

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

***APPLICATION NUMBER:***  
**BLA 125268**

**PHARMACOLOGY REVIEW(S)**

## MEMORANDUM

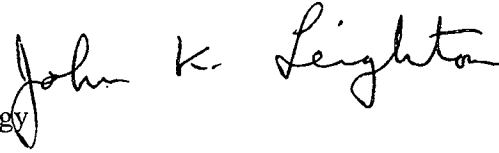
Nplate (romiplostim)

**Date:** May 23, 2008

**To:** File for BLA 125268

**From:** John K. Leighton, PhD, DABT

Associate Director for Pharmacology  
Office of Oncology Drug Products

A handwritten signature in black ink, reading "John K. Leighton". The signature is written in a cursive, flowing style. The first name "John" is written with a large, looped 'J'. The middle initial "K." is written with a simple, bold 'K' followed by a period. The last name "Leighton" is written with a large, looped 'L' and a trailing flourish.

I have examined the labeling and pharmacology/toxicology supporting reviews and memoranda provided by Drs. Kokate and Laniyonu and concur with their conclusions that Nplate may be approved. No additional pharmacology/toxicology studies are necessary.

### Supervisory Pharmacologist Memo

**BLA:** 125268  
**Product:** Romiplostim (Nplate)  
**Sponsor:** Amgen, Inc.

Romiplostim (AMG 531, Amgen Megakaryopoiesis Protein-2 or AMP2) is a thrombopoiesis stimulating fusion protein that increases platelet production through activation of the thrombopoietin receptor (TPO or c-Mpl receptor). It is proposed for the treatment of thrombocytopenia in adult patients with idiopathic thrombocytopenic purpura (ITP) who have had an insufficient response to corticosteroids, immunoglobulins or splenectomy. The recommended maximum clinical dose for AMG 531 is 10 mcg/kg injected subcutaneously once a week.

Dr. Tushar Kokate reviewed the preclinical Pharmacology and Toxicology section of NDA 22-090. This secondary review focusing on pertinent preclinical findings was based on Dr. Kokate's review; please see Dr. Kokate's review for details.

In vivo cardiovascular safety study in cynomolgus monkey produced no evidence that AMG 531 had any remarkable effect on cardiovascular parameters. (hERG assay and cardiac action potential assessments were not conducted because of the protein nature of the product). CNS safety evaluation in rats was considered adequate.

Metabolism studies were not conducted. The bioavailability of AMG 531 after a subcutaneous administration was low in rats and cynomolgus monkeys but relatively high in rhesus monkeys. The Tmax varied from 4 to 16 hours depending on the species. Repeat-dose administration in rats and cynomolgus monkeys showed no significant drug accumulation and no apparent sex differences in PK parameters. AMG 531 was mainly excreted through urine.

Definitive toxicology (single and repeat-dose) studies were conducted in rats, Rhesus and Cynomolgus monkey. In general, adverse effects observed including extramedullary hematopoiesis, splenic enlargement, megakaryocytosis/ megakaryocyte hyperplasia, bone hyperostosis and myelofibrosis were related to the over activation of thrombopoietic system caused by AMG 531.

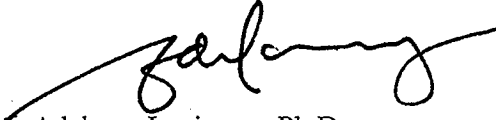
The carcinogenic and mutagenic potential of AMG 531 has not been evaluated. This is consistent with current guidance on biotechnology-derived pharmaceuticals and in view of the nature of AMG 531.

Reproductive toxicology studies were conducted in rats and rabbits and mice. In rats and rabbit developmental toxicity studies, no evidence of fetal harm was observed at AMG531 doses up to 11 times (rats) and 82 times (rabbit) the maximum human dose (MHD, based on systemic exposure). In mice at doses 5 times the MHD, reductions in maternal body weight and increased post-implantation loss occurred.

In a prenatal and postnatal development study in rats, at doses 11 times the MHD, there was an increase in peri-natal pup mortality. AMG 531 crossed the placental barrier in rats and increased platelet counts in fetuses at clinically relevant doses.

Dr. Kokate has concluded that the preclinical package of AMG 531 was complete for this product, and that the studies conducted support the safety and efficacy of AMG 531 from preclinical pharmacology/toxicology perspectives. He recommends approval of the BLA and suggested changes in the label that would more appropriately reflect findings from preclinical studies.

I concur with Dr. Kokate's recommendations.

 5/19/08

Adebayo Laniyonu, Ph.D.

Supervisory Pharmacologist



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

## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER:	125268
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	10/23/07
PRODUCT:	Nplate (Romiplostim)
INTENDED CLINICAL POPULATION:	Idiopathic Thrombocytopenic Purpura (ITP)
SPONSOR:	Amgen, Inc.
DOCUMENTS REVIEWED:	Electronic submission (eCTD)
REVIEW DIVISION:	Division of Imaging and Hematology Drug Products (HFD-160)
PHARM/TOX REVIEWER:	Tushar Kokate, Ph.D.
PHARM/TOX SUPERVISOR:	Adebayo Laniyonu, Ph.D.
DIVISION DIRECTOR:	Rafel Rieves, M.D.
PROJECT MANAGER:	Florence Moore, M.Sc.

## **TABLE OF CONTENTS**

<b>EXECUTIVE SUMMARY .....</b>	<b>3</b>
<b>2.6 PHARMACOLOGY/TOXICOLOGY REVIEW .....</b>	<b>9</b>
<b>2.6.1 INTRODUCTION AND DRUG HISTORY.....</b>	<b>9</b>
<b>2.6.2 PHARMACOLOGY.....</b>	<b>13</b>
2.6.2.1 Brief summary .....	13
2.6.2.2 Primary pharmacodynamics.....	14
2.6.2.3 Secondary pharmacodynamics.....	26
2.6.2.4 Safety pharmacology .....	26
2.6.2.5 Pharmacodynamic drug interactions.....	29
<b>2.6.4 PHARMACOKINETICS/TOXICOKINETICS.....</b>	<b>29</b>
2.6.4.1 Brief summary .....	29
2.6.4.2 Methods of Analysis .....	31
2.6.4.3 Absorption .....	31
2.6.4.4 Distribution .....	33
2.6.4.5 Metabolism .....	35
2.6.4.6 Excretion.....	35
2.6.4.7 Pharmacokinetic drug interactions.....	39
2.6.4.8 Other Pharmacokinetic Studies.....	39
<b>2.6.6 TOXICOLOGY.....</b>	<b>42</b>
2.6.6.1 Overall toxicology summary.....	42
2.6.6.2 Single-dose toxicity .....	46
2.6.6.3 Repeat-dose toxicity .....	49
2.6.6.4 Genetic toxicology.....	77
2.6.6.5 Carcinogenicity.....	77
2.6.6.6 Reproductive and developmental toxicology.....	78
2.6.6.7 Local tolerance.....	100
2.6.6.8 Special toxicology studies .....	100
<b>OVERALL CONCLUSIONS AND RECOMMENDATIONS.....</b>	<b>103</b>
<b>APPENDIX/ATTACHMENTS .....</b>	<b>104</b>

## ***EXECUTIVE SUMMARY***

### **I. Recommendations**

- A. Recommendation on approvability: **Approval**
- B. Recommendation for nonclinical studies: **None**
- C. Recommendations on labeling:

### **Pregnancy (Category C)**

#### **8.1 Pregnancy**

##### **Pregnancy Category C:**

There are no adequate and well-controlled studies of Nplate use in pregnant women. In animal reproduction and developmental toxicity studies, romiplostim crossed the placenta, and adverse fetal effects included thrombocytosis, post-implantation loss, and an increase in pup mortality. Nplate should be used during pregnancy only if the potential benefit to the mother justifies the potential risk to the fetus.

*Pregnancy Registry:* A pregnancy registry has been established to collect information about the effects of Nplate use during pregnancy. Physicians are encouraged to register pregnant patients, or pregnant women may enroll themselves in the Nplate pregnancy registry by calling 1-877-Nplate (1-877-675-2831).

In rats and rabbit developmental toxicity studies, no evidence of fetal harm was observed at romiplostim doses up to 11 times (rats) and 82 times (rabbit) the maximum human dose (MHD) based on systemic exposure. In mice at doses 5 times the MHD, reductions in maternal body weight and increased post-implantation loss occurred.

In a prenatal and postnatal development study in rats, at doses 11 times the MHD, there was an increase in perinatal pup mortality. Romiplostim crossed the placental barrier in rats and increased fetal platelet counts at clinically equivalent and higher doses.

#### **8.3 Nursing Mothers**

It is not known whether Nplate is secreted into human milk; however, human IgG is excreted in human milk. Published data suggest that breast milk antibodies do not enter the neonatal and infant circulation in substantial amounts. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from Nplate, a decision should be made whether to discontinue nursing or to discontinue Nplate, taking into account the importance of Nplate to the mother and the known benefits of nursing.

## Nonclinical Toxicology

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

The carcinogenic potential of romiplostim has not been evaluated. The mutagenic potential of romiplostim has not been evaluated. Romiplostim had no effect on the fertility of rats at doses up to 37 times the MHD based on systemic exposure.

### 13.2 Animal Toxicology and/or Pharmacology

In a 4-week repeat dose toxicity study in which rats were dosed subcutaneously three times per week, romiplostim caused extramedullary hematopoiesis, bone hyperostosis and marrow fibrosis at clinically equivalent and higher doses. In this study, these findings were not observed in animals after a 4-week post-treatment recovery period. Studies of long-term treatment with romiplostim in rats have not been conducted; therefore, it is not known if the fibrosis of the bone marrow is reversible in rats after long-term treatment.

## II. Summary of nonclinical findings

Romiplostim (AMG 531, AMP2) is a thrombopoiesis stimulating fusion protein that increases platelet production through activation of the thrombopoietin receptor (TPO or c-Mpl receptor). Romiplostim is indicated for the treatment of thrombocytopenia in adult patients with chronic immune (idiopathic) thrombocytopenic purpura (ITP) who have had an insufficient response to corticosteroids, immunoglobulins or splenectomy. The recommended maximum clinical dose is 10 mcg/kg injected subcutaneously once a week.

### Brief overview of nonclinical findings

**Pharmacology:** Various in vitro binding studies showed that AMG 531 has high affinity for the TPO receptor (c-Mpl). AMG 531 binds with high affinity to TPO receptors on rat, cynomolgus monkey and human platelets, and stimulates megakaryocyte colony-forming cell proliferation. Platelets incubated in the presence of AMG 531 ( $\geq 10$  ng/mL) showed increased sensitivity to adenosine diphosphate (ADP)-induced platelet aggregation. The concentration at which increased sensitization to ADP occurred (10 ng/ml) was ~10-fold higher than the expected clinical plasma concentration.

Single or repeat dose in vivo administration of AMG 531 in mice, rats, and non-human primates caused dose-dependent increases in platelet counts (3- to 5-fold). Intravenous and subcutaneous administrations of AMG 531 were equally effective in terms of pharmacodynamic response, i.e. increase in platelet counts. Non-human primates were less sensitive (~50-fold less) than rodents to the pharmacodynamic effects of AMG 531.

In BDF1 mice with or without splenectomy, AMG 531 (10-100 mcg/kg) produced similar degree of increase in platelet count (2 to 4-fold) suggesting that the spleen status did not have a critical influence on the pharmacological response of AMG 531.

**Safety Pharmacology:** In cynomolgus monkey, intravenous administration of AMG 531 (300-5000 mcg/kg) did not produce any remarkable effect on cardiovascular parameters including EKG. The NOAEL for the CVS safety study was 300-times the maximum human dose (MHD). In a CNS safety study in rats, no effects on Functional Observational Battery (FOB), body temperature or motor activity were observed at AMP2 doses up to 100 mcg/kg administered subcutaneously (17-times MHD based on systemic exposure).

**Pharmacokinetics:** Single and repeat dose PK/TK studies with AMG 531 were conducted in mice, rats and monkey. The bioavailability of AMG 531 after subcutaneous administration was low (20-25%) in rats and cynomolgus monkey. The T<sub>max</sub> varied from 4 to 16 hours depending on the species. The apparent elimination t<sub>1/2</sub> was 30 to 45 hours in rats but longer (~296 hours) in monkey suggesting a slow absorption process after subcutaneous administration in monkeys. Repeat-dose administration in rats (1 month) and cynomolgus monkeys (6 months) showed no significant drug accumulation and no apparent sex differences in PK parameters. The increase in systemic exposure (AUC) was greater than dose proportional in rats but not in monkeys. AMG 531 distributes widely into the extravascular space as indicated by the high V<sub>d</sub> values (100-200 ml/kg) across the species. AMG 531 was mainly excreted through urine (>80%).

PK and PD (increase in platelet counts) profile of AMG 531 was evaluated in splenectomized, nephrectomized and thrombocytopenic animals. The PK profile and the increase in platelet counts was similar in splenectomized rats as compared to normal rats suggesting that splenectomy did not affect the pharmacodynamic effect of AMG 531 and spleen is not involved in the removal of AMG 531. In nephrectomized rats, the AUC and t<sub>1/2</sub> were significantly higher suggesting kidney plays a major role in the clearance of AMG 531. In thrombocytopenic mice, the C<sub>max</sub> values were similar to normal mice at high dose (30 mcg/kg) of AMG 531 but not at lower concentration (3 mcg/kg, C<sub>max</sub> increased by 10-fold). Therefore, the impact of thrombocytopenia on AMG 531 PK is inconclusive.

Bridging studies were conducted to compare PK profile of AMG 531 using frozen liquid formulation (used in preclinical studies) to that with the clinical lyophilized formulation. Systemic exposure and increase in platelet count was similar for both formulations.

**Repeat-dose toxicity studies:**

**Rats:** In a repeat-dose toxicity study, AMG 531 (10, 30 & 100 mcg/kg, 1- to 37-times MHD based on systemic exposure) was administered subcutaneously three times weekly for 4-weeks. AMG 531 caused dose-dependent increases in platelet counts (2 to 4-fold). In general, adverse effects observed in rats were related to the over activation of thrombopoietic system caused by AMG 531. These effects occurred at clinically relevant doses and included extramedullary hematopoiesis, splenic enlargement, megakaryocytosis/megakaryocyte hyperplasia, femoral and sternal bone hyperostosis and bone marrow myelofibrosis. These effects were reversed by the end of 4-week recovery period. No NOAEL was established since one male died and myelofibrosis in one female was observed at the lowest dose tested (10 mcg/kg).

Mortality was observed at all dose levels tested. In many instances, deaths occurred in conjunction with blood sampling events. Although exact cause of death could not be determined, it is likely to be associated with extreme thrombocytosis, increased blood viscosity and difficulty in obtaining blood samples in these animals.

Rats seem to be the more relevant species in terms of pharmacodynamic and adverse effects observed in clinical studies. However, long-term toxicology studies in rats could not be conducted as AMG 531 produced immunogenic response in rats. Rats developed neutralizing antibodies to AMG 531 in approximately 50% of animals at all dose levels tested resulting in negation of the pharmacodynamic effect (increase in platelet count) of AMG 531.

**Monkeys:** AMG 531 (500, 1000 or 5000 mcg/kg, 30 to 300-times MHD) was administered subcutaneously to cynomolgus monkeys once weekly for 13 or 26 weeks followed by 8 week of recovery period. AMG 531 treatment caused dose-dependent increase in platelet counts (1.5 to 4-fold), megakaryopoiesis in the bone marrow and megakaryocytosis in the submandibular lymph node. These are expected effects based on the mechanism of action of AMG 531. These effects were reversed by the end of the recovery period. The NOAEL for the 6-month repeat-dose toxicity study was 5000 mcg/kg (300-times MHD).

The overall incidence of antibody development for AMG 531 in monkey was 54.2% binding antibodies (antibodies that bind to AMG 531 without affecting the pharmacodynamic response) and 4.2% neutralizing antibodies (antibodies that bind to AMG 531 and negate the pharmacodynamic response). In terms of pharmacodynamic effects, monkey seems to be less sensitive species than rats. In monkey, approximately 50-fold higher dose (5000 mcg/kg) was needed to achieve same response (3-4 fold increase in platelet counts) as seen in rats (100 mcg/kg). Unlike in rats, no myelofibrosis was observed in monkeys after 6 months of repeated dosing. Based on pharmacodynamics and adverse effects profiles, rat seems to be a more relevant species than monkey in terms of predicting these effects in humans.

#### **Reproductive toxicology:**

**Fertility and early embryonic development:** AMG 531 had no effect on male or female fertility in rats at doses up to 100 mcg/kg (37-times MHD based on AUC).

#### **Embryo-fetal development:**

**Rats:** AMG 531 was administered subcutaneously at doses of 10, 30 & 100 mcg/kg. There was no effect on any of the parameters evaluated for maternal and developmental toxicity. The NOAEL for maternal and fetal toxicity was 100 mcg/kg (11-times MHD based on AUC).

AMG 531 crosses the placenta resulting in substantial fetal serum concentration that was approximately 50% of maternal serum concentration. Accordingly, dose-dependent

significant increases in platelet counts (1.7- to 6-fold as compared to baseline values) and binding antibodies to AMG 531 were observed in fetuses. Thus, there is a potential for adverse effects related to thrombocytosis in fetuses due to placental transfer of AMG 531.

**Rabbits:** AMG 531 administered to pregnant rabbits at doses of 10, 30, 60 & 100 mcg/kg (4 to 82-times MHD based on AUC) had no effect on maternal or developmental toxicity at doses up to 60 mcg/kg (39-times MHD). At 100 mcg/kg dose, lower maternal body weight gain, food consumption and total body weight change adjusted for uterine weight were observed. One of 52 fetuses examined in the high dose group had external malformations characterized by gastroschisis, ectrodactyly and cutis aplasia (all in one fetus). This may be an incidental finding rather than treatment-related effect. In terms of pharmacodynamic effects, rabbit may not be relevant species since no significant increase in the platelet count was observed at doses up to 100 mcg/kg (82-times MHD).

**Mice:** AMG 531 (3, 10, 30 & 100 mcg/kg/day, 0.1 to 5-times MHD based on AUC) was administered three times weekly during GD 6-15. The high dose of 100 mcg/kg/day dose was associated with overall reduction in maternal body weight gain (8.4%). Developmental toxicity, as evidenced by increased post-implantation loss, was also attributable to the 100 mcg/kg/day dose of AMG 531. Consistent with the post-implantation loss, the numbers of live fetuses were reduced at the high-dose. NOAEL for maternal and developmental toxicity in mice was 30 mcg/kg/day (2-times MHD).

**Pre-natal and post-natal development:** Treatment of F0 dams with AMG 531 (10, 30 or 100 mcg/kg) caused slight prolongation of the gestation period at all doses and increase in the incidence of peri-natal F1 pup mortality at high dose in rats. For maternal toxicity, no definitive NOAEL was established and a NOAEL for survival and pre- and post-natal development of the F1 offspring was 30 mcg/kg (3-times MHD) of maternal dose.

**Antigenicity:** AMG 531 was immunogenic in mice, rats and monkeys. Neutralizing anti-drug antibodies developed in all species tested. Antibodies binding to endogenous TPO were also observed. However, no endogenous TPO neutralizing antibodies were detected in any of the species tested.

#### **Nonclinical safety issues relevant to clinical use**

**Bone marrow myelofibrosis:** In a 4-week repeat-dose toxicity study in rats, myelofibrosis was seen at clinically equivalent and higher doses, which was reversible by the end of 4-week recovery period. Whether myelofibrosis is reversible after much longer chronic treatment is not known since treatment duration was only 4-weeks. There is a potential for long-term treatment with AMG 531 to result in an irreversible fibrotic state leading to bone marrow dysfunction. In clinical studies, increase in reticulin formation in the bone marrow and one case of myelofibrosis was observed at doses  $\geq 5$  mcg/kg. The label for Romiplostim contains warning for the potential risk of reticulin formation and marrow fibrosis. Romiplostim will be available to patients only through a special restricted distribution program. The sponsor has proposed a risk management program for Romiplostim to monitor reticulin formation

***Carcinogenicity:*** There is a concern related to the tumor stimulation capacity of thrombopoietin and, therefore, AMG 531 ability to stimulate certain types of acute myeloid leukemia cells. However, the ability to assess potential carcinogenicity in a traditional chronic rodent study is negated since AMG 531 is highly immunogenic in rats and mice. Neutralizing antibodies were detected in mice and rats with a corresponding diminution of the platelet response. In a clinical study of Romiplostim administration to patients with a myelodysplastic syndrome (MDS), increased blast cells counts were observed in some patients. The proposed label for Romiplostim contains warning stating that stimulation of the thrombopoietin (TPO) receptor on the surface of hematopoietic cells may increase the risk for hematologic malignancies.

***Patients with renal impairment:*** No clinical studies were conducted in patients with renal impairment. In a PK study in nephrectomized rats, the t/12 was significantly longer and systemic exposure to AMG 531 was ~2-fold higher as compared to normal rats suggesting clearance of AMG 531 is likely to be slower in patients with renal impairment.

***Pregnancy and nursing mothers:*** AMG 531 crosses the placenta resulting in substantial fetal serum concentration that was approximately 50% of maternal serum concentration. Accordingly, dose-dependent significant increases in platelet counts (1.7- to 6-fold as compared to baseline values) were observed in fetuses. AMG 531 is a recombinant protein that has an Fc domain of human IgG. It is known that maternal IgG can be transferred by the FcRn receptor to the fetus and similar mechanism may be responsible for AMG 531. It is likely that placental transfer of AMG 531 may occur in humans. AMG 531 should be administered to pregnant women only if the potential benefit justifies the risk. The Maternal Health Team (MHT) has recommended that sponsor develop and maintain a pregnancy exposure registry for the pregnancy and fetal outcomes of women exposed to Romiplostim during pregnancy as a post-marketing commitment.

No preclinical studies were conducted to evaluate excretion of AMG 531 in breast milk. The presence of the Fc domain in AMG 531 molecule, though, suggests that AMG 531 is likely to be secreted in the breast milk. Caution should be exercised when Romiplostim is administered to a nursing mother. The MHT has recommended that sponsor consider conducting a lactation study in a subset of women enrolled in the registry, who choose to breastfeed their infants despite the potential risks, to assess the presence of Romiplostim in breast milk and potential effects in nursing infants.

## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

AMG 531 (Amgen Megakaryopoiesis Protein-2 or AMP2) is a thrombopoiesis stimulating fusion protein that increases platelet production through activation of the thrombopoietin receptor (TPO or c-Mpl receptor). AMG 531 is expressed recombinantly in *Escherichia coli* and purified as a dimer consisting of 2 Fc-peptide-peptide subunits. The complete molecule is comprised of a human immunoglobulin IgG1 Fc domain, with each single chain subunit covalently linked at the C-terminus to a peptide chain containing two thrombopoietin receptor binding domains. According to the sponsor, the Mpl binding (peptide) domain and Fc carrier domain respectively confer the biological activity and control persistence of the drug in the body. The basic structure of AMG 531 is shown in the figure below.



AMG 531 has no amino acid sequence homology to endogenous thrombopoietin (eTPO) and was designed to stimulate platelet production by mimicking the action of thrombopoietin but without the potential to induce an antibody reaction that could in turn bind to and affect the function of the natural ligand (eTPO). AMG 531 is indicated for the treatment of thrombocytopenia in adult patients with idiopathic thrombocytopenic purpura (ITP) who have had an insufficient response to corticosteroids, immunoglobulins or splenectomy. ITP is an autoimmune disorder characterized by platelet destruction caused by anti-platelet auto-antibodies. In adults, ITP is more common in women than in men (ratio of approximately 2:1) and rarely remits spontaneously. Auto-antibodies produced by patients with ITP have been shown to bind to megakaryocytes and platelets, suggesting that these patients may have impaired thrombopoiesis. The recommended maximum clinical dose for AMG 531 is 10 mcg/kg injected subcutaneously once a week. The drug, due to the nature of ITP disease process is intended for chronic use.

**BLA number:** 125268  
**Review number:** 001  
**Sequence number/date/type of submission:** 10/23/07  
**Information to sponsor:** Yes ( ) No (X)  
**Sponsor and/or agent:** Amgen, Inc.  
 Thousand Oaks, CA 91320  
**Manufacturer for drug substance:** Amgen, Inc.  
  
**Reviewer name:** Tushar Kokate, Ph.D.  
**Division name:** Division of Medical Imaging &  
 Hematology Drug Products  
**HFD #:** 160  
**Review completion date:** 5/06/08  
  
**Drug:**  
     Trade name: Romiplostim/Nplate  
     Code name: AMG-531, AMP-2  
     Chemical name: Fusion protein  
     Molecular formula/molecular weight: 59 kilodalton

**Drug class:** Thrombopoietin receptor analog

**Intended clinical population:** AMG 531 is indicated for the treatment of thrombocytopenia in adult patients with chronic ITP who have had an insufficient response to corticosteroids, immunoglobulins or splenectomy.

**Clinical formulation:** The AMG 531 lyophilized formulation is a white, solid cake that is reconstituted with sterile water for injection. After reconstitution, the solution contains 0.25 or 0.5 mg/mL AMG 531, ~mM histidine, ~mannitol, ~sucrose, and ( ~ polysorbate 20 at pH ~. Each vial of AMG 531 is intended for a single use.

**Route of administration:** Subcutaneous (once a week)

**Disclaimer:** Tabular information was constructed by the reviewer unless cited otherwise. Figures/graphs were copied from the sponsor's submission.

**Studies reviewed within this submission:**

<i>Study</i>	<i>Title</i>
R2006118	A comparison of the binding of AMP2 to hu-Mpl versus mu-Mpl using a Biacore ~
R20070018	AMG 531 and human MPL binding analysis
102177	The binding of AMG 531 and rHu TPO to platelets and stabilized Mpl for multiple species

00-111	In vitro effects of AMP2 on platelet function and intra-platelet signaling
PP01103	The effect of MGDF and/or AMP2 on in vitro MK-CFC derived colony formation from baboon, cynomolgus or rhesus bone marrow cells
PP01104	The effect of MGDF and/or AMP2 on in vitro MK-CFC derived colony formation from human bone marrow cells
PP01114	Similar pharmacodynamic effects of a single injection of AMP2 administered intravenously or subcutaneously
PP01197	Dose titration of AMP2
PP01122	A dose titration of AMP2 intravenous or subcutaneous in normal female CD rats
PP01126	Efficacy of AMP2 in normal mice compared to splenectomized mice
PP01116	AMP2 treatment of an ITP model in W/BF1 mice
100882	Receptor binding assay
101497	Cardiovascular safety pharmacology study in cynomolgus monkeys
101954	A study of the pharmacological effects on the CNS of AMP2 via a single subcutaneous dose in SD rats
100909	A PK/PD study of AMP2 in male SD rats following intravenous or subcutaneous administration
100895	A Pk/PD study of AMP2 following intravenous administration in C57BL/6J mice
100998	A PK/PD study of AMP2 following single dose intravenous or subcutaneous administration in male rhesus monkeys
101604	A PK/PD study of AMP2 following single dose intravenous or subcutaneous administration to male cynomolgus monkeys
101995	Quantitative whole body autoradiography and tissue distribution of radioactivity following intravenous administration of <sup>125</sup> I-AMP2 to female rats
100896	A Pk/PD study of AMP2 following intravenous administration in Fc-Rn Knock-Out and Wild Type mice
100995	Pharmacokinetics of AMP2 following a single intravenous dose administration in control, sham and splenectomized male SD rats
101402	A pharmacokinetic study of AMP2 following intravenous administration in bilaterally nephrectomized male SD rats
101896	A PK study of AMG 531 in normal and thrombocytopenic mice
102342	A low dose PK study of AMG 531 in normal and thrombocytopenic mice
102921	A pharmacokinetic study of AMG 531 in male rats to compare the frozen liquid and the lyophilized formulations following single subcutaneous administration
103126	2-Week subcutaneous injection pharmacology study of AMG 531 in female rats for comparison of platelet counts between two formulations
103974	Single dose subcutaneous injection toxicity study with AMG 531 in rats
100876	4-Week subcutaneous toxicity and toxicokinetic study with AMP2 in rats with a 4-week recovery
100877	A 4-week toxicity study of AMP2 administered by subcutaneous or intravenous injection to rhesus monkeys, with a 4-week recovery period
101158	A 4-week repeated dose toxicity study of AMP2 administered subcutaneously to female cynomolgus and rhesus monkeys followed by a 4-week recovery period
101814	A 3 and 6 month repeated dose toxicity study of AMP2 administered



## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

#### *In vitro studies:*

In vitro binding studies showed AMG 531 has high affinity for the TPO receptor (c-Mpl). The affinity of AMG 531 for c-Mpl measured by Biacore assay suggested that AMG 531 had somewhat higher affinity (Kd: 14 nM) than TPO (Kd: 33 nM) for human TPO receptor (c-Mpl). However, the affinity varied depending upon the assay method employed. Using displacement of radio-iodinated TPO from human platelets, AMG 531 had a 17-fold lower affinity than TPO for human c-Mpl. Therefore, the in vitro study results need to be considered within the context of the assay methods employed.

In vitro binding of AMG 531 to c-Mpl stimulated megakaryocyte colony-forming cell (MK-CFC) proliferation in cynomolgus monkey, baboon, and human hematopoietic progenitor cells and from isolated human CD34+ mobilized peripheral blood stem cells. Tests evaluating competitive binding of AMG 531 versus radio-iodinated recombinant human thrombopoietin (rHuTPO) indicated that AMG 531 binds with high affinity to rat, cynomolgus monkey, and human platelets.

Platelets incubated in the presence of AMG 531 ( $\geq 10$  ng/mL) showed increased sensitivity to adenosine diphosphate (ADP)-induced platelet aggregation. The concentration at which increased sensitization to ADP occurred (10 ng/ml) was ~10-fold higher than the expected clinical plasma concentration.

#### *In vivo studies:*

Pharmacodynamic studies using AMG 531 were performed in mice, rats, and non-human primates. Single or repeat dose administration of AMG 531 in these species caused dose-dependent increases in platelet counts. Intravenous and subcutaneous administrations of AMG 531 were equally effective in terms of pharmacodynamic response, i.e. increase in platelet counts. Non-human primates were much less sensitive (~50-fold less) than rodents to the pharmacodynamic effects of AMG 531.

In mice and rats, increase in platelet counts were seen at doses  $\geq 1.0$   $\mu$ g/kg with maximum increases of 4 to 5-fold occurring at doses of 100 & 300 mcg/kg. By contrast, in monkeys 50-fold higher dose (5000 mcg/kg) was required to achieve similar pharmacodynamic response. The increase in platelet counts peaked at approximately 6 to 10 days after dosing and returned to baseline by approximately Day 20.

In BDF1 mice with or without splenectomy, AMG 531 (10-100 mcg/kg) produced similar degree of increase in platelet count (2 to 4-fold) suggesting that the spleen status did not have a critical influence on the pharmacological response of AMG 531.

**Safety Pharmacology:**

CVS and CNS safety studies were conducted in monkey and rats, respectively. In cynomolgus monkey, intravenous administration of AMG 531 (300-5000 mcg/kg) did not produce any remarkable effect on CVS parameters including EKG. The NOAEL for the CVS safety study was 300-times the maximum human (MHD).

In a CNS safety study in rats, no effects on FOB, body temperature or motor activity were observed at doses of 10-100 mcg/kg (17-times MHD).

**2.6.2.2 Primary pharmacodynamics****Mechanism of action:****In vitro pharmacodynamic studies:****1) Study #R2006118: A comparison of the binding of AMP2 to human-MPL versus murine-MPL**

The objective of this study was to compare binding of AMP2 to human-MPL versus murine-MPL receptors as measured by Biacore assay.

**Methods:****Results:**

The results from the study are summarized in the table below.

	AMP2			TPO		
	Kd (nM)	Ka (1/ms)	Kd (1/s)	Kd (nM)	Ka (1/ms)	Kd (1/s)
Human-MPL	14	$5.72 \times 10^6$	$8.13 \times 10^{-2}$	33	$7.6 \times 10^5$	$2.49 \times 10^{-2}$
Murine-MPL	3.5	$1.61 \times 10^7$	$5.53 \times 10^{-2}$	66	$5.0 \times 10^5$	$3.26 \times 10^{-2}$

**Conclusions:**

The affinity (Kd) of AMP2 to human-Mpl receptor was 4-times lower than that of AMP2 to murine-Mpl receptor (14 nM versus 3.5 nM). This was primarily due to the slower on rate (ka) of AMP2 binding to human-Mpl than the binding to murine-Mpl. In contrast, the affinity of TPO for human-Mpl (Kd: 33 nM) was 2-fold higher than its affinity for

murine-Mpl (K<sub>d</sub>: 66 nM). However, TPO had a lower binding affinity for both human and murine-Mpl receptors as compared to AMG 531.

## 2) Study #R20070018: AMG 531 and human Mpl binding analysis

The objective of this study was to determine the affinity of rhu-Mpl (recombinant human MPL) binding to three AMG 531 samples generated from different lots.


### Methods:

Binding assays were carried out on the immobilized rhu-Mpl surfaces. AMG 531 (2 nM and 6 nM) was incubated with various amounts of rhu-Mpl in sample buffer (0.1 mg/mL BSA and 0.005% P20 in PBS) for over 4 hours before injection over the immobilized rhu-Mpl surfaces.

100% AMG 531 binding signal was determined in the absence of rhu-Mpl in solution. Since only free AMG 531 molecules were able to bind to rhu-Mpl surface, a decreased binding response with increasing concentrations of incubated rhu-Mpl indicated binding of AMG 531 to rhu-Mpl in solution. Thus, binding signal was proportional to the concentration of free AMG 531 at equilibrium with a given rhu-Mpl concentration in solution.

### Results:

The results are summarized in the table below.

Sample	Lot #	K <sub>D</sub> (95% CL, pM)
AMG 531 control	ID 035278	510 (160-1000)
	ID 035280	660 (360-1100)
	ID 035282	650 (320-1100)

### Conclusion:

The data suggests that all three AMG 531 samples bind to rhu-Mpl (recombinant human MPL) with similar affinities (~600 pM).

## 3) Study #102177: The binding of AMG 531 and rHu-TPO to platelets and stabilized Mpl for multiple species

The objective of this study was to compare in vitro binding of AMG 531 and recombinant human TPO (rHu-TPO) to platelets and stabilized Mpl from human, rats, rabbit and monkey. The binding displacement assay was carried out using radio-iodinated TPO.

**Results:**

Both cold TPO and AMG 531 fully displaced the labeled (iodinated)-TPO using human, rat and monkey platelets. For rabbit platelets, the total binding of iodinated-TPO was very low (comparable to the background levels) and there was no displacement of labeled TPO by the cold TPO or AMG 531. The sponsor states that the rabbit platelets used for the study were not of the quality seen with other animal sources. However, sponsor did not provide details or further explanation on the quality of rabbit platelets used in the study.

The half-maximal concentrations (EC50) of displacement for rHu-TPO and AMG 531 are summarized in the table below.

EC50 >>	Human Platelets	Rat Platelets	Monkey Platelets
rHu-TPO	3.6 ng	1.4 ng	4.4 ng
AMG 531	60 ng	82 ng	50 ng

**Conclusions:**

In general, rHu-TPO had much higher affinity than AMG 531 for displacement of iodinated-TPO from platelets derived from various species. For human platelets, AMG 531 had a 17-fold lower affinity than rHu-TPO for human c-Mpl. In general, AMG 531 had similar affinity for human, rat and monkey platelets. There was no specific binding of AMG 531 or rHu-TPO observed for the platelets derived from rabbit.

**4) Study #00-111: In vitro effects of AMP2 on platelet function and intra-platelet signaling**

The objective of this study was to determine the in vitro effects of AMP2 (1-100 ng/ml) on platelet function (platelet aggregation) and intra-platelet signaling (phosphorylation of c-mpl and JAK2 pathways).

**Methods:**

**Effects of AMP2 on human platelet function in vitro:** Blood samples from ten healthy male volunteers were used for the study. To evaluate platelet function, an adenosine diphosphate (ADP) concentration-platelet aggregation response curve was established using concentrations of ADP between 0.1-20  $\mu$ M. The ADP concentration-platelet aggregation response curve was repeated in the presence of AMP2 at 1, 10 and 100 ng/ml.

**Effects of AMP2, TMP and Fc-Leptin on human platelet function in vitro:** Blood samples from ten male volunteers were used for the study. A sub-maximal ADP concentration (0.6-3  $\mu$ M) was selected for each individual, determined by the platelet response to various concentrations of ADP. The platelets were then incubated with either AMP2 (1, 10, 100 ng/ml), PEG-MGDF (100 ng/ml), Fc-Leptin (1  $\mu$ g/ml), the

thrombopoietin mimetic peptide (TMP, 100 ng/ml) or PBS for five minutes at 37°C. The platelet aggregation response to sub-maximal ADP was assessed in each condition.

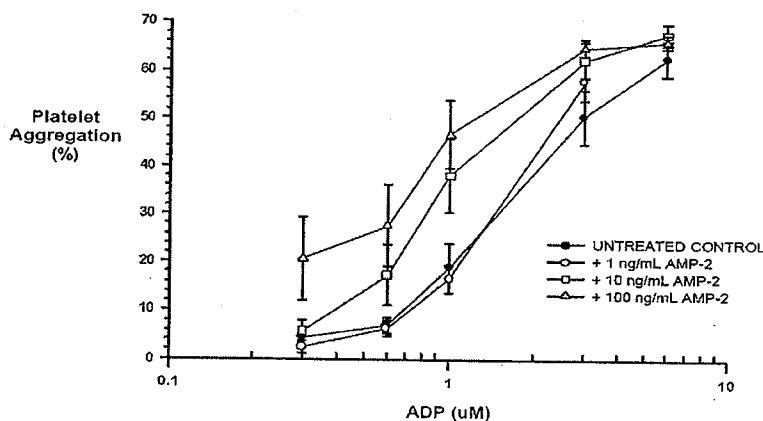
**Effects of AMP2 on rabbit platelet function in vitro:** To assess platelet aggregation, blood samples were collected from eight female New Zealand White rabbits. A sub-maximal ADP concentration (0.6-3  $\mu$ M) concentration was selected for each individual rabbit, determined by the platelet response to various concentrations of ADP. The platelets were then incubated with AMP2 (1, 10, 100 ng/ml) for five minutes at room temperature to determine the platelet aggregation response to sub-maximal ADP concentrations.

**Intra-platelet signaling (phosphorylation of the c-mpl and JAK2):** Blood samples from four healthy male volunteers were used to determine phosphorylation of the c-mpl and JAK2 by AMP2 (1  $\mu$ g/ml) by incubating platelets for 10 minutes at 37°C. Immunoprecipitations of c-mpl and JAK2 were performed on the pre-cleared platelet lysates and electrophoretically separated proteins were transferred onto PVDF membranes for western blot analysis.

## Results:

**Effects of AMP2 on human platelet function in vitro:** Platelet aggregation response to a range of ADP concentrations (0.2-6  $\mu$ M) was determined in the presence of AMP2 (1, 10 and 100 ng/ml). As shown in the figure below, there was a leftward shift in the concentration-response curve in the presence of AMP2 (10 & 100 ng/ml), suggesting platelets are more sensitive to ADP at these concentrations of AMP2. AMP2 at 1 ng/ml did not alter platelet sensitivity to ADP.

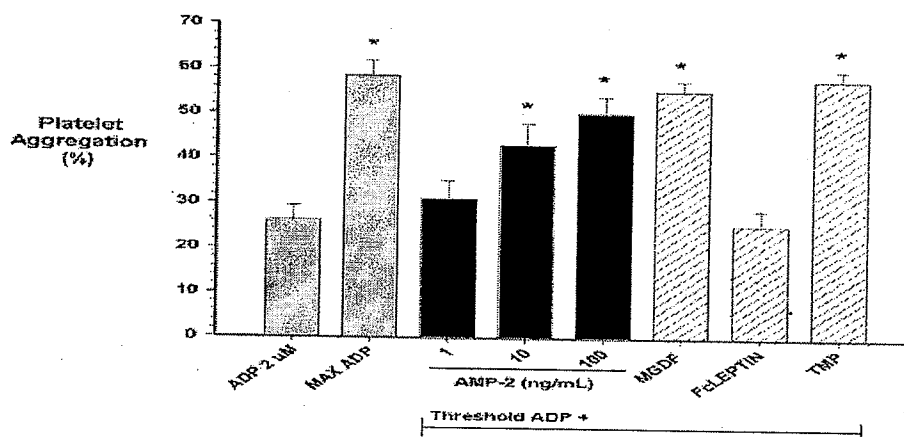
Effects of AMP2 on Human Platelet Function



Co-incubation of threshold concentrations of ADP (2  $\mu$ M) with AMP2 (1, 10 & 100 ng/ml) augmented the platelet aggregation response (see figure below). This enhanced

aggregation response was similar to that observed with  $\alpha$ -MGDF. The sensitization did not appear related to the Fc portion of the AMP2 molecule, as a similar Fc-conjugated molecule, Fc-Leptin, did not affect the platelet response. In contrast, incubation with the thrombopoietin mimetic peptide (TMP) portion of the AMP2 molecule did induce a sensitization response.

#### Effects of AMP2, TMP and Fc-Leptin on Human Platelet Function



Human platelets incubated with 1  $\mu$ g/ml AMP2 induced tyrosine phosphorylation of c-mpl and JAK2. It appears that tyrosine phosphorylation of c-mpl and JAK2 is associated with platelet sensitization in human platelets in vitro

**Effects of AMP2 on rabbit platelet function in vitro:** When threshold concentrations of ADP were used, co-incubation with AMP2 augmented the response of rabbit platelets. The platelet sensitization was concentration dependent between 10 and 100 ng/ml.

#### Conclusions:

AMP2 ( $\geq 10$  ng/ml, 10-times clinical Cmax) enhanced platelet sensitivity to ADP by augmenting ADP-induced platelet aggregation in both human and rabbit platelets in vitro. This platelet sensitization is associated with tyrosine phosphorylation of c-mpl and Jak2 in human platelets in vitro.

#### 5) Study #PP01103: The effect of MGDF and/or AMP2 on in vitro MK-CFC derived colony formation from baboon, cynomolgus or rhesus monkey bone marrow cells

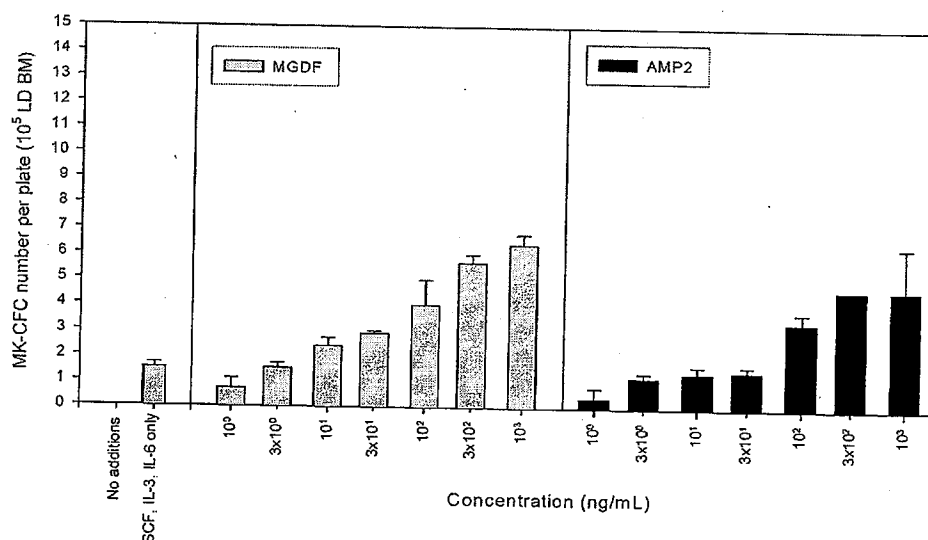
The addition of proteins, which bind mpl, to bone marrow cell cultures results in an increase in the formation of megakaryocyte-colony forming cells (MK-CFC)-derived colony formation. The objective of this study was to evaluate the effects of AMP2 and/or MGDF (megakaryocyte growth & development factor) on MK-CFC derived colony formation by baboon, cynomolgus or rhesus monkey bone marrow cells. The intention was to obtain an indication of the relative potency of AMP2 across primate species.

### Methods:

Bone marrow aspirates were obtained from 3 primate species – baboon (n=2), cynomolgus (n=2) and rhesus (n=1) monkeys. Low density bone marrow (LDBM) cells were plated at either 100,000/mL or 50,000/mL in a serum-depleted fibrin clot based assay system optimized for the growth and development of MK-CFC in the presence of escalating doses of MGDF and/or AMP2.

### Results:

Cynomolgus LDBM cells responded to MGDF (1-1000 ng/ml) and AMP2 (1-1000 ng/ml) by promoting the stimulation of MK-CFC derived colony formation in a dose-dependent fashion (see the figure below). MGDF was more potent colony stimulating factor when compared to AMP2.



Similar to cynomolgus derived LDBM cells, baboon LDBM cells also responded to MGDF and AMP2 by promoting the stimulation of MK-CFC derived colony formation in a dose-dependent fashion. However, baboon LDBM cells were more sensitive to MGDF and AMP2 than were cynomolgus LDBM cells since higher MK-CFC derived colony numbers were obtained in each culture throughout the dose-response range. As compared to doses of either MGDF or AMP2 alone, no additive or synergistic effects were observed on the development of MK-CFC derived colony formation when MGDF (1 & 1000 ng/ml) was added to cultures in combination with various concentrations of AMP2 (1, 10, 100 & 1000 ng/ml) in baboon LDBM cells. Similar results were obtained for LDBM cells derived from rhesus monkey (data not shown).

### Conclusion

All three primate species (cynomolgus, baboon and rhesus) responded to both MGDF and AMP2 in promoting the stimulation of MK-CFC derived colony formation. No additive

or synergistic effects were observed on the development of MK-CFC derived colony formation when MGDF and AMP2 were added to cultures simultaneously.

**6) Study #PP01104: The effect of MGDF and/or AMP2 on in vitro MK-CFC derived colony formation from human bone marrow cells**

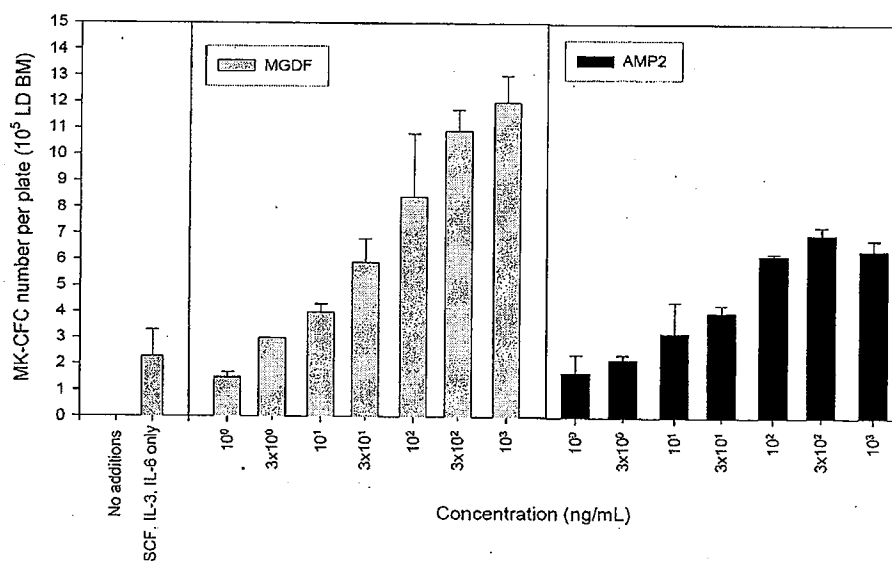
The objective of this study was to evaluate effects of AMP2 and/or MGDF (megakaryocyte growth & development factor) on in vitro MK-CFC (megakaryocyte-colony forming cells) derived colony formation from human bone marrow cells.

**Methods:**

Bone marrow aspirates were obtained from normal healthy volunteers. Either LDBM (low density bone marrow cells) or CD34 + LDBM cells were cultured in a serum-depleted fibrin clot based assay system optimized for the growth and development of MK-CFC in the presence of increasing doses of MGDF and/or AMP2.

**Results:**

Both human LDBM and CD34 + LDBM cells responded to MGDF (1-1000 ng/ml) and AMP2 (1-1000 ng/ml) by promoting the stimulation of MK-CFC derived colony formation in a dose-dependent fashion. The figure below shows human LDBM MK-CFC growth in response to graded doses of MGDF and AMP2. MGDF was a more potent colony stimulating factor than AMP2. No additive or synergistic effect occurred when MGDF, at suboptimal (1 ng/mL) or optimal (1000 ng/mL) concentrations was added to increasing doses of AMP2 (1-1000 ng/ml).



**Conclusion:**

Both MGDF and AMP2 promoted the stimulation of MK-CFC derived colony formation; MGDF was more potent than AMP2 in stimulating the MK-CFC derived colony formation.

**7) Study #100882: Receptor binding assay ( )**

To assess selectivity, AMG 531 was screened *in vitro* in a \_\_\_\_\_ assay for receptor binding affinity to sixty-three different receptors, enzymes, or ion channel targets. AMG 531 was tested at concentrations of 0.2 and 2  $\mu$ M in duplicate for each of the receptor/enzyme targets included in the profile. The highest concentration chosen was at least 10-fold higher than the highest concentration found in the monkey plasma following administration of AMG 531 at a dose of 5000 mcg/kg (300-times MHD). Using the criterion that inhibition of  $\geq 50\%$  constitutes activity at a given receptor, AMG 531 did not show activity at any of the receptors or enzymes tested. Based on these results, the sponsor concluded that AMG 531 is not anticipated to cause any substantive pharmacologic activity *in vivo* other than the expected pharmacodynamic effect, i.e. activation of TPO receptor.

**In vivo pharmacodynamic studies:****8) Study #PP01114: Similar pharmacodynamic effects of a single injection of AMP2 administered intravenously or subcutaneously**

This study was conducted to determine if the route of injection, subcutaneous or intravenous, alters the pharmacodynamic profile of AMP2 in mice. AMP2 produces a pharmacodynamic effect as measured by increased platelet numbers in the *in vivo* studies.

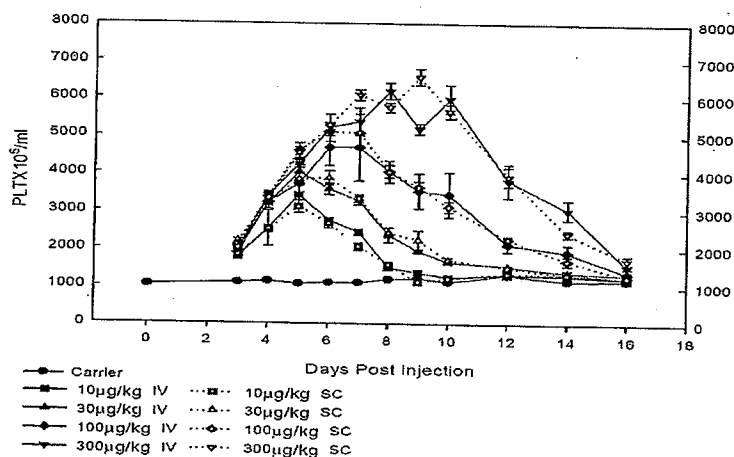
**Methods:**

Normal female BDF1 mice were divided into 9 groups of 20 mice each, and injected either subcutaneous or intravenous with 0, 10, 30, 100 & 300 mcg/kg doses of AMP2. Platelet levels were monitored for 16 days post-dosing. Blood samples were obtained on Days 0 (control only), 3-10, 12, 14 and 16 post-administration. Complete and differential blood count analyses were conducted for each blood sample.

**Results:**

As shown in the figure and table below, the platelet levels in mice treated acutely with AMP2 increased in a dose-dependent manner regardless of route of administration (subcutaneous or intravenous). The time-to-peak for platelet levels seem to increase with the increasing doses of AMP2.

Acute dose (mcg/kg, iv or sc)	Peak increase as compared to baseline values	Time-to-peak (Days post-administration)
10	3-fold	Day 5
30	4-fold	Days 5-6
100	4.5-fold	Days 6-7
300	5-fold	Days 8-10



### Conclusions:

Dose- and time-dependent increases in platelet count (3 to 5-fold) were observed in response to an acute injection of AMP2 (10-300 mcg/kg) in mice. The route of injection (intravenous or subcutaneous) had no effect on the platelet response.

### 9) Study #PP01197: Dose titration of AMP2

This study was similar to the earlier in vivo study in mice (# PP01114) except that AMP2 was administered subcutaneously at lower doses (0.1, 0.3, 1, 3, 5, 10 & 100 mcg/kg, n=5/group) for dose titration purpose to establish a lowest dose level of AMP2 that generates an effect on platelet number.

### Results:

Similar to the findings in study # PP01114, high dose of 100 mcg/kg AMP2 caused ~5-fold increase in platelet levels and the values returned to normal around Day 17. At doses below 1 mcg/kg, mice did not respond to the AMP2 treatment. The threshold for platelet response appears to be between 1 and 3 mcg/kg. Below this, the platelet increase was minimal. The table below shows the dose-dependent increase in platelet levels.

Acute dose (mg/kg, sc)	Peak increase as compared to baseline values	Time-to-peak (Days post-administration)
0.1-0.3	No increase	---
1	<50%	Day 5
3	2-fold	Day 5

5	2.5-fold	Days 5
10	3-fold	Days 5
100	5-fold	Days 6

**Conclusions:**

Dose-dependent increases in platelet count were observed in response to an acute injection of AMP2 in mice. Platelet count approximately doubled after treatment with 3 mcg/kg of AMP2. The threshold for platelet response appears to be between 1 and 3 mcg/kg.

**10) Study #PP01122: A dose titration of AMP2 intravenous or subcutaneous in normal female CD rats**

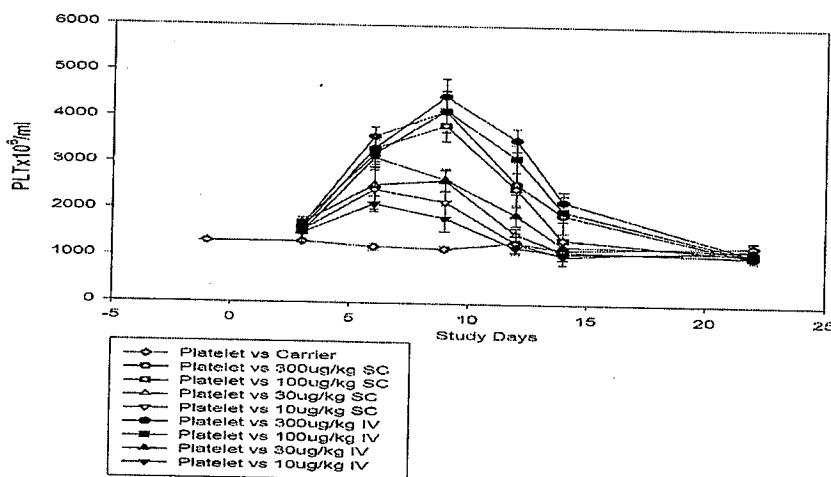
The purpose of this study was to evaluate the effect of route of administration and various doses of AMP2 on platelet count in rats.

**Methods:**

Each cohort of 4 normal female CD rats was treated with a single dose of AMP2 administered via subcutaneous or intravenous bolus injection. Treatment groups received doses of 0 (vehicle control, PBS), 10, 30, 100 or 300 mcg/kg. Blood samples were collected on Days -1, 3, 6, 9, 12, 14 & 21 post-injection.

**Results:**

As shown in the figure below, animals treated with AMP2 showed a dose-dependent increase in peripheral blood platelet levels. Platelet levels peaked by Days 6-9 post-injection, and returned to normal by Day 21. The time-to-peak appears to increase with increasing doses of AMP2.



At the lowest dose tested (10 mcg/kg, iv or sc), there was ~2-fold increase in the platelet levels as compared to baseline and at the highest dose (300 mcg/kg, iv or sc), the maximum increase was ~4.5-fold. The response was similar for both subcutaneous and intravenous routes of injection for each dose of AMP2.

**Conclusion:**

AMP2 (10-300 mcg/kg) caused 2 to 5-fold increase in the platelet levels in rats. As seen in mice, intravenous or subcutaneous administration did not show any clear advantage in terms of pharmacodynamic response or bioavailability of AMP2. Dose response was equivalent in either case.

**11) Study #PP01126: Efficacy of AMP2 in normal compared to splenectomized mice**

Splenectomy is an accepted treatment in ITP patients that are refractory to steroid or other therapies. In this study, efficacy of AMP2 was compared in splenectomized and normal mice.

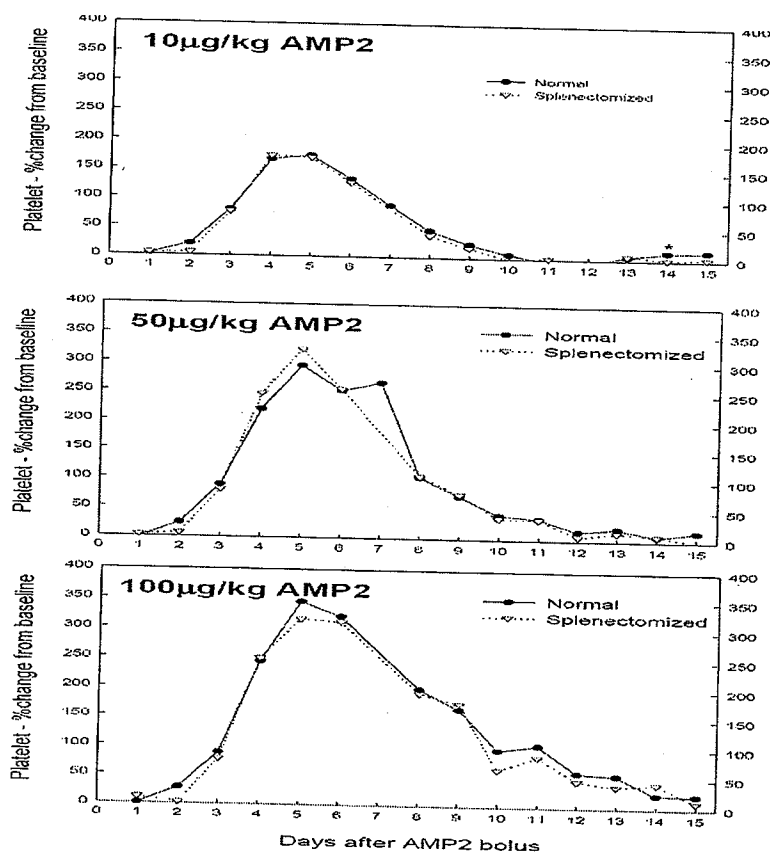
**Methods:**

Normal and splenectomized (spleen surgically removed) female BDF1 mice were treated with a single subcutaneous dose of AMP2 (0, 10, 50 & 100 mcg/kg, n=5/group) and platelet response was monitored for 2 weeks. A normal baseline platelet range was established for both normal and splenectomized mice by taking daily blood samples from 5 control mice per day throughout the study.

**Results:**

The normal platelet range for splenectomized mice was higher than for normal mice. Mean platelet counts in normal mice were  $1343 \times 10^9/L \pm 129$  and in splenectomized mice  $1532 \times 10^9/L \pm 142$ .

Splenectomized mice and normal mice responded to AMP2 in a similar dose-dependent manner. Normal and splenectomized mice given the same dose of AMP2 had similar platelet responses (see the figure below).



Acute dose (mcg/kg, sc)	Peak increase (% baseline)		Time-to-peak (post-dosing)	
	Normal	Splenectomized	Normal	Splenectomized
10	170%	170%	Day 4	Day 5
50	300%	325%	Day 5	Day 5
100	325%	350%	Day 6	Day 5

\* Depending on the dose, platelet counts remained significantly elevated as compared to the respective controls from Days 2-14 and returned to baseline by Days 10-15. Higher doses took longer time for return to baseline.

### Conclusion:

AMP2 was equally effective in terms of increase in platelet count in splenectomized mice as it was in the normal mice.

### 12) Study #PP01116: AMP2 treatment of an ITP model in W/BF1 mice

The W/BF1 (NZW x BXSB) is a mouse model of generalized autoimmune disease with many features in common with systemic lupus erythematosus. According to the sponsor, male (but not females) W/BF1 mice develop auto-antibodies to platelets leading to an immune mediated thrombocytopenia similar in some aspects to human ITP. The objectives of this study were to determine if thrombocytopenic W/BF1 mice respond to

AMP2 treatment and to examine the effects of AMP2 treatment pre- and post-splenectomy in these animals.

**Results:**

*Development of thrombocytopenia in W/BF1 mice:* Male W/BF1 mice showed a progressive thrombocytopenia over time. At 8-10 weeks of age, platelet counts were within a normal range ( $900-1500 \times 10^9/L$ ) but progressively decreased over the time. By 23 weeks of age, all surviving animals had platelet counts below  $500 \times 10^9/L$  and were considered thrombocytopenic.

*Splenectomy treatment:* Splenectomy was performed after thrombocytopenia was established in majority of the animals (18-23 weeks) and platelet counts were determined one week post-surgery. The response to splenectomy was variable, with some animals showing 2 to 5-fold increase in platelet count (splenectomy responders: ~70%), while others failed to respond (splenectomy non-responders). During the 20 weeks following surgery, splenectomy-responders relapsed and re-developed thrombocytopenia.

*AMP2 treatment:* AMP2 (50 mcg/kg, sc) administration to thrombocytopenic mice resulted in 2 to 7-fold increase in platelet counts. Splenectomy non-responders and animals that re-developed thrombocytopenia following splenectomy showed a significant increase in platelet counts in response to AMP2 administration. Although, AMP2 administration provided symptomatic relief in terms of elevated platelet counts, it did not offer any survival advantage in this generalized autoimmune disease animal model.

**Conclusion:**

AMP2 treatment was effective in increasing the platelet count for both pre- and post-splenectomy thrombocytopenia in this model of generalized autoimmune disease.

**2.6.2.3 Secondary pharmacodynamics: N/A****2.6.2.4 Safety pharmacology:****Neurological effects:*****Study title:***

**A study of the pharmacological effects on the central nervous system of AMP2 via a single subcutaneous dose in the Sprague-Dawley rat**

**Key study findings:**

There were no remarkable behavioral effects following a single subcutaneous administration of AMP2 at doses up to 100 mcg/kg (17-times MHD based on systemic exposure) in rats.

**Study no.:** 101954

**Volume # and page #:** 4.2.1.3.1-101954

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** Nov 16, 2001

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Lot # and bioactivity:** 1111269M9, 94% of standard potency

**Methods:**

The objective of this study was to evaluate the effects of AMP2 on the CNS following a single subcutaneous administration in the Sprague-Dawley rats (main study: 8/sex/dose, TK satellite group: 4/sex/dose).

**Dose:** 0 (vehicle control: 10 mM sodium acetate & 5% sorbitol), 10, 30 & 100 µg/kg

**Parameters:** FOB, motor activity & grip strength and body temperature

**Frequency of observation:** Pre-dose, 12 & 48 hours and 8 days post-dose

**Toxicokinetic time-points:** 2, 12 & 48 hours and 5 days post-dosing. Serum levels of AMP2 measured using a validated ELISA assay.

**Results:**

***Behavioral effects:***

No remarkable treatment related changes noted during the FOB and there was no apparent effect on body temperature or motor activity.

***Toxicokinetics:***

AMP2 concentration was below the assay quantification limit (0.27 ng/ml) in the 10 mcg/kg dose group animals. At 30 & 100 mcg/kg doses, the C<sub>max</sub> and AUC values were 4.35 & 31.8 ng/ml and 106 & 1100 ng.h/ml, respectively. The T<sub>max</sub> was 12 hours post-dosing. At 5 days post-dosing, AMP2 concentration was not detectable at 10 & 30 mcg/kg doses and <1 ng/ml at 100 mcg/kg.

***Reviewer's comments:***

Based on the data provided, the NOAEL was 100 mcg/kg (17-times MHD based on AUC). It should be noted that high dose produces a maximal response (~4-fold increase) in the primary pharmacologic endpoint, i.e. an increase in peripheral platelet count.

**Cardiovascular effects:**

***Study title:***

**Cardiovascular evaluation of AMP2 in cynomolgus monkeys via bolus intravenous injection**

**Key study findings:**

AMP2 (500-5000 mcg/kg) did not affect CVS parameters including EKG. The NOAEL for the CVS safety study in monkey was established at 5000 mcg/kg (300-times MHD).

**Study no.:** 101497

**Volume # and page #:** 4.2.1.3.1-101497

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** Feb 19, 2001

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug lot # and % purity/potency:** 1111269M9, 94%

**Methods:**

The objective of this study was to evaluate the effects of a single slow intravenous bolus injection (dosing duration: 1 min) of AMP2 on the cardiovascular function in conscious telemetered Cynomolgus monkeys (males, 3/dose, 2-5 years old).

*Dose:* 0 (vehicle control: 10 mM sodium acetate & 5% sorbitol), 500, 1000 & 5000 µg/kg

*Parameters evaluated:* Systemic blood pressure (systolic, diastolic and mean), heart rate, EKG (RR, PR, QRS & QT/QTc intervals), body temperature and daily clinical observations.

*Frequency of CVS data collection:* At 3-minute interval up to Day 10.

*Data analysis:* Pre-dosing, at 10 minute interval for the first 4 hours post-dosing, every 1 hour from 4 to 24 hours post-dosing, and thereafter, every 24 hours up to Day 10. For EKG measurements, following time-points were selected for analysis: 15, 30, 45, 60, 75, 90, 105, 120 minutes, 4 & 24 hr, Days 7 & 10 post-dosing.

*Clinical pathology:* Pre-dosing and Days 7 and 10 post-injection.

*Cmax determination:* 4 & 12 hours post-dosing.

**Results:**

*CVS parameters and body temperature:* There were no significant effects on systemic BP, heart rate or EKG including QT interval.

*Clinical signs and body weight:* No adverse clinical signs or body weight changes were observed.

*Clinical pathology:* Platelet count increased by 1.8, 1.6 and 2.5-fold as compared to the baseline at 500, 1000 & 5000 µg/kg, respectively on Day 10 post-dosing. The platelet count was somewhat higher in the low-dose group as compared to the mid-dose group (1.8- versus 1.6-fold). There were no other significant effects.

*C<sub>max</sub>*: The mean AMP2 concentrations were 1050, 2140 and 11,100 ng/ml at 500, 1000 & 5000 mcg/kg, respectively at 4 hours post-injection. At 12 hours, the mean C<sub>max</sub> values were 77, 156 & 753 ng/ml, respectively.

***Reviewer's comments:***

Based on the data provided, there were no physiologically significant changes in the CVS parameters following acute administration of AMP2. There were no alterations in ECG morphology, rhythm or ECG intervals including QT/QTc interval. The NOAEL was established at 5000 µg/kg (300-times MHD).

Effect on EKG parameters was also evaluated in the repeat-dose toxicology study in monkey. There were no significant effects on EKG parameters including QT/QTc at doses up to 5000 mcg/kg.

In terms of pharmacodynamic response, i.e. increase in platelet counts; monkey seems to be a less sensitive species than rats. In rats, 100 mcg/kg produced ~4-fold increase in the platelet counts, while in monkey 50-fold higher dose (5000 mcg/kg) produced only 3-fold increase in the platelet counts.

Pulmonary effects: No studies conducted to evaluate pulmonary effects.

Renal effects: Renal effects were evaluated in the repeat-dose toxicity studies in rats and monkey. No adverse renal effects were observed.

Gastrointestinal effects: N/A

Abuse liability: N/A

**2.6.2.5 Pharmacodynamic drug interactions:**

No pharmacodynamic drug interaction studies were conducted.

**2.6.4 PHARMACOKINETICS/TOXICOKINETICS**

**2.6.4.1 Brief summary**

Single and repeat dose PK/TK studies with AMG 531 were conducted in mice, rats, rhesus and cynomolgus monkeys. Doses were administered by the intravenous or subcutaneous route.

***Single dose administration***: The bioavailability of AMG 531 after a subcutaneous administration was low in rats (21-28%) and cynomolgus monkeys (19%) but relatively high in rhesus monkeys (45-74%, decreasing with increasing dose). The T<sub>max</sub> varied from 4 to 16 hours depending on the species. The apparent elimination t<sub>1/2</sub> was 30 to 45 hours in rats but much longer in cynomolgus (~296 hours) and rhesus (110-195 hours)

monkeys suggesting a slow absorption process after subcutaneous administration in monkeys. In general, the increase in C<sub>max</sub> and AUC was linear/dose-proportional in rats and monkeys.

**Repeat dose administration:** PK profile of AMG 531 after repeat dose administration was evaluated as part of the toxicology studies in rats and cynomolgus monkeys. Three times weekly subcutaneous dosing for one month in rats and once weekly for 6 months in cynomolgus monkeys showed no significant drug accumulation and there were no apparent sex differences in PK parameters. The increase in systemic exposure (AUC) was greater than dose proportional in rats but not in monkeys.

**Distribution:** AMG 531 distributes widely into the extravascular space as indicated by the high V<sub>d</sub> values (100-200 ml/kg) across the species. AMG 531 also transferred readily across the placenta, possibly through the neonatal Fc receptor (FcRn). The fetal serum exposure was ~50% of the maternal serum exposure and the exposure in amniotic fluid was 11%-44% of the maternal exposure.

**Metabolism:** No metabolism studies were conducted.

**Excretion:** AMG 531 was mainly excreted through urine. Over 168 hours post-dosing, the recovery of total radioactivity in urine and feces was 88% and 7%, respectively in rats. About 11% and 48% of the total radioactivity in urine and feces, respectively, was TCA-precipitable, suggesting that the excreted radioactivity was primarily free iodide or small peptide fragments.

**Mechanistic PK studies:** PK and PD (increase in platelet counts) profile of AMG 531 was evaluated in splenectomized, nephrectomized and thrombocytopenic animals. The PK profile and the increase in platelet counts was essentially similar in splenectomized rats as compared to normal rats suggesting that splenectomy did not affect the pharmacodynamic effect of AMG 531 and the spleen is not involved in the removal of AMG 531.

In nephrectomized rats, the systemic exposure (AUC) and t<sub>1/2</sub> were significantly higher as compared to normal rats suggesting that the kidney plays a major role in the clearance of AMG 531. Clearance of AMG 531 may likely be lower in patients with kidney dysfunction.

In a Fc-Rn receptor Knock-Out (KO) mice, AMG 531 was eliminated much faster (16- to 24-fold increase in clearance) than in Wild Type (WT). In addition t<sub>1/2</sub> was markedly shorter in KO mice (1.3-2.2 hrs) relative to WT mice (6.5-12.5 hrs) and the increase in platelet count was much less pronounced in KO mice suggesting Fc-Rn receptor appears to act as a salvage receptor for AMG 531 similar to that seen in maintaining human IgG in serum.

In thrombocytopenic mice, the C<sub>max</sub> and AUC parameters were essentially similar to normal mice when AMG 531 was intravenously administered at concentration of 30

mcg/kg. However, at lower concentration (3 mcg/kg), the serum concentration of AMG 531 were 10-fold higher in thrombocytopenic mice. Therefore, the impact of thrombocytopenia on AMG 531 PK is inconclusive. How thrombocytopenia affects the increase in platelet count by AMG 531 was not evaluated.

**PK profile using liquid and lyophilized formulation:** A frozen liquid formulation was used in the preclinical and early clinical studies. However, the lyophilized formulation was used in the pivotal clinical studies. Therefore, bridging studies were conducted to compare PK and PD (increase in platelet counts) profile of the frozen liquid and lyophilized formulations of AMG 531 following subcutaneous administration. The frozen liquid formulation and the lyophilized formulation were found to yield comparable systemic exposures and increase in platelet counts. Based on these studies, use of frozen liquid formulation in the pre-clinical studies instead of clinical lyophilized formulation did not appear to affect the PK-PD profile of AMG 531. No further bridging studies are necessary.

#### 2.6.4.2 Methods of Analysis

[see under individual study reviews]

#### 2.6.4.3 Absorption

Single and repeat-dose PK/TK studies were conducted in rats, mice, cynomolgus monkeys and rhesus monkeys.

#### Single dose administration:

The table below summarizes the PK profile of AMG 531 after single dose intravenous and subcutaneous administration in various species at different dose levels.

Species Dose	Cmax (mcg/ml)		AUC (mcg.h/ml)		CL (ml/h/kg)		Vd (ml/kg)	Tmax (h)		T1/2 (h)		F (%)
	iv	sc	iv	sc	iv	sc		iv	sc	iv	sc	
<b>Study # 100909:</b> <b>SD Rats</b>												
10 mcg/kg	0.69	0.019	2.85	0.793	7.7	27.7	139	16	13.2	45.2	28	
100 mcg/kg	2.68	0.084	12.5	3.35	6.3	23.5	118	8	14.8	33.3	27	
300 mcg/kg	7.24	0.234	35.6	7.31	8.4	41.0	114	12	13.5	29.6	21	
<b>Study # 100998:</b> <b>Rhesus monkey</b>												
500 mcg/kg	8.97	1.45	67.2	49.5	7.4	10.2	196	8	115	110	74	
2000 mcg/kg	34.1	4.08	283	135	7.6	17.0	184	8	102	169	48	
5000 mcg/kg	117	4.90	660	294	7.8	17.3	184	4	143	195	45	
<b>Study # 101604:</b> <b>Cynomolgus monkey</b>												
500 mcg/kg (iv)	6.58	---	25.6	---	20.1	---	198	--	68.1	---	---	
5000 mcg/kg (iv & sc)	88.1	3.02	291	56	17.3	99.2	110	4	96.1	296	19.2	

<b>Study # 100895:</b>											
<b>C57BL/6J Mice</b>											
30 mcg/kg (iv)	0.18	---	0.58	---	43.3	---	161	---	6.1	---	---
100 mcg/kg (iv)	1.33	---	5.09	---	19.6	---	87.5	---	9.8	---	---
300 mcg/kg (iv)	3.04	---	26.1	---	11.5	---	87.4	---	15	---	---

--- Not tested/applicable

The bioavailability of AMG 531 after subcutaneous administration was somewhat lower in rats (21-28%) and cynomolgus monkeys (19%) across the dose range. In rhesus monkeys, bioavailability was relatively higher but it decreased from 74% at 500 mcg/kg to 45% at 5000 mcg/kg.

In mice, the pharmacokinetics of AMP2 was nonlinear; while in rats and monkeys the increase in C<sub>max</sub> and AUC was relatively dose-proportional in the dose ranges studied. The T<sub>max</sub> varied from 4 to 16 hours depending on the species. The apparent elimination t<sub>1/2</sub> was 30 to 45 hours in rats but much longer in cynomolgus (~296 hours) and rhesus (110-195 hours) monkeys suggesting a slow absorption process after subcutaneous administration in monkeys. The volume of distribution was higher across the species suggesting significant distribution in extracellular space.

The pharmacodynamic response (increase in platelet count) was also evaluated as part of the PK studies in above species. In general, there was a dose-dependent increase in the platelet counts across the species. The increase in platelet count was similar irrespective of route of administration (iv or sc). Rats and mice showed more robust response as compared to cynomolgus or rhesus monkeys. The maximum increase in platelet counts occurred 5 to 10 days post-dosing and the count returned to the baseline values 13 to 27 days post-dosing depending on the species. The PD data is summarized in the table below.

Species and Dose	Increase in platelet count (P <sub>max</sub> )	T <sub>max</sub> for the peak increase in platelet count	Duration for the increase in platelet count
<b>Study # 100909:</b> SD Rats 30-300 mcg/kg (iv or sc)	2.2 to 4.6-fold	7-10 Days	16-21 Days
<b>Study # 100998:</b> Rhesus monkey 500-5000 mcg/kg (iv or sc)	1.6 to 2.9-fold	7-10 Days	17-24 Days
<b>Study # 101604:</b> Cynomolgus monkey 500-5000 mcg/kg (iv or sc)	1.8 to 4.6-fold	8-10 Days	21-27 Days
<b>Study # 100895:</b> C57BL/6J Mice 30-300 mcg/kg (iv)	4.9 to 6.3-fold	5-8 Days	13 Days

#### **Repeat-dose administration:**

PK profile of AMG 531 after repeat-dose administration was evaluated as part of the toxicology studies in rats and monkeys. Three times weekly subcutaneous dosing for one month in rats and once weekly for 3 or 6 months in cynomolgus monkeys showed no

significant drug accumulation and there were no apparent sex differences in PK parameters. The increase in systemic exposure (AUC) was greater than dose proportional in rats but not in monkeys. The following table summarizes the PK profile of AMG 531 after repeat-dose subcutaneous administration in rats and monkey. Please refer to the toxicology study section for more details.

Species and Dose	Cmax (ng/ml)			AUC (ng.hr/ml)		
<b>SD Rat</b> (4-Week repeat-dose, sc)	Week 1	Week 4		Week 1	Week 4	
30 mcg/kg	7.2	9.6		232	217	
100 mcg/kg	75.2	69.4		1950	2450	
<b>Cynomolgus Monkey</b> (3 & 6 Month repeat-dose, sc)	Week 1	Week 13	Week 26	Week 1	Week 13	Week 26
500 mcg/kg	179	186	138	2230	2710	1920
1000 mcg/kg	359	349	311	4510	4600	4190
5000 mcg/kg	1640	1610	1410	16700	23000	20100

#### 2.6.4.4 Distribution:

##### *Study # 101995:*

**Quantitative whole body autoradiography and tissue distribution of radioactivity following intravenous administration of  $^{125}\text{I}$ -AMP2 to female rats**

##### **Methods:**

The tissue distribution was evaluated by quantitative whole-body autoradiography (QWBA) and by tissue excision. Two separate groups of rats (n=8/group) were administered  $^{125}\text{I}$ -labeled AMP2 (300 mcg/kg, intravenous) and radioactivity in blood & various tissues was measured up to 168 hours post-dosing. In the first group of rats, the radioactivity was determined using QWBA method (2 rats/time-point: 0.5, 12, 72 & 168 hours post-dosing). In the second group, animals were sacrificed at various time-points (2/time-point, 0.5, 12, 72 & 168 hours) and total radioactivity was measured in various tissues (tissue excision). In addition, TCA-precipitable activity was analyzed in heart, kidneys, liver, ovaries, spleen and serum.

Urine and feces was collected from three animals (2 from Group 1 & 1 from Group 2) up to 168 hours post-dose. Urine and feces were analyzed for total radioactivity and TCA-precipitable radioactivity.

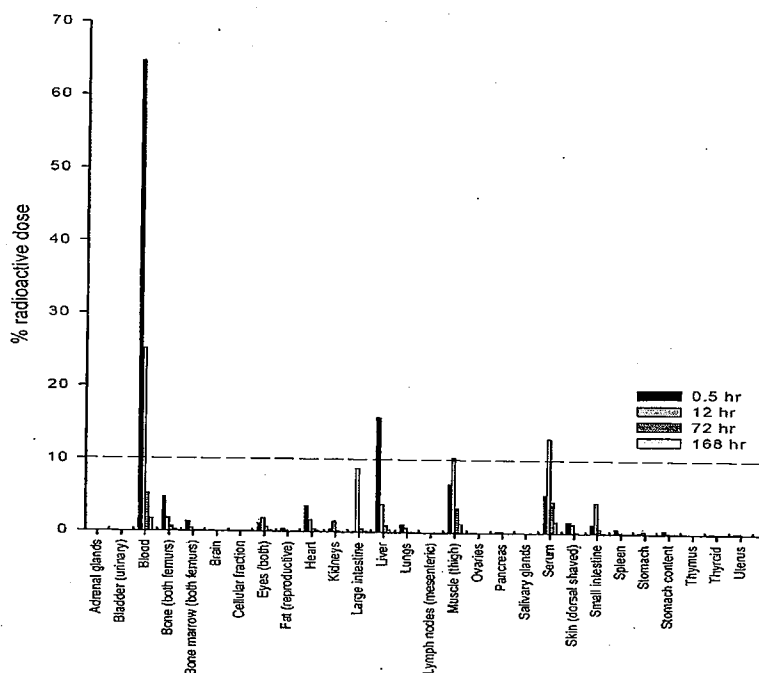
##### **Results:**

The distribution of  $^{125}\text{I}$ -AMP2-derived radioactivity following a single intravenous administration was extensive. Most of the administered radioactivity was found in blood at all collection time points. The Cmax values were reached at 0.5 to 12 hours post-

dosing for all tissues. The tissues with highest % of radioactive dose were blood (65%), liver (18%) and muscle (15%). Tissues with highest concentrations of radioactivity (ng-

eq/g tissue) were thyroid, blood components, kidney, bone marrow and liver. The tissue distribution data obtained by QWBA method were generally consistent with those from tissue excision. The QWBA results also showed high levels in lung, ovary, and adrenal gland in addition to the above noted organs. By 168 hours post-dose, only blood, skin, and muscle contained levels greater than 0.37% of the administered radioactivity.

The figure below shows the distribution of radiolabeled AMG 531 in various tissues. For all excised tissues, the TCA-precipitable radioactivity was 84-95% of the total radioactivity, suggesting that the tissue radioactivity represented intact AMG 531 or large fragments of AMG 531. In all tissues, no accumulation was observed at 168 hours post-dose based on the total radioactivity.



Urine was the primary route of elimination of  $^{125}\text{I}$ -AMP2-derived radioactivity with 87.7% of the administered dose excreted in the urine and 6.6% excreted in the feces. The overall recovery of dosed radioactivity was 96%. TCA-precipitable radioactivity accounted for only 10.7 to 11.3% of the radioactivity in urine and 45.9 to 49.2% in feces, suggesting AMP2 is probably degraded into small peptide fragments before elimination. The elimination data is summarized in the table below.

Collection interval (hours)	Percent of radioactive dose (mean)	
	Urine	Feces
0-24	51.3	2.68
0-48	67.4	4.54
0-72	75.0	5.39
0-96	80.1	5.88
0-120	83.5	6.17
0-144	86.1	6.45
0-168	87.7	6.56

**Reviewer's comments:**

The distribution of AMG 531 in tissues was extensive; consistent with the high Vd values observed in the PK studies. The tissues with high levels of distribution were blood, liver, lung and muscle. In all tissues, no accumulation was seen at 168 hours post-dose and most of the drug was eliminated. Urine was the primary route of elimination.

**2.6.4.5 Metabolism:**

No metabolism studies were conducted. Based on ICH-S6 guidance for biopharmaceutical derived products, biotransformation studies are not required for therapeutic proteins, such as AMG 531, designed to interact with a specific target. Proteases are likely involved in the degradation of AMG 531.

**2.6.4.6 Excretion:**

As noted in study # 101995 (Distribution Study), AMG 531 is mainly excreted through urine. Over 168 hours post-dosing, the recovery of total radioactivity in urine and feces was 88% and 7%, respectively. The majority of radioactivity was collected during the first 24 hours. About 11% and 48% of the total radioactivity in urine and feces, respectively, was TCA-precipitable, suggesting that the excreted radioactivity was primarily free iodide or small peptide fragments.

**Mechanistic PK studies:****a) Study # 100995:**

**Pharmacokinetics of AMP2 following a single intravenous dose administration to control, sham and splenectomized male Sprague-Dawley rats**

A single dose of 100 or 1000 mcg/kg AMP2 was administered intravenously to normal, sham-operated or splenectomized male SD rats (n=6/group) and blood samples were collected at various time-points for up to 160 hrs for PK and PD analysis.

The PK data is summarized in the sponsor's table below.

Parameter	Control		Sham-Operated		Splenectomized	
Dose ( $\mu\text{g/kg}$ )	100	1000	100	1000	100	1000
n	6	6	6	6	6	6
n/timepoint	3	3	3	3	3	3
C <sub>max</sub> (ng/mL)	1910	21300	1840	24800	2540	24700
t <sub>1/2</sub> (h)	24.9	24.5	15.3	29.9	18.6	25.7
AUC <sub>(0-∞)</sub> (ng·h/mL)	10700	101000	11100	132000	16100	99900
CL (mL/h/kg)	9.33	9.88	8.98	7.60	6.21	10.0
V <sub>ss</sub> (mL/kg)	124	153	96.5	113	92.3	185
MRT <sub>(0-∞)</sub> (h)	13.3	15.5	10.7	14.9	14.9	18.5

**Conclusion:**

The PK and PD (increase in platelet counts) profiles of AMP2 in control, sham-operated and splenectomized rats were similar, suggesting the spleen was not involved in the removal of AMP2 at doses of 100 and 1000  $\mu\text{g/kg}$  during the first 168 hours after a single intravenous bolus dose of AMP2 in rats.

**b) Study # 101402:**

**A pharmacokinetic study of AMP2 following intravenous administration in bilaterally nephrectomized male Sprague Dawley rats**

The objective of this study was to investigate the effect of a bilateral nephrectomy on the PK of AMP2 in male SD rats. A total of 24 rats were used in the study (n = 4/group). Rats in Groups 1 & 2 served as control and did not have surgery. Animals in Group 3 & 4 underwent a sham procedure (kidney manipulation) and groups 5 & 6 were bilaterally nephrectomized. All animals received a single intravenous dose of AMP2 (Groups 1, 3 & 5: 30  $\mu\text{g/kg}$  and Groups 2, 4 & 6: 300  $\mu\text{g/kg}$ ) and blood samples were collected at pre-dose, 3, 15 and 30 minutes, and 1, 2, 4, 8 and 12 hours post-dose for PK analysis.

The PK parameters are summarized in the table below:

Group	Treatment	Dose ( $\mu\text{g/kg}$ )	n	C <sub>max</sub> (ng/mL)	t <sub>1/2</sub> (h)	AUC <sub>(0-12h)</sub> (ng·h/mL)	V <sub>e</sub> (mL/kg)
1	Control	30	4	492 (40)	5.91 (0.78)	1900 (220)	61.3 (5.0)
2		300	4	8350 (680)	5.29 (0.76)	23800 (1900)	36.1 (2.8)
3	Sham-Operated	30	3*	528 (106)	4.74 (0.32)	1970 (260)	58.2 (10.7)
4		300	4	7370 (190)	4.59 (0.32)	22700 (1500)	40.8 (1.0)
5	Nephrectomized	30	4	613 (87)	7.43 (1.22)	2490 (180)	49.8 (7.6)
6		300	4	7040 (690)	6.62 (1.01)	40900 (4600)	42.9 (4.2)

All values are reported as mean (SD)

Mean values rounded to 3 significant figures whenever possible.

\*Animal 9 in the 30  $\mu\text{g/kg}$  sham-operated group was excluded from the analysis due to abnormally high AMP2 serum levels observed in this animal.

**Conclusion:**

No difference was observed in AMP2 PK between control and sham-operated animals. The exposure to AMP2 as measured by AUC was 1.5 to 2-fold higher in bilaterally-

nephrectomized rats as compared to sham-operated animals. In addition, the  $t_{1/2}$  was significantly longer after nephrectomy. This suggests that the kidney plays a significant role in the clearance of AMP2. Clearance of AMP2 is likely to be slower in patients with compromised kidney function.

**c) Study # 100896:**

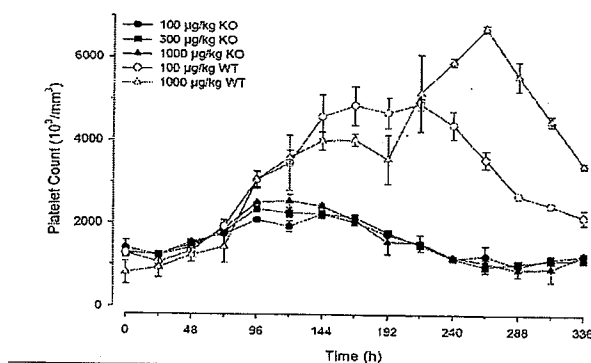
**A pharmacokinetic/pharmacodynamic study for AMP2 following intravenous administration in Fc-Rn Knock-out and wild-type mice**

AMP2 (100-1000 mcg/kg) was administered to Fc-Rn receptor Knock-Out (KO) and Wild Type (WT) mice and blood samples were obtained from mice at various time-points (3 mice/time point) up to 96 hours for PK and PD evaluation in Fc-Rn KO mice.

The PK parameters and PD data are summarized in the sponsor's table and figure below:

Parameter	Knock-Out			Wild Type	
	Group 1	Group 2	Group 3	Group 4	Group 5
Nominal Dose ( $\mu\text{g/kg}$ )	1000	300	100	1000	100
Actual Dose ( $\mu\text{g/kg}$ ) <sup>a</sup>	1204	421	136	1204	136
n/timepoint	3	3	3	3	3
$T_{\text{max}}$ (h)	0	0	0	0	0.333
$C_{\text{max}}$ (ng/mL)	25400	2630	485	26400	1570
$t_{1/2}$ (h)	1.27	2.20	1.54	12.5	6.50
$\text{AUC}_{(0-\infty)}$ (ng $\cdot$ h/mL)	9380	1980	427	146000	10400
CL (mL/h/kg)	128	212	318	8.23	13.1
$V_{\text{ss}}$ (mL/kg)	193	464	406	158	104
$\text{MRT}_{(0-\infty)}$ (h)	1.51	2.19	1.28	19.2	7.94
$V_c$ (mL/kg)	47.5	160	280	45.6	86.5

<sup>a</sup> Actual doses were determined from posterior dose solution analysis and were used in the noncompartmental data analysis.



**Conclusion:**

AMP2 was eliminated much faster in Fc-Rn KO mice than in WT mice with an increase of 24- and 16-fold in clearance in KO mice at 100 and 1000 mcg/kg, respectively.  $T_{1/2}$  was markedly shorter in KO mice (1.3-2.2 hrs) relative to WT mice (6.5-12.5 hrs). The increase in platelet count was more pronounced in WT mice compared to KO mice.

These results suggest that in mice the Fc-Rn receptor appears to act as a salvage receptor for AMP2 similar to that seen in maintaining human IgG in serum.

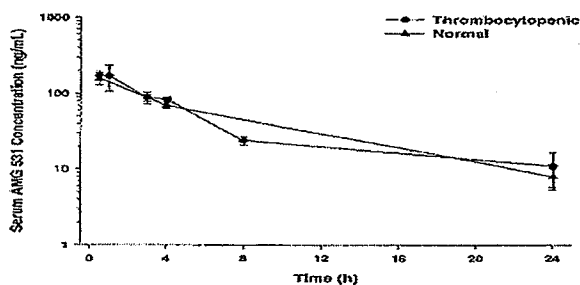
**d) Study # 101896:**

**PK study of AMG 531 in normal and thrombocytopenic mice**

The objective of this study was to investigate influence of thrombocytopenia on the PK profile of AMG 531 (30 mcg/kg) in thrombocytopenic (n=35) and normal mice (n=25) after single intravenous administration. Thrombocytopenia in mice was induced by an intraperitoneal injection of 62.5 mg/kg of carboplatin. Four hours later, mice were exposed to 5 Grey whole body radiation. Eleven days post-radiation, AMG 531 was administered intravenously and blood samples were collected up to 24 hours post-dosing for PK analysis using ELISA method.

**Results:**

The figure below shows the mean concentration-time profiles of AMG 531 in thrombocytopenic and normal mice (n=5/time-point). The C<sub>max</sub> (175 & 178 ng/ml) and AUC<sub>0-24 h</sub> (1010 & 1250 ng\*hr/ml) values were similar in thrombocytopenic and normal mice, respectively.



**Reviewer's comments:**

Overall, the composite PK profile and exposure parameter estimates (AUC and C<sub>max</sub>) were similar in thrombocytopenic and normal mice suggesting thrombocytopenia had no impact on AMG 531 PK within the first 24 hours post-administration. However, the study results are limited since the effects on PK after repeat administration and pharmacodynamic response (increase in platelet counts) were not evaluated. Also an additional study conducted using 10-fold low dose of AMG 531 (3 mcg/kg) showed significant differences in the PK profile in normal and thrombocytopenic mice. This study is reviewed below.

**e) Study # 102342:**

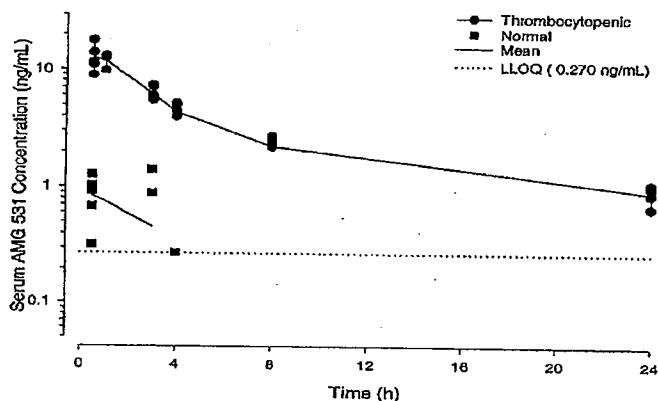
**A low dose pharmacokinetic study of AMG 531 in normal and thrombocytopenic mice**

The objective of this study was to evaluate the influence of thrombocytopenia on the PK profile of AMG 531 (3 mcg/kg) in thrombocytopenic (n=35) and normal mice (n=25)

after single intravenous administration. PK samples were collected up to 24 hours post-dosing and analyzed using ELISA method.

**Results:**

As shown in the figure below, the mean serum levels of AMG 531 in thrombocytopenic mice were significantly higher than those observed in normal mice.

**Reviewer's comments:**

In contrast to previous study, this study suggests that thrombocytopenia had a significant impact on AMG 531 PK profile. The serum concentrations of AMG 531 in thrombocytopenic mice were ~10-fold higher than in normal mice. Therefore, whether thrombocytopenia affects the PK profile of AMG 531 remains inconclusive. In addition, how this affects the pharmacological response is not known since platelet count measurements were not conducted.

**2.6.4.7 Pharmacokinetic drug interactions:** Not conducted**2.6.4.8 Other Pharmacokinetic Studies:**

All pre-clinical studies and early clinical studies were carried out using a frozen liquid formulation of AMG 531. Subsequently, the sponsor developed a lyophilized formulation (to be marketed formulation) that was used in Phase 3 clinical studies. Bridging studies were conducted to compare pharmacokinetic and pharmacodynamic (increase in platelet counts) profile of the frozen liquid and lyophilized formulations of AMG 531. These studies are reviewed below.

**a) Study # 102921:**

**PK study of AMG 531 in male rats to compare the frozen liquid and the lyophilized formulations following single subcutaneous administration**

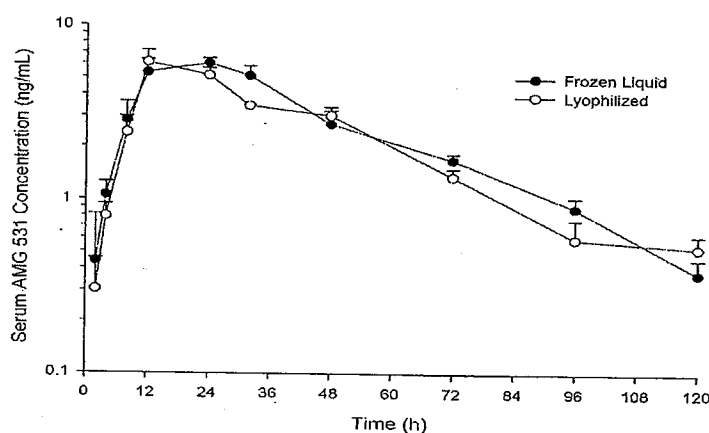
**Methods:**

This study was conducted in male SD rats to compare the PK of the frozen liquid formulation to the lyophilized formulation. Two groups of rats (n=24/group) were

injected with the two formulations of AMG 531 (30 µg/kg, sc) and plasma concentrations of AMG 531 were monitored for 120 hours post-dosing. Blood samples were collected at following time-points: 2, 4, 8, 12, 24, 32, 48, 72, 96 & 120 hours post-dosing (n=8/time-point).

### Results:

Two formulations achieved comparable exposures in rats with no meaningful differences in the PK profile. The figure and table below summarize the PK profile of AMG 531 for two formulations:



Cohort	Dose (mcg/kg, sc)	Tmax (h)	Cmax (ng/ml)	AUC <sub>0-∞</sub> (ng.h/ml)	t <sub>1/2</sub> (h)
Frozen liquid	30	24	6.04	316	25.5
Lyophilized	30	12	6.12	285	27.6

### Reviewer's comments:

This was a bridging study to compare PK profile for the two formulations (liquid and lyophilized). The PK profile appears essentially similar for both formulations. Based on the results, use of frozen liquid formulation in the pre-clinical studies instead of lyophilized formulation (clinical formulation) did not significantly affect the PK profile of AMG 531. Please note the sponsor also conducted a pharmacodynamic study evaluating increase in platelet counts for the two formulations (see below).

### b) Study # 103126:

**2-Week subcutaneous injection pharmacology study of AMG 531 in female rats for comparison of platelet counts between two formulations**

### Methods:

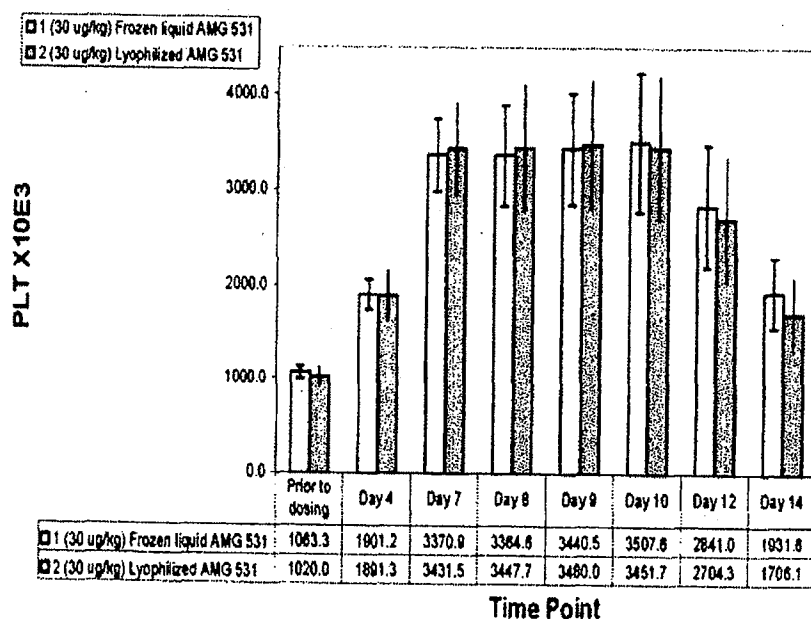
The purpose of this study was to compare platelet count response using frozen liquid formulation (lot # A0102140000, same as used in pre-clinical studies) and lyophilized

formulation (lot # 46A014855), following a single subcutaneous administration of AMG 531 (30 mcg/kg) to female SD rats (n=10/formulation).

Blood samples were collected from all animals prior to dosing on Day 1, and post-dosing on Days 4, 7, 8, 9, 10, 12 and 14 for measuring platelet counts. The sampling analysis was conducted to determine the concentration of AMG 531 injected as a frozen liquid formulation (29.7 mcg/ml) and lyophilized formulation (33.4 mcg/ml).

### Results:

Acute administration of the two different formulations to female rats resulted in no statistically significant differences in platelet counts at various time-points. The maximum increase in platelet counts was 330% (Tmax: Day 10) and 341% (Tmax: Day 9) as compared to baseline values for frozen liquid and lyophilized formulations, respectively. The figure below shows the platelet counts at different time-intervals.



### Reviewer's comments:

Based on the bridging studies conducted to compare two formulations (frozen liquid and lyophilized AMG 531), the PK and PD profile was similar for both formulations. Based on the results, use of frozen liquid formulation in the pre-clinical studies instead of clinical lyophilized formulation did not appear to affect the PK-PD profile of AMG 531. No further bridging studies are necessary.

## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

AMG 531 was evaluated in following toxicology studies:

Study type	Species	Route of administration
Acute dose toxicity	Rat	Subcutaneous
4-Week repeat-dose toxicity	Rat and Rhesus monkey	Subcutaneous & intravenous
4-Week repeat-dose toxicity	Rhesus and Cynomolgus monkey	Subcutaneous
3 & 6-Month repeat-dose toxicity	Cynomolgus monkey	Subcutaneous
Fertility study	Rat	
Embryo-fetal development	Mice, rat & rabbit	Subcutaneous
Peri- and post-natal toxicity	Rat	Subcutaneous

#### Single-dose toxicity studies:

In an acute dose toxicity study in rats, AMG 531 (100, 300 & 1000 mcg/kg, sc) caused marked increase in platelet counts (92-217%) at all dose levels, increased spleen-to-terminal body weight ratios and increased extramedullary hematopoiesis in the spleen (300 & 1000 mcg/kg). These effects are consistent with the expected pharmacologic activity of AMG 531. One female in the 100 mcg/kg dose group was found dead following the clinical pathology bleed. There were no clinical signs or macroscopic/microscopic findings. The death was attributed to blood collection procedure in these animals with increased blood viscosity due to thrombocytosis.

#### Repeat-dose toxicity studies:

**Rats:** In rat repeat-dose toxicity study, AMG 531 (10, 30 & 100 mcg/kg, 1- to 37-times MHD based on AUC) was administered subcutaneously three times weekly for 4-weeks. As expected, AMG 531 caused dose-dependent increases in platelet counts (2 to 4-fold). In general, adverse effects observed in rats were related to the over activation of thrombopoietic system caused by AMG 531. These effects occurred at clinically relevant doses and included extramedullary hematopoiesis, splenic enlargement, megakaryocytosis/ megakaryocyte hyperplasia, bone hyperostosis and myelofibrosis. These effects (including myelofibrosis) were reversed by the end of recovery period. No NOAEL was established since one male died and myelofibrosis in one female was observed at the lowest dose tested (10 mcg/kg).

In rats, mortality was observed at all dose levels tested. Deaths occurred in conjunction with blood sampling events. When requisite blood collections were eliminated from studies, there were no deaths observed. Thus, the deaths were considered consequences

of the handling and blood collection procedures. Although exact cause of death could not be determined, it is likely to be associated with the events due to extreme thrombocytosis, increased blood viscosity and difficulty in obtaining blood samples in these animals.

Myelofibrosis was seen at all dose levels and was reversible by the end of recovery period/after discontinuation of the drug. It is not known whether myelofibrosis is reversible after chronic treatment since rats were administered only 4-weeks of repeat doses in the study. There is a potential for long-term treatment with AMG 531 to result in an irreversible fibrotic stage leading to bone marrow dysfunction. In clinical studies, increase in reticulon formation in the bone marrow and one case of myelofibrosis was observed at doses  $\geq 7$  mcg/kg. Based on pre-clinical and clinical findings, myelofibrosis remains a significant concern. The sponsor has proposed clinical management program for AMG 531 to monitor reticulon formation and potential for myelofibrosis.

In terms of pharmacodynamic effects and adverse effect observed in clinical studies, rat seems to be a more relevant species. However, long-term toxicology studies in rats could not be conducted as AMG 531 produces immunogenic response in rats. In the present study, rats developed neutralizing antibodies to AMG 531 in approximately 50% of animals at all dose levels tested. Antibodies binding to endogenous TPO were also observed. However, no TPO neutralizing antibodies were detected.

**Monkeys:** AMG 531 (0, 500, 1000 or 5000 mcg/kg, 30-300 times MHD based on AUC) was administered subcutaneously to cynomolgus monkeys once weekly for 13 or 26 weeks followed by 8 weeks recovery period. There were no treatment-related effects on mortality, clinical observations, body weight, food consumption, ECG, ophthalmology, urinalysis, plasma biochemistry, gross necropsy and organ weights. AMG 531 treatment caused dose-dependent increase in platelet counts (1.5 to 4-fold), accompanied by low mean platelet volume. In the microscopic evaluation, megakaryopoiesis in the bone marrow and megakaryocytosis in the submandibular lymph node were observed. These effects were reversed at the end of the recovery period. At the injection sites, slight perivascular mononuclear cell infiltration was observed in AMG 531-treated animals with a trend towards recovery noted at the end of treatment-free period.

Overall incidence of antibody development towards AMP2 was 54.2%. The incidence of anti-AMP2 neutralizing antibodies was 4.2%. Anti-TPO antibodies were observed in one animal in the 500  $\mu$ g/kg group. However, no TPO neutralizing antibodies were detected.

The NOAEL for the 6 month repeat-dose toxicity study in cynomolgus monkey was 5000 mcg/kg (300-times MHD). Findings in the 4-week studies in monkeys were similar to the longer-term toxicity studies (3/6 months) in cynomolgus monkeys.

In terms of pharmacodynamic effects, monkey seems to be a much less sensitive species than rats. In monkey, approximately 50-fold higher dose (5000 mcg/kg) was needed to achieve same response (3-4 fold increase in platelet counts) as seen in rats at 100 mcg/kg. Monkey was also less immunogenic than rats. Unlike in rats, no myelofibrosis was

observed in monkeys after 6 months of repeated dosing. Based on pharmacodynamic and adverse effects seen, the rat seems to be a more relevant species than monkey in terms of predicting adverse effects in humans.

#### **Genotoxicity and Carcinogenicity:**

Due to the protein-based nature of AMG 531 and in accordance with available regulatory guidance for biotechnology-derived pharmaceuticals, ICH S6, genotoxicity studies were not conducted. AMG 531 is a recombinant protein composed of an Fc domain and an Mpl receptor binding domain. It is unlikely that AMG 531 would react directly with DNA or other chromosomal material.

Carcinogenicity studies were not conducted. Based on the guidance for biotechnology-derived pharmaceuticals, carcinogenicity studies are generally not required for protein-based drugs such as AMG 531. Also AMG 531 was highly immunogenic in rats and mice. Neutralizing antibodies were observed in both species making it difficult to assess carcinogenicity potential in traditional rodent models.

#### **Reproductive toxicology:**

***Fertility and early embryonic development:*** AMG 531 (10, 30 & 100 mcg/kg) was administered 3 times weekly by subcutaneous injection to rats from before mating to mid-gestation. Mortality was observed at all dose levels (2/dose group). The deaths were attributed to thrombocytosis induced increase in blood viscosity resulting in difficulties in obtaining blood samples and handling of the animals. AMG 531 caused 1.2 to 3-fold increases in platelet count. AMG 531 treatment at 30 and 100 mcg/kg doses caused decreases in mean body weight, body weight gain and food consumption. AMG 531 had no effect on male or female fertility at doses up to 100 mcg/kg (37-times MHD based on AUC).

***Embryo-fetal development:*** Embryo-fetal studies were conducted in mice, rats, and rabbits.

***Rats:*** For the developmental toxicity study in rats, AMG 531 was administered subcutaneously at doses of 10, 30 & 100 mcg/kg. There was no effect on any of the parameters evaluated for maternal and developmental toxicity. The NOAEL for maternal and fetal toxicity was 100 mcg/kg (11-times MHD based on AUC). However, this NOAEL should be interpreted with caution since AMG 531 crosses the placenta and fetal serum concentrations were substantial; approximately 50% of maternal serum concentration. Accordingly, dose-dependent significant increases in platelet counts (1.7- to 6-fold as compared to control/baseline values in fetus) and binding antibodies to AMG 531 were observed in fetuses. Consequently, there is a potential for adverse effects related to thrombocytosis in fetuses. AMG 531 is a recombinant protein that has an Fc domain of human IgG. It is known that maternal IgG can be transferred by the FcRn receptor to the fetus and similar mechanism may be responsible for AMG 531. It is likely that placental transfer of AMG 531 occurs in humans. AMG 531 should be administered to pregnant women only if the potential benefit justifies the risk.

**Rabbits:** AMG 531 administered to pregnant rabbits at doses of 10, 30, 60 & 100 mcg/kg (4-82 times MHD based on AUC) had no effect on maternal or developmental toxicity at doses up to 60 mcg/kg (39-times MHD). At 100 mcg/kg dose, lower maternal body weight change, lower food consumption and lower total body weight change adjusted for uterine weight were observed. There were no effects on fetal body weights. Fetal external examinations was normal. However, one of 52 fetuses examined in the high dose group had external malformations characterized by gastroschisis, ectrodactyly and cutis aplasia (all in one fetus). This may be an incidental finding rather than treatment-related effect. The overall incidence of anti-AMPG 531 antibodies in dams was 44%. There were no AMG 531 neutralizing antibodies detected.

In terms of pharmacodynamic effects, rabbit seems to be a much less sensitive species than rats since no significant increase in the platelet counts was observed at any of the doses tested. These results are consistent with the in vitro binding displacement assay, where AMG 531 did not show any specific binding to platelets derived from rabbits. Based on these assessments, it seems rabbits do not respond to AMG 531 and no definitive embryo-fetal toxicity study was conducted in rabbits. Nevertheless, adequate exposure to AMG 531 (4-82 x MHD) was obtained in this study for assessment of embryo-fetal toxicity that formed the basis for the recommendation in the label. In lieu of rabbit reproductive toxicity study, the sponsor conducted a developmental toxicity study in mice.

**Mice:** AMG 531 (3, 10, 30 & 100 mcg/kg/day, 0.1-5 times MHD) was administered three times weekly during GD 6-15. The high dose of 100 mcg/kg/day dose was associated with overall reduction in maternal body weight gain (8.4%). Developmental toxicity, as evidenced by increased post-implantation loss, was also attributable to the 100 mcg/kg/day dose of AMG 531. Consistent with the post-implantation loss, the numbers of live fetuses were reduced at the high-dose. NOAEL for maternal and developmental toxicity in mice was 30 mcg/kg/day (2-times MHD).

Only fetal external examination was conducted; visceral and skeletal examinations were not conducted. Therefore, fetal NOAEL should be interpreted with caution.

**Pre-natal and post-natal development:** Treatment of F0 dams with AMG 531 (10, 30 or 100 µg/kg, 3-11 times MHD) in rats, induced neutralizing antibodies to AMG 531 in high number (70-80%) of dams. Therefore, antibody-positive (Ab+, ~30 dams/group) and antibody-negative (Ab-, 9-14 dams/group) dams were evaluated separately.

Four Ab- dams (F0) died at the end of the lactation period (3 in mid-dose group and 1 in high-dose group). Cause of death could not be determined but it was speculatively attributed to cerebrovascular accident ('stroke'), induced by increased blood viscosity resulting from high platelet count. There was a slight prolongation of the gestation period observed in all dose groups, regardless of antibody status (Ab+ or Ab-). The normal pattern for this strain (SD rats), as reflected by the control group, is ~50% of dams with a 22-day gestation period and the remainder a 21-day period. In AMG-531 treated animals,

there was a shift with ~70% having a 22-day period. In addition, 3 dams had a 23-day gestation period.

Regardless of maternal antibody status, there was an increase in the incidence of perinatal F1 pup mortality at high dose. However, there was no effect on overall live litter size. Physical and functional development up to sexual maturity, including fertility and general reproductive function were not affected.

For maternal toxicity, no definitive NOAEL was established due to effects of AMG 531 on gestation period and mortality seen in mid- and high-dose groups. Based on increased perinatal pup mortality at the highest dose, a NOAEL for survival and pre- and post-natal development of the F1 offspring was 30 mcg/kg (3-times MHD) of maternal dose.

#### **2.6.6.2 Single-dose toxicity**

##### **a) Single-dose subcutaneous injection toxicity study with AMG 531 in rats**

##### **Key study findings:**

Administration of AMG 531 as a single subcutaneous injection to rats at dose levels of 100, 300, or 1000 µg/kg (11-110 times MHD) resulted in a lower body weight gain (11%-27% lower) in females at all dose levels. However, there was no dose-response relationship for this effect and no effect on body weight was observed in males. One female in the 100 mcg/kg dose group was found dead following the clinical pathology bleed on Day 9 post-dosing. All other animals survived to the scheduled termination on Day 16. There were no clinical signs or macroscopic/microscopic findings in the dead animal. The sponsor considered cause of death likely to be related to the blood collection procedure.

Higher platelet count (92%-217%) was seen at all dose levels consistent with the pharmacologic activity of AMG 531. Other clinical pathology findings were relatively minor but treatment-related (lower RBC count, hemoglobin & hematocrit, lower triglycerides and higher gamma glutamyltransferase) or secondary to an increase in platelet count (increased potassium & phosphorus). Clinical pathology findings observed at Day 9 were reversed by Day 16. There was an increase in the mean spleen-to-terminal body weight at 1000-µg/kg and increased extramedullary hematopoiesis in the spleen at 300 or 1000 µg/kg. These are consistent with the expected pharmacologic activity of AMG 531.

No clear NOAEL was established since reduced body weight gain in females was seen at all dose levels and there were minor but treatment-related clinical pathology effects.

**Study no.: 103974**

**Volume # and page #: eCTD 4.2.3.1.1-103974**

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** Jan 13, 2004

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug lot # and % potency:** 46A014855, 105%

**Objective:**

The aim of this study was to evaluate the toxicity of AMG 531 when administered as a single dose subcutaneous injection to rats.

**Methods:**

Doses: 0, 100, 300 & 1000 µg/kg, sc

Species/strain: CD(SD)IGS BR rats

Number: 5/sex/group

Route and dose volume: subcutaneous acute administration, 0.2-2 ml/kg

Age: 8 to 9 weeks old

Weight: males: 230-280 g, females: 178-241 g.

Mortality: Twice daily

Clinical signs: Cage-side observations: Once daily for 2 hr post-dosing

Detailed observations: Once weekly

Body weights: Weekly

Food consumption: Weekly

Ophthalmoscopy: Not conducted

Hematology: Hematology: Days 9 & 16 post-dosing, Coagulation tests: Day 16

Clinical chemistry: Days 9 & 16 post-dosing

Urinalysis: Not conducted

Gross pathology: Days 16 and for animals that died at unscheduled interval.

Organ weights: Adrenals, brain, heart, kidney, liver, lungs, ovary, pituitary, prostate, spleen, testis, thyroid with parathyroid & thymus.

Bone marrow smear was prepared from femur and preserved for future examination.

Histopathology: Control & high dose group and animals that died at unscheduled interval. Spleen and macroscopic lesions were examined at all dose levels.

Adequate Battery: yes (X), no ( )

Peer review: yes (X), no ( )

Toxicokinetics: Not conducted.

**Results**

**Mortality:**

One female in 100 mcg/kg dose group was found dead following the clinical pathology bleed on Day 9. There were no clinical signs and no macroscopic or microscopic

findings. The sponsor considers the death was due to the blood collection procedure and not treatment-related. No further details were provided.

All other animals including those in the high dose group survived to the scheduled termination on Day 16 post-dosing.

**Clinical signs:** No remarkable observations.

**Body weights:**

Decrease in the body weight gain seen in females (11%, 27% & 20%, at 100, 300 & 1000 mcg/kg, respectively). However, there was no dose-response relationship for the lower body weight gain observed and there were no effects on the body weight in males.

**Food consumption:** No remarkable effects on food consumption.

**Ophthalmoscopy:** Not conducted

**Clinical pathology:**

***Day 9 post-dosing:*** Treatment-related effects observed at Day 9 post-dosing included markedly ***higher platelet count*** at all dose levels (92% to 217% higher than respective controls) and moderately higher inorganic phosphorus (12% to 22% higher), potassium (17% to 35% higher), and gamma glutamyltransferase (up to 19 IU/L higher than respective controls) for males and females at all dose levels. Platelet counts were higher in females than males, and the effect on platelet count, especially for the females, did not appear to be dose-related.

According to the sponsor, higher inorganic phosphorus and potassium may represent normal release from the increased platelet numbers during clot formation in the serum chemistry sample. The mechanism for higher gamma glutamyltransferase was not clear but there were no residual histopathologic findings at Day 16 typically associated with an increase in this enzyme activity (e.g., biliary hyperplasia). Higher gamma glutamyltransferase on Day 9 was considered likely the result of the high platelet counts and not an indication of cholestasis or other hepatobiliary injury.

Additional findings at Day 9 that were minor but treatment-related included lower RBC count at all dose levels in females (9%-12%); and at 1000 mcg/kg in males (7%), lower hemoglobin and hematocrit for males and females given 300 or 1000 µg/kg (<10%), mildly higher mean corpuscular volume and mean corpuscular hemoglobin for females at all dose levels, and mildly lower triglycerides for females at all dose levels. Although these effects were minor with no dose-response relationship, they were treatment-related.

***Day 16:*** All treatment-related effects on Day 9 were reversed by Day 16, although females given 1000 mcg/kg dose continued to have somewhat higher (35%) platelet count. Males in 1000 mcg/kg had higher absolute reticulocyte count that was considered a normal response to the previously reduced RBC mass.

**Gross pathology:**

No remarkable macroscopic findings.

**Organ weights:**

26% increase over the control in mean spleen-to-body weight percentage at 1000 mcg/kg (males) correlating with the increased microscopic extramedullary hematopoiesis observed in spleen. These are expected pharmacologic effects of AMG 531.

Males in 100 mcg/kg group had a significant increase in mean heart and kidney weights as a percent of each to the terminal group mean body weight. No correlating macroscopic changes were present for either of these findings and effects were not seen at high dose of 1000 mcg/kg. These changes were not considered pathologically significant.

**Histopathology:**

Increased *extramedullary hematopoiesis* was observed in males given 300 or 1000 µg/kg and in females given 1000 µg/kg. Minimal extramedullary hematopoiesis was present in the liver of some animals (controls and AMG 531-treated); however, the small foci were never greater than the degree which might be seen as background with young rats.

**Reviewer's comments:**

The goal of this acute toxicity study was to establish a gradient of treatment-related toxic effects of AMG 531 and select appropriate dose-levels for the repeat-dose toxicology study in rats reviewed below.

**2.6.6.3 Repeat-dose toxicity****a) 4-Week subcutaneous toxicity and toxicokinetic study with AMP-2 in rats with a 4-week recovery****Key study findings:**

AMP-2 was administered three times weekly by subcutaneous injection at 10, 30 and 100 mcg /kg/dose, 1-37 times MHD based on AUC) or by intravenous injection at a dose level of 100 mcg/kg/dose for 4 weeks. AMP2 treatment caused dose-dependent increase in mortality. The exact cause of death could not be determined; there were no overt signs of toxicity noted at any dose level. Effects on clinical pathology endpoints included increases in platelet counts, a generalized stimulatory effect on leukocyte production, and a decrease in red blood cell count, hematocrit and hemoglobin. The effects on platelets and red blood cells were noted at all dose levels. Consistent with the stimulus for increased platelet production were findings of megakaryocyte hyperplasia in the spleen, liver, bone marrow, and megakaryocytosis in the lung. Other significant findings included myelofibrosis of bone marrow, hepatic extramedullary hematopoiesis, splenic lymphocytic depletion and bone hyperostosis. These lesions generally exhibited dose-related increased incidences and/or severity and tended to be more pronounced in males than in females. The clinical pathology effects were more pronounced and more related to

dose level early in the study (after 4 doses) than late in the study (after 12 doses). This correlated with the presence of anti-AMP-2 neutralizing antibodies. In addition, the severity of histopathology findings was generally reduced for animals with positive antibody titers. All treatment-related effects were reversed following recovery. No NOAEL was established since one male died and myelofibrosis was observed in one female at the lowest dose tested (10 mcg/kg).

**Study no.: 100876**

**Volume # and page #:** eCTD 4.2.3.2.1-100676

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** Jan 11, 2000

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug, lot #, and % potency:** 1111269M9, AMP2 bioactivity: 94%

The aim of this study was to assess the toxicity and the toxicokinetics of AMP-2 administered 3 days/week for 4 weeks to rats via subcutaneous and intravenous injection, and to assess the reversibility of any effects after a 4-week recovery period.

#### **Methods:**

Doses: subcutaneous: 0, 10, 30 & 100 mcg/kg/day, 3 days/week for 4 weeks

intravenous: 100 mcg/kg/day, 3 days/week for 4 weeks

Days of injection: 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24 & 26

Species/strain: CD(SD)IGS BR rats

Number: main study: 10/sex/group

recovery phase [control & high dose (iv and sc) only]: 5/sex/group

Route and dose volume: subcutaneous & intravenous, 1 ml/kg

Satellite groups used for TK: 12/sex/group

Age: 45 to 50 days old

Weight: males: 151-235 g, females: 120-178 g

Unique study design or methodology (if any):

--Serum antibody analysis conducted at the end of treatment and recovery phase.

Mortality: Twice daily

Clinical signs: Cage-side observations: Once daily for 2 hr post-dosing

Detailed observations: Once weekly

Body weights: Weekly

Food consumption: Weekly

Ophthalmoscopy: Pre-dose and on Day 26 (main study) & Day 54 (recovery group)

EKG: Not conducted

Hematology: Hematology & coagulation test: Days 10, 29 and 58 for all animals  
Platelet aggregation test: Days 9, 30, 31 and 57 (5/sex/group). Platelet aggregation test performed under stimulated (ADP and collagen) & unstimulated conditions.

Clinical chemistry: Days 29 and 58

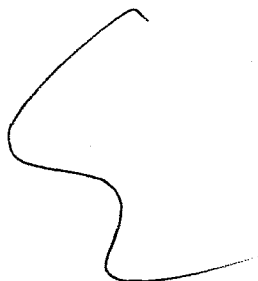
Urinalysis: Days 29 and 58

Gross pathology: Days 29 (main study) & 58 (recovery group) and for animals that died at an unscheduled interval.

Organ weights: Adrenals, brain, heart, kidney, liver, lung, ovary, pituitary, prostate, salivary gland, seminal vesicle, spleen, testis, thymus, thyroid with parathyroid & uterus.

Histopathology: Adequate Battery: yes (X), no ( )

Peer review: yes (X), no ( )



Sampling time-point for macrophage function test: Days 30 and 57 (4/sex/group from animals designated for platelet aggregation test).

Serum antibody analysis:

Day 1 (3/sex) and Days 29 & 58 (for all animals)

Toxicokinetics:

3/sex/group/time-point: Pre-dose, 0.5, 2, 4, 8, 12, 24 & 48 hours on Days 1 and 26.

In addition, blood was collected from 4/sex/group prior to dosing on Days 8, 15 & 22 and on Days 33, 40, 47 & 54.

Parameters evaluated were C<sub>max</sub>, AUC<sub>last</sub>, T<sub>max</sub>, C<sub>av</sub> and elimination half-life on Days 1 & 26 using non-compartmental analysis.

## Results

### Mortality:

There were 14 treatment-related **deaths** before scheduled termination. There were no clinical signs of toxicity prior to death. Although the causes/exact mechanisms could not be established, based on their occurrence in the test article treated groups, the deaths appear to be treatment-related with the exception of one male (10 mcg/mL dose group)

that according to the sponsor died of incidental causes unrelated to treatment. Mortality observed was dose-dependent. The data is summarized in the table below.

Dose group	Males	Females	% Mortality*
Control	--	--	0%
10 mcg/kg (sc)	1** on Day 25		5%
30 mcg/kg (sc)	--	2 on Day 30	9%
100 mcg/kg (sc)	1 on Day 15	2 on Day 10	14%
100 mcg/kg (iv)	5 on Days 10, 14, 15 & 30	2 on Days 10 & 22	32%

\*Includes animals from main study group plus satellite group

\*\*2 males died in the low-dose group. However, one died of incidental causes

**Clinical signs:** No remarkable observations.

**Body weights:**

There were no statistically significant differences in mean body weights. However during 4-weeks of treatment, *slight reduction in body weight gain* was apparent at all doses, with no dose proportionality. Weight gains were similar during the recovery period.

**Food consumption:**

No significant effects except that males in the intravenous high-dose group had lower values during the treatment phase.

**Ophthalmoscopy:** No remarkable ophthalmic observations.

**EKG:** Not conducted.

**Hematology:**

**Days 10 & 29:** Administration of AMP-2 was associated with several effects on hematology parameters. In general, the effects at Day 10 were more pronounced than those at Day 29. All of the clinical pathology findings were reversed by Day 58 (recovery phase). Treatment-related adverse clinical pathology findings and magnitude of these effects at various dose levels (Groups 2-5: 10, 30, 100 (sc) & 100 (iv) mcg/kg) are summarized in the sponsor's table below.

Parameter	Sex	Day 10	Day 29
Red blood cell count	M	-	4, 5 ↓
	F	-	3, 4, 5 ↓
Hemoglobin	M	2, 3, 4, 5 ↓ to ↓↓	4, 5 ↓
	F	2, 3, 4, 5 ↓ to ↓↓	3, 4, 5 ↓
Hematocrit	M	2, 3, 4, 5 ↓ to ↓↓	2, 3, 4, 5 ↓
	F	2, 3, 4, 5 ↓ to ↓↓	3, 4, 5 ↓
Mean corpuscular volume	M, F	3, 4, 5 ↓↓ to ↓↓↓	-
Mean corpuscular hemoglobin	M, F	3, 4, 5 ↓	-
Mean corpuscular hemoglobin concentration	M, F	2, 3, 4, 5 ↓ to ↓↓	-
Platelet count	M, F	2, 3, 4, 5 ↓↓↓	2, 3, 4, 5 ↓ to ↓↓↓
Mean platelet volume	M	2, 3, 4, 5 ↓ to ↓↓	2, 3, 4, 5 ↓ to ↓↓
	F	2, 3, 4, 5 ↓ to ↓↓	4, 5 ↓↓
Fibrinogen	M, F	4, 5 ↓↓	-
White blood cell count	M, F	3, 4, 5 ↓ to ↓↓	-
Absolute neutrophil count	M, F	3, 4, 5 ↓ to ↓↓	3, 4, 5 ↓ to ↓↓
Absolute lymphocyte count	M	4, 5 ↓ to ↓↓	-
	F	3, 4, 5 ↓ to ↓↓	-
Absolute monocyte count	M	3, 4, 5 ↓ to ↓↓	4, 5 ↓
	F	3, 4, 5 ↓ to ↓↓	-
Absolute eosinophil count	M	4, 5 ↓	4, 5 ↓
	F	3, 4, 5 ↓	-
Inorganic phosphorus	M	ND	2, 3, 4, 5 ↓ to ↓↓
	F	ND	2 ↓
Potassium	M	ND	2, 3, 4, 5 ↓ to ↓↓
	F	ND	2 ↓

a Groups affected are listed by the group number (i.e., 2, 3, 4, or 5); magnitude of effects are designated by arrows (e.g., ↓, ↓↓, and ↓↓↓ correspond to mildly, moderately, and markedly higher, respectively, than the control group).

M = male, F = female; ND = not determined; dash (-) = no effect.

The most prominent effect was dose-related **marked increase in platelet count** at Day 10 (~2- to 4-fold higher than control values) for all AMP-2 treated groups. Platelet counts remained high at Day 29 for many treated animals, especially for those administered low 10 mcg/kg/dose (mean counts for this group were 2- to 2.5-fold higher). Mean platelet volumes were increased in conjunction with the higher platelet counts. These are expected pharmacologic effects of AMP-2.

According to the sponsor, **reduced platelet aggregation** observed in all treatment groups at Day 10 probably resulted from difficulty with the analytical procedure due to the markedly high numbers of platelets in blood samples from the treated animals. Following modification of the procedure for blood sample processing (the platelet rich plasma samples diluted with 300 mcL of autologous platelet poor plasma), platelet aggregation at

Day 30/31 was not significantly affected by AMP-2. However, males in the mid- & high-dose groups still had somewhat lower values than those for the control group.

**Erythrocyte effects** were characterized by lower RBC count (Day 29 only), lower hemoglobin and hematocrit, lower mean corpuscular volume and higher mean corpuscular hemoglobin concentration (Day 10 only). The reasons/mechanisms for erythrocyte effects were unclear.

AMP-2 appeared to have a generalized stimulatory effect on leukocyte production as peripheral blood counts for neutrophils, lymphocytes, monocytes, and eosinophils were increased, especially at Day 10. According to the sponsor, moderately higher fibrinogen observed in 100 mcg/kg dose group animals also suggested the possibility of mild inflammation, perhaps at the injection sites.

#### **Clinical chemistry:**

The clinical chemistry findings associated with the administration of AMP-2 were **higher inorganic phosphorus and potassium** at Day 29. These were considered to be secondary to the increased platelet counts as these analytes are released from platelets in vitro. These effects were reversed by Day 58 during the recovery phase.

**Urinalysis:** No remarkable findings.

#### **Gross pathology:**

**Spleen enlargement** was observed in the high-dose (100 mcg/kg: subcutaneous & intravenous routes) group animals. There were no other remarkable macroscopic findings.

Macroscopic findings in the recovery group animals were unremarkable.

#### **Organ weights:**

Treatment-related organ weight findings were **limited to spleen** and characterized by statistically significant increases in spleen absolute weights (30 & 100 mcg/kg), spleen-to-body weight ratios (10, 30 & 100 mcg/kg) and spleen-to-brain weight ratios (10, 30 & 100 mcg/kg).

In the high-dose group, increases in spleen absolute weights (intravenous route) and spleen-to-body weight percentages (subcutaneous & intravenous routes) persisted during the recovery period. There were no other toxicologically relevant changes in the organ weights.

#### **Histopathology:**

There were several treatment-related microscopic findings. These included *spleen megakaryocyte hyperplasia and lymphocytic depletion; liver megakaryocytosis and extramedullary hematopoiesis; lung megakaryocytosis; bone marrow megakaryocyte hyperplasia and myelofibrosis; and femoral and sternal bone hyperostosis.*

The lesions noted above generally exhibited dose-related increased incidences and/or severity with males showing more pronounced effects. The incidences and severity of histopathological findings were higher after intravenous injection as compared to the subcutaneous route.

The *megakaryocyte proliferation* observed in various organs was a direct treatment related effect. It was not determined whether the other findings were direct test article related effects or secondary to the megakaryocyte proliferation. However, none of these lesions were considered to be direct cause of death in various treatment groups.

***Spleen megakaryocyte hyperplasia:***

Megakaryocyte populations in the red pulp clearly exceeded the occasional, scattered cells which are within normal limits for the rat spleen. In affected spleens, increased numbers of megakaryocytes diffusely infiltrated and expanded the red pulp, often resulting in grossly observed spleen enlargement. An additional splenic red pulp feature was *splenic extramedullary hematopoiesis*, consisting primarily of small erythroid precursor-like cells with scanty cytoplasm and hyperbasophilic round nuclei.

***Bone and bone marrow findings:***

The adverse microscopic findings were characterized by *marrow megakaryocyte hyperplasia and myelofibrosis, and bone hyperostosis*. The epiphyseal and diaphyseal bone marrow cavities exhibited variably increased numbers of megakaryocytes which disrupted the normal architecture of adjacent hematopoietic cell populations.

***Bone marrow myelofibrosis:*** Myelofibrosis in the marrow cavities of affected femurs was characterized by fibrous connective tissue which distorted/replaced the normal architecture. The fibrous stroma was well-vascularized and contained abundant fibrocytes cavity; hyperostotic bone trabeculae often appeared to extend downward from these linear foci into the distal diaphysis.

***Bone hyperostosis:*** Bone hyperostosis in affected femurs was characterized by foci of closely spaced bone trabeculae which were composed of paler, more fibrillar osteoid than control trabeculae. Few, if any, hyperostotic foci were associated with the epiphyseal plate growth zones in the proximal diaphysis (femoral epiphyseal growth zones in treated animals were similar to those in controls).

Sternal megakaryocyte hyperplasia, myelofibrosis and hyperostosis were morphologically similar to the femoral lesions.

***Recovery group animals:***

In the recovery group, the liver, lung, femoral and sternal bone and bone marrow lesions including myelofibrosis were no longer evident. Incidences and mean severity of splenic hematopoiesis in the recovery group were similar in control and treated groups and were considered incidental and unrelated to treatment.

***Microscopic findings at injection site (subcutaneous):***

There were dose-related increased incidences of *dermal chronic inflammation* in males. Other subcutaneous injection site lesions that occurred almost exclusively in AMP-2 treated animals were *muscle and subcutis chronic inflammation and myofiber regeneration* in both males and females.

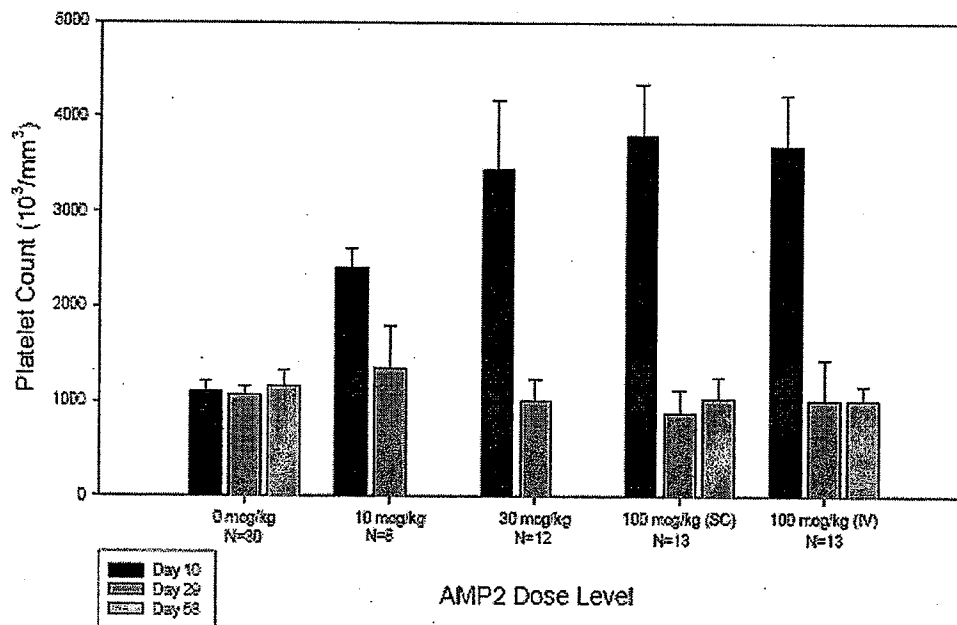
did not exhibit alterations in functional activity as measured by the production of the pro-inflammatory cytokine, TNF $\alpha$ . There were no significant differences in the production of TNF $\alpha$  (basal and stimulated) between control and AMP-2 treated animals.

**Serum antibody analysis:**

In the AMP-2 treated groups, a total of 51 of 94 (54%) animals were positive for development of antibodies to AMP-2 and 46 of 94 (49%) had neutralizing antibodies to AMP-2. In the control group, all animals were negative for the development of antibodies to AMP-2. The 10 mcg/kg dose group had a 17% incidence of antibody formation. At  $\geq 30$  mcg/kg, relatively high incidence of antibody formation of 31% to 55% was observed. The design of this study did not allow for determination of antibody presence in the toxicokinetic rats. However, it is likely that antibodies were also present in the toxicokinetic satellite animals considering the high antibody incidence in the toxicity main study rats. It is possible that antibodies contributed to the variability observed in the toxicokinetic data. The table below summarizes the antibody analysis data.

Dose (mcg/kg, route)	Reactive (% reactive)	Total
0 (sc)	0 (0%)	30
10 (sc)	3 (17%)	18
30 (sc)	11 (55%)	20
100 (sc)	8 (31%)	26
100 (iv)	12 (41%)	29

As expected, rats that developed neutralizing antibodies had less significant increase in platelet counts (Day 29) as shown in the figure below.



All animals including those treated with AMP-2 were negative for antibodies to endogenous thrombopoietin (eTPO) in the biosensor immunoassay. All animals tested in the anti-TPO neutralizing antibody bioassay were also negative for neutralizing antibodies.

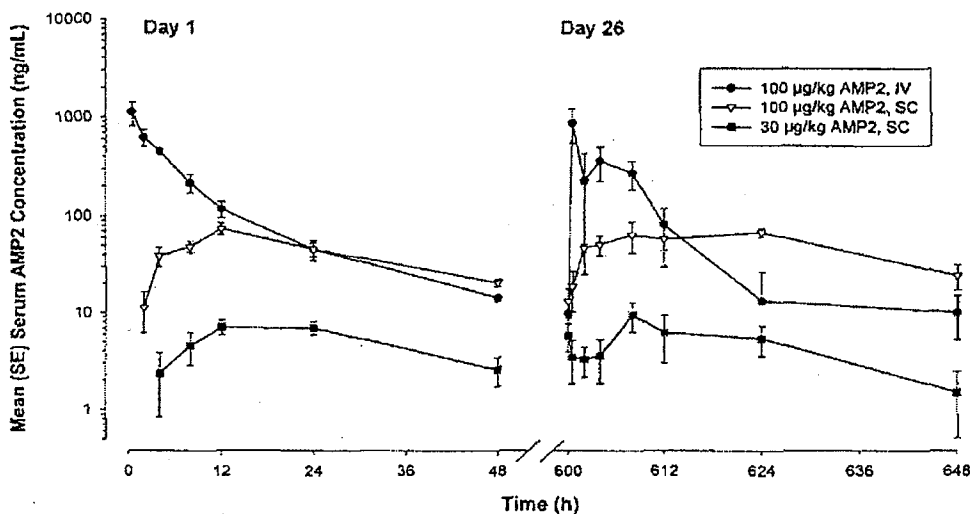
#### **Toxicokinetics:**

There was no remarkable difference in the TK between males and females. Therefore, mean profile of six animals (3 males + 3 females)/group/time-point was used for TK analysis. The sponsor's table below summarizes the TK profile.

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On Original

Table 4. Mean (SD) Toxicokinetic Parameter Estimates<sup>a</sup> from Averaged Serum AMP2 Concentrations

TIW Dose	Day	T <sub>max</sub> (h)	C <sub>max</sub> (ng/mL)	AUC <sub>(0-48)</sub> (ng·h/mL)	T <sub>1/2</sub> (h)	CL or CL/F (mL/h/kg)	V <sub>ss</sub> (mL/kg)	V <sub>c</sub> (mL/kg)	F <sub>rel</sub> (%)	AR <sub>AUC</sub>
30 µg/kg SC <sup>b</sup>	1	12	7.17	232	16.8	nc <sup>c</sup>	nc	nc	nc	nc
	26	8	9.59	217	13.0	122	nc	nc	nc	0.934
100 µg/kg SC	1	12	75.2	1950	20.9	nc	nc	nc	nc	nc
	26	24	69.4	2450	16.0	33.2	nc	nc	nc	1.25
100 µg/kg IV	1	0.5	1120	6490	12.2	nc	nc	nc	30.0	nc
	26	0.5	896	4570	13.5	21.0	209	73.3	53.5	0.704

<sup>a</sup>Calculated estimates were rounded to 3 significant figures whenever possible.<sup>b</sup>TK parameters were not calculated for the 10 µg/kg SC group because the concentrations were BLQ for the majority of animals.<sup>c</sup>nc- Not calculated due to insufficient toxicokinetic data or if parameter not relevant.

The majority of the AMP2 concentrations in the 10 µg/kg SC dose group were BLQ, thus profile not shown.

Quantifiable AMP-2 concentrations were not detected at low dose (10 mcg/kg). On Day 1 after a single SC dose, the peak serum concentrations were observed at 12 hours for the 30 & 100 mcg/kg. The exposure level (AUC) over 48 hours, after a single dose was 232 ng·h/mL and 1950 ng·h/mL for the 30 & 100 mcg/kg, respectively. There was a nonlinear increase in the C<sub>max</sub> (10-fold) and AUC (8-fold) when the dose was increased by 3.3-fold from 30 to 100 mcg/kg. On Day 26, this nonlinearity was still apparent with minimal accumulation ratios relative to Day 1 (0.934 and a 1.25, respectively). The initial

volume of distribution was 73.3 mL/kg and the volume of distribution at steady state after intravenous administration (100 mcg/kg) was 209 mL/kg suggesting AMP-2 is distributed extravascularly after dosing. After multiple dosing, peak levels did not increase significantly and trough levels were consistent between Days 1 and 26 suggesting no significant accumulation of the drug.

There was a high incidence of antibodies to AMP-2 (31 to 55% reactive) observed at doses 30 mcg/kg or higher. Direct assessment of the effects of antibodies on the TK was not possible since samples for antibody analyses and TK analyses were taken from different animals.

#### Conclusion:

Based on the red blood cell findings for two males given 10 mcg/kg/dose (and the death of one of these) and the finding of myelofibrosis in one female given 10 mcg/kg/dose, a no-observable-adverse-effect level was not determined for this study.

#### **Reviewer's comments:**

Administration of AMP2 was associated with dose-dependent increase in mortality. There were no AMP2-related findings attributable as the cause of death. According to the sponsor, the mortality seen was due to combination of extreme thrombocytosis and increase in blood viscosity resulting from platelet increase making blood sampling and handling of these animals difficult. Mortality was consistently observed in other toxicology studies conducted in rats. It is likely that marked thrombocytosis/increase in platelets (3 to 4-fold over baseline) in these normal animals with normal baseline platelet count may have resulted in thrombotic events leading to death. However, exact cause of death was not determined.

Increases in platelet counts consistent with the pharmacologic activity of AMP-2 were apparent at all dose levels. Mechanisms for a generalized stimulatory effect on leukocyte production and a decrease in red blood cell count, hematocrit and hemoglobin were uncertain. Decrease in RBC count may be due to inhibition of RBC production in the bone marrow secondary to the stimulation of platelet production by AMP2, i.e. stem cell competition.

Histopathological findings were characterized by myelofibrosis of bone marrow, megakaryocyte hyperplasia/megakaryocytosis bone hyperostosis and extramedullary hematopoiesis. These effects are likely due to expected activation of thrombopoietic system caused by AMP2. These effects are toxicological consequences of the expected pharmacological action of the drug. These adverse effects, in particular, myelofibrosis were observed at clinically relevant doses. The sponsor states that myelofibrosis was clearly reversible at the end of recovery period/after discontinuation of the drug. However, in the present study, rats were administered only 4-weeks of repeat doses, it is likely that long-term dosing/treatment with AMG 531 can result in irreversible fibrotic

stage resulting in bone marrow function failure. In clinical studies, increase in reticulin formation in the bone marrow and one case of myelofibrosis was observed at doses  $\geq 7$  mcg/kg. Based on pre-clinical and clinical findings, myelofibrosis remains a significant concern. However, the sponsor has proposed clinical management program to monitor reticulin formation and potential for myelofibrosis.

In clinical studies, some ITP patients had rebound thrombocytopenia after cessation of treatment with AMP2. In the present study in rats, no thrombocytopenia was developed during the recovery period. However, these studies were carried out in normal animals with normal platelet count and effects after chronic treatment were not evaluated. Therefore, the results of present study (no thrombocytopenia during recovery phase) should be interpreted with caution.

In terms of pharmacodynamic effects and adverse effect observed in clinical studies, rat seems to be a more relevant species than monkey (see review of the toxicology studies conducted in monkey). However, long-term toxicology studies in rats were not conducted as AMP2 produces immunogenic response in rats. In the present study, rats developed neutralizing antibodies to AMP2 in approximately 50% of animals at all dose levels tested. In these animals with AMP2 neutralizing antibodies, the pharmacodynamic response (increase in platelet count) was completely reversed. In clinical studies, antibodies binding to AMP2 (antibodies that bind to AMP2 but do not affect AMP2-induced increase in platelet count) and AMP2 neutralizing antibodies (antibodies that bind to AMP2 and negate the AMP2-induced increase in platelet count) have been observed. Antibodies binding to endogenous TPO were also observed in various toxicology studies conducted in rats and monkeys. However, no TPO neutralizing antibodies were observed in any of the toxicology studies conducted. In clinical studies, antibodies binding to TPO were detected but no neutralizing antibodies were observed.

**Study title:**

**b) A 4-week toxicity study of AMP2 administered by subcutaneous or intravenous injection to rhesus monkeys, with a 4-week recovery period**

**Key study findings:**

Administration of AMP2 to rhesus monkeys three times per week for four weeks, at subcutaneous dose levels of 500, 1000, and 5000 mcg/kg or at an intravenous dose level of 5000 mcg/kg caused marked thrombocytosis and megakaryocyte hyperplasia, increase in platelet aggregation and increase in spleen weight; effects secondary to marked increase in platelet counts. Platelet counts were increased approximately 4-fold above baseline for the low and middle dose groups and approximately 6-fold for the 5000 mcg/kg dose groups (intravenous and subcutaneous). There was also a dose-dependent decrease in red blood cell counts and related parameters. The degree of anemia was not clinically significant at any dose level.

Other treatment-related histomorphologic alterations included an indication of a dose-dependent immunogenic reaction to AMP2 at the subcutaneous injection sites. The presence of follicular cysts and/or multiple cystic follicles in the ovaries (seen in at least one female from each AMP2-treated group) was judged to be of possible relationship to administration of the test article.

**Study no.: 100877**

**Volume # and page #: eCTD 4.2.3.2.1-100877**

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** Jan 24, 2000

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug, lot #, and % potency:** 1111269M9, AMP2 bioactivity: 94%

**Objective:**

The aim of this study was to determine toxicity of AMP2 when administered by subcutaneous or intravenous injection to rhesus monkeys three times/week for 4 weeks, and to evaluate recovery from any effects of AMP2 over a 4-week treatment-free period.

**Methods:**

The overall study design is summarized in the sponsor's table below.

Group No.	Number of Males/Females	Dose Level (µg/kg)	Dose Vol. (mL/kg)	Dose Solution Conc. (mg/mL)	Route	No. M/F Sacrificed on:	
						Day 29	Day 56
1	5/5	0 (control)	0.321	0	SC/IV*	3/3	2/2
2	3/3	500	0.321	1.56	SC	3/3	
3	3/3	1000	0.321	3.12	SC	3/3	
4	5/5	5000	0.321	15.6	SC	3/3	2/2
5	3/3	5000	0.321	15.6	IV	3/3	

SC = subcutaneous IV = intravenous

\*The control group received the control article by both the SC and IV routes at the same schedule (3 times/week) as the other study groups.

Doses: 0, 500, 1000 & 5000 (sc & iv) mcg/kg/day, 3 days/week for 4 weeks

Species/strain: Rhesus monkey (*Macaca mulatta*)

Number: main study: 3/sex/group, recovery group (control & high dose, sc): 2/sex/group

Route and dose volume: subcutaneous & intravenous, 0.321 mL/kg

Satellite groups used for TK: No satellite group but blood samples from all main study animals were obtained for TK analysis.

Time-points for TK analysis: Pre-dosing and on Days 1 & 26

Age: 2 to 7 years old

Weight: Males: 3.1-4.5 kg, females: 2.8-4.3 kg

Unique study design or methodology (if any):

--Antibody analysis for main study group & recovery phase animals:

Analysis for antibodies to AMP2: Pre-dosing and on Days 14, 28 & 55.  
Analysis for antibodies to endogenous TPO: Pre-dosing and on Day 28

--Hormone assay: Analysis for FSH, LH and estradiol.

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Weekly

Food consumption: Daily

Ophthalmoscopy: Pre-dose and during Weeks 4 (main study) & 8 (recovery group)

EKG: Pre-dosing and during Weeks 4 (main study group, last week of dosing) & 8 (recovery group, last week of recovery period)

Hematology: Hematology: Pre-dosing, Days 7, 10, 14, 21, 28 & 55

Coagulations tests: Days 14, 28 & 55

Clinical chemistry: Pre-dosing, Days 14, 28 & 55

Urinalysis: Pre-dosing, Days 28 & 56

Gross pathology: Days 29 (main study, 3 days after the last dose on Day 26) & 56 (4-week recovery group) and for animals that died at an unscheduled interval.

Organ weights: Adrenals, brain, heart, kidney, liver, lung, ovary, pituitary, prostate, salivary gland, seminal vesicle, spleen, testis, thymus, thyroid with parathyroid & uterus.

Histopathology: Adequate Battery: yes (X), no ( )

Peer review: yes (X), no ( )

Uterus, cervix, vagina and ovaries for all females, including both the terminal sacrifice and recovery sacrifice, were examined by additional pathologists.

Toxicokinetics:

Blood samples were obtained from all animals in the main study groups prior to dosing and at following time-points following dosing on Days 1 and 26: 1, 4, 8, 12, 24 & 48 hours post-dosing.

Time-points for recovery group animals: 72, 144, 192 & 288 hours after last dosing.

## Results

Mortality: None

Clinical signs: No remarkable observations.

Body weights: No treatment-related effects on body weights.

Food consumption: No remarkable effects on food consumption.

Ophthalmoscopy: No ocular abnormalities. No remarkable findings.

EKG: No effects on EKG parameters including QT interval.

Hematology:

Treatment-related hematologic changes mainly reflected the expected thrombopoietic activity of AMP2:

--Dose-dependent **increase in platelet counts** (3- to 6-times baseline counts), with the peak response occurring on Days 14, 28 & 21 for 500, 1000 & 5000 mcg/kg dose group animals, respectively. The increase in platelet counts was of similar magnitude (~6-fold) after 5000 mcg/kg subcutaneous or intravenous administration.

Mean platelet counts returned to near baseline in the high-dose recovery group animals by Day 55.

--**Mean platelet volume (MPV) decreased** (10-19%), non-dose-dependently, in all AMP2-treated groups by Day 14, with complete recovery by Day 55. In contrast to the decrease in overall MPV, microscopic examination of platelets in the peripheral blood smear revealed large platelets in all AMP2-treated animals on Days 14 and 28.

--Dose-dependent **decrease in RBC counts** (and associated parameters, hemoglobin and hematocrit) was observed at all dose levels, with complete recovery by Day 55. AMP2-treated groups had 10-16% lower mean RBC count, whereas in control the count was lower by 6% as compared to respective pre-dosing values.

--Evaluation of **platelet aggregation** revealed AMP2-related increase in the change in impedance and a decrease in lag time for collagen-activated platelet aggregation on Days 14 and 28 in all AMP2-treated groups. These changes indicate an increase in platelet aggregability, and are likely related to the marked thrombocytosis (expected pharmacological effect) observed in the animals.

--A slight increase in plasma fibrinogen in the AMP2-treated groups was evident on Day 28. However, fibrinogen values for individual animals remained within the normal range.

#### **Clinical chemistry:**

No remarkable effects, except a slight increase in LDH in the AMP2-treated groups. The increase in LDH diminished in parallel with the reduction in platelet counts during the recovery phase. The apparent changes in plasma LDH were attributed to release from platelets occurring either in vivo or ex vivo during the preparation of plasma from blood samples containing high platelet counts. It has been reported that large increases in platelet counts are associated with spurious increases in LDH due to high content of this enzyme in platelets.

**Urinalysis:** No remarkable effects on urinalysis parameters.

#### **Gross pathology:**

***Enlarged ovaries*** were noted in several AMP2-treated females. Ovarian enlargement corresponded histologically to the presence of ***follicular cysts and/or multiple cystic follicles***.

***Focal reddening at the injection site***, which corresponded histologically to subcutaneous hemorrhage, was noted in animals from all groups including the control group, but the incidence of this finding was greater in AMP2-treated groups.

Microscopically, there was a dose-related increase in the incidence and severity of perivascular mononuclear cell infiltrates in the subcutaneous tissue, which is consistent with an immunogenic reaction to the administration of a foreign peptide.

**Organ weights:**

Small *increases in spleen weight* were observed in all AMP2-treated groups. However, the increase in spleen weight was not associated with any corresponding macroscopic (splenic enlargement) or microscopic (extramedullary hematopoiesis) findings that were seen in the acute and repeat dose studies in rats.

**Histopathology:**

Dose-dependent *megakaryocytic hyperplasia* of the bone marrow, a consequence of the pharmacologic action of AMP2, was observed in all of the bones examined. Female animals had a slightly greater severity than males. Following the recovery period, megakaryocytic hyperplasia was markedly reduced.

Ovarian enlargement seen at necropsy corresponded histologically to the presence of *follicular cysts and/or multiple cystic follicles*. In the females that were observed to have lesions in the ovaries, there was a tendency for these animals to have elevated levels of estradiol, follicle stimulating hormone, and/or luteinizing hormone, however, there was no clear relationship between the levels of these hormones and administration of AMP2. *Following a pathology peer review of the ovary changes, a panel of consultants collectively concluded that the cystic alterations were most likely a result of physiological or developmental influences, although a relationship to the test article could not be ruled out.*

Following recovery period, 1 of 2 female from the AMP high dose group still had ovarian cystic follicles graded as mild, suggesting that complete recovery of an AMP2 effect on the ovaries had not occurred.

The severity of the cellular infiltrates at the subcutaneous injection sites was reduced during the recovery period.

**Toxicokinetics:**

Since a sex difference was not detected for the toxicokinetic (TK) analysis of AMP2, the mean concentration-time profiles and mean TK parameters were combined for the males and females. On Day 1, after subcutaneous (SC) administration, the C<sub>max</sub>, occurred in the range of 4-8 hours post-dose with mean values 1080 and 7810 ng/mL at 500 mcg/kg and 5000 mcg/kg, respectively. A less than dose-proportionate increase in AUC from the 500 to the 5000 mcg/kg dose occurred in SC dose groups, from 25100 to 161000 ng\*h/mL, possibly related to decreased bioavailability at higher doses. For Day 1, a relative bioavailability of 28.2% was calculated after SC dosing of 5000 mcg/kg relative to the 5000 mcg/kg intravenous group. Over the 26-day dosing period, accumulation of AMP2 was approximately 2-fold for all the four dose groups.

**Antibody analysis:**

A total of 17 of the 28 (60.7%) AMP2-dosed animals were positive for development of antibodies to AMP2 in the biosensor immunoassay of which 5 of the 28 (18%) AMP2-dosed animals tested positive for the development of neutralizing antibodies to AMP2.

***Reviewer's comments:***

Most of the effects observed were related directly or indirectly to the thrombopoietic activity of AMP2. Marked thrombocytosis (3-6 fold increase in platelet counts) resulted in secondary effects such as megakaryocyte hyperplasia, increase in platelet aggregation and increase in spleen weight. There was also a dose-dependent decrease in red blood cell counts and related parameters. The degree of anemia was not clinically significant at any dose level. The anemia observed may be due to inhibition of RBC production in the bone marrow secondary to the stimulation of platelet production by AMP2 (i.e., stem cell competition).

There was a dose-related increase in the incidence and severity of perivascular mononuclear cell infiltrates in the subcutaneous tissue injection site, which suggests an immunogenic reaction due to AMP2 administration.

Increase in ovary weight was observed that correlated histopathologically with follicular cysts in the ovaries. Although, cystic alterations may have resulted from physiological or developmental influences, a pathology peer review did not completely rule out the test article effects. To evaluate further, the sponsor conducted another 4-week repeat-dose study in rhesus and cynomolgus monkeys reviewed below.

**Study title:**

**c) A 4-week repeated dose toxicity study of AMP2 administered subcutaneously to female cynomolgus and rhesus monkeys followed by a 4-week recovery period**

**Key study findings:**

This study was a follow-up toxicity study to evaluate further the findings of ovarian cystic follicles noted in a repeat-dose study in rhesus monkeys (Study no.: 100877). AMP2 was administered subcutaneously to female cynomolgus (100, 300, 500 and 5000 mcg/kg) and female rhesus (5000 mcg/kg) monkeys three times weekly for 4 weeks followed by a 4-week recovery period.

Dose-dependent increase in platelet counts accompanied by an increase in megakaryocytes in the bone marrow was seen at all dose levels. The degree of increase in platelet counts was similar in cynomolgus and rhesus monkeys. The following findings were considered to be secondary to the increase in platelets: anemia with an increase in reticulocyte count and an increase in erythroblasts, increase in lactate dehydrogenase and

creatinine phosphokinase, increase in spleen weight, increase in megakaryocytes in the lungs and liver and eosinophilic substance in the red pulp of the spleen and in the blood vessels of the lungs. In addition, increased leukocyte count and prolongation of coagulation parameters were noted in the high-dose group. Urinalysis showed increase in P2-microglobulin (cynomolgus and rhesus) and N-acetyl-P-D-glucosaminidase (rhesus) in the high-dose group but there were no histopathological correlates for these findings. Unlike earlier repeat-dose study in rhesus monkeys, no ovarian follicular cysts were noted in both species. All treatment-related effects including increase in platelet counts returned to baseline during the recovery period. The NOAEL was 500 mcg/kg (30-times MHD).

AMP2 was more immunogenic in rhesus monkeys. The overall incidence of anti-AMP2 antibodies was 31% (cynomolgus) and 100% (rhesus) with no AMP2-neutralizing or antibodies to endogenous TPO detected for both species. In cynomolgus monkeys, dose-dependent increase in Cmax and AUC were observed with Cmax ranging from 26-2920 ng/mL on Day 28. In rhesus, Cmax and AUC were twice as high as seen in cynomolgus monkeys.

**Study no.: 101158**

**Volume # and page #:** eCTD 4.2.3.2.1-101158

**Conducting laboratory and location:** S Wakayama, Japan

**Date of study initiation:** Aug 15, 2000

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug, lot # and % potency:** 1111269M9, 99%

#### Methods:

The overall study design is summarized in the sponsor's table below.

Group	Test Article	Species	Dose Level (µg/kg/dose)	Dose Volume (mL/kg/dose)	AMP2 Concentration (mg/mL)	Number of Animals (Animal No.)*	
						Female	
1	Control	Rhesus	0	0.321	0	5+3*	(1 – 8)
2	Control	Cynomolgus	0	0.321	0	4+2*	(9 – 14)
3	AMP2	Cynomolgus	100	0.321	0.312	4+2*	(15 – 20)
4	AMP2	Cynomolgus	300	0.321	0.935	4+2*	(21 – 26)
5	AMP2	Cynomolgus	500	0.321	1.56	4+2*	(27 – 32)
6	AMP2	Cynomolgus	5000	0.321	15.6	5+3*	(33 – 40)
7	AMP2	Rhesus	5000	0.321	15.6	5+3*	(41 – 48)

Animals were administered AMP2 or AMP2 placebo three times per week.

\*: 5 or 4 animals/group were necropsied on the day after the final dose and 3 or 2 animals/group following a 28-day recovery period. Animals for recovery study were Nos. 1-3, 9, 10, 15, 16, 21, 22, 27, 28, 33-35 and 41-43.

Doses: Cynomolgus: 0, 100, 300, 500 & 5000 mcg/kg/dose, 3 days/week for 4 weeks

Rhesus: 0 & 5000 mcg/kg, 3 days/week for 4 weeks

Species/strain: Rhesus (4-8 years old, 4-6 kg) and cynomolgus (4-5 years old, 2.5-3.8 kg) monkeys

Number: main study: 4-5/dose, recovery group: 2-3/dose

Route and dose volume: subcutaneous, 0.321 ml/kg

Satellite groups used for TK: No satellite group but blood samples from all main study animals were obtained for TK analysis.

Time-points for TK analysis: Pre-dosing and on Days 1 & 26

Unique study design or methodology (if any):

--Antibody analysis for main study group & recovery phase animals: Pre-dosing and on Days 14, 28 & 55 (BIACORE assay).

--Hormone analysis: Analysis for FSH, TSH, LH, progesterone and estradiol was conducted during Week 4 (main study) and Week 8 (recovery).

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Weekly

Food consumption: Daily

Ophthalmoscopy: Pre-dose and during Weeks 4 (main study) & 8 (recovery group)

Hematology: Hematology: Pre-dosing, Days 6, 13, 20 & 27 & during week 4 of recovery

Clinical chemistry: Pre-dosing and during Weeks 4 (main study) & 8 (recovery group)

Urinalysis: Pre-dosing and during Weeks 4 & 8

Gross pathology: Days 29 (main study) and 56 (4-week recovery group)

Organ weights: Adrenals, brain, heart, kidneys, liver, lungs, ovary, pituitary, prostate, salivary gland, seminal vesicle, spleen, testis, thymus, thyroid with parathyroid & uterus.

Histopathology: Adequate Battery: yes (X), no ( )

Peer review: yes (X), no ( )

Toxicokinetics:

Day 1: 1, 4, 8, 12, 24 & 48 hours after dosing

Days 7, 14 & 21: Prior to dosing

Day 28: Prior to dosing and 1, 4, 8, 12 & 24 hours post-dosing

Recovery group: 48, 72, 96, 120, 192, 264 & 456 hours after dosing on Day 28.

## Results

Mortality: None

Clinical signs: No remarkable observations in both species.

Body weights: No treatment-related effects on body weights in both species.

Food consumption: No remarkable effects on food consumption.

Ophthalmoscopy: No ocular abnormalities. No remarkable findings.

**Hematology:**

Treatment-related hematologic changes mainly reflected the expected thrombopoietic activity of AMP2:

***Cynomolgus monkeys:***

--Dose-dependent **increase in platelet counts** (2 to 6-fold higher) was noted in all treatment groups. On day 6, the mean platelet counts were 1.5 to 3.15-fold higher than pre-dosing values. At the end of dosing (Day 27), the mean values were further increased and were 1.85 to 6.9-fold higher. At 5000 mcg/kg, decrease in platelet volume was noted on Days 13, 20 & 27 of dosing.

-- A significant **increase in leukocyte count** (with an increase in lymphocyte count) was noted on Day 20 of dosing in high-dose group. Also significant **increase in reticulocyte count** was noted in 500 and 5000 mcg/kg dose group animals.

--In high-dose group **prolongation of prothrombin time** was noted on Day 27.

--Return to baseline values was noted at the end of recovery period.

***Rhesus monkeys:***

-- A significant **increase in platelet counts**. At 5000 mcg/kg dose (only dose tested), platelet counts were 2.73-fold and 7.16-fold higher than baseline values on Days 6 & 27, respectively. A significant decrease in platelet volume was also observed.

--Other parameters affected were **decreases in erythrocyte count, hematocrit value and hemoglobin concentration**, increase in reticulocyte count, increase in leukocyte count (with an increase in lymphocyte count), increase in monocyte count, **increase in erythroblast and prolongation of prothrombin and aPTT time**.

--Return to baseline values was observed at the end of recovery period.

**Clinical chemistry:**

--*Cynomolgus*: Increase in LDH in the high-dose group, likely to be secondary to the increase in platelet counts.

--*Rhesus*: Increase in LDH and CPK was observed. Slight but significant increase in globulin fraction (decrease in A/G ratio) was also noted.

--*Recovery group*: No treatment-related changes in both species.

**Urinalysis:**

*Cynomolgus*: **Increase in beta2-microglobulin** was noted in Week 4 of dosing in the 5000 mcg/kg dose group animals (2-fold increase). A tendency for an increase (but not statistically significant) in beta2-microglobulin was also noted in the 300 and 500 mcg/kg dose groups. There were no other remarkable effects on urinalysis parameters.

*Rhesus*: Increase in NAG (1.5-fold increase as compared to baseline value) and beta2-microglobulin (2.5-fold increase) noted in the high-dose group animals. There were no associated microscopic findings for the increases in NAG and beta-microglobulin.

*Recovery group*: Increased beta2-microglobulin noted at the end of the dosing period recovered to pre-dosing values in Week 4 of recovery in both species.

#### **Hormone analysis:**

*Cynomolgus*: No treatment-related changes in FSH, LH, TSH, E2 or progesterone.

*Rhesus*: Increase in progesterone and estradiol (E2) concentration noted during Week 4 of the treatment. The values returned to baseline during recovery period.

#### **Bone marrow examination:**

Although, individual values were within the range of the background data, a significant **decrease in bone marrow nucleated cell count** was noted at the end of dosing period in cynomolgus (500 & 5000 mcg/kg) and rhesus (5000 mcg/kg) monkeys. The cell count returned to baseline during recovery period.

#### **Gross pathology:**

*Cynomolgus*: Only finding noted was the presence of several red foci in the lung of one animal at 5000 mcg/kg dose.

*Rhesus*: No remarkable gross findings. In particular, there were **no enlarged ovaries** as observed in the earlier repeat-dose study in rhesus monkey.

#### **Organ weights:**

**Increase in spleen weight** (both absolute and relative) was noted in rhesus monkeys; secondary to increase in platelet counts. No remarkable effects on organ weights in cynomolgus monkey.

#### **Histopathology:**

*Cynomolgus*:

--**Increase in megakaryocytes** in bone marrow (at all dose levels) and liver (5000 mcg/kg).

--Slight **eosinophilic substance** was observed in the red pulp of the **spleen** (300-5000 mcg/kg) and blood vessels of the lung (5000 mcg/kg).

-- In the lung of one animal of the 5000 mcg/kg dose group that showed red foci in gross pathology, hemorrhage accompanied by fibrous thickening of the pleura was observed.

--Minor extramedullary hematopoiesis was observed in the endometrium of the uterus of the 100 and 5000 mcg/kg dose group animals.

--**Slight neutrophilia** was noted in the liver of the 5000 mcg/kg dose group animals.

--*Recovery group*: No changes induced by the test article at the end of recovery period were noted.

*Rhesus*:

Histopathological findings in the rhesus monkey included increase in megakaryocytes in bone marrow and lungs, slight eosinophilic substance in the red pulp of spleen and blood

vessels of the lungs and slight increase in extramedullary hematopoiesis in bone marrow and spleen.

#### **Ovaries (histopathology):**

**No treatment-related changes** were noted in the ovaries in both rhesus and cynomolgus monkeys.

#### **Toxicokinetics:**

Peak AMP2 concentration was observed 4 hours after SC administration in female cynomolgus monkeys. C<sub>max</sub> ranged from 59.5 ng/mL (100 mcg/kg) to 2720 ng/mL (5000 mcg/kg) on Day 0, and from 26.4 ng/mL (100 mcg/kg) to 2920 ng/mL (5000 mcg/kg) on Day 28.

On Day 0 and Day 28, the observed C<sub>max</sub> and AUC in rhesus monkeys were approximately twice as high as those observed in cynomolgus monkeys that received the same dose (5000 mcg/kg). The mean T<sub>1/2</sub> ranged from 4.49 to 10.4 hours in cynomolgus monkeys, and was 9.10 hours in rhesus monkeys. Repeated dosing of AMP2 resulted in an accumulate ratio of 1.47 in rhesus monkeys. In cynomolgus monkeys, reduced exposure to AMP2 upon multiple dosing was observed for the 100 mcg/kg dose group. It is possible that elevated platelets may contribute to the accelerated AMP2 removal at lower doses upon repeated dosing.

Dose (µg/kg) Species	n	Day	C <sub>max</sub> (ng/mL)	T <sub>max</sub> <sup>a</sup> (h)	AUC <sub>(0-24)</sub> <sup>b</sup> (ng·h/mL)	t <sub>1/2</sub> (h)		Accumulation Ratio <sup>e</sup>
						t <sub>1/2β</sub> <sup>c</sup> All	t <sub>1/2γ</sub> <sup>d</sup> Recovery	
100 Cynomolgus	6	0	59.5 (25.0)	4	690 (373)	6.05 (1.69)	NA	0.346
		28	26.4 (9.0)	4	239 (101)	NA	418	
300 Cynomolgus	6	0	120 (56)	4	1290 (534)	4.49 (1.40)	NA	1.31
		28	127 (73)	4	1700 (978)	NA	7	
500 Cynomolgus	6	0	149 (106)	6	1710 (1280)	4.83 (2.40)	NA	1.70
		28	230 (182)	6	2900 (2350)	NA	65.4	
5000 Cynomolgus	8	0	2720 (1410)	4	24500 (10900)	10.4 (2.40)	NA	1.64
		28	2920 (1370)	4	40200 (29100)	NA	67.2, 324	
5000 Rhesus	8	0	4880 (2080)	4	47500 (22900)	9.10 (1.78)	NA	1.47
		28	5720 (2230)	6	69800 (26200)	NA	215, 128	

<sup>a</sup>Mean values rounded to 2 significant figures whenever possible.

#### **Antibody analysis:**

All Rhesus and Cynomolgus monkeys administered AMP2 in this study were negative for development of antibodies to TPC. The overall incidence of anti- AMP2 antibodies in Cynomolgus and Rhesus monkeys was 30.8 04 and 100%, respectively. The observed anti-AMP2 antibodies were non-neutralizing. A comparison of the incidence of antibodies to AMP2 at the 5000 mcpg/kg dose given by SC administration demonstrates that AMP2 is more immunogenic in Rhesus monkey than Cynomolgus monkeys (see the sponsor's table below).

Group	Dose (µg/kg) & Route	Total No. of Monkeys	No. Positive for anti-AMP2 Binding Antibodies	No. Positive for anti-TPO Binding Antibodies	Percent Incidence of anti-AMP2 Binding Antibodies	Percent Incidence of anti-TPO Binding Antibodies
2	0 (SC)	6	0	0	0%	0%
3	100 (SC)	6	1	0	17%	0%
4	300 (SC)	6	4	0	67%	0%
5	500 (SC)	6	2	0	33%	0%
6	5000 (SC)	8	1	0	13%	0%
Overall incidence		26 animals dosed	8	0	30.8%	0%

**Reviewer's comments:**

This study was a follow-up repeat-dose toxicity study to evaluate further the findings of ovarian cystic follicles noted in an earlier 4-week repeat-dose study in rhesus monkeys. Ovarian cysts are sometimes observed in wildy grown monkeys. However, the occurrence of ovarian cysts was higher than seen in the historical control. According to the sponsor, this study was conducted in cynomolgus monkeys to include animals that were ovulating since rhesus monkeys are seasonal breeders and were quiescent at the time toxicity study was being conducted. Unlike earlier repeat-dose study in rhesus monkeys, no ovarian follicular cysts were noted in both rhesus and cynomolgus strains. It is likely ovarian cysts seen in the earlier study are incidental findings, and not AMG 531 treatment-related effect.

Similar degree of increase in platelet counts (3-6 fold increase) was observed in rhesus and cynomolgus monkeys; accompanied by megakaryocytosis in the bone marrow, lung and liver. Anemia with an increase in reticulocyte count and increase in erythroblasts was observed at mid and high doses. In addition, eosinophilic substance in the red pulp of the spleen and in the blood vessels of the lungs was noted in histopathology. It is not clear if these effects are definitively due to high increase in platelet counts. These effects were seen at doses >100-times the exposure expected in humans.

AMP2 was more immunogenic in rhesus than cynomolgus monkey. The overall incidence of anti-AMP2 binding antibodies in cynomolgus and rhesus monkeys was 30.8% and 100%, respectively. These were non-neutralizing antibodies.

In terms of pharmacodynamic response (increase in platelet count), monkeys seem to be less sensitive than rats, even though in vitro studies indicated similar binding affinities for TPO receptors. Approximately 50-times higher plasma concentrations were required to achieve similar responses as in rats.

**d) A 3 and 6 month repeated dose toxicity study of AMP2 administered subcutaneously in cynomolgus monkeys followed by 8-week recovery period**

AMP2 (0, 500, 1000 or 5000 mcg/kg, 30-300 times MHD based on AUC) was administered subcutaneously to cynomolgus monkeys once weekly for 13 weeks or 26 weeks followed by 8 week of recovery period. There were no AMP2 treatment-related effects on mortality, clinical observations, body weight, food consumption, ECG, ophthalmology, urinalysis, plasma biochemistry, gross necropsy and organ weights.

Dose-dependent increases in platelet counts were observed from Week 2 through Week 25, accompanied by low mean platelet volume in females at 500 and 5000 mcg/kg dose. In the microscopic evaluation, increase of megakaryopoiesis in the bone marrow; and megakaryocytes in the submandibular lymph node were noted at 1000 and 5000 mcg/kg doses. These effects are due to the pharmacological action of AMP2, and were reversed at the end of the recovery period. At the injection sites, slight perivascular mononuclear cell infiltration was observed in AMP2-treated animals with a trend towards recovery noted at the end of treatment-free period.

The TK profile of AMP2 was similar in both male and females and no accumulation of the drug was observed after repeated dosing. There was a dose-dependent increase in C<sub>max</sub> (138-1410 ng/ml) and AUC (1920-20,100 ng\*hr/ml). Overall incidence of antibody development towards AMP2 was 54.2%. The incidence of anti-AMP2 neutralizing antibodies was 4.2%. Anti-TPO antibodies were observed in one animal in the 500 µg/kg group. No TPO neutralizing antibodies were detected.

The NOAEL for this 6 month repeat-dose toxicity study in cynomolgus monkey was 5000 mcg/kg (300-times MHD).

Study no.:	101814
Volume # and page #:	eCTD 4.2.3.2.1-10814
Conducting laboratory and location:	<u>  </u>
Date of study initiation:	August 20, 2002
GLP compliance:	Yes
QA report:	yes (X) no ( )
Drug, lot #, and % purity:	A0102140000, 98%

The purpose of this study was to evaluate the toxicity and toxicokinetics of AMP2 when administered subcutaneously to cynomolgus monkeys once weekly for 3 or 6 months, and to assess the reversibility of any effects following an 8-week recovery period.

**Methods**

Doses: 0, 500, 1000 or 5000 µg/kg once weekly for 13 weeks or 26 weeks  
 Species/strain: Cynomolgus monkey  
 Number/sex/group or time point (main study): 6/sex/dose: 3/sex/dose each were necropsied at the end of the 13-week and 26-week dosing period  
 Recovery group: 2/sex/dose after 8-weeks of recovery period following the 26-week dosing period.  
 Route and dose volume: Subcutaneous, 0.25 mg/mL.  
 Age: 2.5-6 years old at dosing  
 Weight: 2.8-4.78 kg for males and 2.4-2.92 kg for females  
 Unique study design or methodology (if any): none

The study design is shown in the sponsor's table.

Group	Test or Control Article	Dose Level (µg/kg/dose)	Concentration (mg/mL)	Dose Volume <sup>a</sup> (mL/kg)	Number of Animals Male: female (Study specific number) <sup>b, c, d</sup>
1	AMP2 Placebo	0	0	0.25	8:8 (1-16)
2	AMP2	500	2	0.25	8:8 (17-32)
3	AMP2	1000	4	0.25	8:8 (33-48)
4	AMP2	5000	20	0.25	8:8 (49-64)

Animals were administered AMP2 or placebo once per week.

a: Individual dose volume (mL) was based on the most recent body weight.

b: Three male and 3 female animals per group were necropsied at the end of the 13-week dosing period (study specific number 1-6, 17-22, 33-38, 49-54).

c: Three male and 3 female animals per group were necropsied at the end of the 26-week dosing period (study specific number 7-12, 23-28, 39-44, 55-60).

d: Two male and 2 female animals per group were used for evaluation of the reversibility of test article-related toxicity (study specific number 13-16, 29-32, 45-48, and 61-64).

**Observations and times:**

Mortality/Morbidity: Twice daily

Clinical signs: Full observations once daily

Body weights: Weekly

Food consumption: Weekly

Ophthalmoscopy: Prior to treatment, once at Week 13 & 25 of dosing and at the end of recovery period.

EKG: Pre-dosing, once at Weeks 12 & 25 of dosing and once at Weeks 4 and 8 of the recovery period.

Hematology: Once at weeks 2, 4, 8, 16 & 20, prior to the necropsies (weeks 13 & 26), and once at Weeks 4 and 8 of the recovery period.

Clinical chemistry: Pre-dosing, once at Week 4 and prior to necropsy (Weeks 13 & 26) and during recovery period (Weeks 4 & 8)

<u>Urinalysis:</u>	Once at Weeks 12 and 25 and once at Week 8 of the recovery period and at necropsy of the recovery animals.
<u>Gross pathology:</u>	Weeks 13 & 26 and recovery period: 8 weeks, unscheduled death/morbidity.
<u>Organ weights:</u>	Adrenal (2), brain, heart, kidney (2), liver (with gallbladder), lung, ovary (2), spleen, testis with epididymis (2), pituitary, prostate, spleen, thyroid with parathyroid, thymus and uterus.
<u>Histopathology:</u>	Adequate Battery: yes (X); Peer review: no (X)

Blood gas & respiraory rate: Pre-dosing, Days 1, 10 & 83

Toxicokinetics (main study animals):

Sampling time-points: Prior to dosing and 2, 4, 8, 24 and 48 hours after dosing on Day 1, Day 85 (Week 13) and Day 176 (Week 26). Trough level samples were collected on Day 8 (Week 2), Day 36 (Week 6) and Day 120 (Week 18) prior to dosing and 48, 72, 168, 264, 432 and 600 hours after dosing during the recovery period.

Antibody measurements:

Once at Weeks 2, 4, 12 & 25, and at Weeks 4 and 8 of the recovery period.

## Results

Mortality: No unscheduled deaths or in moribund condition.

Clinical signs: No remarkable clinical observations. Mild swelling and/or erythema were periodically observed at the injection sites

Body weights and food consumption: No significant effects on body weights or food consumption.

Ophthalmoscopy: There were no treatment-related ophthalmic observations.

ECG: No significant effect on ECG parameters, including QT/QTc interval.

Hematology:

Dose-dependent 1.6 to 3.7-fold increase in platelet counts.

The peak of increase in platelet count was observed at Week 2, 4, 8 or 12 depending on the dose. Peak levels in male and female were similar: 500 mcg/kg: 1.58 and 1.70 fold increase, 1000 mcg/kg: 1.65 and 1.59 fold increase and 5000 mcg/kg: 2.87 and 3.65 fold increase; accompanied by low mean platelet volumes in females at 500 & 5000 mcg/kg.

Large or giant platelets were observed in all dose groups including control throughout acclimation and dosing and recovery periods.

Blood gas and respiratory rate:

No effects on pCO<sub>2</sub>, pO<sub>2</sub> or respiratory rates

Clinical chemistry: No treatment-related significant clinical pathology findings

Urinalysis: No treatment-related effects.

Gross pathology: There were no remarkable findings.

Organ weights: Organ weights were not affected.

Histopathology:

**13 weeks:** Mild increase of *megakaryopoiesis* in the bone marrow in 1 male at 1000 mcg/kg and 2 males & 2 females at 5000 mcg/kg. One male from 1000 mcg/kg dose also showed megakaryocytosis in the submandibular lymph nodes

**26 weeks:** Moderate increase in *megakaryopoiesis* in the bone marrow (1 female at 1000 mcg/kg and 1 male & 1 female at 5000 mcg/kg). Megakaryocytes in submandibular lymph node were also observed in one female at high dose.

**Recovery period:** The above findings are likely to be due to the pharmacological action of the drug related to the increase in platelet production. These effects (megakaryopoiesis) were reversed at all dose levels at the end of 8-week recovery period.

**Injection site:** At the injection sites, mild perivascular mononuclear cell infiltration was observed at all dose levels at Weeks 13 and 26. There were no other remarkable histopathological findings.

**Toxicokinetics:**

The toxicokinetics of AMP2 was linear and similar in both males and females. The presence of binding anti-AMP2 antibodies had no apparent impact on the toxicokinetic profile. No appreciable accumulation was observed upon multiple weekly dosing for up to 26 weeks post dose. TK data is summarized in the sponsor's table below.

Dose (µg/kg)	Week 1				Week 13				Week 26					
	n	T <sub>max</sub> (h)	C <sub>max</sub> (ng/mL)	AUC <sub>(0-24)</sub> (ng·h/mL)	n	T <sub>max</sub> (h)	C <sub>max</sub> (ng/mL)	AUC <sub>(0-24)</sub> (ng·h/mL)	AR <sup>b</sup>	n	T <sub>max</sub> (h)	C <sub>max</sub> (ng/mL)	AUC <sub>(0-24)</sub> (ng·h/mL)	AR <sup>b</sup>
500	15 <sup>c</sup>	4 (4-8)	179 (128)	2230 (1480)	16	4 (2-8)	186 (144)	2710 (2450)	1.29 (0.898)	10	4 (4-8)	138 (158)	1920 (2090)	1.05 (0.498)
1000	16	4 (2-8)	359 (239)	4510 (2750)	16	4 (2-8)	349 (228)	4600 (2780)	1.10 (0.591)	10	4 (4-8)	311 (252)	4180 (3650)	1.19 (0.809)
5000	16	4 (2-4)	1640 (805)	18700 (7310)	15 <sup>d</sup>	4 (2-8)	1610 (835)	23000 (11800)	1.55 (1.00)	9 <sup>e</sup>	4 (2-8)	1410 (837)	20100 (12200)	1.16 (0.728)

<sup>a</sup>Mean values rounded to 3 significant figures, except for T<sub>max</sub> values which are shown as median (range).

<sup>b</sup>AR (Accumulation Ratio) = AUC<sub>(0-24)</sub>, Week 13 or Week 26 / AUC<sub>(0-24)</sub>, Week 1.

<sup>c</sup>Animal 17 was excluded from toxicokinetic analysis because all of the values during Week 1 were BLQ.

<sup>d</sup>In the 5000 µg/kg dose group, Animal 60 was excluded from toxicokinetic analysis at Weeks 13 and 26 because of the presence of anti-AMP2 neutralizing antibodies.

### Antibody measurement:

Overall incidence of antibody development towards AMP2, TPO and human Fc was 54.2%, 2.1% and 14.6% respectively. The incidence of anti-AMP2 neutralizing antibodies was 4.2% in the study. Anti-TPO antibodies were observed in one animal at 500 µg/kg. However, no neutralizing TPO antibodies were observed.

Incidence of anti-AMP2 antibodies is summarized in the sponsor's table below.

Group	Dose (µg/kg)	Day -1	Day 14	Day 28	Day 83-84	Day 175	Day 203	Day 231
1	0	0/16	1/16	0/16	1/16	0/10	0/4	0/4
2	500	1/16	0/16	1/16	5/16	5/10	2/4	0/4
3	1000	3/16	0/16	4/16	5/16	5/10	3/4	3/4
4	5000	2/16	0/16	4/16	7/16	8/10	3/4	2/4

### Reviewer's comments:

Based on the results of this study, the NOAEL for the 6-month repeat-dose toxicity study in monkey was 5000 mcg/kg (300-times MHD). AMG 531 caused dose-dependent increases in platelet count (1.6 to 3.7-fold). The main effect observed was megakaryopoiesis in the bone marrow at mid- and high-doses. This is an expected effect at super-pharmacological doses and was reversed at the end of recovery period. At the injection sites, perivascular mononuclear cell infiltration was observed at all doses.

TK analysis showed dose-dependent increase in C<sub>max</sub> and AUC of AMG 531 with no apparent accumulation after repeat dosing. The presence of binding anti-AMP2 antibodies had no apparent impact on the TK profile. Binding antibodies to AMG 531 were observed in 54% of animals with 4.2% of animals developing neutralizing antibodies.

In terms of pharmacodynamic effects (increase in platelet counts), monkey seems to be much less sensitive species than rats. In monkey, approximately 50-fold higher dose (5000 mcg/kg) was needed to achieve same response (3-4 fold increase in platelet counts) as seen in rats (100 mcg/kg). Monkey was also less immunogenic than rats. Unlike in rats, no myelofibrosis was observed in monkeys after 6 months of repeated dosing. Based on the Pharmacodynamic and adverse effects seen, rat seems to be more relevant species than monkey in terms of predicting effects in humans.

#### **2.6.6.4 Genetic toxicology:**

Genotoxicity testing is not required based on current guidance for biotechnology-derived pharmaceuticals. AMG 531 is a recombinant protein composed of an Fc domain and an Mpl receptor binding domain with \_\_\_\_\_

#### **2.6.6.5 Carcinogenicity:**

Carcinogenicity studies were not conducted. Based on the guidance for biotechnology-derived pharmaceuticals, carcinogenicity studies are generally not required for protein-based drugs such as AMG 531. Also AMG 531 was highly immunogenic in rats and mice. Neutralizing antibodies were observed in both species making it difficult to assess carcinogenicity potential in traditional rodent models. No hyperplastic lesions were observed in the repeat-dose toxicity in monkey at doses up to 5000 mcg/kg (300-times MHD). However, the duration of this toxicity study was limited to 6 months.

There is a concern related to the tumor stimulation capacity of thrombopoietin and, therefore, AMG 531 ability to stimulate certain types of acute myeloid leukemia cells. There are published studies indicating increased expression of TPO receptors in certain subset of myelogenous leukemia cells and TPO has been noted to participate in the in vitro proliferation of these cells. The label for AMG 531 contains warning stating stimulation of the thrombopoietin (TPO) receptor on the surface of hematopoietic cells may increase the risk for hematologic malignancies. In a clinical study of AMG 531 administration to patients with a myelodysplasia syndrome (MDS), increased blast cells counts were observed in some patients.

#### **2.6.6.6 Reproductive and developmental toxicology**

##### **Fertility and early embryonic development:**

###### ***Study title:***

**A study to determine the effects of AMG 531 via subcutaneous administration on fertility in Sprague Dawley rats**

###### **Key study findings:**

AMG 531 (10, 30 & 100 mcg/kg, 1-37 times MHD based on AUC from 4-week repeat-dose toxicity study) was administered three times weekly by subcutaneous injection to rats from before mating to mid-gestation. Mortality was observed at all dose levels tested (1 male & 1 female each died at 10, 30 & 100 mcg/kg). The deaths were attributed to thrombocytosis induced increase in blood viscosity resulting in difficulties in obtaining blood samples and handling of these animals. AMG 531 caused 1.2 to 3-fold increases in platelet count.

AMG 531 treatment at 30 and 100 mcg/kg doses caused decreases in mean body weight (3-6%), body weight gain (maximum 43-50%) and food consumption (6-13%). Enlarged spleens were also observed at these doses, an expected pharmacological effect.

AMG 531 had no effect on male or female fertility at doses up to 100 mcg/kg (37-times MHD). There were no effects on estrous cycle, sperm density, morphology & motility, mating behavior and reproductive performance.

AMG 531 neutralizing antibodies were observed in 56% of treated animals. 6% of animals also had endogenous TPO binding antibodies but no TPO neutralizing antibodies were detected.

**Study no.: 102970**

**Volume # and page #: eCTD 4.2.3.5.1**

**Conducting laboratory and location:**

**Date of study initiation:** 18 Feb, 2003

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, and % potency:** A0102140000, 100%

The aim of this study was to determine the effects of AMG 531 on fertility, when administered three times per week by subcutaneous injection to rats from prior to mating to mid-gestation.

##### **Methods**

Doses: 0, 10, 30 & 100 mcg/kg

Species/strain: SD rats (9-10 weeks old, male: 274-367 g, female: 220-309 g)

Number/sex/group: Main study: 25/sex/group + Satellite group: 5/sex/group  
Route and volume: Subcutaneous, 3-times/week, 1 ml/kg

*Satellite groups used for toxicokinetics:*

Toxicokinetics was not performed.

5/sex/group, designated as satellite animals, had blood collected for platelet count and clinical immunology (antibody analysis) testing pre-dose, prior to cohabitation, and prior to termination. Antibody analysis was conducted using biosensor immunoassay.

*Study design:*

Animals were dosed three times weekly, beginning four weeks prior to cohabitation (males) or two weeks prior to cohabitation (females), until the day prior to necropsy.

After four weeks of dosing for males and two weeks of dosing for females, animals were cohabitated, one male to one female within the same dose group. The animals were separated upon confirmation of mating (sperm positive and/or copulatory plug), or after 14 days of cohabitation, whichever came first. The day of confirmation of mating was designated Gestation Day (GD) 1. Females were sacrificed on GD 14-16 or 14-16 days after the end of cohabitation and necropsy was conducted. Males were sacrificed and necropsied after completion of all female necropsies.

*Parameters and endpoints evaluated:*

Endpoints evaluated included mortality, clinical observations, body weight (twice weekly), food consumption (twice weekly), gross necropsy findings, vaginal cytology (daily), pregnancy status, fertility, estrous cycling, reproductive organ weights, number of corpora lutea, number & position of dead/live fetuses, number & position of early/late resorptions, sperm analysis, clinical pathology (platelet count), clinical immunology (antibody analysis), and histopathology (reproductive organs).

## Results

### Mortality:

**Main study group:** There were **3 deaths** (all males). One male each in the 10 and 100 µg/kg dose groups were found dead on Days 9 and 12, respectively. The dead animals exhibited no clinical symptoms prior to death. There were no gross necropsy findings except spleen enlargement, which is an expected pharmacological effect due to increase in platelet counts.

One male in the 30 µg/kg dose group was euthanized on Day 18 due to morbidity. Prior to euthanasia, the moribund male exhibited hunched posture, squinting, rough hair coat, nasal and eye discharge and severe weight loss.

**Satellite group:** One female in each of the 10, 30 and 100 µg/kg satellite dose groups died on Day 12 following pre-cohabitation blood collection.

These deaths were believed to be due to a combination of the pharmacological effect of AMG 531 (thrombocytosis) in high dose, increased blood viscosity due to large increase in platelet counts making it difficult to obtain blood sample and the bleeding procedure. The sponsor stated that blood collection procedures were amended (no details provided) and no other deaths associated with blood collection occurred. All other animals survived to scheduled termination.

**Clinical signs:** No remarkable clinical observations in surviving animals.

**Body weight:**

*Mean body weights* were significantly **lower (3%-6%)** than controls for 30 & 100 mcg/kg males on Days 15, 18 & 22, in addition to significantly lower mean body weight gains (~50%.) observed at these doses. There was no dose-response relationship for the effects on body weight. Females were less affected than males with lower mean body weight gains (~43%) observed in the 30 mcg/kg group only.

There were no effects on body weight parameters at 10 mcg/kg.

**Food consumption:**

AMG 531 treatment (30 & 100 mcg/kg) resulted in 6-13% **decrease** in the total food consumption in males and 6-8% decrease in females. There was no dose-response relationship for the effect on food consumption. However, lower body weights were consistent with the lower total food consumption.

No effects on food consumption were observed at 10 mcg/kg.

**Vaginal cytology:**

*No effects on the estrous cycles* were observed at any of the dose levels.

**Sperm analysis:**

There were **no effects** on sperm density, morphology or sperm motility at any of the dose levels tested.

**Reproductive performance:**

*Mating behavior was unaffected* by AMG 531 treatment. All females, with the exception of one female each in the 10, 30 and 100 mcg/kg dose groups, were confirmed to have mated. There was no significant difference in the pre-coital interval among the dose groups.

Pregnancy rate for the 0, 10, 30 and 100 mcg/kg dose groups were 96.7, 93.1, 93.1, and 96.6%, respectively. Females treated with 30 mcg/kg ovulated significantly fewer eggs than controls as evidenced by the number of corpora lutea, 14.2 versus 16.7, respectively. This resulted in a significantly lower number of implantations (13.5 versus 15.7, for 30 mcg/kg and controls, respectively). However, the lower number of corpora lutea were within the historical range (12.9 to 21.7) and were considered not treatment-related. No effects were seen at high dose of 100 mcg/kg.

There were no differences in pre-implantation loss, post-implantation loss or the mean number of live fetuses among groups. The sponsor's table below summarizes the reproductive performance in females.

	Group 1	Group 2	Group 3	Group 4
No. of females paired	30	29	29	29
No. of females mated	30	28	28	28
Pre-coital period (Mean) (S.D.)	2.7 2.8	3.9 3.3	2.7 2.6	2.5 2.4
Copulation Index (%)	100.0	96.6	96.6	96.6
Not pregnant (%)	1 (3.3)	2 (6.9)	2 (6.9)	1 (3.4)
Died/Killed	0	0	0	0
Survived to scheduled kill	1	2	2	1
Pregnant (%)	29 (96.7)	27 (93.1)	27 (93.1)	28 (96.6)
Died/Killed/Aborted	0	0	0	0
with total resorption	0	0	0	0
with live fetuses at scheduled kill	24	23	23	24

Nominal Dose: Group 1 - 0 µg/kg    Group 2 - 10 µg/kg    Group 3 - 30 µg/kg    Group 4 - 100 µg/kg

#### **Gross pathology and organ weights:**

***Spleen enlargement*** was observed in both the 30 & 100 mcg/kg treated males and females. This is an expected pharmacological effect at high doses due to increased platelet count. There were no other macroscopic findings.

There were no effects on mean organ weights or mean organ-to-body weight ratios of male reproductive tissues. Organ weights for females were not evaluated.

#### **Histopathology:**

No effects on the testes and epididymides were observed.

#### **Clinical pathology (platelet counts):**

Increased platelet counts (118-223%) were observed in males at all dose levels and in females at 30 & 100 mcg/kg doses. There was no clear dose-response relationship for the increase in platelet counts. This is likely due to development of neutralizing antibodies for AMG 531 after repeated dosing.

#### **Clinical immunology:**

Overall incidence of antibodies in animals dosed with AMG 531 is summarized in the table below. The distribution of binding and neutralizing antibodies among females and males was comparable. 56% of animals had neutralizing antibodies against AMG 531. 7% of the animals also had TPO binding antibodies. However, no TPO neutralizing antibodies were detected.

Detection of antibodies	% of animals
Abs binding to the whole AMG 531 molecule	62.8%
Abs that bound to TMP portion of the molecule	56.8%
Anti-TPO (endogenous TPO) binding antibodies	5.6%
Abs that bound to the whole AMG 531 molecule at baseline (control)	1 animal
Abs with neutralizing activity against AMG 531	56%
Abs with neutralizing activity against TPO	None
Neutralizing Abs against AMG 531 or TPO at baseline	None

**Toxicokinetics:** Not conducted.

**Sponsor's conclusions:**

The LOAEL was 30 mcg/kg (3-times MHD) based on effects on body weight and food consumption. The NOAEL was established at 10 µg/kg (1-times MHD). Treatment at doses up to and including 100 µg/kg (37-times MHD) AMG 531 had no effect on fertility.

**Reviewer's comments:**

AMG 531 had no effect on male or female fertility at doses up to 100 mcg/kg (37-times MHD based on AUC from the 4-week repeat-dose toxicity study in rats).

Mortality was observed at all dose levels tested. Although there was no dose-response relationship (1 male & 1 female each died at 10, 30 & 100 mcg/kg), deaths were clearly treatment-related. This is consistent with the mortality seen in the 4-week repeat-dose toxicity study in rats. The sponsor claims that the deaths are due to thrombocytosis induced increase in blood viscosity resulting in difficulties in obtaining blood samples and handling of these animals. It is quite likely that the deaths are due to exaggerated pharmacological effects of the drug but no clear cause of death was established.

**Embryo-fetal development:**

**Study title:**

**a) A dose range-finding study to determine the effects of subcutaneous administration of AMP2 on embryo-fetal development and placental transfer in Sprague Dawley rats**

**Key study findings:**

Treating pregnant rats subcutaneously every-other-day from gestation day 7 to 19 with AMG 531 at 10, 30, 60 & 100 mcg/kg (0.25-11 times MHD based on AUC obtained in this study) had no effect on maternal or developmental toxicity.

AMG 531 crosses the placenta resulting in dose-dependent increases in fetal blood levels (~50% of blood concentration in dams) and amniotic fluid (11-44%), and significant increase in platelet counts in fetuses. AMG 531 was immunogenic; AMG 531 binding antibodies were observed in dams (overall 46.7% of total animals) as well as fetuses (7.5%). All antibody-positive fetal samples originated from antibody positive dams.

**Study no.: 101948**

**Volume # and page #: eCTD 4.2.3.5.2.1-101948**

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** Nov 9, 2001

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug lot # and % purity:** A0102140000, 99.8%

**Methods:**

AMG 531 was administered to pregnant rats (n=5/dose) via subcutaneous injection at doses of 0, 10, 30, 60 & 100 mcg/kg during the period of organogenesis. Dosing was performed on gestation day (GD) 7, 9, 11, 13, 15, 17 & 19.

*Maternal evaluations:* The parameters evaluated included mortality, clinical observations, body weights, gross pathology (GD 22), pregnancy status, live and dead fetuses, gravid uterine weight, early & late resorptions, abnormalities of the placenta or embryonic sac, number & type of implantations and number of corpora lutea (ovary).

*Fetal Evaluations:* All live and dead fetuses were examined externally and individual sex and body weights were recorded.

Amniotic fluid, maternal blood and fetal blood were analyzed to determine platelet counts, clinical immunology, toxicokinetics and placental transfer in a satellite group of animals (n=15/dose). The time-points for sampling were as follows:

*Clinical immunology (dams):* Prior to dosing on GD 7, 14, and 19.

*TK (dams):* Pre-dose, and 4, 12, 24 and 48 hours post-dose of GD 7 & 19 treatment.

*Placental transfer (amniotic fluid, maternal & fetal blood):* Samples were collected for test article analysis following the last TK sample on GD 19.

*Platelet count:* From main study animals on GD 22.

## **Results**

### **Maternal data:**

*Mortality:* None in main study animals.

According to the sponsor due to the procedure of blood collection, five animals from TK satellite group died: two on GD 14 (10 mcg/kg) and three on GD 19 (two in 10 mcg/kg and one in 30 mcg/kg group). No further explanation was provided. However, it is likely that the deaths were due to combination of extreme thrombocytosis that causes increase in blood viscosity making it difficult to withdraw frequent blood sampling from the animals. It should be noted there were no deaths at any dose levels in the main study group animals since no blood was withdrawn from these animals.

*Clinical observations:* No remarkable clinical observations.

*Body weights:* No significant effect on body weight, body weight gain and body weight gain adjusted for gravid uterine weight.

*Food consumption:* No effect on food consumption.

*Gross pathology findings:* Not remarkable (main study group). No gross necropsy was performed on animals that died in the satellite group.

*Pregnancy status:* No effect on pregnancy status.

*Uterine weight:* Mean gravid uterine weights of all AMG 531-treated females were comparable to control animals.

*Corpora lutea:* The mean number of corpora lutea were similar among all groups.

*Implantation Number and Type:* AMG 531 treatment had no effect on implantations.

### **Fetal Data**

AMG 531 treatment had no effect on fetal weight and external examination findings were unremarkable.

### **Platelet counts:**

Both maternal and fetal platelet counts were affected. Platelet counts were dose-dependently increased in dams (1.5- to 3-fold as compared to control group) and in fetuses (1.7- to 6-fold as compared to control values in fetuses).

### **Clinical immunology:**

The overall incidence of development of antibodies was as follows:

*Anti-AMG 531 antibodies (dams):* 46.7%

*Anti-AMG 531 antibodies (fetuses):* 7.5%. All antibody-positive fetal samples originated from antibody positive dams.

*Anti-AMG 531 neutralizing antibodies (dams):* 11.1%

*Antibodies to endogenous TPO or anti-TPO neutralizing antibodies:* None.

The summary of anti-AMG 531 antibodies in dams and fetuses is summarized in the sponsor's table below.

**Table 13**  
Summary of Overall Incidence of antibodies per Dosing Group as Detected by a Biosensor Immunoassay in the Dams for Study 101948

Group	Dose (µg/kg)	Total No Of Rats	No. of Animals Positive for anti-AMP2 Binding Antibodies	No. of Animals Positive for anti-TPO Binding Antibodies	Percent Incidence of anti-AMP2 Binding Antibodies	Percent Incidence of anti-TPO Binding Antibodies
6	0	15	4	0	26.7%	0
7	10	15	6	0	40.0%	0
8	30	15	8	0	53.3%	0
9	100	15	7	0	46.7%	0
Overall Incidence		45	21	0	46.7%	0

**Table 14**  
Summary of Overall Incidence of antibodies per Dosing Group as Detected by a Biosensor Immunoassay in the Fetuses for Study 101948

Group	Dose (µg/kg)	Total No Of Rats	No. Positive for anti-AMP2 Binding Antibodies	No. Positive for anti-TPO Binding Antibodies	Percent Incidence of anti-AMP2 Binding Antibodies	Percent Incidence of anti-TPO Binding Antibodies
6	0	15	0	0	0%	0
7	10	13	1	0	7.7%	0
8	30	14	2	0	14.3%	0
9	100	13	0	0	0%	0
Overall Incidence		40	3	0	7.5%	0

#### **TK and placental transfer:**

AMG 531 concentrations in maternal blood, fetal blood and amniotic fluid increased with increasing dose with no accumulation observed after multiple dosing. In all dose groups, the average serum concentrations in fetal animals were ~50% of those in dams. The concentrations in amniotic fluid were relatively low and variable (11%-44%) as compared to fetal serum concentrations. The TK data obtained on GD 19 is summarized in the sponsor's table below.

Dose Group	Parameter <sup>a</sup>	Dam	Fetus	Amniotic Fluid
10 µg/kg	C <sub>max</sub> (ng/mL)	0.715	0.313	0.308
	T <sub>max</sub> (h)	4	4	24
	AUC <sub>(0-48)</sub> (ng·h/mL)	17.0	8.30	7.46
	C <sub>avg</sub> (ng/mL)	0.354	0.173	0.155
30 µg/kg	C <sub>max</sub> (ng/mL)	10.5	2.65	1.11
	T <sub>max</sub> (h)	12	12	48
	AUC <sub>(0-48)</sub> (ng·h/mL)	184	79.9	31.7
	C <sub>avg</sub> (ng/mL)	3.83	1.66	0.660
100 µg/kg	C <sub>max</sub> (ng/mL)	25.0	11.5	3.40
	T <sub>max</sub> (h)	12	24	48
	AUC <sub>(0-48)</sub> (ng·h/mL)	733	378	83.3
	C <sub>avg</sub> (ng/mL)	15.3	7.83	1.74

<sup>a</sup>Parameter values except T<sub>max</sub> are rounded to 3 significant figures.

#### Reviewer's comments:

The dose levels selected were based on the pharmacodynamic response (increase in platelet counts) expected in rats. The super-pharmacologic doses produce extreme thrombocytosis and associated toxicity; thus limiting the administration of AMG 531 at very high doses.

AMG 531 crosses the placenta and significant levels of AMG 531 were detected in fetal blood (~50% of blood concentrations in dams) and amniotic fluid (11-44%). Accordingly, platelet counts were increased in dams (maximum 3-fold) and fetuses (maximum 6-fold as compared to baseline values in fetus) in a dose-dependent manner. Anti-AMG 531 antibodies were detected in dams (46.7%) as well as fetuses (7.5%) at all dose levels. Possible implications of these findings are discussed below in the 'Definitive rat embryo-fetal developmental toxicity study' section.

AMG 531 is a recombinant protein that has an Fc domain of human IgG. It is known that maternal IgG can be transferred by the FcRn receptor to the fetus. It is possible that a similar mechanism is responsible for the transfer of AMG 531 from the dam to the fetus.

#### Study title:

b) A Study to determine the effects of subcutaneous administration of AMG 531 on embryo-fetal development in Sprague Dawley rats

#### Key study findings:

The potential maternal and developmental toxicity of AMG 531 in pregnant rats was evaluated at 10, 30 & 100 mcg/kg doses (0.25-11 times MHD). There was no effect on

any of the parameters evaluated for maternal and developmental toxicity. Only effect observed was enlargement of the spleen in one 30 µg/kg and four 100 µg/kg female dams. This is an expected pharmacological effect of AMG 531 due to increase in platelet counts. The NOAEL for maternal and fetal toxicity was 100 mcg/kg (11-times MHD).

**Study no.: 102273**

**Volume # and page #:** eCTD 4.2.3.5.2.1-102273

**Conducting laboratory and location:**

**Date of study initiation:** April 28, 2002

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug lot # and % purity:** A0102140000, 99.8%

### Methods:

The study design is summarized in the sponsor's table below.

Group		Dosage (µg/kg)	Test Article Concentration (µg /mL)	Volume (mL/kg)	Treatments (GD)	Number of Mated Females	Animal Numbers
1	Control	0	0	1	7,9,11,13,15,17,19	25	7691-7715
2	Low	10	10	1	7,9,11,13,15,17,19	25	7716-7740
3	Mid	30	30	1	7,9,11,13,15,17,19	25	7741-7765
4	High	100	100	1	7,9,11,13,15,17,19	25	7766-7790

**Mortality and cageside observations:** Twice daily

**Clinical signs:** Detailed clinical observations were recorded prior to each dosing

**Body Weights:** GD 1, 7, 9, 11, 13, 15, 17, 19 & 22

**Food consumption:** Daily

**Gross necropsy:** On GD 22, all dams were weighed and necropsy was performed.

Macroscopic lesions or tissues with significant findings were preserved for microscopic evaluation.

**Toxicokinetics:** Not conducted

**Antibody analysis:** Not conducted

**Maternal evaluations:** The parameters evaluated included pregnancy status, live and dead fetuses, gravid uterine weight, early & late resorptions, abnormalities of the placenta or embryonic sac, number and type of implantations and number of corpora lutea.

**Fetal Evaluations:** Parameters evaluated included fetal body weight, sex and external, visceral, head and skeletal examinations.

## Results

### Maternal data:

**Mortality:** None

**Clinical signs:** No remarkable clinical observations at any dose levels.

**Body weights:** AMG 531 produced no effect on body weight, body weight gain or body weight gain adjusted for gravid uterine weight.

**Food consumption:** No effect on food consumption.

**Gross pathology:** AMG 531 treatment resulted in enlargement of the spleen in one 30 µg/kg and four 100 µg/kg females. This is an expected pharmacological effect due to increase in platelet counts.

**Pregnancy status:** AMG 531 treatment had no effect on pregnancy status.

**Uterine weight:** Mean gravid uterine weights of all AMG 531-treated females were comparable to that of the controls.

**Corpora lutea:** The mean number of corpora lutea for pregnant females was similar among groups including control.

**Implantation Number and Type:** AMG 531 treatment had no effect on implantations. The mean numbers of implantations, resorptions and live fetuses were similar for control and AMG 531-treated females. There were no dead fetuses in any group.

### Fetal Data

AMG 531 treatment had no effect on numbers of male and female fetuses or fetal weights. The numbers of male and female fetuses, total litter weights and individual fetal weights of the AMG 531-treated animals were comparable to those of the controls.

AMG 531 had no effect on fetal development. There were 3 malformations observed: situs inversus in one 10 µg/kg fetus, unossified vertebra in one 10 µg/kg fetus, and a retinal fold in one 100 µg/kg fetus. Retinal fold is a common artifact of Bouin's fixation and the incidence was within historical control levels. As each of these malformations was only observed in one fetus, and were not dose related, they were not considered treatment related effects.

### ***Reviewer's comments:***

Based on the data provided, the NOAEL for maternal and fetal toxicity was 100 mcg/kg (11-times MHD based on AUC). However, this NOAEL should be interpreted with caution for following reasons.

AMG 531 crosses the placenta and fetal serum concentrations were substantial; approximately 50% of maternal serum concentration. Accordingly, dose-dependent significant increase in the platelet counts (1.7- to 6-fold as compared to control/baseline values in fetus) was observed in fetuses.

In a 4-week repeat dose study in SD rats, AMG 531 caused bone marrow myelofibrosis and other adverse events related to toxicological consequences of the pharmacodynamic effects (increase in platelet counts) at all dose levels tested (10, 30 & 100 mcg/kg). In the present embryo-fetal toxicity study in rats, microscopic evaluation was not conducted (not required based on guidance) to see if increase in reticulin/myelofibrosis or any other expected pharmacological adverse effects (extramedullary hematopoiesis, megakaryocytosis, megakaryocyte hyperplasia, bone hyperostosis etc.) were observed in fetuses due to high increase in platelet counts caused by AMG 531. Since increased reticulin formation and/or fibrosis was observed in pre-clinical as well as clinical studies and there is a potential for significant exposure of AMG 531 during embryo-fetal development, AMG 531 should be administered to pregnant women only if the potential benefit justifies the risk.

**Study title:**

**c) A dose-range finding study to determine the effects of subcutaneous administration of AMP2 on embryo-fetal development in New Zealand white rabbits**

**Key study findings:**

There was no effect on maternal or developmental toxicity at doses up to 60 mcg/kg (39-times MHD). There were no effects on maternal mortality, clinical observations, gross pathology, pregnancy status, number of corpora lutea and implantations, fetal body weights and fetal external examinations. At 100 mcg/kg dose, lower maternal body weight change and total body weight change adjusted for uterine weight were observed.

**Study no.: 101949****Volume # and page #: eCTD 4.2.3.5.2.1-101949****Conducting laboratory and location:****Date of study initiation:** Nov 19, 2001**GLP compliance:** Yes**QA reports:** yes (X) no ( )**Drug lot # and % purity:** A0102140000, 99.8%**Methods:**

The study design is summarized in the sponsor's table below.

Group		Dosage (µg/kg)	Test Article Concentration (µg/mL)	Volume (mL/kg)	Treatments (GD)	Number of Mated Females	Animal Numbers
1	Control	0	0	1	7,9,11,13,15,17,19	5	6128-6132
2	Low	10	10	1	7,9,11,13,15,17,19	5	6133-6137
3	Low-Mid	30	30	1	7,9,11,13,15,17,19	5	6138-6142
4	Mid-High	60	60	1	7,9,11,13,15,17,19	5	6143-6147
5	High	100	100	1	7,9,11,13,15,17,19	5	6148-6152

**Maternal evaluations:** The parameters evaluated included mortality, clinical observations, body weight, food consumption, gross pathology, pregnancy status, live and dead fetuses, gravid uterine weight, number & placement of uterine implantation sites, early & late resorptions, abnormalities of the placenta or embryonic sac and number of corpora lutea (ovary).

**Fetal Evaluations:** All live and dead fetuses were examined externally and individual sex and body weights were recorded.

**Toxicokinetics, clinical immunology & platelet count:** Toxicokinetics and clinical immunology parameters were evaluated using a satellite group of animals at 0, 10, 30, 60 & 100 mcg/kg dose levels (n=3/dose). Platelet counts were analyzed in the main study group animals. The time-points for sampling were as follows:

**Clinical immunology (dams):** Prior to dosing on GD 7, 14, and 21.

**TK (dams):** Pre-dose, and 1, 4, 8, 12, 24 and 48 hours post-dose of GD 7 & 19.

**Platelet count (dams & fetus):** On GD 14 & 21 for dams and on GD 21 for fetuses.

## Results

### Maternal data:

**Mortality:** None

**Clinical signs:** No remarkable clinical observations at any dose levels.

**Body weights:** The total body weight change adjusted for gravid uterine weight for the high dose group was significantly lower than control (37.7 g for control versus -26.5 g for high dose group). This effect is more likely due to a combination of somewhat lower mean body weight gain (control: +16.2% versus high-dose: +12.4%) and higher mean gravid uterine weight (17%) for the high dose group.

**Food consumption:** For the high dose group, the mean food consumption was lower than the control during the intervals of GD 12-13, 13-14 & 26-27. No effect on food consumption was observed in other dose group animals.

**Gross pathology:** No remarkable gross pathology findings.

**Pregnancy status:** AMG 531 treatment had no effect on pregnancy status.

**Uterine weight:** No effect on gravid uterine weight.

**Corpora lutea:** No effect on the mean number of corpora lutea.

**Implantation Number and Type:** AMG 531 treatment had no effect on implantations. The mean numbers of implantations, resorptions, live and dead fetuses were similar for control and AMG 531-treated females.

### Fetal Data

**Body weights:** AMG 531 treatment did not affect fetal weights.

**External examinations:** One of the total 52 fetuses examined in the 100 mcg/kg dose group was malformed. Malformation findings in this fetus included gastrochisis, ectrodactyly and cutis aplasia. This may be an incidental finding. External examination of fetuses at other dose levels was unremarkable.

### Platelet counts (dams & fetus):

There was *no significant increase in platelet counts* at any dose levels. There were no significant differences among groups in either the maternal or fetal platelet counts. About 50% mean increase in platelet counts was seen at the high dose in dams. However, there was high variability among individual animals and the mean increase was not statistically significant as compared to control.

### Clinical immunology (dams):

The overall incident of anti-AMP2 antibodies in dams was 44.4% (4 of total 9 dams examined: 1 of 3 at 10 mcg/kg, 2 of 3 at 30 mcg/kg and 1 of 3 at 100 mcg/kg).

All dams were negative for anti-AMP2 neutralizing antibodies, antibodies binding to endogenous TPO and anti-TPO neutralizing antibodies. Antibody analysis was not conducted for the fetuses.

### Toxicokinetics (dams):

There was a dose-proportional increase in C<sub>max</sub> and AUC and no accumulation was observed after multiple dosing. The maximum serum concentration was observed at 12 hours post-dosing on both GD 7 and GD 19. The TK data for dams is summarized in sponsor's table below.

Gestation Day	Parameter <sup>a</sup>	10 µg/kg	30 µg/kg	100 µg/kg
GD 7	C <sub>max</sub> (ng/mL)	14.1 (2.52)	48.4 (7.91)	168 (19.3)
	T <sub>max</sub> <sup>b</sup> (h)	12 (12-24)	12 (12-12)	12 (12-12)
	AUC <sub>(0-48)</sub> (ng <sup>h</sup> /mL)	359 (87.8)	1310 (169)	4520 (327)
GD 19	C <sub>max</sub> (ng/mL)	9.04 (1.11)	40.9 (2.37)	172 (24.7)
	T <sub>max</sub> <sup>b</sup> (h)	12 (8-12)	12 (8-12)	12 (8-24)
	AUC <sub>(0-48)</sub> (ng <sup>h</sup> /mL)	288 (73.3)	1290 (251)	5440 (767)
	Accumulation Ratio	0.821 (0.280)	1.01 (0.288)	1.21 (0.227)

<sup>a</sup>Parameter values except T<sub>max</sub> are rounded to 3 significant figures.

<sup>b</sup>T<sub>max</sub> values are reported as median (range).

**Reviewer's comments:**

High dose of AMG 531 (100 mcg/kg, 82-times MHD based on AUC) caused lower maternal body weight gain, lower total body weight change adjusted for gravid uterine weight, sporadic lower food consumption and 1 of total 52 fetuses examined in this dose group had malformations characterized by gastroschisis, ectrodactyly & cutis aplasia (likely to be an incidental finding rather than treatment-related effect).

Lower food consumption and changes in body weight parameters at high dose were not significant except for the lower body weight change adjusted for uterine weight. Therefore, no clear maternal toxicity was observed at any dose levels tested. However, high dose tested did provide adequate exposure to AMG 531 (82-times MHD). Conservatively, the NOAEL for maternal and fetal toxicity was 60 mcg/kg (39-times MHD) based on minor effects seen on the body weight parameters in dams and malformation (likely to be incidental) seen in one of the fetus at 100 mcg/kg. No fetal visceral or skeletal examination was conducted.

In terms of pharmacodynamic effects, rabbit seems to be much less sensitive species than rats since no significant increase in the platelet counts was observed at any of the doses tested. These results are consistent with the in vitro binding displacement assay, where AMG 531 did not show any specific binding to platelets derived from rabbits. Based on these assessments, it seems rabbits do not respond to AMG 531 and no definitive embryo-fetal toxicity study was conducted in rabbits. Nevertheless, adequate exposure to AMG 531 (4 to 82 times MHD) was obtained in this study for assessment of embryo-fetal toxicity that formed the basis for the recommendation in the label. In lieu of rabbit reproductive toxicity study, the sponsor conducted a developmental toxicity study in mice reviewed below.

**Study title:**

**d) Subcutaneous developmental toxicity study of AMG 531 in mice**

**Key study findings:**

AMG 531 (3, 10, 30 & 100 mcg/kg/day, 0.1 to 5-times MHD) treatment caused dose-dependent increase in platelet counts (1.4 to 3-fold). The high dose of 100 mcg/kg/day dose was associated with overall reduction in maternal body weight gain (8.4%). The greatest weight gain decrease (53.8%) occurred during gestation days 6 to 9. Developmental toxicity, as evidenced by increased post-implantation loss, was also attributable to the 100 mcg/kg/day dose of AMG 531. NOAEL for maternal and developmental toxicity in mice was 30 mcg/kg/day (2-times MHD).

**Study no.: 103322**

**Volume # and page #: eCTD 4.2.3.5.2.1-103322**

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** Aug 12, 2003

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug lot # and % purity:** A0102140000, 99.8%

**Methods:**

AMG 531 was administered subcutaneously three times weekly (GD 6, 9, 12 & 15) at doses of 0, 3, 10, 30 & 100 mcg/kg (n=8/dose).

**Mortality and clinical observations:** Daily

**Body Weights and food consumption:** Weekly

**Food consumption:** Not monitored

**Gross necropsy:** GD 18 (3 days after the last dose)

**Platelet counts:** Day of necropsy (GD 18)

**Toxicokinetics and antibody analysis:** Not conducted

**Maternal and fetal evaluations:** Uteri were examined for pregnancy, number and distribution of corpora lutea and implantation sites, live and dead fetuses and early and late resorptions. Fetuses were weighed and examined for gross external alterations and sex.

**Results**

**Maternal data:**

**Mortality and clinical observations:** None

**Body weights:** Overall body weight gain was reduced (8.4% decrease) in the high dose group for the entire dosing and gestation period. The greatest weight gain decrease (53.8%) occurred during GD 6 to 9. The reduction in body weight gains resulted in slight but not significant reduction (~5%) in maternal body weight beginning GD 11. There were no effects on body weight parameters at other doses.

**Food consumption:** Not evaluated

**Gross pathology:** No remarkable necropsy findings.

**Platelet counts:** Dose-dependent increase (1.4- to 3-fold) in platelet counts observed. Platelet counts at 3, 10, 30 & 100 mcg/kg/day dose groups were 139.2%, 157.2%, 227.8% and 299.1%, respectively, of vehicle control group values.

**Caesarean-sectioning and litter observations:**

There were 7 to 8 pregnant female mice with one or more live fetuses per dose group. In the 100 mcg/kg/day dose group, the average number of early resorptions (1.1), the % of dams with any resorptions (71.4%) and the % dead or resorbed conceptuses per litter (12.2%) were increased, as compared with control group (control: 0.2, 50% and 4.4%, respectively). The number of live fetuses was reduced in the 100 mcg/kg/day dose group (70 fetuses, mean: 10/litter) as compared to control (95 fetuses, mean: 11.9/litter).

No other parameters were affected. There was one dead fetus in the 3 mcg/kg/day dose group. No dam had a litter consisting of only resorbed conceptuses. All placentae appeared normal.

**Fetal gross observations:**

One fetus in the 30 mcg/kg dose group had an open right eye lid. Although this is an uncommon finding in this species, this gross external alteration was considered unrelated to treatment since the observation occurred in only one fetus at mid-dose level. No other fetal gross external alterations were identified. Fetal weights were not affected.

***Reviewer's comments:***

The dose levels for this study were based on the pharmacodynamic responses (increase in platelet count) seen in mice. The doses used were anticipated to cover an adequate range, while avoiding a dose likely to induce extreme thrombocytosis that might have compromised the study. The high dose of AMG 531 (100 mcg/kg) was expected to produce a maximal response in the primary endpoint, an increase in peripheral platelet counts (3 to 5-fold over baseline). The predicted exposure at the high-dose is ~5-times the maximum exposure in humans.

Based on the study results, the NOAEL for maternal and developmental toxicity was 30 mcg/kg (2-times MHD based on AUC). At high-dose (100 mcg/kg), reduction in maternal body weight and increased post-implantation loss was observed. Consistent with the post-implantation loss, the numbers of live fetuses were reduced at the high-dose. It is pertinent to note that only fetal external examination was conducted; visceral and skeletal examinations were not conducted. Therefore, fetal NOAEL should be interpreted with caution.

**Pre-natal and post-natal development:**

***Study title:***

**A study to determine the effects of subcutaneous administration of AMG 531 on pre- and post-natal development and maternal function in rats**

**Key study findings:**

Treatment of F0 dams with AMG 531 (10, 30 or 100 µg/kg, 3-11 times MHD) over the period GD 6 to PND 21, by subcutaneous injection on alternate days, induced neutralizing antibodies to AMG 531 in high number (70-80%) of dams in each dose group. For Ab- dams, ~4-fold increase in platelet counts was observed, whereas Ab+ dams had a lower increase in platelet counts (≤1.4-fold).

Four Ab- dams (F0) died at the end of the lactation period; 3 in mid-dose group and 1 in high-dose group. Exact cause of death could not be determined. Slight prolongation of

the gestation period was observed in all dose groups, regardless of antibody status (Ab+ or Ab-).

Regardless of maternal antibody status, there was an increase in the incidence of perinatal F1 pup mortality at 100 µg/kg dose (3 total litter losses). There was no effect on overall live litter size. No treatment-related abnormalities in morphology or behavior of the offspring were observed. Physical and functional development up to sexual maturity, including fertility and general reproductive function were not affected.

In F0 dams, only treatment-related necropsy findings were splenic enlargement (moderate to extreme) in 30 & 100 µg/kg Ab- animals; an expected pharmacological effect. There were no necropsy findings or reproductive organ weight findings in F1 offspring.

For maternal toxicity, no definitive NOAEL was established due to effects of AMG 531 on F0 gestation period and mortality seen in mid- and high-dose groups. Based on increased peri-natal pup mortality at the highest dose, a NOAEL for survival and pre- and post-natal physical and functional development of the F1 offspring was 30 mcg/kg (3-times MHD) of maternal dose.

**Study no.:** 102969

**Volume # and page #:** eCTD 4.2.3.5.3.1-10269

**Conducting laboratory and location:** \_\_\_\_\_

**Date of study initiation:** July 18, 2003

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug lot # and % purity:** A0102140000, 98%

**Objective:** The objective of this study was to evaluate potential effects on the pregnant/lactating female rat and on development of the conceptus and offspring, following subcutaneous administration of AMG 531 to the dam from implantation through weaning of the F1 offspring.

## Methods

Doses: 0, 10, 30 & 100 mcg/kg (every-other-day GD 6 through PND 20/21)

Species/strain: SD rats

Number/sex/group: 44/group

Route of administration and dosing volume: Subcutaneous, 1 ml/kg

Satellite groups used for toxicokinetics: TK not conducted.

In rats, ~50% of treated animals develop antibodies to AMG 531. Therefore, potential toxicity in antibody-positive (Ab+) and antibody-negative (Ab-) animals was evaluated separately. To achieve this, the study was conducted in two replicates (R1 and R2) separated by a 13-week interval. The first replicate provided information on the pharmacological response and enabled prediction of the proportion of animals that might

form antibodies in the second replicate; the general objective being to obtain 16-20 F0 females per group for each of 2 subsets: antibody positive and antibody negative dams.

Group	Group Designation	Doses (on alternate days)			Days of treatment	Number of animals			
		Dose	Concentration	Volume		Replicate 1		Replicate 2	
		µg/kg	µg/mL	mL/kg		F <sub>0</sub> females	F <sub>1</sub> weanlings (untreated)	F <sub>0</sub> females	F <sub>1</sub> weanlings (untreated)
1	Control	0	0	1	GD 6 - PND 20/21	24	1/sex/litter	20	1/sex/litter
2	Low	10	10	1	GD 6 - PND 20/21	24	1/sex/litter	20	1/sex/litter
3	Middle	30	30	1	GD 6 - PND 20/21	24	1/sex/litter	20	1/sex/litter
4	High	100	100	1	GD 6 - PND 20/21	24	1/sex/litter	20	1/sex/litter

F0 females were bled for platelet analysis on PND 21 (Replicate 1) or PND 25 (Replicated 2). Antibody analysis was conducted pre-dose and on PND 24 or 25 (R1 & R2). One F1 weanling of each sex from (nominally) each litter was retained for evaluation of post-weaning physical and functional development, including reproductive function. The mated F1 females were sacrificed for evaluation at GD 14, the F1 males soon after, including evaluation of antibody status for both sexes.

#### ***Parameters and endpoints evaluated:***

***F0 females:*** Mortality, clinical observations, body weights, food consumption, platelet count, antibody analysis and gross necropsy (PND 25).

***F1 generation*** (pre- and post-weaning): Physical examinations, body weight and sex, growth and maturation, open field evaluation, locomotor activity, learning & memory (T-water maze), reproductive function, antibody analysis and gross necropsy (GD 14).

## **Results**

### ***Antibody and platelet analysis:***

***F0 dams: Neutralizing anti-AMG 531 antibodies*** occurred in high number of treated animals (70-80%) as shown in the table below (Group 2-4: 10, 30 & 100 mcg/kg); it exceeded the 50% incidence rate expected from earlier toxicity studies in rats. One control animal (Group 1) was also found to have positive neutralizing antibodies against AMG 531 and was eliminated from consideration in the study. Binding antibodies to endogenous TPO were detected in AMG 531-treated (6.8-11.4%) animals at all dose levels and in control group (9.3%). However, no neutralizing antibodies to TPO were observed in AMG 531-treated or control group animals.

The table below summarizes the data for development of neutralizing antibodies to AMG 531 in F0 dams at various dose levels.

Antibody Status	Number of Pregnant Dams			
	Control	10 mcg/kg	30 mcg/kg	100 mcg/kg
Ab+	1 (2%)	34 (79%)	30 (70%)	29 (67%)
Ab-	41	9	13	14
Total	42	43	43	43

**F1 generation:** AMG 531 binding antibodies were detected in one low-dose animal from F1 generation (none in control); whether these were neutralizing antibodies is not known since no analysis for neutralizing antibodies in the F1 generation was conducted. F1 generation also had endogenous TPO binding antibodies. However, antibodies binding to TPO were detected in both AMG 531-treated (4.5-9.3%) and control group (Females: 9.3% & males: 15.9%) animals.

**Platelet count:** In F0 dams as expected, treatment with AMG 531 produced less increase (maximum 1.3-fold) in platelet counts in Ab+ animals (neutralizing anti-AMG 531 antibodies), while Ab- dams had 3 to 4-fold increase in the count. There was no clear dose-response relationship for the increase in platelet counts in Ab- dams. This may be due to the high number of neutralizing antibodies observed in all AMG 531-treated dose groups. Platelet count in F1 generation was not monitored.

Mean platelet volume was increased by 4% & 16% at 30 & 100 mcg/kg doses in Ab- dams. No effect on platelet volume was observed in Ab+ animals.

#### **F0 maternal data:**

##### ***Survival and pregnancy:***

The overall pregnancy rate was 41-43 pregnancies per group. There were no maternal deaths during the gestation period. However, ***four dams were found dead*** around the end of the lactation period, all ***in Ab- animals*** (3 in mid-dose & 1 in high-dose). There were no necropsy findings. The exact cause of death was not determined. The sponsor suggests that deaths may have occurred due to cerebrovascular accident (stroke), induced by increased blood viscosity resulting from markedly elevated platelet counts. ***Three dams in the high dose Ab- subgroup and one in the middle dose Ab+ subgroup were sacrificed early, following total litter loss.***

##### ***Clinical observations:***

No remarkable clinical observations in any of the treated animals (Ab+ or Ab-), including those that died prematurely.

##### ***Body weights:***

No remarkable effects on body weight parameters.

**Food consumption:**

No significant effect on food consumption in either sub-group of dams (Ab+ & Ab-) during gestation. During lactation, there was an overall trend towards slightly less food consumption in Ab- dams in the high-dose group.

**Parturition, litter size and F1 survival to weaning:**

There was a slight *prolongation of the gestation period* in all AMG 531-treated groups (Ab+ and Ab-) when compared to the control, as shown in the table below. A gestation length of 21 to 22 days is the normal range for this strain of rats, with approximately half the animals showing a 21-day gestation period and the other half a 22-day period. This trend was seen in control animals. In AMG 531-treated group, the gestation period was somewhat increased: ~70% dams had a 22-day gestation period and remaining 30% had 21-day gestation period. This increase in gestation period was similar in magnitude and incidence for all the Ab+ subgroups, and for the middle and high dose Ab- subgroups. In addition, **3 dams had a 23-day gestation period and suffered total litter loss** (2 at high-dose in Ab- subgroup and 1 at mid-dose in Ab+ subgroup). These dams were sacrificed early, following total litter loss. The gestation length data is summarized in the table below.

Gestation Length (Days)	Gestation Length Incidence and %						
	Antibody Negative				Antibody Positive		
	Control	10 mcg/kg	30 mcg/kg	100 mcg/kg	10 mcg/kg	30 mcg/kg	100 mcg/kg
21	21	5	3	2	10	9	9
22	20	4	10	10	24	20	20
23	0	0	0	2	0	1	0
Total litters:	41	9	13	14	34	30	29
21	51%	56%	23%	14%	29%	30%	31%
22	49%	44%	77%	71%	71%	67%	69%
23	0%	0%	0%	14%	0%	3%	0%

Irrespective of maternal antibody status, there was an *increase in the incidence of perinatal F1 pup mortality* at high-dose with 3 total litter losses. The live-birth index was decreased from 99.5% in control to 92.7% and the % of stillborn pups was increased from 0.5% in control to 7.3% in the high-dose group animals. In addition, for the Ab-subgroup only, there was an increase in pup loss during the early lactation period (viability index for the period PND 0-4: control: 97.4% versus high-dose Ab-: 87.6%). The increase in pup mortality in this subgroup was mainly due to 3 total litter losses. A total litter loss also occurred in one animal in the middle dose, Ab+ subgroup.

Although, there were increases in peri-natal pup mortality, overall live litter size was not affected at any doses. Offspring sex ratio was not affected; there was no sex bias among the pups lost during lactation.

**F1 offspring development to weaning:**

**Clinical observations:** No remarkable observations.

**Body weights:** No remarkable effect on pup weight at birth, or on subsequent body weight development up to weaning.

**F1 offspring development post-weaning:**

**Mortality:** There were no premature decedents among the F1 males and females retained for post-weaning evaluations.

**Clinical observations:** There was no evidence of treatment-related incidence of any clinically overt abnormality among the F1 generation after weaning, including the F1 females' gestation period.

**Body weights:** No remarkable differences in post-weaning body weight performance of the F1 generation, regardless of dose level or F0 maternal antibody status.

**Post-weaning functional development evaluation:**

**Open field test:** No behavioral abnormalities observed in the open field tests for the F1 generation.

**Motor activity:** No meaningful differences in the spontaneous, exploratory motor activity (total activity or the pattern of activity) of the F1 generation.

**Learning & memory:** No remarkable effect on the learning and memory profile of the F1 generation in water T-maze test.

**Attainment of puberty:** No effect on the mean age of attainment of puberty. There was also no change in the normal pattern of estrous cycling in the F1 females.

**Mating performance and fertility:** Mating performance or fertility in F1 generation was not affected, regardless of the F0 antibody status.

**Pregnancy performance:** No effect on the pregnancy performance of the F1 generation, up to GD 14. Numbers of corpora lutea, implants, and both pre- and post-implantation losses, were comparable across all the subgroups.

**Necropsy and macroscopic findings (F0 & F1):**

**F0 necropsy:**

**Ab- subgroups:** 11 of the 14 dams that received 100 µg/kg had enlarged spleen (moderate to extreme). Two dams that had enlarged spleen (extreme) also showed enlarged lymph nodes. One dam in 30 mcg/kg dose group also showed slight splenic enlargement.

For the 4 Ab- dams that died on PND 21 or 22 (3 in the mid-dose & 1 in high-dose), no obvious cause of death was detected at necropsy. Sponsor has speculated that it may have been due to cerebrovascular accident (stroke), induced by increase in blood viscosity resulting from elevated platelet levels.

**Ab+ subgroup:** No remarkable macroscopic findings. .

***F1 necropsy:***

No remarkable findings, regardless of F0 maternal antibody status, for either the pre-weaning animals or at post-weaning termination.

***Reviewer's comments:***

No definitive NOAEL for maternal toxicity was established due to increase in gestation length at all doses (10-100 mcg/kg, 0.25-11 times MHD based on AUC) and mortality seen in mid- and high-dose groups. Mortality may be due to thrombotic events caused by increased blood viscosity due to extreme thrombocytosis. In these animals, platelet count was increased 3 to 5-fold over the normal baseline count. Mortality was seen only in Ab- animals with high platelet counts but not in Ab+ animals that had relatively less increase (maximum 1.4-fold) in platelet counts. Nevertheless, exact cause of death could not be determined.

There was an increase in the incidence of peri-natal F1 pup mortality at high-dose (100 mcg/kg, 11-times MHD) that was not dependent on the maternal antibody status. There were no treatment-related abnormalities in morphology or behavior of the F1 offspring, and no remarkable findings were observed for various measures of F1 physical and functional development up to sexual maturity, including fertility and general reproductive function. Based on the increased peri-natal pup mortality at the high dose (100 mcg/kg), a NOAEL for survival and pre- and post-natal physical and functional development of the F1 offspring was 30 µg/kg (3-times MHD).

**2.6.6.7 Local tolerance:**

Local tolerance studies were conducted as part of the repeat-dose toxicity studies in rats and monkey. In rats, subcutaneous administration of AMG 531 produced muscle and subcutis chronic inflammation and myofiber regeneration at the site of injection. In monkey, mild perivascular mononuclear cell infiltration was observed at the injection site.

**2.6.6.8 Special toxicology studies:**

***a) Study #PP01113 & PP01108:***

**Induction of anti-AMP2 antibodies in mice after exposure to AMP2 and effectiveness of AMP2 dose escalation**

The objective of this study was to evaluate induction of anti-AMP2 antibodies in mice and to determine if the antibodies were neutralizing to AMP2 and endogenous TPO, and

to determine if the loss in efficacy of AMP2 due to antibodies could be overcome by increasing the dose of AMP2.

**Methods:**

*Evaluation of induction of anti-AMP2 antibodies:* Normal BDF1 mice were injected subcutaneously approximately every 21 days for four cycles with 50 or 100 mcg/kg (n=5/group) AMP2 and generation of antibodies was determined after 4 cycles of treatment. Blood samples were collected once a week for determination of platelet count. At the end of study, serum from treated mice was tested for the presence of antibodies by Biacore analysis. Antibodies to endogenous TPO or various components of AMP2 were screened.

Further studies were performed to determine if the loss of efficacy of AMP2 due to anti-AMP2 antibodies could be overcome by escalating doses of AMP2. In these studies, mice were treated initially (first cycle) with 50 mcg/kg AMP2 resulting in the development of anti-AMP2 antibodies. Subsequently, AMP2 (50, 100, 500 & 1000 mcg/kg) was administered every 21 days for 6 additional cycles and platelet response after each cycle was determined to evaluate efficacy of AMP2. At the end of study (Day 154), blood samples were collected and analyzed for serum antibodies to AMP2, TMP and endogenous TPO.

**Results:**

*Induction of anti-AMP2 antibodies:* Mice responded to the first cycle of AMP2 administration (50 & 100 mcg/kg) with ~3 to 4-fold increase in platelet counts as compared to baseline values, but in the subsequent cycles of administration, platelet count increased only by 1.2-1.6 fold over baseline. After 4 cycles, neutralizing antibodies to AMP2 were detected in 60% and 80% of mice treated with 50 and 100 mcg/kg AMP2, respectively. No antibodies to endogenous TPO were detected.

To determine the timing of antibody production, mice were injected with a single dose of 50 mcg/kg AMP2 and five mice were sacrificed every day for 21 days and tested for the presence of antibodies. On Day 6 post-dosing, antibodies to the TMP (thrombopoietin mimetic peptide) component of the AMP2 molecule were first detected, while antibodies to the entire AMP2 molecule (neutralizing antibodies) were detected on Day 14 onwards.

*Effect of AMP2 dose escalation in anti-AMP2 antibody positive mice:* Mice receiving the lower doses (50 or 100 mcg/kg AMP2) showed little or no platelet increase in the 2<sup>nd</sup> and subsequent cycles of AMP2 administration. Mice treated with the higher dose (500 mcg/kg) maintained a platelet response (3 to 4-fold increase in platelet count) that was equivalent to the initial treatment with 50 mcg/kg AMP2. The highest dose group, 1000 mcg/kg, exceeded the initial platelet response (~5-fold increase) in each of the six subsequent cycles of AMP2 administration. Anti-AMP2 antibodies were detected in 80%-100% of animals per dose group. None of the mice showed reactivity against endogenous TPO.

**Conclusion:**

Although 60-80% of the mice generated an antibody response to AMP2 within 2 weeks of a single exposure, endogenous platelet production was not impacted. Dose-escalation of AMP2 was effective in overcoming the antibody response resulting in increase in platelet counts even in the presence of anti-AMP2 antibodies.

**Reviewer's comments:**

Although increasing doses of AMP2 were able to overcome the loss of efficacy due to induction of anti-AMP2 antibodies, it is not clear if this can be sustained over a long-term period.

**b) Study #101815:**

**Preliminary studies of AMP2 cross-reactivity testing on selected human and cynomolgus monkey tissues and a human megakaryoblastic cell line**

**Methods:**

This study report described multiple preliminary studies conducted to determine the appropriate positive controls, concentration and conjugation/form of test article (AMP2), and fixation and staining conditions, which would be useful and reproducible for immunohistochemical detection of potential cross-reactivities of the AMP2. AMP2 (1-50 mcg/ml) was applied to cryosections of selected human and cynomolgus monkey tissues, human buffy coat, and human megakaryoblastic leukemia MEG-01 cells and to human peripheral blood smears to evaluate AMP2 binding and potential cross-reactivity. Various indirect immunoperoxidase procedures were used in an attempt to demonstrate reactivity of the unconjugated or fluoresceinated forms of AMP2 with the cryosections or blood smears. In some staining runs, various cations were included in the binding buffer to enhance potential ligand-receptor interactions. As an alternative detection system, a rabbit polyclonal anti-AMP2 antibody was used to examine binding to MEG-01 cells and various human or cynomolgus monkey tissues.

**Results:**

No reactivity was observed that was judged specific for the test article. According to the sponsor, the results of these preliminary studies indicated that the immunohistochemical methods available were not sufficient to allow detection of the binding of the FITC-AMP2 ligand to its receptor on human or cynomolgus monkey tissues, or cultured cells. It has been reported that normal and neoplastic human hematopoietic cells have 50-2500 c-Mpl binding sites per cell, and according to the sponsor, immunohistochemical methodologies are only capable of detecting antigen (epitope)-antibody (CDR) binding if there are at least 10,000 binding sites per cell. The sponsor states that further immunohistochemistry studies will not provide useful information regarding the potential cross-reactivity of AMP2, probably as a result of low-receptor display of potential binding sites.

**Reviewer's comments:**

Tissue cross-reactivity studies are generally not required for protein products. No further studies are necessary to characterize potential cross-reactivity of AMG 531.

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

The BLA application for Romiplostim (AMG 531) is approvable from Pharmacology and Toxicology perspective. There are no outstanding nonclinical issues. The major nonclinical findings were as follows:

- AMG 531 binds with high affinity to TPO receptors on rat, monkey and human platelets, and stimulates proliferation of megakaryocyte progenitor cells derived from monkeys and humans. Single or repeat dose administration of AMG 531 in mice, rats, and non-human primates caused dose-dependent increases in platelet counts (3- to 5-fold). The presence or absence of the spleen had no effect on platelet response in mice.
- No adverse effects on CNS and cardiovascular function were observed in rats and monkey.
- Repeat dose toxicity studies were conducted in rats (1 month) and cynomolgus monkey (3 and 6 months). In general, adverse effects observed were related to the over activation of thrombopoietic system caused by AMG 531. In rats (10, 30 & 100 mcg/kg, 1-37 times MHD), AMG 531 caused extramedullary hematopoiesis, femoral and sternal bone hyperostosis and bone marrow myelofibrosis at all doses tested. These effects were reversed by the end of 4-week recovery period. Longer-term toxicology studies in rats could not be conducted as rats developed neutralizing antibodies to AMG 531. In terms of pharmacodynamic effects, monkey seems to be less sensitive species than rats. In monkey, approximately 50-fold higher dose (5000 mcg/kg, 300-times MHD) was needed to achieve same response (4-fold increase in platelet counts) as seen in rats (100 mcg/kg). Unlike in rats, no myelofibrosis was observed in monkeys after 6 months of repeated dosing. Based on pharmacodynamic response and adverse effect profile seen in humans, rat seems to be a more relevant species than monkey in terms of predicting these effects in humans. Anti-drug antibodies were seen in rats and monkey but did not cross-react with endogenous thrombopoietin.
- There is a concern related to the tumor stimulation capacity of thrombopoietin and, therefore, AMG 531 ability to stimulate certain types of acute myeloid leukemia cells. However, the ability to assess potential carcinogenicity in a traditional chronic rodent study is negated since AMG 531 is highly immunogenic in rats and mice. Neutralizing antibodies were detected in mice and rats with a corresponding diminution of the platelet response.
- AMG 531 had no effect on fertility in rats. In developmental toxicity studies in rats (1 to 11-times MHD) and rabbits (3 to 82-times MHD), there was no evidence of fetal harm. In mice at doses 5-times the MHD, reduction in maternal body weight and increased post-implantation loss occurred. In a prenatal and

postnatal development study in rats (1 to 10-times MHD), there was slight prolongation of the gestation period at all dose levels tested and an increase in peri-natal pup mortality at doses 10-times the MHD.

- AMG 531 crossed the placental barrier in rats and increased platelet counts (1.7 to 6-fold) in fetuses at clinically equivalent and higher doses. Thus, there is a potential for adverse effects related to thrombocytosis in fetuses due to placental transfer of AMG 531.

**Recommendation:** From the perspective of nonclinical pharmacology and toxicology, Romiplostim is recommended for approval.

Signatures (optional):

Reviewer Signature Tushar Kokate

Supervisor Signature [Signature] Concurrence Yes ☒ No ☐

**APPENDIX/ATTACHMENTS**

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

BLA Number: 125268/0

Applicant: Amgen

Submitted Date: 10/23/07

Drug Name: Nplate  
(romiplostim)

BLA Type: Original

Stamp Date: 10/23/07

On initial overview of the NDA application for RTF:

	Content Parameter	Yes	No	Comment
1	On its face, is the pharmacology/toxicology section of the NDA organized (in accord with 21 CFR 314 and current guidelines for format and content) in a manner to allow substantive review to begin?	√		
2	Is the pharmacology/toxicology section of the NDA indexed and paginated in a manner allowing substantive review to begin?	√		
3	On its face, is the pharmacology/toxicology section of the NDA legible so that substantive review can begin?	√		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted in this NDA (carcinogenicity, mutagenicity*, teratogenicity*, effects on fertility, juvenile studies, acute and repeat dose adult animal studies*, animal ADME studies, safety pharmacology, etc)?	√		
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).	√		
6	On its face, 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 sponsor <u>submitted</u> a rationale to justify the alternative route?	√		

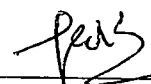
# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

	Content Parameter	Yes	No	Comment
7	Has the sponsor <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?	√		
8	Has the sponsor submitted all special studies/data requested by the Division during pre-submission discussions with the sponsor?	--		No special studies/data were requested
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?	√		
10	If there are any impurity – etc. issues, have these been addressed? (New toxicity studies may not be needed.)	√		
11	Has the sponsor addressed any abuse potential issues in the submission?	--		N/A
12	If this NDA is to support a Rx to OTC switch, have all relevant studies been submitted?	--		N/A
13	From a pharmacology/toxicology perspective, is the NDA/BLA fileable? If "no" please state below why it is not.	√		Fileable from a pharmacology/toxicology perspective

Any Additional Comments:

Tushar Kokate  
Reviewing Pharmacologist

11/16/07  
Date



Team Leader/Supervisor

Date