCTD 4.2.3.4.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	3/14/2008
GLP compliance:	Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	AF37702, Lot PLI039-06, 99.3%
ECAC concurrence:	Yes (Exec. CAC meeting of 1/30/2008)

Key Study Findings*Neoplastic findings:*

- There were no drug-related tumors
- Increased incidences of splenic hemangiosarcoma/hemangiomas were statistically significant in male mice
- Pulmonary adenomas were observed at higher incidences in all male AF37702-treatment groups

Non-neoplastic findings:

- Hyperactivity, erythema, and urinary incontinence appear to be AF37702-related in female mice. Thinness was observed in males.
- Findings in the spleen (increased weight, enlarged, discoloration and hematopoiesis) were expected given the pharmacology of the drug and were supported by increased red blood cells, hemoglobin, and hematocrit levels
- Increase white blood cells and differentials were increased up to 16-fold, however, findings were not supported microscopically

Methods

Doses: Vehicle control (VC), 1 g/kg urethane (positive control), 0.1, 0.25, 0.5 mg/kg AF37702

Frequency of dosing: AF37702: Every 3 weeks for 10 doses, IV; Urethane: Days 1, 3, 5; IP

Dose volume: 10 mL/kg: Positive control group, all other groups: 5 mL/kg

Route of administration: Control and AF37702: IV (tail vein); Urethane: IP

Formulation/Vehicle: 20 mM phosphate and 0.003% Tween 20 in 4.7% sorbitol, pH range 6.0

Basis of dose selection: General toxicology studies and Exec. CAC recommendations

Species/Strain: Mouse/CB6F1Jic-Tg.rasH2@Tac

Number/Sex/Group: 25/sex/group

Age: 8 weeks

Animal housing: Individual

Paradigm for dietary restriction: As lib food and water

Dual control employed: No

Interim sacrifice: No

Satellite groups: No

Observations and Results**Mortality**

Sex	No. of animals affected (day of death)				
	Males				
Dose (mg/kg)	PC	VC	0.1	0.25	0.5
Found dead	13 (9-112)	-	1 (108)	1 (154)	-
Moribound		1 (167)	-	-	-
Early Sacrifice -positive control	12 (114)	-	-	-	-
Terminal sacrifice	-	24	24	24	25

PC: positive control; VC vehicle control; -: not observed; NOTE: deaths were pooled for PC animals and surviving PC animals were sacrificed on day 114

Summary: Clinical signs of these early death animals include thin (VC), rapid and shallow breathing (VC), hunched (VC & 0.1 mg/kg), and decreased motor activity (0.1 mg/kg). No clinical signs were recorded for the 0.25 mg/kg group death. The mortalities observed in males are dose-independent as no deaths occurred in the high-dose (0.5 mg/kg). Female mortalities only observed in the positive control group.

Clinical signs

Sex	No. of animals affected							
	Males				Females			
Dose (mg/kg)	VC	0.1	0.25	0.5	VC	0.1	0.25	0.5
No. of animals	25	25	25	25	25	25	25	25
Hyperactive	-	-	-	-	1	3	3	5
Thin	1	1	1	3	-	-	-	-
Erythema	-	-	-	-	4	1	8	16
Urinary incontinence	-	-	-	-	-	-	-	3

VC vehicle control; -: not observed

Body Weights

Unremarkable

Food Consumption

Unremarkable

Hematology

	Males				Females			
	VC	% change			VC	% change		
Dose (mg/kg)	0	0.1	0.25	0.5	0	0.1	0.25	0.5
Reticulocytes (abs)	305	↑4.8 fold*	↑5 fold*	↑6.4 Fold*	-	-	↑80	↑10.8 fold*
RBC	11	-	↑11*	↑23*	11	↑15	↑14	↑33*
HGB	17	↑18*	↑27*	↑32*	17	↑14	↑9	↑32*
HCT	52	↑18*	↑24*	↑29*	50	↑13	↑7	↑31*
Mean platelet volume	5.4	↑16	↑21*	↑28*	-	-	-	-
WBC	6.16	-	-	↑6.7 fold*	-	-	-	-
Segmented neutrophils	0.8	↑26	48	↑3.9 Fold*	-	-	-	-
Basophils	0.002	-	-	↑11.5 Fold*	-	-	-	-
monocytes	0.10	↑86	↑145	↑16.3 Fold*	0.16	-	↑28	↑67*

VC: vehicle control; -: no change, *: statistically significant compared to controls (P≤0.05)

Summary: Increased white blood cells and differentials were not supported microscopically. No signs of infection or inflammation present. Increased red blood cells (with corresponding hemoglobin and hematocrit levels) are expected with drugs of

this class. Levels in females were not dose-dependent but HD (0.5 mg/kg) levels were statistically significant compared to controls.

Organ Weight

Relative to bodyweight	Males				Females			
	VC	% change			VC	% change		
Dose (mg/kg)	0	0.1	0.25	0.5	0	0.1	0.25	0.5
Heart	0.85	-	-	↑16	-	-	-	-
Spleen	0.39	↑83	↑3 fold*	↑9 fold*	0.69	↑20	↑97	↑175*
Testis	2.05	↓37	↓37	↓38	NA	NA	NA	NA

VC: vehicle control; -: no change; *: statistically significant compared to controls (P≤0.05)

Summary: Increased spleen weights are relative to increase hematopoiesis observed microscopically. No correlative findings were noted to support increased heart and testis weights in male mice.

Gross Pathology

Sex	Dose (mg/kg)	No. of animals affected							
		Males				Females			
		VC	0.1	0.25	0.5	VC	0.1	0.25	0.5
Liver	Discoloration, dark	-	1	2	15	-	-	-	-
Spleen	Enlarged	-	2	18	25	-	5	11	24
	Discoloration, dark	-	2	-	1	-	-	1	2

VC: vehicle control; -: no change,

Summary: liver and spleen findings likely due to increased hematopoiesis observed microscopically

Histopathology

Peer Review: Yes, statement included and signed. Following review of the microscopic findings reported by the study pathologist, the results were discussed and appropriate terminology and the diagnosis mutual agreed on. Differences of opinion between the study and reviewing pathologists were resolved with agreement on the diagnosis.

Neoplastic findings:

Sex	Dose (mg/kg)	No. of animals affected									
		Males					Females				
		PC	VC	0.1	0.25	0.5	PC	VC	0.1	0.25	0.5
	No. animals	25	25	25	25	25	25	25	25	25	25
Hemangiosarcoma	Bone marrow	-	-	-	-	1	-	-	-	-	-
	Spleen	23	-	1	6	4	22	1	4	3	4
	Salivary gland	-	-	-	-	-	-	-	-	1	-

Sex		No. of animals affected									
		Males					Females				
Dose (mg/kg)		PC	VC	0.1	0.25	0.5	PC	VC	0.1	0.25	0.5
No. animals		25	25	25	25	25	25	25	25	25	25
	Stomach	-	-	-	-	-	-	-	1	-	-
	Subcutis	-	-	-	-	-	-	-	1 ¹	-	-
Hemangioma	Ileum	-	-	-	-	-	-	-	-	1	-
	Total	23	-	1	6	5	22	1	6	4	4
Adenoma	Harderian gland	NA	-	4	1	1	NA	-	2	2	1
	Lung	3	3	4	8	4	25	2	-	3	-
	Liver	-	-	-	-	-	-	-	-	-	1
Carcinoma	Harderian gland	-	-	-	-	-	-	-	1	-	-
	Total	3	3	8	9	5	25	3	4	5	2
Lymphoma (multicentric)	Lymphoid tissue	1	-	1	-	-	-	-	-	-	-
	Total	1	-	1	-	-	-	-	-	-	-

PC: positive control; VC: vehicle control; -: no change; NA: not applicable (harderian gland was not evaluated in positive control animals); 1: subcutis hemangiosarcoma data should be carefully interpreted as the subcutis was only evaluate in this one animals; thus, data was not included in total tumor count.

Statistical Analysis: There were no drug-related tumor findings. See the statistical review by Dr. Min.

Non-neoplastic findings:

			No. of animals affected							
			Males				Females			
Dose (mg/kg)			VC	0.1	0.25	0.5	VC	0.1	0.25	0.5
No. of animals			25	25	25	25	25	25	25	25
Tissue	Findings	Severity								
Bone marrow, femur	Erythropoiesis	Minimal	-	-	-	-	-	1	-	-
		Mild	-	-	-	1	-	5	3	-
		Moderate	-	24	24	24	-	16	22	25
		Total	-	24	24	25	-	22	25	25
Bone marrow, sternum	Erythropoiesis	Minimal	-	-	-	-	-	1	-	-
		Mild	-	-	-	1	-	5	3	7
		Moderate	-	24	25	24	-	16	22	25
		Total	-	24	25	25	-	22	25	25
Spleen	Extra-medullary hematopoiesis	Minimal	-	9	1	-	-	3	8	1
		Mild	1	14	3	-	1	-	3	11
		Moderate	-	1	19	1	-	-	3	11
		Marked	-	-	1	24	-	1	1	2
		Total	1	24	24	25	1	4	15	25
Liver	Extra-medullary hematopoiesis	Minimal	-	1	10	-	-	-	1	3
		Mild	-	1	-	25	-	-	-	-
		Moderate	-	-	-	-	-	1	-	-
		Total	-	2	10	25	-	1	1	3
Pancreas	Atherosclerosis; artery	Moderate	-	-	1	1	-	-	-	-
		Total	-	-	1	1	-	-	-	-
Uterus	Angiectasis	Mild	NA	NA	NA	NA	-	-	1	1
		Moderate	NA	NA	NA	NA	-	2	1	3
		Total	NA	NA	NA	NA	-	2	2	4

VC: vehicle control; -: no change; NA: not applicable

Summary: Findings are expected from the pharmacology of the drug.

Toxicokinetics

Not done

Summary of individual study findings:

Adequacy of carcinogenicity study and Appropriateness of test models:

Tg.rasH2 is a standard rodent model used for carcinogenicity studies. The positive control results demonstrated the appropriateness of the test model. The Applicant followed Exec. CAC's recommendations.

Evaluation of Tumor Findings:

There are no drug-related tumors.

Splenic hemangiosarcomas were not dose-dependent, while they were statistically significant.

Pulmonary adenomas were seen in higher incidences amongst AF37702-treated groups. The mid-dose group (0.25 mg/kg) findings (8 males) were above the ^{(b) (4)} historical control range of 0 – 6/25), however, the statistical analysis indicated that these findings were not dose- or treatment-dependent. In females, a numerical increase in pulmonary adenomas was noted in the 0.1 mg/kg group only. Toxicokinetic analyses were not done so variations in exposure to AF37702 are not known. Other findings (carcinoma in Harderian gland/lymphoid tissue) were sporadic.

Both lesions reported are amongst the most common tumors found in control Tg ras H2 mice. See table from Morton et al. 2002. *The Tg rasH2 Mouse in Cancer Hazard Identification*. Toxicol Pathol 2002 30:139.

Copyright Material

8.3 Discussion and Conclusions:

The carcinogenic potential of peginesatide was evaluated in a 28-week Tg.rasH2 mouse study and a standard 2-year rat bioassay. The design of both studies was acceptable and based on previous toxicology studies and/or the guidance of the Exec. CAC. As expected with the pharmacology of peginesatide, increases in splenic hematopoiesis were observed with corresponding increases in organ weight (mice only), red blood cells, hemoglobin, and hematocrit levels. There were no peginesatide-related tumors.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Study for Effects of AF37702 on Fertility and General Reproductive Performance in Rats (SEG I)

Study no.: AF058-020 (Affymax); 0325RA33.001 (b) (4)

Study report location: eCTD 4.2.3.5.1

Conducting laboratory and location:

(b) (4)

Date of study initiation: 1 Nov 05

GLP compliance: Statement included and signed

QA statement: Statement included and signed

Drug, lot #, and % purity: AF37702, 031114A, 98.7%

Key Study Findings

- Mortalities occurred in males at doses of ≥ 1 mg/kg and in females at the 5 mg/kg dose.
- Fertility index was reduced in males and females starting at the peginesatide dose of 0.1 mg/kg; however, effects were most evident at doses of ≥ 1 mg/kg.
- Reduced weight of seminal vesicles was seen at peginesatide doses of ≥ 0.1 mg/kg and was statistically significant at ≥ 1 mg/kg. Reduced weight of prostate was reported at ≥ 1 mg/kg. Smaller testes were noted at all dose levels.
- Sperm count was reduced by 12% at 5 mg/kg. Increased morphological abnormalities in sperm were evident at ≥ 0.1 mg/kg of peginesatide.
- There was a decrease in viable fetuses at ≥ 0.1 mg/kg, which appears to be due to pre- and post-implantation losses.
- There was an increased incidence of non-gravid females at ≥ 1 mg/kg.

Methods

Route of administration: IV, Bolus

Formulation/Vehicle: 10mM acetate in 0.9% sodium chloride

Species/Strain: Rat, Sprague-Dawley

Age: 18 – 19 weeks old

Weight: 313 – 459 g (males); 238 – 300 g (females)

Group No./Treatment	Dose Level ¹ (mg/kg/dose)	Concentration (mg/ml)	Dose Volume (ml/kg)	No. of Animals ^{2,3}	
				Males	Females
1/VehicleControl	0	0	1.0	24 + 10	24 + 10
2/AF37702	0.05	0.05	1.0	24	24
3/AF37702	0.1	0.1	1.0	24	24
4/AF37702	1.0	1.0	1.0	24	24
5/AF37702	5.0	5.0	1.0	24 + 10	24 + 10

¹ Male rats were dosed once weekly for a total of seven weeks: four weeks prior to cohabitation and a maximum of three weeks during and following the cohabitation period. Female rats received 4 – 7 doses as follows: once weekly for two weeks prior to cohabitation, once weekly for a maximum of three weeks during cohabitation, and once on Gestation Days 0 and 7.

² An attempt at mating 24 females was made in order to obtain a minimum of 20 gravid females, determined at necropsy.

³ In order to differentiate between the sexual performance of treated males and treated females at the high dose of 5 mg/kg/dose, 10 males from Group 1 were mated with 10 females from Group 5; and 10 males from Group 5 were mated with 10 females from Group 1.

Observations and Results

Mortality

Animals were checked twice daily (am/pm).

Animal #	Dose (mg/kg)	Sex	Day of Death	Observations	
				Reason	General (include pathology)
8089	1	M	46	Euthanized	Decreased activity, brown staining, cold to touch, moribound, extremities cold to touch; enlarged spleen, dark pancreas, liver, kidneys, testes & GI tract
8092	1	M	38	Euthanized	Abnormal gait and stance, cold to touch decreased activity, moribound, enlarged prostate and spleen, dark GI tract and kidney
8094	1	M	49	Euthanized	Abnormal gait, body drop, constant rolling, increased respiration, enlarged spleen, dark pancreas & liver, stomach distended
8727	5	F	GD	Found Dead	No clinical signs, enlarged spleen and kidney, red fluid in urinary bladder, dark discoloration of GI tract, kidneys, pancreas, and ovaries; lungs with black spots
8110	5	M	41	Found Dead	No clinical signs, enlarged spleen and heart, dark kidneys, GI tract, multiple black spots throughout glandular region, stomach filled with black fluid, lungs with black spots
8112	5	M	40	Found Dead	Decreased activity, extremities cold to touch, spleen enlarged, dark kidneys and GI tract
8113	5	M	40	Found Dead	Decreased activity, red discharge (penis)

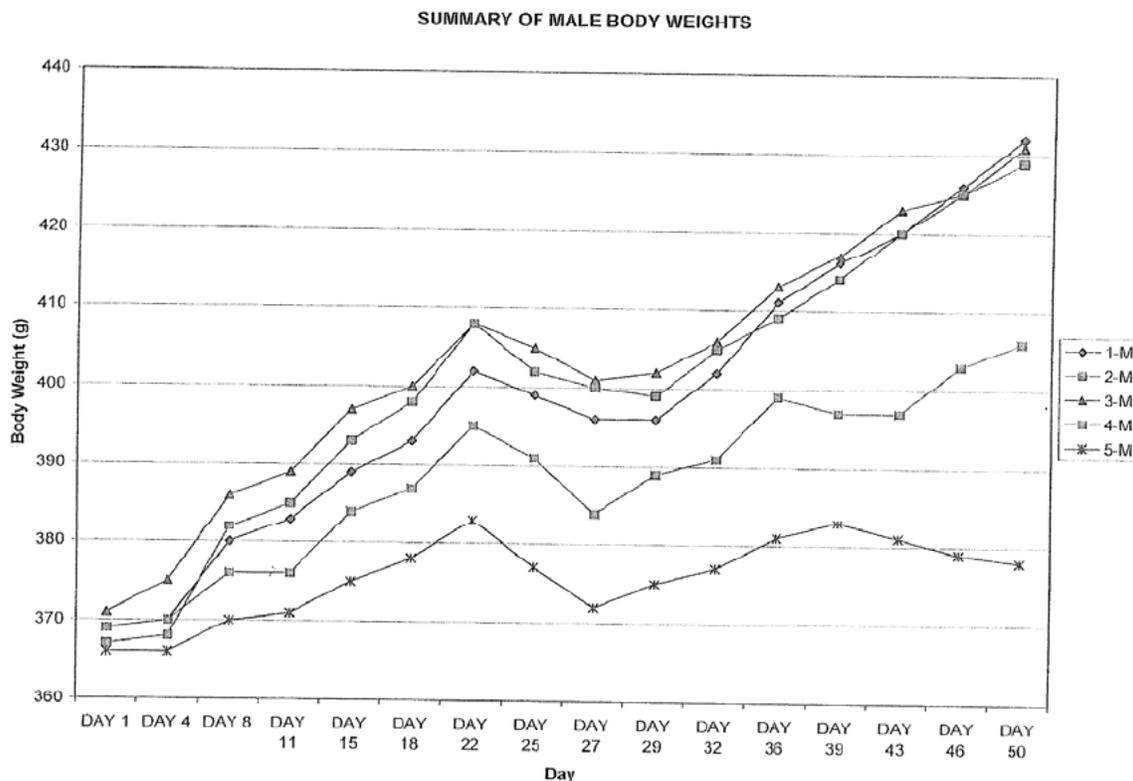
Animal #	Dose (mg/kg)	Sex	Day of Death	Observations	
				Reason	General (include pathology)
					stomach distended with black discoloration, dark kidneys and GI tract
8117	5	M	48	Found Dead	Extremities cold to touch, enlarged spleen dark pancreas, liver, kidneys, & lower GI tract
8118	5	M	51	Found Dead	Decreased activity, extremities cold to touch, enlarged spleen, dark pancreas, lungs, distended bladder with red fluid, dark liver and pancreas
8122	5	M	49	Found Dead	No clinical signs, enlarged spleen, kidney, and heart, dark pancreas, lungs, kidneys & Stomach, urinary bladder distended bladder with red fluid
8123	5	M	34	Euthanized	Abnormal gait and stance, cold to touch, decreased activity, extremities cold to touch, thin, pitted kidneys, enlarged spleen
8124	5	M	35	Found Dead	Extremities cold to touch, prostate enlarged, enlarged spleen, pitted kidneys, dark red GI tract and peritoneal cavity
8125	5	M	36	Euthanized	Decreased activity, extremities cold to touch, thin, enlarged spleen, dark red kidneys, GI tract, & peritoneal cavity
8126	5	M	48	Found Dead	Decreased activity, enlarged prostate, dark testes, enlarged spleen with multiple white spots, pancreas dark pink, kidney enlarged, liver dark, lower GI tract dark red with multiple black spots
8127	5	M	48	Found Dead	Decreased activity, enlarged prostate, dark testes, GI, & pancreas, enlarged spleen, lungs surrounded by white mucus
8128	5	M	48	Found Dead	Decreased activity, dark testes, liver, pancreas, & GI tract, enlarged spleen, bladder distended
8130	5	M	51	Found Dead	Decreased activity, dark testes, liver, pancreas, & GI tract, enlarged spleen and bladder, stomach distended
8137	5	M	40	Found Dead	No clinical signs, enlarged prostate and spleen, dark kidneys & GI tract, stomach distended

Clinical Signs

No clinical signs observed other than those listed in the mortality section

Body Weight

(Excerpted from Applicant's submission)



Food Consumption

Unremarkable

Estrous cycles

Unremarkable

Hematology

	Male					Female				
	Control	% change from control				Control	% change from control			
Dose (mg/kg)	0	0.05	0.1	1	5	0	0.05	0.1	1	5
No. animals	10	10	10	10	10	10	10	10	10	10
RBC	8.98	↑40	↑43	↑60	↑70	6.98	↑34	↑48	↑93	↑102
HCT	49.8	↑40	↑45	↑64	↑64	39.7	↑41	↑58	↑89	↑95
HGB	16	↑43	↑49	↑69	↑57	13.4	↑43	↑58	↑77	↑78

Morphology Data

	Male					Female				
	Incidence					Incidence				
Dose (mg/kg)	0	0.05	0.1	1	5	0	0.05	0.1	1	5
No. animals	10	10	10	10	9 ^a	10	10	10	10	10
Anisocytosis	-	10	10	10	9	-	3	8	9	10
Polychromasia	-	-	-	2	9	-	-	-	2	7

a: 1 sample was clotted in the 5 mg/kg group

Necropsy

Animals were necropsied at the end of treatment.

Macroscopic findings		Male					Female				
Dose (mg/kg/day)		0	0.05	0.1	1	5	0	0.05	0.1	1	5
Seminal vesicles	Small	-		-	-	2	-	-	-	-	-
Kidney	Enlarged, dark, bilaterally	-	-	-	3	6	-	-	-	-	1
Liver	Enlarged, dark	-	1	-	3	5	-	-	-	-	-
	Dark	-	-	-	-	-	-	-	-	1	1
Spleen	Enlarged, dark	-	-	-	4	8	-	-	-	-	-
	Dark	-	-	-	-	-	-	-	-	1	2
Testis	Small (left)	-	1	-	1	-	-	-	-	-	-
	Small (right)	-	1	-	-	-	-	-	-	-	-

Organ Weights

	Male				
	Control	% change from control			
Dose (mg/kg)	0	0.05	0.1	1	5
No. animals	10	10	10	10	10
Prostate	0.207	-	-	↓22	↓22
Seminal vesicles	0.312	-	↓11	↓24*	↓33*
HGB	16	↑43	↑49	↑69	↑57

*: statistically significant compared to controls $P \leq 0.01$

Fertility Parameters

(Excerpted from Applicant's submission)

SUMMARY OF SAME GROUP MATING INCIDENCE/COPULATORY INDICES						
SEX: FEMALE						
PERIOD	DOSE: (mg/kg) GROUP:	0	0.05	0.1	1.0	5.0
		1-F	2-F	3-F	4-F	5-F
NO MATING OBSERVED:		0	0	1	3	3
MEAN NO. OF DAY IN COHABITATION:#		4	3	4	4	6
NO. OF MALE/FEMALE PAIRS		24	24	24	25	25
NO. OF CONFIRMED MATED PAIRS		24	24	23	22	22
MEAN MATING DAY/MATED PAIR		3.8/24	3.3/24	3.8/23	4.2/22	6.0/22
COPULATORY INDICE (%):@		100	100	96	88	88
FERTILITY INDICES (%):@@		92	92	83	64	39

Reflects the mean value of mated females only
 @ (No. of confirmed mated pairs/No. of male/female pairs) x 100
 @@ (No. of gravid females/No. of presumed gravid females) x 100

Copulation index (%): $\frac{\text{no. presumed pregnant animals}}{\text{no. paired animals}} \times 100$

Fertility index (%): $\frac{\text{no. pregnant animals}}{\text{no. presumed pregnant animals}} \times 100$

Pregnancy Parameters

(Excerpted from Applicant's submission)

Weekly Dose (mg/kg) [@]	0 (VehicleControl)		0.05 (AF37702)	0.1 (AF37702)	1.0 (AF37702)	5.0 (AF37702)	
	SGM*	CM*	SGM*	SGM*	SGM*	SGM*	CM*
Number of Females	24/34	10/34	24/24	24/24	24/24	24/34	10/34
Necropsy Observations							
Dark/reddened tissues/organs	-	-	a	a	a	a	a
Estrous Cycles	-	-	-	-	-	-	-
Cesarean Data							
Nongravid Dams	-	-	-	-	a	a	a
Gravid Dams	-	-	-	-	e	e	e
Corpora Lutea	-	-	-	-	-	-	-
Total Implantations	-	e	-	-	-	-	-
Viable Fetuses	-	e	-	b	b	b	b
Nonviable Fetuses	-	a	-	d	d	d	d
Pre-Implantation Loss	-	a	a	a	a	a	-
Post-Implantation Loss	-	a	-	d	d	d	d
Reproductive Indices							
Mean number of days to mating	-	a	-	-	-	a	-
Fertility Indices	-	-	-	-	e	e	-
Co-pulatory Indices	-	-	-	-	-	-	-

- * = SGM = Same Group Matings; CM = Cross-Matings (Group 1 females mated with Group 5 males; Group 5 females mated with Group 1 males)
- @ = The number of doses in parentheses reflect the number of cumulative doses administered from treatment initiation
- = No noteworthy findings
- a = Higher incidence with higher dose group
- b = Significant decreases noted
- d = Significant increases noted
- e = Lower incidence for test article-treated group

Male Sperm Parameters

(Excerpted from Applicant's submission)

Group:	1	2	3	4	5
Level: AF37702 (MG/KG/DOSE)	0	0.05	0.1	1.0	5.0
MOTILITY (%)					
Mean	90	83	88	89	88
SD	7	18	14	8	18
N	34	23	24	20	20
EPIDIDYMAL COUNT (MILLION SPERM/GRAM)					
Mean	1081.5	1042.4	1108.9	1030.8	951.5
SD	284.1	358.3	303.6	167.2	211.7
N	34	24	24	21	20
SPERM MORPHOLOGY (% ABNORMAL of INTACT SPERM)^a					
Mean	2.1	1.7	1.4	1.5	1.3
SD	1.8	1.6	1.0	1.3	1.5
N	34	23	24	21	20

a=MEAN AND STANDARD DEVIATIONS WERE CALCULATED USING THE NUMBER OF ABNORMAL INTACT SPERM AS A PERCENTAGE OF THE NUMBER OF INTACT SPERM EXAMINED.

NONE STATISTICALLY DIFFERENT FROM CONTROL GROUP (GROUP 1).

Stability and Homogeneity

(Excerpted from Applicant's submission)

AF37702 dosing formulations prepared for (b) (4) Study No. 0325RA33.001 were formulated within 1.5 to 10.1% of their targeted nominal concentrations of 0.05, 0.3, 1.0 and 5.0 mg/ml. The vehicle control samples were devoid of test article.

Study Summary

AF37702 was administered to male and female rats via bolus injection at doses of 0, 0.1, 0.5, 1, and 5 mg/kg. Eighteen mortalities were reported (3/24 at 1 mg/kg and 15/48 at 5 mg/kg). General clinical signs included decreased activity, cold to touch, cold extremities, and poor grooming. These clinical signs were also observed in surviving animals. Decreased body weights were observed in 1 and 5 mg/kg males compared to controls. Hematology findings were consistent with the pharmacology of this drug (decreased RBC, HCT, HGB). Necropsy findings included darkened or red tissues at doses ≥ 0.05 mg/kg. Enlarged liver, spleen, and kidneys were also observed at necropsy. No effects on the number of estrous cycles were observed. Reduced seminal vesicles and prostate weights were observed at ≥ 0.1 and 1 mg/kg, respectively. Small testes sizes and reduced sperm count were also noted. Increased numbers of non-gravid females were observed at doses ≥ 1 mg/kg. Decreased fertility indices were also noted in these groups. Increased non-viable fetuses were observed at ≥ 0.1 mg/kg while decreases in viable fetuses were observed at ≥ 0.05 mg/kg.

9.2 Embryonic Fetal Development

Study title: A study for effects of AF37702 on embryo-fetal development in rats (Seg II)

Study no.:	AF04-017
Study report location:	eCTD 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	09 May 06
GLP compliance:	Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	AF37702, Lot #12AB1, 87.7%
Route of administration:	Intravenous

Key Study Findings

- Pharmacological effects of the drug were observed at all doses
- Increased total resorptions, dead fetuses, post-implantation loss, and litters with all dead/resorbed fetuses at ≥ 1 mg/kg
- Decreased live fetuses at ≥ 10 mg/kg and decreased fetal and gravid uterine weight at ≥ 1 mg/kg

- No NOAEL for embryofetal or teratogenic effects could be identified
- Adverse embryo-fetal effects included the following: Cleft palate, variations in major blood vessels, sternum anomaly, and unossification of sternebrae and metatarsals
- Malformations and variations were observed at ≥ 1 mg/kg
- The dose of 1 mg/kg in rats resulted in an AUC comparable to that in subjects receiving intravenous doses of peginesatide at the highest dose

Design: This study was designed to assess the maternal and fetal effects of AF37702 when administered to pregnant rats. One-hundred-twenty-three female Sprague-Dawley rats (Crj:CD(SD)IGS) weighing 207-289 g and 13 weeks of age, were naturally impregnated by 70 breeder males and randomly distributed among 7 treatment groups. Females were administered daily IV doses of either acetate/saline IV solution (control) or AF37702 on days 6, 9, 12, 15 and 18 of presumed gestation. Dosages of 0, 1, 10 and 50 mg/kg/day of AF37702 were used in Groups 1-4 (toxicology groups), and dosages of 1, 10 and 50 mg/kg/day of AF37702 were used in Groups 5-7 (Toxicokinetic groups). Doses for all groups were equal in volume at 5.0 mL/kg. The study design is shown in the following table (*Excerpted from Applicant's submission*).

Group	Dose of AF37702 (mg/kg)	Number Females	
		Assigned	Pregnant
Toxicology			
1	0 (Vehicle)	24	19
2	1	24	20
3	10	24	23
4	50	24	23
Toxicokinetics			
5	1	9	Omitted
6	10	9	Omitted
7	50	9	Omitted

All rats were observed daily for survival and clinical signs. Body weights were recorded on Days 0 and 3, and daily thereafter up to Day 20 of presumed gestation. Food consumption values were recorded for each 3-day interval. Dams were bled for hematology evaluation and then sacrificed by lethal injection on Day 20 of presumed gestation. The thoracic, abdominal and pelvic viscera were examined and gross lesions were preserved in formalin. The number and distribution of corpora lutea, implantations, early and late resorptions, and live and dead fetuses were recorded. All fetuses were weighed and evaluated for external, visceral and skeletal alterations and sex recorded. Indices for pre-implantation loss and post-implantation loss were calculated.

Observations and Results

Mortality

No treatment-related mortalities

Clinical Signs

Unremarkable

Body Weights

Unremarkable

Food Consumption

Unremarkable

Hematology

Statistically significant increases were observed in RBCs, HGB, and HCT:

	Females			
	VC	% change		
Dose (mg/kg)	0	1	10	50
No. of Animals	10	10	10	10
RBC	6.85	↑37**	↑40**	↑40**
HGB	14	↑33**	↑29**	↑33**
HCT	41.2	↑47**	↑43**	↑50**
*Polychromasia				
- rare – slight	2	-	-	-
- moderate	-	9	7	5
- marked	-	1	3	5

* Incidence of finding

Gross Necropsy

Animals were necropsied at time of death or the end of treatment (GD 21).

		Females			
		VC	% change		
Dose (mg/kg)		0	1	10	50
No. of Animals		24	24	24	24
Amniotic fluid	Dark	-	-	1	2
Spleen	Enlarged	-	22	23	21

Pregnancy Parameters and Fetal Examinations

(Excerpted from Applicant's submission)

PARAMETER	TREATMENT GROUP			
	1 (Control)	2 (1 mg/kg)	3 (10 mg/kg)	4 (50 mg/kg)
Dams Assigned	24	24	24	24
Dams Died	0	0	0	0
Aborted	0	0	0	0
Pregnancies	19	20	23	23
Live Litters	19	20	23	23
Corpora Lutea Mean Litter	18.2	16.5	19.1	18.5
Implants Mean Litter	14.8	14.8	14.1	15.0
Total Live Fetuses	265	263	282	259
Live Fetuses Mean Litter	13.8	13.1	12.3*	11.3**
Total Resorbed Fetuses	17	31*	39*	83**
Total Dead Fetuses	0	2	4	4
Pre-Implant Loss (%) ^A	16.5	8.9	25.3	16.6
Post-Implant Loss (%) ^B	6.0	11.5*	14.6**	24.7**
# Litters with all dead or resorbed	0	2 *	4**	4**
Fetal Wts (M/F)	4.38/4.10	3.67**/3.44**	3.59**/3.43**	3.53**/3.38**
Sex Ratio (M/F)	0.99	0.92	0.80	0.93
Dam live body wt (g)	369	363	358	347*
Gravid uterus wt (g)	86.3	73.2*	67.3**	62.3**
Corrected body wt. (g) ^C	283	290	287	284
Dams with spleen enlarged	1	22	23	21
Corrected Body wt gain (g) ^D	54	56	54	54

^A Pre-implant Loss: No. of Corpora Lutea – No. Implants/No. Corpora Lutea x 100

^B Post-implant Loss: Number of Live fetuses/Number Implants x 100

^C Final body weight – Gravid uterus weight

^D Initial body weight – Corrected body weight

Fetal Malformations

Dose (mg/kg)	Fetus				Litter			
	0	1	10	50	0	1	10	50
External Malformations								
Numbers Examined	251	263	272	248	18	20	22	22
Cleft palate	-	-	1	1	-	-	1	1
Visceral Malformations								
No. Examined	128	132	132	122	19	20	23	23
Major blood vessel – variation	-	1	1	2	-	1	1	2
Skeletal malformations								
No. Examined	137	131	150	137	19	20	23	23
Sternoschisis	-	-	1	2	-	-	1	2
Sternum anomaly	-	-	1	2	-	-	1	2
Sternebrae unossified (1–3, and/or 4)	1	5	5	5	1	3	4	5
Sternebrae unossified (5 and/or 6)	1	17	16	17	1	12	11	10
Metatarsals unossified	-	32	30	18	-	13	16	10
Vertebral centra unossified (or reduced)	-	8	8	7	-	3	5	5
Vertebral arches – reduced ossification	-	11	8	7	-	4	5	5
Total malformation	-	2	4	5	-	2	3	3
Total variation	20	56	54	50	12	17	21	20

Toxicokinetics

Methods

Twenty-seven female rats, presumed pregnant, were assigned to their respective dose groups. Each animal received an IV dose of AF37702 at doses of 1, 10 or 50 mg/kg once daily on GDs 6, 9, 12, 15 and 18. On GDs 6 and 18 (after 5 doses), toxicokinetic samples were collected from 9 animals/sex/time-point at pre-dose, 0.25, 1, 4, 8, 24, 48, and 72 h post-dose.

Results

- AUC increases were greater than dose proportional between all doses on Days 6 and 18
- C_{max} and AUC values were higher on Day 18 compared to Day 6, suggesting drug accumulation

(Excerpted from Applicant's submission)

Plasma pharmacokinetic parameters of AF37702 following single and multiple IV administration of AF37702 in pregnant rats.

Day	Dose (mg/kg)	C _{max} (µg/mL)	t _{max} (h)	AUC _{0-t} (µg·h/mL)	AUC _{0-inf} (µg·h/mL)	t _{1/2} (h)	CL/F (mL/h·kg)	V _{ss} (mL/kg)	AI ^a C _{max}	AI ^b AUC
GD6	1	43.47	0.25	1003.4	1170.8	25.9	0.854	29.97	NA	NA
	10	511.3	0.25	13026.1	17423.1	37.5	0.574	29.54	NA	NA
	50	2219.4	0.25	92395.4	ND	75.7	ND	ND	NA	NA
GD18	1	73.28	0.25	1409.7	1427.1	11.2	0.701	ND	1.69	1.40
	10	1110.6	1	28587.0	29548.4	13.5	0.338	ND	2.17	2.19
	50	3905.8	0.25	143429	182070	32.5	0.275	ND	1.76	1.55

^a C_{max} accumulation index = C_{max} (GD18)/ C_{max} (GD6)

^b AUC accumulation index = AUC(0-72h, GD18)/AUC(0-72h, GD6)

ND: not determined, large extrapolated AUC and AUMC

NA: not applicable

Animal to Human Exposure Multiples

Dose (mg/kg)	AUC 0-∞ (µg·/mL)	Multiples of IV Human Exposure	Multiples of SC Human Exposure
1	1427	1.14	4.23
10	29548	23.5	87.7
50	182070	145	540

Study Summary

AF37702 was administered to male and female rats via bolus injection at doses of 0, 1, 10, and 50 mg/kg. The primary effects observed in the dams were consistent with the pharmacological action of known erythropoiesis-stimulating agents. Although not dose-dependent, all groups receiving AF37702 had significantly elevated RBC, HGB and HCT. Polycythemia, which increased in severity with dose, was also observed as well as enlarged spleens. Other than the expected pharmacological effects of the drug, there was no maternal toxicity observed in the dams of any group, and all of the effects were embryo-fetal effects.

Fetal examinations show increases in post-implantation loss and the number of dead fetuses. These corresponded to decreases in the number of live fetuses, and total resorbed fetuses. Decreases in fetal weights were also observed. Embryo-fetal lethality was observed in all groups receiving AF37702. Cleft palates, sternum anomalies, and sternoschisis were noted at the 10 and 50 mg/kg dose. Reduced bone ossification characterized by unossified and/or reduced ossification in the sternebrae, metatarsals, and the vertebral arches/centra was observed at doses ≥ 1 mg/kg. Due to fetal toxicities in all AF37702 doses, no NOAEL was established.

Study title: Effects of AF37702 on embryo-fetal development in rats (Supplemental Study)

Study no.: BA06123
 Study report location: eCTD 4.2.3.5.2
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 16 June 06
 GLP compliance: Statement included and signed
 QA statement: Statement included and signed
 Drug, lot #, and % purity: AF37702, Lot # 12AD2, 99.1%

Key Study Findings

- Decreased fetal weights were observed at 0.25 mg/kg
- Decreases in the number of ossified sacral and caudal vertebra were observed at doses \geq 0.05 mg/kg but was statistically significant at 0.25 mg/kg
- NOAEL for embryo-fetal toxicity was 0.01 mg/kg

NOTE: This study was designed to supplement study AF04-017, in which a NOAEL for the embryo-fetal development was not identified. Toxicities associated with the pharmacology of AF37702 (\uparrow RBC parameters and enlarged spleen) were similar in both studies.

Design:

(Excerpted from Applicant's submission)

Group	Test article	Dosage level (mg/kg/3 days)	Concentration (mg/mL)	Dosage volume (mL/kg)	No. of mated females	Animal Nos.
Control	Vehicle	0	0	1	20	1F01-1F20
Low	AF37702	0.01	0.02	0.5	20	2F01-2F20
Mid	AF37702	0.05	0.05	1	20	3F01-3F20
High	AF37702	0.25	0.25	1	20	4F01-4F20

Observations and Results**Hematology**

Statistically significant increases were observed in RBCs, HGB, and HCT:

	Females			
	VC	% change		
Dose (mg/kg)	0	0.01	0.05	0.25
No. of Animals	20	20	20	20
RBC	6.05	↑8*	↑30*	↑48
HGB	12.0	↑11*	↑32*	↑33*
HCT %	32.6	↑12*	↑36*	↑45*
# Polychromatosis	0	0	19**	20**

*p≤0.05 ** p≤0.01

Incidence of finding

Gross Necropsy

	Females				
	VC	% change			
Dose (mg/kg)	0	0.01	0.05	0.25	
No. of Animals	20	20	20	20	
Spleen	Enlarged	-	1	19	20

Pregnancy Parameters

Unremarkable

Fetal Examinations

Dose (mg/kg)	0	0.01	0.05	0.25	
No. of Dams	20	20	20	20	
Fetal Weight	Male	4.11	-	-	↓14*
	Female	3.93	-	-	↓14*

Fetal Malformations

	Fetus			
	0	0.01	0.05	0.25
External Malformations – unremarkable				
Visceral Malformations – unremarkable				
Skeletal malformations/variations				
No. Examined	148	156	150	144
Sacral and caudal vertebra ossified (mean)	8.5	8.5	8.2	7.7*

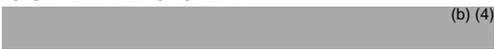
Toxicokinetics

Not done

Study Summary

AF37702 was administered to male and female rats via bolus injection at doses of 0, 0.01, 0.05, and 0.25 mg/kg. Decreased fetal weights were observed at 0.25 mg/kg. Decreases in the number of ossified sacral and caudal vertebra were observed at doses ≥ 0.05 mg/kg. NOAEL for embryo-fetal toxicity is 0.01 mg/kg.

Study title: A study for effects of AF37702 on embryo-fetal development in rabbits (Seg II)

Study no.:	AF05-003
Study report location:	eCTD 4.2.3.5.2.1
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	1 Mar 05
GLP compliance:	Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	AF37702, Lot #031114A, 98.7%

Key Study Findings

- Maternal toxicity attributed to exaggerated pharmacology of AF37702
- Embryo-fetal toxicity observed at all doses
- NOAEL for maternal toxicity and embryo-fetal toxicity could not be established
- Increased incidences of unossified hyoid arches and/or bones were observed at all doses
- Developmental toxicities included decreased fetal weight ≥ 0.5 mg/kg and malformations/variations, which included bend hyoid arches (≥ 0.5 mg/kg/day), and delay bone ossification (50 mg/kg)

Design:

This study was designed to assess the effects of AF37702 when administered to pregnant rabbits. Seventy six female New Zealand White rabbits (Kbl:JW (SPF) weighing 3.1-4.8 kg and 8 months of age, were mated with breeder bucks and equally distributed among 4 treatment groups, as shown in the following table. There were 19 females assigned to each group in an attempt to assure 16 pregnancies per group. They were administered daily intravenous doses in the marginal ear vein of either saline/acetate IV solution or AF37702 on days 6, 11 and 18 of presumed gestation. Scheduled sacrificed was on GD 29.

(Excerpted from Applicant's submission)

Group No./Treatment	Dose Level (mg/kg/dose)	Concentration (mg/ml)	Dose Volume (ml/kg)	No. of Females*
1/Vehicle-Control	0	0	5	19
2/AF37702	0.5	0.1	5	19
3/AF37702	5.0	1	5	19
4/AF37702	50.0	10	5	19

* Nineteen female rabbits were mated, in an attempt to ensure that a minimum of 16 females were confirmed gravid during the cesarean sections.

Observations and Results

Mortality

Animal #	Dose (mg/kg)	Sex	Day of Death	Observations	
				Reason	General (include pathology)
4044	0.5	F	29	Found Dead	Hair mass in stomach
4059	0.5	F	26	Sacrificed	Premature delivery. Animal showed ↓ food intake, weight gain, altered fecal output at GD 14-26. Nine-dead fetuses observed
4067	5	F	29	Found dead	Reduced food consumption, decreased body weight,
4092	50	F	6	Sacrificed	Physical injury

Clinical Signs

Unremarkable

Body Weight

	% change from control			
	Control	0.5	5	50
Dose (mg/kg)	0	0.5	5	50
Body weight gain	0.3	↓67	↓67	↓67

Food consumption

Unremarkable

Hematology

	Females			
	VC	% change		
Dose (mg/kg)	0	0.5	5	50
RBC	6.17	↑29**	↑33**	↑34**
HGB	13.4	↑25**	↑30**	↑26**
HCT	41.3	↑40**	↑82**	↑77**
*Polychromasia				
- rare – slight	-	6	-	-
- moderate	-	2	9	6
- marked	-	-	1	4

* Incidence of finding

Gross Necropsy

		Females			
Dose (mg/kg)		0	0.5	5	50
Liver	Congested	-	-	4	7
Spleen	Enlarged	-	1	6	4

Pregnancy Parameters

Dose (mg/kg)	0	0.5	5	50
No. Pregnant Dams				
Corpora lutea	9.5	10.3	10.1	10.6
Implantation sites	7.9	8.4	8.3	9.1
Pre-implantation loss	1.6	1.9	1.9	1.5
Post-implantation loss	0.3	1.4	0.3	0.9
Live fetuses	7.6	7.1	7.9	8.2
Dead fetuses	0	0	0	0
Early resorptions	0.1	0.4	0.2	0.4
Late resorptions	0.2	1	0.1	0.5
Gravid uterine weight	470	390	391	421

Embryo-Fetal Examinations

Dose (mg/kg/day)	Fetal weight (g)				% change from control		
	0	0.5	5	50	0.5	5	50
Male	43.2	34.8**	35.7**	31.4**	↓19	↓17	↓27
Female	42.2	35.3**	33.6**	33**	↓16	↓20	↓22
Male + female	43.2	35.3**	35.3**	32.3**	↓18	↓18	↓25

**: statistically significant compared to controls $p \leq 0.01$

Fetal Malformations

Dose (mg/kg)	Fetus				Litter			
	0	0.5	5	50	0	0.5	5	50
External Malformations								
Numbers Examined	129	113	119	115	17	16	15	14
Anophthalmia	-	-	-	1	-	-	1	1
Carpal/Tarsal	-	1	-	1	-	1	-	1
Visceral Malformations								
No. Examined	128	132	132	122	19	20	23	23
Hydrocephaly	-	1	-	-	-	1	-	-
Heart and/or great vessel – anomaly	2	1	-	1	2	1	-	1
Skeletal malformations								
No. Examined	137	131	150	137	19	20	23	23
Rib anomaly	1	-	1	1	1	-	1	1
Skull anomaly	-	2	-	1	-	2	-	1
Sternum anomaly	-	-	1	2	-	-	1	2
Sternebrae fused	-	-	1	1	-	-	1	1
Costal cartilage	-	1	-	-	-	1	-	-
Hyoid body/Arch Unossified	-	17	11	12	-	6	3	6
Hyoid Arches bent	7	22	24	25	5	9	10	11
Skull bones – reduced ossification	-	-	-	3	-	-	-	1
Pubis unossified	-	1	-	1	-	1	-	1
Metacarpals unossified	-	-	-	1	-	-	-	1
Total malformations	1	3	3	5	1	2	3	3

Toxicokinetics

Not done

Stability and Homogeneity

Adequate

Study Summary:

AF37702 was administered female rabbits via intravenous administration at doses of 0, 0.5, 5, and 50 mg/kg on gestation days 6, 11, and 16. Toxicokinetics were not done in this study. A total of 4 mortalities at doses \geq 0.5 mg/kg were reported in this study. Two animals were in the 0.5 mg/kg group. One death was likely due to a hair mass found in the lungs and the other was sacrificed due to premature delivery. This animal had decreased food intake and low body weight. This was likely test-article related. One animal in the 5 mg/kg dose group was found dead on Day 29 with clinical signs of reduced food consumption and decreased body weight. This death was also test-article

related. The animal in the 50 mg/kg was sacrificed due to a physical injury, likely not test-article related. Increased RBCs and differentials, polycythemia, and enlarged spleen are expected with drugs of this class and were noted all at doses. No other signs of maternal toxicity were noted. Developmental toxicities included decreased fetal weight at doses ≥ 0.5 mg/kg and malformations/variations, which included bend hyoid arches (≥ 0.5 mg/kg/day), and delay bone ossification (50 mg/kg). Due to malformations at the 0.5 mg/kg dose, a NOAEL could not be determined for embryo-fetal toxicity.

Study title: Effects of AF37702 on embryo-fetal development in rabbits (Supplemental Study)

Study no.: BA06124
 Study report location: eCTD 4.2.3.5.2
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 16 June 06
 GLP compliance: Statement included and signed
 QA statement: Statement not included
 Drug, lot #, and % purity: AF37702, Lot # 12AD2, 99.1%

Key Study Findings

- Maternal effects consistent with drug pharmacology
- Increased pre-mature deliveries at ≥ 0.05 mg/kg/dose and post-implantation loss starting at the mid-dose
- Embryo-fetal toxicities/malformations observed included absent gallbladder and fused sacral/causal arches/certrum at the 0.25 mg/kg

NOTE: This study was designed to supplement study AF05-003, in which a NOAEL for the embryo fetal development was not identified. Toxicities associated with the pharmacology of AF37702 (\uparrow RBC parameters) were similar in both studies.

Design:

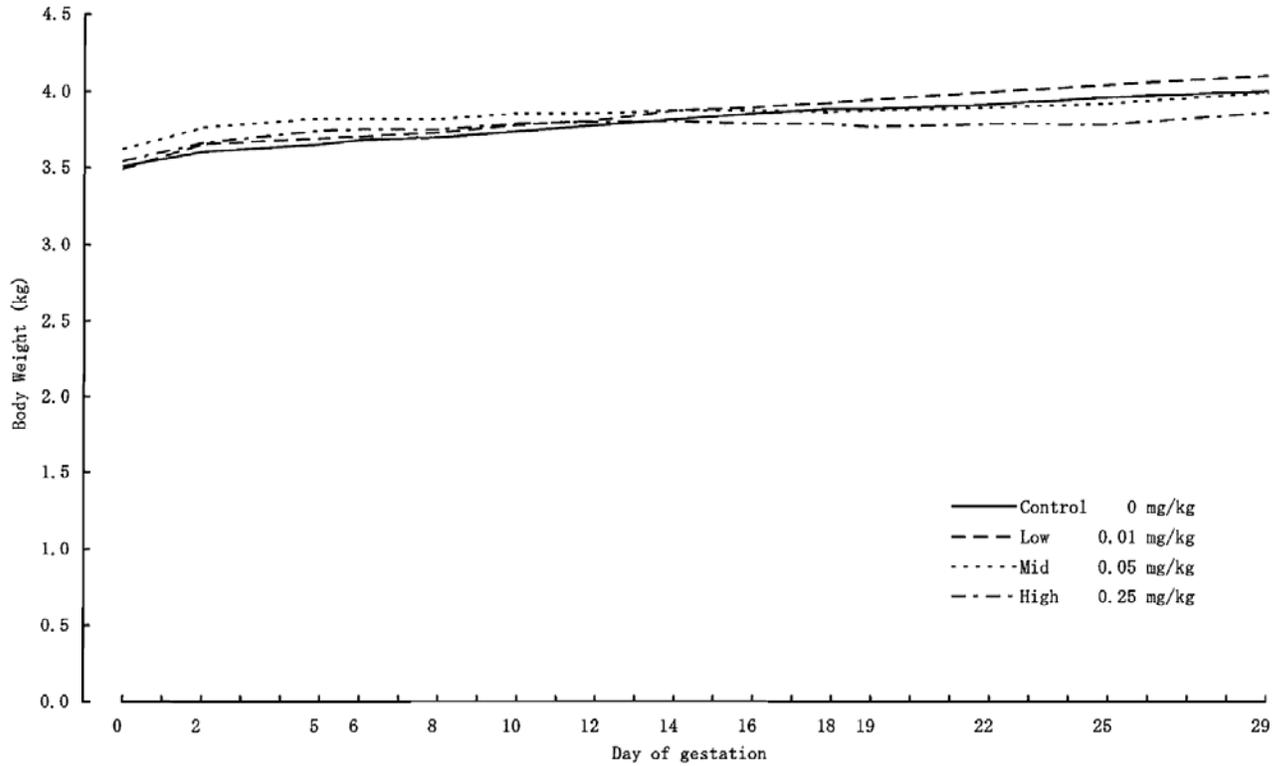
(Excerpted from Applicant's submission)

Group	Test article	Dosage level (mg/kg/5 days)	Concentration (mg/mL)	Dosage volume (mL/kg)	No. of mated females	Animal Nos.
Control	Vehicle	0	0	1	20	1F01-1F20
Low	AF37702	0.01	0.02	0.5	20	2F01-2F20
Mid	AF37702	0.05	0.05	1	20	3F01-3F20
High	AF37702	0.25	0.25	1	20	4F01-4F20

Observations and Results

Body Weight

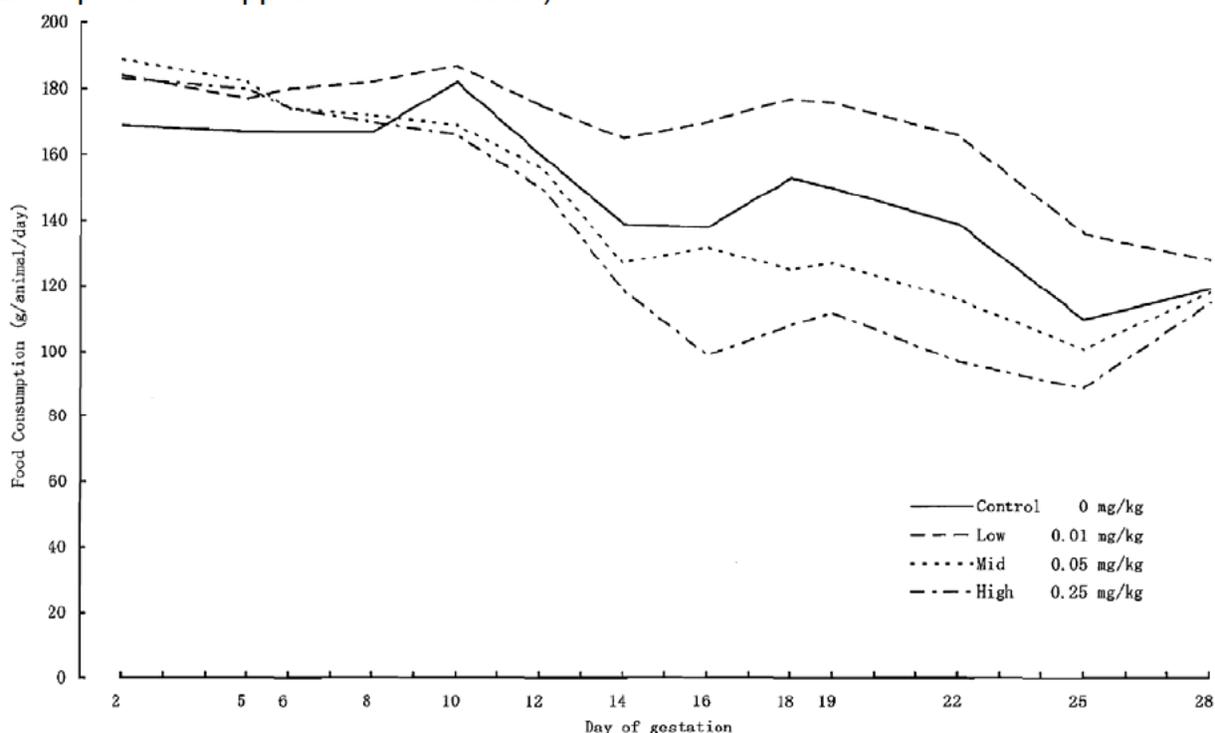
(Excerpted from Applicant's submission)



Effects of AF37702 on embryo-fetal development in rabbits -supplemental study-
Body Weight (Female)

24

(Excerpted from Applicant's submission)



Effects of AF37702 on embryo-fetal development in rabbits -supplemental study-
Food Consumption (Female)

Hematology

Statistically significant increases were observed in RBCs, HGB, and HCT:

	Females			
	VC	% change		
Dose (mg/kg)	0	0.01	0.05	0.25
No. of Animals	18	19	17	16
RBC	6.15	↑12*	↑23*	↑25*
HGB	13.5	↑16*	↑26*	↑26*
HCT %	38.9	↑17*	↑33*	↑38*
# Polychromatosis	0	0	0	2

*p≤0.05 ** p≤0.01
Incidence of finding.

Pregnancy Parameters

	Control	% Change from control		
	0	0.01	0.05	0.25
No. Pregnant Dams	18	19	18	18
Post-implantation loss	11	13	16	22*
Pre-mature deliveries	-	-	1	2

Fetal Examinations

		Control	% Change from control		
Dose (mg/kg/day)		0	0.01	0.05	0.25
No. Pregnant Dams		18	19	18	18
Fetal Weight	Male	41	-	-	↓15*
	Female	38.9	-	↓10	↓15*

Fetal Malformations

Dose (mg/kg)	Fetus			
	0	0.01	0.05	0.25
External Malformations – unremarkable				
Visceral Malformations				
No. Examined	130	143	139	127
Absent gallbladder	-	-	-	2
Skeletal malformations/variations				
No. Examined	130	143	139	127
Fused sacral arch	-	-	-	2
Fused sacral centrum	-	-	-	2
Fused caudal arch	-	-	-	3
Fused caudal centrum	-	-	-	3
Fused sternbrae	-	6	6	6

Toxicokinetics

Not done

Study Summary

AF37702 was administered intravenously every 5 days to male and female rabbits via bolus injection at doses of 0, 0.01, 0.05, and 0.25 mg/kg. Maternal toxicity effects included decreased body weights and food consumption and increases in RBC parameters. Premature deliveries occurred at doses \geq 0.05 mg/kg and were likely due to decreased food consumption. Increased post implantation loss was noted in groups \geq 0.05 mg/kg. Fetal toxicity included decreased fetal weights, which were observed at 0.05 and 0.25 mg/kg in females and 0.25 mg/kg in males. Developmental toxicities included absent gallbladders (0.25 mg/kg), fused sacral/caudal arch and/or centrum (0.25 mg/kg).

9.3 Prenatal and Postnatal Development

Study title: Intravenous Developmental and Perinatal/Postnatal Reproduction Toxicity Study of AF37702 in Rats, Including a Postnatal Behavioral/Functional Evaluation

Study no.: AF08-006
 Study report location: eCTD 4.2.3.5.3.1
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 20 Jan 08
 GLP compliance: Statement included and signed
 QA statement: Statement included and signed
 Drug, lot #, and % purity: AF37702, Lot #PLI039-06, 9.3%

Key Study Findings

- ↑ pups found dead or cannibalized in F1 litters at ≥ 3 mg/kg
- ↓ bodyweights in F1 rats at ≥ 3 mg/kg
- No changes in preputial separation in males or vaginal patency in females
- No changes in response inhibition, retention, or learning in males and females
- Mating, fertility, and pregnancy were unaffected
- F2 generation pups were unaffected

Design:

Doses: 0, 0.5, 3 and 15 mg/kg
 Frequency of dosing: F0: GDs 5 & 18; lactation day (LD): 13
 Dose volume: 1 mg/ML
 Formulation/vehicle: 20 mM sodium phosphate and 0.003% Tween® 20 in 4.7% sorbitol, pH range 6.0 ± 0.3
 Species/strain: Crl:CD(SD)
 Number/sex/group: F0: 25
 F1: 25
 Satellite groups: 6
 Study Design: **F₀ females** dosed on gestation days (GD) 5 and 18; and LD 13, with the day of mating noted as GD 0. Females delivered naturally, then euthanized on PND 21.
F₁ litters were culled on postnatal day (PND) 4 to 8 pups (3/sex/group if possible). External exams were completed on culled pups. On PND 14, the air righting test was performed. On PND 21, one pup/sex/litter was chosen for further evaluation (ophthalmoscopic, locomotor, and behavioral examination) and gestation. Mated females were euthanized on GD 14.

Tests performed: air righting (AR)-PND 14-17,
 pass on 3 consecutive days
 Straight Channel swim test
 Cincinnati water maze (CWZ)-PND 65-75, 2
 trials/day, 4 days
 Passive avoidance (PA)-PND 70-80, training
 trial and 24 hr. retention
 Acoustic Startle Response (ASR)- PND 80-90

Observations and Results

F0 Generation

Mortality

No drug-related mortalities

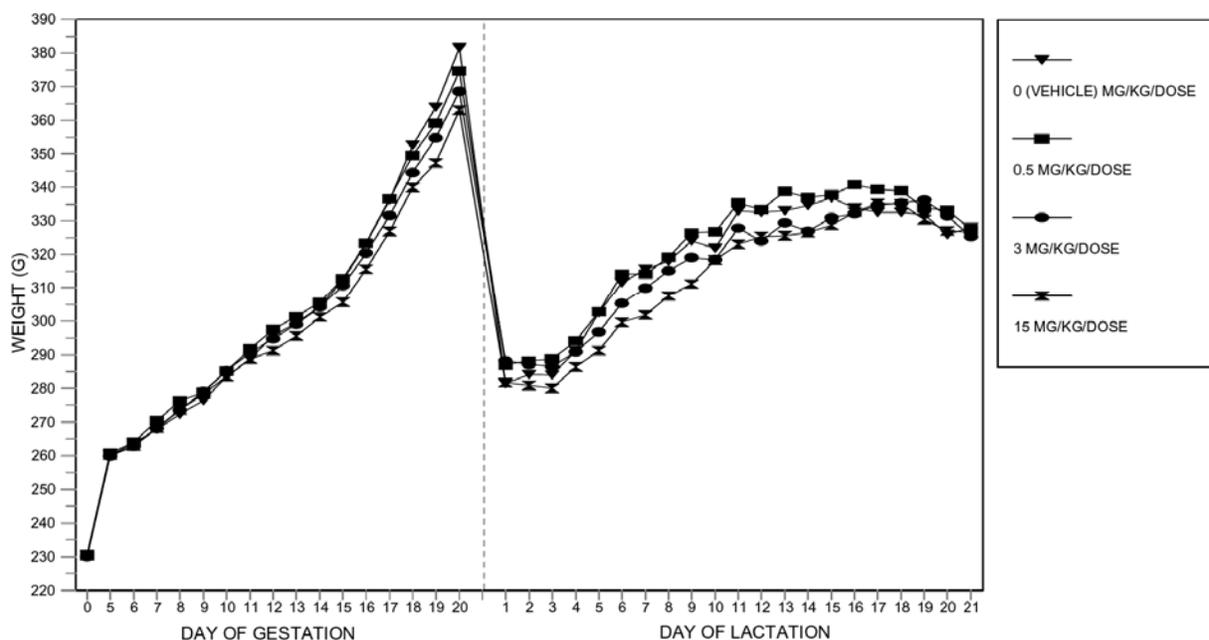
Clinical Signs

Unremarkable

Body Weight

(Excerpted from Applicant's submission)

MATERNAL BODY WEIGHTS - F0 GENERATION FEMALE RATS



Food consumption

No drug-related changes during gestation.

	Females			
	Control	% change from control		
Dose (mg/kg/day)	0	0.5	3	15
LD 1 – 14	53.5	-	↓10**	↓14**

LD: lactation day

Hematology

	Females			
	VC	% change		
Dose (mg/kg)	0	0.5	3	15
Gestation Day 10				
RBC	7	-	↑11*	↑14*
HGB	14	↑15*	↑15*	↑16*
HCT	42	↑22*	↑24*	↑25*
WBC	9.4	↑38*	↑60*	↑63*
Lactation Day 2				
RBC	6	↑28*	↑53*	↑61*
HGB	12	↑26*	↑24*	↑25*
HCT	36	↑33*	↑54*	↑57*

** : statistically significant compared to controls $p \leq 0.01$

Gross Necropsy

	Females			
	0	0.5	3	15
Dose (mg/kg)	0	0.5	3	15
Spleen Enlarged	-	1	24	25

Pregnancy Parameters

No drug-related changes in the following:

- # pregnancies, deliveries, implantation sites, dams with still born pups
- gestation index and duration of gestation

F1 Generation

Mortality

No. Pregnant Dams	23	24	23	23	% change from control		
Dose (mg/kg)	0	0.5	3	15	0.5	3	15
Pups found dead or cannibalized (days 2 – 4)	8	8	15**	22**	-	↓88**	↓175**
Viability index	98.4	98.4	95.4	94.5	-	↓3**	↓4**

** : statistically significant compared to controls $p \leq 0.01$

No differences in drug-related mortalities after weaning

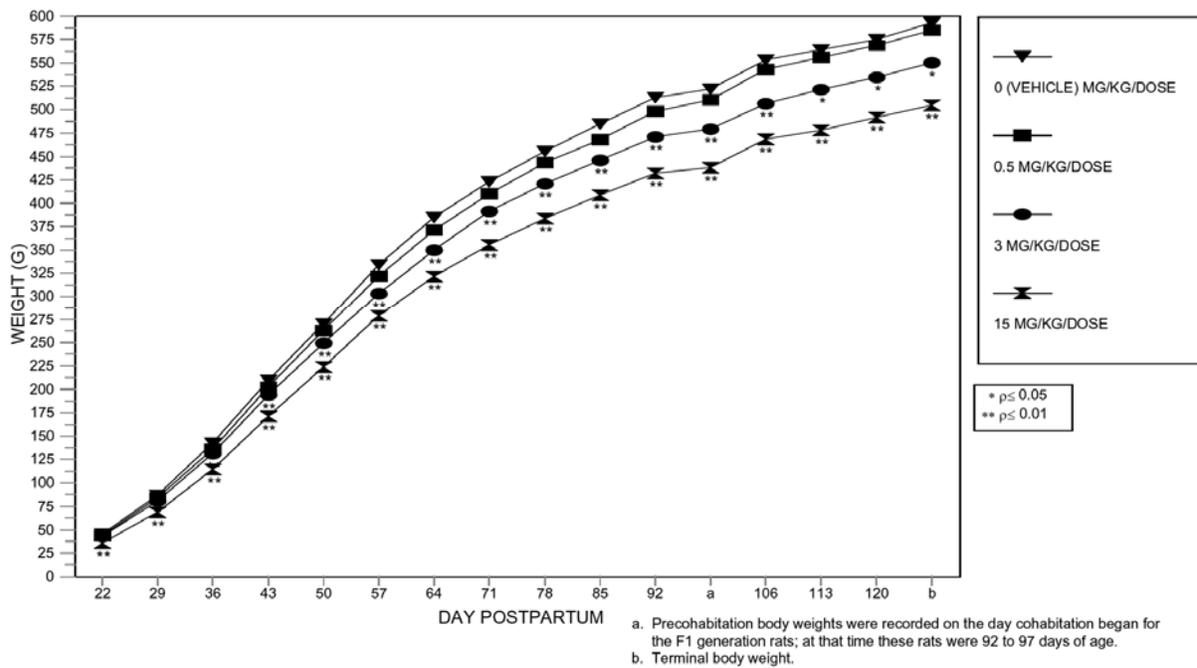
Clinical signs

Unremarkable

Body weight

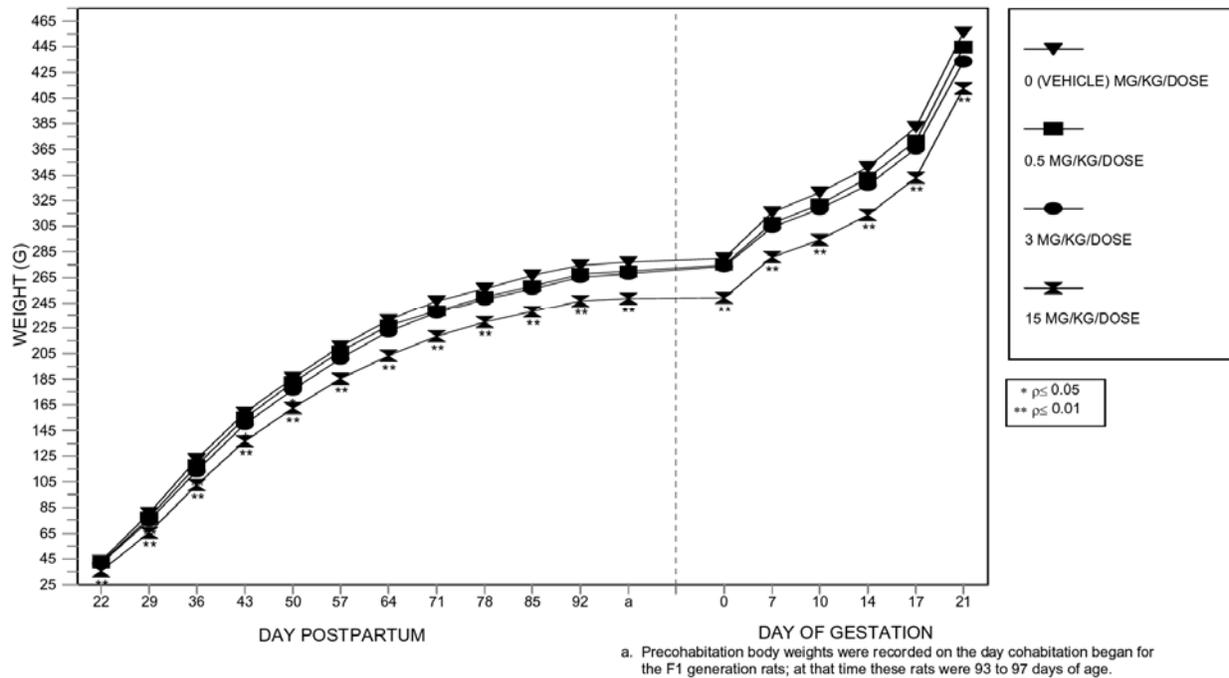
(Excerpted from Applicant's submission)

BODY WEIGHTS - F1 GENERATION MALE RATS



(Excerpted from Applicant's submission)

BODY WEIGHTS - F1 GENERATION FEMALE RATS



Food consumption

Unremarkable

Gross Necropsy

Unremarkable

Sexual maturation

No drug-related changes in preputial separation in males or vaginal patency in females,

Passive avoidance

No drug-related changes in response inhibition, retention, or learning in males and females

Watermaze performance

No drug-related changes in response inhibition, retention, or learning in males and females

Mating and Fertility

No drug-related changes in mating and fertility

Caesarean-section

No drug-related changes in the following:

- corpora lutea, implantations, litter sizes, # of live/dead fetuses, resorptions
- dams with any resorptions, dams with all conceptuses resorbed, dams with viable fetuses, and placentas

F2 Generation

No drug-related changes in the following:

- live fetuses, live male fetuses, fetal body weights, resorbed conceptuses

No remarkable gross observations observed

Toxicokinetics

- AF37702 exposure in maternal and fetal plasma increased with dose
- Since AUCs are not provided, animal:human exposure data could not be obtained

(Excerpted from Applicant's submission)

Mean (SD) toxicokinetic parameters in pregnant rats and fetuses following IV administration of AF37702 (N=6 per group)

Day	Group	Dose (mg/kg)	Maternal Plasma (ng/mL)	Fetal Plasma (ng/mL)	Fetal/Maternal Ratio	Amniotic Fluid (ng/mL)	Amniotic/Maternal Ratio
DG8,24h	I	0	BLQ	BLQ	NA	BLQ	NA
DG8,24h	II	0.5	2674±306.9	14.29±15.81	0.005±0.006	BLQ	0.000
DG8,24h	III	3	23480±3963	60.21±39.74	0.003±0.002	22.91±27.54	0.001±0.001
DG8,24h	IV	15	126000±16350	406.0±374.4	0.003±0.003	160.6±130.8	0.001±0.001

BLQ : below limit of quantification (< 25 ng/mL)

Stability and Homogeneity

Adequate

Study Summary

AF37702 was administered to female rats via bolus injection at doses of 0, 0.5, 3, and 15 mg/kg on gestation days 5 and 18 (all animals) and lactation day 13 (main animals only). AF37702 exposure in maternal and fetal plasma increased with dose. Maternal

toxicity included decreases in body weight and food consumption at doses ≥ 3 mg/kg. Increased RBC and RBC indices with enlarged spleens were expected due to the pharmacology of AF37702. There were no differences in the number of pregnancies, deliveries, implantation sites, and dams with still born pups. No changes in gestation index and duration of gestation were noted. There was an increase in pups found dead or presumed cannibalized observed at doses ≥ 3 mg/kg, which correlated to the decrease in viability index observed.

Decreased body weights were also observed in the F1 generation rats. There were no drug-related changes in preputial separation in males or vaginal patency in females. No changes in response inhibition, retention, or learning in males and females were also noted. Mating, fertility, and pregnancy parameters were unaffected. Changes in the F2 generation litters were unremarkable.

Discussion and Conclusions:

When administered intravenously to male and female rats at weekly intervals prior to and during mating, fertility was reduced at ≥ 0.1 mg/kg and was most evident at toxic doses ≥ 1.0 mg/kg of peginesatide. Adverse effects in males included reduced weight of seminal vesicles and prostate, increased morphological abnormalities of the sperm, and reduced sperm count. Decreased viable fetuses at ≥ 0.1 mg/kg in females appeared to be due to pre- and post-implantation losses. There was no apparent drug-related effect on estrous cycles or number of corpora lutea.

Administration of peginesatide by intravenous injection to rats and rabbits during organogenesis was associated with embryofetal deaths and malformations. Dosing was every third day in rats for a total of 5 doses and every fifth day in rabbits for a total of 3 doses. Adverse embryofetal effects included reduced fetal weight, increased resorptions, dead fetuses, cleft palate, sternum anomalies, unossification of sternbrae and metatarsals, reduced ossification of some bones, and variations in major blood vessels. These effects were evident in rats at peginesatide doses of ≥ 1 mg/kg. The dose of 1 mg/kg results in exposures (AUC) comparable to those estimated in humans after intravenous administration at the high dose of 0.35 mg/kg. Reduced fetal weight and ossification were also seen in a separate embryofetal developmental study in rats at a lower dose of 0.25 mg/kg. Adverse embryofetal effects in rabbits were observed at ≥ 0.5 mg/kg/dose of peginesatide. In a separate embryofetal developmental study in rabbits, adverse findings were observed at lower doses and included increased incidence of premature delivery at ≥ 0.05 mg/kg/dose in addition to those described above. The effects in rabbits were observed at doses lower (5%-50%) than the highest dose in patients.

Thus, administration of peginesatide during pregnancy may pose a risk to the human fetus.

10 Special Toxicology Studies

These studies were reviewed by Dr. Ke Zhang, Ph.D. in the Division of Gastroenterology Products on April 13, 2007.

10.1 Impurities

Study title: A Four-week Intravenous toxicity study of AF37702 containing (b) (4) in rats followed by a six-week recovery

Study no.: AF08-012
Study report location: eCTD 4.2.3.7
Conducting laboratory and location: (b) (4)
Date of study initiation: 13 Feb 08
GLP compliance: Statement included and signed
QA statement: Statement included and signed
Drug, lot #, and % purity: AF37702: SF353/6AF1, 99.1%
(b) (4) 5024-54-1, 98%
(b) (4) 4988-16-2, 98.5%

Key Study Findings

- One mortality report at the 5 mg/kg dose
- Effects consistent with the pharmacology of AF37702
- Other target organ effects seen in the heart and kidney
- Toxicities were comparable to those seen with peginesatide

Methods

Design: This study was designed to determine the toxicity of AF37702 containing increased levels of (b) (4) when given IV once weekly for 5 doses to SD rats. The vehicle was 20mM sodium phosphate and 0.003# TWEEN 20 in 4.7% sorbitol. Animals were assigned to the dose levels as shown in the table below:

(Excerpted from Applicant's submission)

Toxicology Groups

Group	Dose Level ¹ (mg/kg/day)	Calculated Composition of the Dosing Formulation (mg/kg)			Conc. ¹ (mg/mL)	Dose Volume (mL/kg)	Number of Animals	
		AF37702	(b) (4)	(b) (4)			Male	Female
1. Vehicle-Control	0	0	0	0	0	5	15	15
2. Low-dose	0.5	0.32	0.16	0.02	0.1	5	15	15
3. High-dose	5	3.2	1.6	0.2	1	5	15	15

¹Dose level and Concentration reflect combined mg/kg peptide dose and combined mg/mL of all 3 test articles (i.e. AF37702, (b) (4), (b) (4))

Toxicokinetic Groups

Group	Dose Level ¹ (mg/kg/day)	Calculated Composition of the Dosing Formulation (mg/kg)			Conc. ¹ (mg/mL)	Dose Volume (mL/kg)	Number of Animals	
		AF37702	(b) (4)	(b) (4)			Male	Female
4. Vehicle-Control	0	0	0	0	0	5	3	3
5. Low-dose	0.5	0.32	0.16	0.02	0.1	5	9	9
6. High-dose	5	3.2	1.6	0.2	1	5	9	9

¹Dose level and Concentration reflect combined mg/kg peptide dose and combined mg/mL of all 3 test articles (i.e. AF37702, (b) (4), (b) (4))

Observations and Results

Mortality

Animal #	Dose (mg/kg)	Sex	Day of Death	Reason	Observations General (include pathology)
887	5 (AF37702)	F	32	Found dead	Dark pink extremities, dark discoloration in liver and spleen (congestion), enlarged spleen (hematopoiesis, multi-organ congestion (liver spleen, lung, adrenal), hypercellularity in bone marrow

Clinical observations

Dark pink extremities both AF37702 treated groups

Bodyweight

Unremarkable

Food consumption

Unremarkable

Ophthalmoscopy

Unremarkable

Hematology

Dose (mg/kg)	Control 0	Male % change		Control 0	Female % change	
		0.5	5		0.5	5
<i>RBC</i>	8.6	↑40**	↑48**	8.4	↑37**	↑55**
<i>HGB</i>	-	-	-	17.6	↑24.5**	↑25**
<i>HCT</i>	48	↑49**	↑54**	47.3	↑54**	↑62**
<i>RET</i>	2.1	↑457**	↑533**	2.4	↑442**	↑463**

NA: not applicable

Coagulation

Dose (mg/kg)	Control 0	Male % change		Control 0	Female % change	
		0.5	5		0.5	5
<i>PT</i>	17	↑12**	↑18**	-	-	-
<i>APTT</i>	9	↑44**	↑56**	8	↑45**	↑95**

NA: not applicable

Clinical chemistry

Dose (mg/kg)	Control 0	Male % change		Control 0	Female % change	
		0.5	5		0.5	5
<i>BUN</i>	20	↑12**	↑34*	22.6	↑14	NA
<i>TBIL</i>	0.11	↑150**	↑195**	0.21	↑52	NA
<i>FE</i>	99.6	↓76**	↓19*	200	↓73*	NA

NA: not applicable (due to high hemoconcentration, adequate serum samples could not be collected)

Troponin

Blood samples were obtained at pre-dose, day 5, weeks 13, 26, 39, and 43 (recovery). No drug-related changes were observed

Urinalysis

Unremarkable

AF37702 Antibody Evaluation

Unremarkable

Gross Pathology

Dose (mg/kg)		Male			Female		
		Cont rol	% change		Cont rol	% change	
		0	0.5	5	0	0.5	5
		10	10	10	10	10	9
Liver	Dark	-	7	7	-	10	9
	Enlarged	-	2	5	-	-	1
Spleen	Enlarged	-	10	10	-	10	9
Stomach	Black foci	-	-	1	-	-	1
	Dark discoloration (red)	-	-	1	-	-	-
	Dark red foci	-	3	5	-	-	-
	Thickened	-	1	3	-	-	-
	Multiple dark foci	-	-	-	-	-	2
	Kidney	Dark	-	8	8	-	-

Histopathology

Adequate Battery (yes)

Peer Review (yes)

Histological Findings:

Dose (mg/kg)		Male			Female		
		Cont rol	% change		Cont rol	% change	
		0	0.5	5	0	0.5	5
		10	10	10	10	10	9
BM, sternum	Hypercellularity; hematopoietic						
	-minimal		6	1		8	-
	-mild		3	8		2	9
	-moderate	-	-	1	-	-	-
	Focal degeneration,	-	-	1	-	-	-

		Male			Female			
		Cont rol	% change		Cont rol	% change		
Dose (mg/kg)		0	0.5	5	0	0.5	5	
		10	10	10	10	10	9	
Heart	mild Hemorrhage, focal							
	minimal	1	2	2	-	-	4	
	Necrosis, focal, minimal	-	-	1	-	-	3	
	Cardiomyopathy -minimal		1	8		1	1	
	-mild		1	-		-	-	
Kidney	-moderate	-	-	1	-	-	-	
	Chronic progressive nephropathy -minimal	7	3	-	3	6	3	
	-mild	-	6	6	1	2	6	
	-moderate	-	-	4	-	-	-	
	Infarction, marked	-	-	1	-	-	-	
	Congestion, -minimal	-	4	5	-	6	8	
	-mild	-	-	1	-	-	1	
	Thrombosis, artery, moderate	-	-	1	-	-	-	
	Mineralization, tubular, minimal	-	-	1	-	-	-	
	Congestion, -minimal		3	5		8	8	
Liver	-mild	-	-	4	-	-	-	
	Hematopoiesis, extramedullary, minimal	-	9	10	-	10	9	
	Spleen	Hematopoiesis, extramedullary, -minimal		1	1		-	-
		-mild		9	1		-	-
		-moderate		-	8		3	7
Stomach	-marked	-	-	-	-	7	2	
	Erosion -mild		1	1		1	2	
	-moderate	-	1	2	-	-	3	
	Hemorrhage, focal, -minimal		-	3		-	3	
	-mild		2	-		-	1	
	-moderate		-	2		-	2	
	-marked	-	-	1	-	-	-	

Recovery sacrifice

Unremarkable

Toxicokinetics**Methods**

Whole blood samples (approximately 0.5 mL/sample) were collected from 3 animals/sex in Groups 5 and 6 only on Days 1 and 29, via retro-orbital puncture according to the following approximate time points: pre-dose, 0.25, 1, 4, 8, 24, 48, and 72 hours post-dose.

Additionally, on Days 1 and 29, 3 animals/sex in Group 4 had whole blood samples (approximately 0.5 mL/sample) collected via retro-orbital puncture at only the 0.25 hour post-dose time point.

Animals were bled no more than 3 times per collection period, and the total volume of samples collected did not exceed 1% of body weight for a two-week period. Animals were anesthetized by CO2 inhalation prior to collection.

Results

Plasma toxicokinetic parameters in rats following weekly IV administration of AF37702 containing (b) (4)

Day	Dose (mg/kg)	Sex	C _{max} (µg/mL)	t _{max} (h)	AUC ₀₋₁ (µg·h/mL)	AUC _{0-inf} (µg·h/mL)	t _{1/2} (h)	Rc ^a
1	0.5	M	17.51	0.25	429.6	478.4	21.95	NA
		F	16.78	0.25	436.8	478.6	22.31	NA
	5	M	153.8	4	4986	5662	23.15	NA
		F	183.6	0.25	4944	5827	26.18	NA
29	0.5	M	21.48	0.25	371.7	381.0	13.37	0.865
		F	18.04	4	342.9	348.4	12.19	0.785
	5	M	198.8	0.25	5547	5919	17.84	1.11
		F	247.1	0.25	4977	5277	16.92	1.01

a: Rc = AUC₀₋₁(Day 29)/AUC₀₋₁(Day 1)

Study Summary:

AF37702 containing (b) (4) was administered to male and female rats via IV administration) at doses of 0, 0.5 and 5 mg/kg once weekly for a total of 5 doses. AF37702 C_{max} and AUC values increased proportionally between doses and no signs of drug accumulation were observed. Concentrations were similar between males and females.

There was one mortality noted in a female rat at the 5 mg/kg dose. There were no clinical signs that lead to death. Pathologic examinations show toxicities associated with AF37702 pharmacology i.e. multi-organ congestion, enlarged spleen with increased hematopoiesis, and hypercellularity in bone marrow. Effects of AF37702

administration in surviving animals were consistent with its pharmacology. Other toxicities were in the heart (cardiomyopathy) and kidney (nephropathy). These results show that administration of AF37702 with (b) (4) elicited responses consistent with other toxicity studies.

10.2 Antigenicity

Study title: Antigenicity study of AF37702 in guinea pigs: systemic anaphylaxis

Study no.: AF03-41

Study report location: eCTD 4.2.3.7

Methods: A systemic anaphylaxis test was conducted in male Hartley guinea pigs. For the sensitization phase, guinea pigs (10/group) were given AF37702 intravenously at 0.2 or 2 mg/kg or subcutaneously at 0.2 or 2 mg/kg with Complete Freund's Adjuvant (CFA). Control animals were given either vehicle control (0.9% sodium chloride) or positive control, ovalbumin (OVA), at 2 mg/kg subcutaneously with CFA. These animals were dosed once a week for 4 weeks. On day 35, these animals were challenged with 2 mg/kg AF37702 by intravenous injection. The animals in the positive control group were challenged with 4 mg/kg OVA by intravenous injection. The signs of anaphylactic response were then observed. Followings were the anaphylactic reactions assessed:
Slight: Piloerection, scratching of nose, sneezing and tremors
Moderate: Urination defecation, cyanosis, dyspnea, wheezing, lobored respiration, staged gait
Severe: convulsions, prostration or death.

Results: The results indicated that all animals in the positive control group had severe signs of anaphylaxis and died 4-6 minutes after being challenged. Sneezing was noted in two AF37702 treated animals (i.v., 2 mg/kg). All other AF37702 treated animals appeared normal.

Conclusion: AF37702 is not antigenic under the conditions tested

Study title: AF37702: guinea pigs sensitization – maximization test

Study no.: AF03-42

Study report location: eCTD 4.2.3.7

Methods: The purpose of this study is to determine if AF37702 induced the delayed dermal contact hypersensitivity response in guinea pigs. There were 10 animals/sex in the treatment group, 5 animals/sex in the vehicle control group, and 3 animals/sex in the positive control (1-chloro-2,4-dinitrobenzene-DNCB) group.

For the intradermal induction phase (Day 1), each guinea pig received intradermal injections (0.1 ml each) at six sites between the shoulders according to the following table (*Excerpted from Applicant's submission*):

Sites	Vehicle Control Group	Test Article Group	Positive Control Group
1&2	*FCA (1:1)	*FCA (1:1)	*FCA (1:1)
3&4	Saline	5% AF37702** in saline	0.1% DNCB in saline
5&6	5% saline (in 1:1 FCA)	5% AF37702** (in 1:1 *FCA)	0.1% DNCB (in 1:1 *FCA)

*FCA-Freund's Complete Adjuvant

**5% of 10 mg/ml AF37702 stock solution in 10 mM acetate

On Day 7, all animals in the vehicle control and test article groups were pretreated dermally with 10% Sodium Lauryl Sulfate (SLS). On Day 8, the test article at 10 mg/ml was spread over a 2 x 4 cm filter paper (0.3 ml) and applied to the injection site areas. BlendermCI tape was used to occlude the injection area. The dressings were removed following 48 hours of exposure.

Two weeks after the topical induction, the hair was removed from right and left flanks. On Day 22, all test article-treated, vehicle control, and positive control animals were challenged with occluded patches for 24 hours on the left flank and right flanks. A 2 x 2 cm filter paper was saturated (0.2 ml) with the test or positive control article and applied to the left flank. Another 2 x 2 cm filter paper was saturated (0.2 ml) with the vehicle (10 mM acetate in 0.9% saline), 0.9% Sodium Chloride or Petrolatum and applied to the right flank. The same occlusive technique was employed as for topical induction. After 24 hours, sites were unwrapped and wiped clean. Twenty-one hours after unwrapping, the sites were depilated with Nair Lotion Hair Remover. Three hours later the sites were graded for elicited skin reactions (24-hour grade). Approximately 24 hours later the sites were graded a second time (48-hour grade).

Results: There were no treatment-related clinical signs of toxicity. There were no deaths. The incidences of dermal irritation scores were summarized in a table on page 99 in Volume 5.23. This table is attached below (*Excerpted from Applicant's submission*).

Incidence of Dermal Irritation Scores at Challenge

Induction Treatment	Challenge Treatment	Dermal Irritation Scores									
		24-hour Grade					48-hour Grade				
		0	1	2	3	4	0	1	2	3	4
Vehicle Control	AF37702	10	0	0	0	0	10	0	0	0	0
	0.9% sodium chloride	10	0	0	0	0	10	0	0	0	0
Test Article	AF37702	20	0	0	0	0	20	0	0	0	0
	10 mM acetate in 0.9% sodium chloride	20	0	0	0	0	20	0	0	0	0
Positive Control	0.05% DNCB in petrolatum	0	0	2	4	0	0	1	2	3	0
	Petrolatum	6	0	0	0	0	6	0	0	0	0

Conclusion: The results indicated an intradermal induction of AF37702 did not elicit any dermal sensitization response at 24 and 48 hours.

10.3 Other Toxicity Studies

Study title: Evaluation of AF37702 to induce hemolysis in human blood

Study no.: AF03-43

Study report location: eCTD 4.2.3.7

Methods: Human blood (1 mL) was mixed with 0.1, 1, and 10 mg/mL of AF37702 to have final concentrations of 0.05, 0.5, and 5 mg/mL. The mixture was incubated at 37° C for 45 minute and then centrifuged at 1000 g for 5 minute. The supernatant was examined spectrophotometrically at 540 nm.

Results: The results indicated that the percent hemolysis was -2.95, 7.71, and 10.42% at concentrations of 0.05, 0.5, and 5 mg/mL, respectively. However, the percent hemolysis was -0.06, -0.12, and -0.16% at concentrations of 0.05, 0.5, and 5 mg/mL, respectively, in the repeated experiment. Therefore, AF37702 did not cause any significant hemolysis in human blood.

Conclusion: AF37702 is compatible with human blood at concentrations as high as 5 mg/mL.

Study title: Evaluation of AF37702 to induce flocculation in human plasma and blood

Study no.: AF03-44

Study report location: eCTD 4.2.3.7

Methods: Human blood (1 mL) was mixed with 0.1, 1, and 10 mg/mL of AF37702 to have final concentrations of 0.05, 0.5, and 5 mg/mL. The mixture was incubated at 37°

C for 30 minute and then the tubes were examined macroscopically and microscopically for precipitation or coagulation.

Results: The results indicated that there was no precipitation in the human plasma but precipitation was noted in the serum samples in the first assay. No precipitation was noted in either plasma or serum in the repeated experiment.

Conclusion: AF37702 is compatible with human plasma and serum a concentrations as high as 5 mg/mL.

10.3 Discussion and Conclusions:

Peginesatide with (b) (4) elicited a similar toxicological profile as the general toxicity studies in rats. Therefore, these data suggest that increased levels of (b) (4) did not alter the toxicological profile of peginesatide. Peginesatide is compatible with human blood, plasma, and serum. Peginesatide did not cause antigenicity or dermal sensitization in the guinea pig.

11 Integrated Summary and Safety Evaluation

The non-clinical studies of peginesatide support the use of peginesatide intravenously or subcutaneously for the treatment of anemia due to chronic kidney disease in adult patients on dialysis.

See the EXECUTVE SUMMARY, Page 4, for an overall summary of nonclinical findings.

12 Appendix/Attachments

None

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

BRENDA J GEHRKE
02/02/2012

HALEH SABER
02/02/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 202799 **Applicant: Affymax, Inc**

Stamp Date: 27 June 2011

Drug Name: Peginesatide

NDA Type: Standard Review

PDUFA Date: 12 March 2012

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			NA
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement
010908

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?			This is a review issue.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			This is a review issue
11	Has the applicant addressed any abuse potential issues in the submission?			NA
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			NA

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? YES

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None

Kimberly Ringgold

7/7/2011

Reviewing Pharmacologist

Date

Team Leader/Supervisor

Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

KIMBERLY R RINGGOLD
07/18/2011

HALEH SABER
07/20/2011