CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-311

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Mozobil (plerixafor injection)

Date: December 3, 2008
To: File for NDA 22-311
From: John K. Leighton, PhD, DABT
Associate Director for Pharmacology
Office of Oncology Drug Products

I have examined the labeling and pharmacology/toxicology supporting reviews and memoranda provided by Drs. Shwu-Luan Lee and Haleh Saber and concur with their conclusions that Mozobil may be approved. No additional pharmacology/toxicology studies are necessary for the proposed indication.

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/s/

John Leighton
12/3/2008 09:55:45 AM
PHARMACOLOGIST

MEMORANDUM

Date: December 2, 2008
From: Haleh Saber, Ph.D.
Pharmacology Acting Team Leader
Division of Drug Oncology Products
To: File for NDA #22,311
Mozobil (plerixafor injection)
Re: Approvability for Pharmacology and Toxicology

Nonclinical studies that investigated the pharmacology and toxicology of plerixafor were provided in support of the NDA. Mozobil (plerixafor injection) is a hematopoietic stem cell mobilizer, and is indicated in combination with granulocyte-colony stimulating factor (G-CSF) to mobilize stem cells to the peripheral blood for collection and subsequent autologous transplantation in patients with non-Hodgkins lymphoma and multiple myeloma. "Hematopoietic stem cell mobilizer" is used in the highlight section of the label to describe the pharmacologic class of this drug. Selection of this term was based on a CDER initiative to use terms to provide scientifically valid information that will be meaningful to prescribers. Plerixafor blocks the interaction between CXCR4 receptor and its ligand, stromal-derived factor-1 (SDF-1). SDF-1 and CXCR4 are involved in the trafficking and homing of CD34+ cells to the marrow. Plerixafor enhanced G-CSF-induced mobilization and engraftment of hematopoeitic stem cells and progenitor cells in irradiated mice and dogs.

Pharmacology, safety pharmacology, pharmacokinetic/ ADME, and toxicology studies supporting the marketing application of plerixafor for the proposed indication were conducted in *in vitro* systems and in animal species. The general toxicology studies were conducted using the administration route, and dosing schedule and duration that adequately addressed safety concerns for the indicated patient population and the intended duration of administration. General toxicology studies of longer than 4 weeks were not conducted and are not required, since the maximum duration of plerixafor treatment in patients will be 7 days.

Non-clinical studies defined the target organs/tissues of plerixafor as the hematopoietic system (leucocytosis), bone (bone mineral and/or volume loss), liver (increased hematopoiesis), spleen (increased weight, increased hematopoiesis), and cardiovascular (e.g. changes in the blood pressure and heart rate) and central nervous systems. The effects in the respiratory system (reduced tidal volume and respiratory rate) and in the cardiovascular system, may have been at least partially CNS-related. Injection site reactions included hemorrhage and inflammation. Plerixafor was negative for evidence of genetic toxicity in the standard battery of tests described by ICH S2. Carcinogenicity studies were not conducted, nor are they needed for use in this patient population or for this short period of clinical administration (generally 4 days, maximum of 7 days). Plerixafor was teratogenic in rats; therefore, Pregnancy Category D is recommended for Mozobil label. Teratogenic effects were also observed in rabbits in a dose range-finding

developmental toxicity study. Embryo-fetal toxicities appear to be a direct pharmacologic effect of plerixafor; the role of SDF-1/CXCR4 in embryo-fetal development has been described in the literature. If the indication changes, a GLP-compliant reproductive toxicology study in rabbits may be needed, to fully explore the effects of plerixafor on embryo-fetal development. Fertility and pre- and post-natal reproductive toxicity studies have not been conducted and are not required for the proposed indication.

The nonclinical studies were reviewed in detail by Dr. Shwu-Luan Lee. The nonclinical findings are summarized in the "Executive Summary" and "Discussion and Conclusions" of the review, and reflected in the product label.

Recommendation: I concur with Dr. Lee's conclusion that pharmacology and toxicology data support the approval of NDA 22,311 for Mozobil. There are no outstanding non-clinical issues related to the approval of Mozobil for the proposed indication.

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

/s/

Haleh Saber
12/3/2008 09:44:24 AM
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

NDA NUMBER:	**22,311**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**6/16/2008**
PRODUCT:	**Mozobil (plerixafor) subcutaneous injection**
INTENDED CLINICAL POPULATION:	**Enhancing mobilization of hematopoietic stem cells in combination with granulocyte-colony stimulating factor (G-CSF) in patients with non-Hodgkins lymphoma and multiple myeloma**
SPONSOR:	**Genzyme Corporation**
DOCUMENTS REVIEWED:	**Pharmacology/Toxicology**
REVIEW DIVISION:	**Division of Drug Oncology Products**
PHARM/TOX REVIEWER:	**Shwu-Luan Lee, Ph.D.**
PHARM/TOX SUPERVISOR:	**Haleh Saber, Ph.D.**
DIVISION DIRECTOR:	**Robert Justice, M.D., M.S.**
PROJECT MANAGER:	**Susan Jenney**

Date of review submission to Division File System (DFS): November 14, 2008

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability
There are no pharmacology/toxicology issues which preclude approval of plerixafor (Mozobil®) for the requested indication.

B. Recommendation for nonclinical studies
No additional non-clinical studies are required for the proposed indication and duration of administration.

C. Recommendations on labeling
Recommendations on labeling have been provided within team meetings and communicated to the sponsor.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Plerixafor (AMD3100) is a hematopoietic stem cell mobilizer. Plerixafor demonstrated the capacity to mobilize and repopulate hematopoietic stem cells in mice, dogs and humans. Such effects were due to the interruption of interaction between CXCR4 and its ligand, stromal cell-derived factor-1 (SDF-1). Addition of G-CSF enhanced the mobilization effects of plerixafor.

Subcutaneously (SC) administered plerixafor was absorbed completely and rapidly (T_{max} 0.5-1 hr) . The plasma protein binding of 1-10 μg/mL plerixafor was moderate (~35-55% in animals and in humans). After SC administration of radiolabeled plerixafor, high and/or sustained levels of radioactivity were detected in liver, kidney, spleen, injection site, epiphyseal plate and cartilage. Tissues rich in melanin, such as skin and uveal tract showed small but notable radioactivity retention. Considering the affinity for melanin containing tissues, phototoxicity assessment of plerixafor may be necessary if plerixafor can absorb the light (within ~300-700 nm) and if duration of plerixafor treatment is increased (in the present NDA, treatment generally involves 4 doses of the drug). Plerixafor showed penetration through the blood-brain barrier. No significant levels of metabolites were detected in *in vitro* studies using liver microsomes of mouse, rat, dog or human. Parent drug was the major component in urine and plasma samples collected after SC administration of plerixafor. Three non-parent components detected in these samples appeared to be Cu^{2+} complexes of plerixafor. The main excretion route after SC doses was urinary. Under the conditions of studies, plerixafor, up to 100 μM, was not a direct or metabolism-dependent inhibitor of major CYP enzymes tested, including 1A2, 2C9, 2C19, 2D6 and 3A4. The systemic exposure to plerixafor was mostly dose proportional at doses tested in the SC repeat-dose toxicology studies (up to 4 weeks) in rats and dogs. Animal studies suggest the potential for accumulation of the parent drug and/or drug-related radioactivity upon repeated SC dosing.

The safety pharmacology studies in mouse, rat and dog, and general toxicology studies in rat, and dog identified liver, bone, injection sites, spleen, cardiovascular and central nervous system as the target organs/tissues. The major findings are as follows:

- Hematopoietic/lymphoid system:
  The most prominent effect of plerixafor in rats following 4-week repeat-dose treatment was leukocytosis (increased total and differential white blood cell counts). The increase was dose-dependent (at doses $\geq$ 18 mg/m^2) and was reversible. Leukocytosis is at least partially due to an exaggerated pharmacological effect of plerixafor. In rats, increased hematopoiesis in spleen and liver and increased spleen weight, were also observed. It was not certain, whether lymphoid atrophy in spleen and thymus was a direct effect of plerixafor treatment. The hematological or lymphoid changes were not remarkable in dogs.
- Bone and mineral loss in urine:
  Elevated urinary calcium and magnesium levels were found in rats and dogs. Seral magnesium levels were decreased, but changes in calcium levels were not consistent. Reduced bone mineral content of tibia and humerus, and reduced bone volume in fumerus indicated increased bone mineral loss. This finding may be also an exaggerated pharmacological effect of plerixafor. Disruption of CXCR4-related cellular activity was reported to enhance bone loss. The finding was recoverable.
- Central nervous system and respiratory system:
  In mice and rats, plerixafor treatment was associated with CNS suppressive effects. These effects included decreased in-place and locomotor activity, alertness, and startle response, dilated pupils and ptosis. Of note, a CNS stimulant-like response was observed when animals were handled. The suppressive CNS effects may be the underlying mechanism of observed decreases in tidal volumes and respiratory rates in rats. Distribution studies showed that plerixafor or plerixafor-related compunds can cross the blood-brain barrier. In addition, small but measurable amounts of plerixafor were detected in the cerebro-spinal fluid of dogs in a toxicology study. Of note, plerixafor was shown to bind to adrenergic receptors as well as dopamine D_2 receptor at micromolar ranges.
- Cardiovascular system:
  Plerixafor did not inhibit hERG channel currents or induce ECG changes in telemetered dogs. Histopathological findings in cardiovascular system were limited to fibroid necrosis of the myocardial blood vessel wall found in one study conducted in dogs. However, in anesthetized rats, cardiodepression (i.e., decreased blood pressure, heart rates and myocardial contractility) was fatal. On the contrary, plerixafor treatment in conscious dogs induced tachycardia and hypertension. These effects coincided with clinical signs of salivation, pupil dilatation and non-sustained convulsion, suggesting a CNS-related phenomenon. Plerixafor was shown to inhibit angiotensin II-induced vasoconstriction, to deplete intracellular level of calcium, and to bind to adrenergic receptors. These findings provide additional meachanisms for cardiovascular dysfunction observed in animals treated with plerixafor.
- Other toxicities:
  Plerixafor treatment in dogs caused GI clinical signs, such as diarrhea, emesis, increased defecation and salivation, with no histopathological correlate. Lesions at the injection sites (subcutaneous hemorrhage and inflammation, thickening of the skin) were

attributable to repeated needle penetration and/or direct local irritation caused by plerixafor.

Plerixafor was not mutagenic in bacterial Ames test or clastogenic in a chromosome aberration test in V79 Chinese hamster cells. Plerixafor did not increase micronucleus formation in rats after subcutaneous doses up to 25 mg/kg (150 mg/m^2).

Reproductive and developmental toxicities of plerixafor were investigated in rats and rabbits. Plerixafor was teratogenic in these studies. In rats, embryofetal toxicities (at 90 mg/m^2) included: increased resorption and post-implantation loss, as well as decreased fetal weight. Plerixafor also induced external (reduced or absent eyes, shortened limb digits), visceral (cardiac interventricular septal defect, ringed aorta, globular heart), and head (hydrocephaly, dilatation of olfactory ventricles) malformations and variations. The embryofetal toxicities were seen at doses that caused maternal toxicities. The study in rabbits was non-GLP and was not reviewed. Based on the summary information provided by the sponsor, drug-related effects in rabbits were similar to those in rats and included increased post-implantation loss, reduced litter size, and head and external malformations, at SC doses ≥ 36 mg/m^2. The effect of plerixafor on human fertility is unknown.

B. Pharmacologic activity

The antagonistic effects of plerixafor on CXCR4 chemokine receptor were characterized in *in vitro* and/or *in vivo* systems. Plerixafor inhibited the binding of stromal cell-derived factor-1 (SDF-1) to CRCX4, and inhibited SDF-1 stimulated cellular activities, such as calcium flux, chemotaxis and GTPγS binding.

Subcutanous treatment of plerixafor induced leukocytosis (increased total and differential white blood cell counts) in mice, dogs, and humans. Plerixafor enhanced G-CSF-induced mobilization and engraftment of hematopoeitic stem cells and hematopoietic progenitor cells in irradiated mice and in dogs (autologous and allogeneic transplantation).

C. Nonclinical safety issues relevant to clinical use

The toxicities in the target organs identified in the animals, i.e., hematopoietic (leukocytosis and increased spleen size), GI tract (diarrhea) and injection site (erythema and pruritus), were also reported in the patients.

All toxicities reported in animals, including cardiovascular disorders, CNS effects, and teratogenic effects of plerixafor, should be considered as potential risks to humans.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

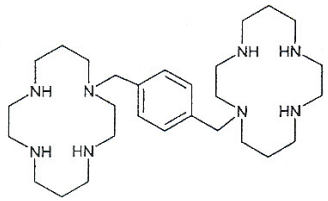
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22311
Review number: 1
Sequence number/date: 000/16 June, 2008
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Genzyme Corporation
500 Kendall Street, Cambridge, MA 02142
Manufacturer for drug substance: (b) (4) (b) (4)

Reviewer name: Shwu-Luan Lee, Ph.D.
Division name: Drug Oncology Products
Review completion date: November 14, 2008

Drug:

Trade name: Mozobil®
Generic name: Plerixafor
Code name: AMD3100, SDZ SID-791 (free base), SDZ SID-791-ch (Octahydrochloride Dihydrate), SDZ 282-791, AME-2
Chemical name: 1,1'-[1,4-phenylenebis (methylene)]-bis-1,4,8,11-tetraazacyclotetradecane
CAS registry number: 110078-46-1
Molecular formula: $C_{28}H_{54}N_8$ (base), $C_{28}H_{54}N_8 \cdot 8\,HCl$ (salt)
Molecular weight: 502.79/794.51 gm/mole (base/salt, 1:1.58); $C_{28}H_{54}N_8 \cdot 8\,HCl \cdot 2H_2O$ 830.51 gm/mole (base/salt 1: 1.65)
Structure:



Relevant INDs/NDAs/DMFs: IND 55851

Pharmacologic class: Hematopoietic stem cell mobilizer (plerixafor blocks the SDF-1/ CXCR4 interaction)

Intended clinical population: in combination with granulocyte-colony stimulating factor (G-CSF) to mobilize hematopoietic stem cells to the peripheral blood for collection and

subsequent autologous transplantation in patients with non-Hodgkins lymphoma and multiple myeloma

Clinical formulation: 20 mg/mL sterile, isotonic, aqueous solution for injection. The unit formula for the dosage form is as the following table (from the sponsor):

Component	Quality Standard	Function	Quantity per Millilitre	Quantity per Vial[b]	Quantity per Batch
Plerixafor[a]	In-house	Drug substance	20.0 mg	24.0 mg	(b) (4)
Sodium chloride	PhEur and USP-NF	(b) (4)	(b) (4)	5.9 mg	
Hydrochloric acid, concentrated	PhEur and USP-NF	pH adjustment	Sufficient to adjust to pH 6.0-7.5	Sufficient to adjust to pH 6.0-7.5	Sufficient to adjust to pH 6.0-7.5
Sodium hydroxide	PhEur and USP-NF	pH adjustment	Sufficient to adjust to pH 6.0-7.5	Sufficient to adjust to pH 6.0-7.5	Sufficient to adjust to pH 6.0-7.5
Water for injection	PhEur and USP-NF	Diluent	Sufficient to reach (b) (4)	Sufficient to reach 1.20 ml	Sufficient to reach (b) (4)
(b) (4)	PhEur and USP-NF	(b) (4)	Sufficient	Sufficient	Sufficient

USP-NF: United Stated Pharmacopoeia - National Formulary; PhEur – European Pharmacopoeia
[a] The weight of plerixafor used is corrected for water content and purity.
[b] These values are calculated for the label claim of 1.2 ml. (b) (4)
[c] (b) (4) b) (4)
(b) (4)

Route of administration: Subcutaneous injection

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Study Number	Study Title
Pharmacology *In vitro* studies	
AOM0053*	Inhibition of SDF-1 Ligand Binding to CXCR4 by AMD3100
AOM0056*	Inhibition of SDF-1 Stimulated GTPγS Binding by AMD3100
AOM0055*	Inhibition of SDF-1 Stimulated Chemotaxis by AMD3100
AOM0054*	Inhibition of SDF-1 Stimulated Calcium Flux by AMD3100
AOM0059*	AMD3100 Has No Significant Effect on CCR5 Mediated Calcium Flux
AOM0057*	The Effects of AMD3100 on IP10 Stimulated Calcium Flux
AOM0058*	The Effects of AMD3100 on MCP-1 Stimulated Calcium Flux
AOM0052*	Inhibition of LTB4 Ligand Binding to the Receptor by AMD3100
AOM0051*	Cross Screening AMD3100 for CCR4 and CCR7
	Chemokine receptor inhibition by AMD3100 is strictly confined to CXCR4: Haste *et al.*, FESB Letters 527: 255-262, 2002.
	Mutation of Asp^{171} and Asp^{262} of the chemokine receptor CXCR4 impairs its co-receptor function for Human Immunodeficiency Virus-1 entry and abrogates the antagonistic activity of AMD3100: Haste *et al.*, Molecular Pharmacology 60: 164-173, 2001
In vivo studies	
AOM0033*	Hematological Effects of AMD11070 in Mice

Study Number	Study Title
	Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist: Broxmeyer *et al.*, J Exp Med, 201: 1307-1318, 2005
AOM0045	AMD3100 for Hematopoietic Stem Cell Mobilization in a Canine Autologous Transplantation Model
AOM0046	AMD3100 for Hematopoietic Stem Cell Mobilization in a Canine Allogeneic Transplantation Model: Follow-up Report
AOM0049	AMD3100 Antagonism of CXCR4 Expressed on Cultured Canine PBMCs
	Durable engraftment of AMD3100 mobilized autologous and allogeneic peripheral-blood mononuclear cells in a canine transplantatin model: Burroughs *et al.*, Blood, 106: 4002-4008, 2005
	Leukocytosis and mobilization of CD34+ hematopoietic progenitor cells by AMD3100, a CXCR4 antagonist: Hubel *et al.*, Supportive Cancer Therapy, 1: 165-172, 2004
Safety Pharmacology	
Neurological effects	
107-031	Receptor Affinities (Binding) of SDZ SID 791
1009304 (2 Nov 2000)	Receptor Binding (Adrenergic, GABA)
1009304 (14 Dec 2000)	Angiotensin Converting Enzyme (ACE) and Neutral Endopeptidase Enzyme Assays
1009304 (15 Dec 2000)	Neuropeptide Y Receptor Functional Assays (agonist and antagonist activity)
POT/M/92/10	The Effects of SDZ 282-791 in the Primary Observation Test in Mice
POT/R/94/5	The Investigation of Potential CNS Effects of Subcutaneously Administered SID 791 Using the Rat Primary Observation Test
GT-249-TX-1	Effects of Plerixafor in the Irwin Test in Rats
Cardiovascular effects	
051128.BOP	Effects of AMD3100 on Cloned hERG Potassium Channels Expressed in Mammalian Cells
9608.0228.01	Dilatation of Rat Aortic Smooth Muscle Cells, Possible Implication of Free Calcium
107-028	Cardiovascular Effects of SDZ SID 791 (282-791) in Anaesthetized Rats after Intravenous and Subcutaneous Administration
(b) (4) 93226*	A Pilot Cardiovascular Profile Study Following a Single Intravenous Infusion of AMD3100 Free Base in the Conscious Restrained Beagle Dog
(b) (4) 93227*	A Cardiovascular Profile Study Following an Intravenous Infusion of AMD3100 Free Base in the Conscious Unrestrained Beagle Dog
Pulmonary effects	
GT-249-TX-2	Effects of Plerixafor on Respiration Rate and Tidal Volume in Conscious Rats
Other studies	
107-029	Effect of SDZ 282-791 on Different Endocrine Parameters in Male Rats
Pharmacokinetics/ ADME	
3167/DrCH	SDZ 282-791: Absorption, Distribution, Metabolism, and Excretion Following Single 20 mg/kg Subcutaneous Doses of [^{14}C]SDZ 282-791-ch in Rat
9608.0258	SDZ SID 791: Absorption and Disposition in Rats Following Single and Multiple Subcutaneous Doses of [^{14}C]SDZ SID 791-ch
9608.026	SDZ 282-791: Absorption, Distribution, Metabolism, and Excretion Following Single 20 mg/kg Oral and 2 mg/kg Intravenous Doses of [^{14}C]SDZ 282-791-ch in Rat
9608.0256	SDZ SID 791: Absorption and Disposition in Dogs Following Single Subcutaneous and Intravenous Doses of [^{14}C]SDZ SID 791-ch
GT-249-PK-3	Stability of Plerixafor in Human, Dog and Rat Whole Blood
GT-249-PK-4	Determination of Red Blood Cell Partitioning of Plerixafor in Rat, Dog and Human Whole Blood
AOM0036	Interspecies Protein Binding of AMD3100 using Ultrafiltration Analysis
7686-108 CMS81280A	Pharmacokinetics, Excretion, Mass Balance, and Quantitative Whole-Body Autoradiography Following Subcutaneous Administration of ^{14}C-AMD3100 to Rats
9608.0251	SDZ SID 791 (SDZ 282-791): Whole-body Autoradiography in Rats Following Single

Study Number	Study Title
	and Multiple Subcutaneous Doses of [^{14}C]SDZ SID 791-ch
AOM0038	*In Vitro* Interspecies Metabolism Profile of AMD3100
GT-249-PK-1	*In Vitro* Metabolic Stability of Plerixafor in Rat, Dog and Human Liver Microsomes
GT-249-PK-5	Metabolite Profile of Plerixafor in Rat Plasma and Urine Following Subcutaneous Administration to Rats
AOM0067	Inhibition of CYP450 Isoforms by AMD3100 Using Fluorometric Substrate Detection
XT055036	*In Vitro* Evaluation of AMD3100 as an Inhibitor of Human Cytochrome P450 Enzymes
AOM0069	Pharmacokinetics of AMD3100 Following a Single 5 mg/kg Subcutaneous Injection in Mice
DMPK08-R001	*In Vitro* Assessment of the Induction Potential of Plerixafor in Primary Cultures of Human Hepatocytes
AOM0073	Noncompartmental Pharmacokinetic Analysis of Plasma Concentration Versus Time Data for AMD3100 and Radioactivity in Rats from Sandoz Studies 3167/DrCH, 9608.0258, and 9608.026.
AOM0074	Noncompartmental Pharmacokinetic Analysis of Plasma Concentration Versus Time Data for AMD3100 and Radioactivity in Dogs from **(b) (4)** Study 9608.0256
Toxicology	
Single dose toxicology	
RCC 380586	SDZ SID 791: Acute Subcutaneous Toxicity Study in Mice
RCC 380564	SDZ SID 791: Acute Intravenous Toxicity Study in Mice
RCC 380575	SDZ SID 791: Acute Subcutaneous Toxicity Study in Rats
RCC 379787	SDZ SID 791: Acute Intravenous Toxicity Study in Rats
Repeat dose toxicology	
Once-daily regimen	
428R-tox 428R-tk	SDZ SID 791: A 4-Week Subcutaneous Toxicity Study in Rats
432R	SDZ SID 791: An Additional 4-Week Subcutaneous Toxicity Study in Rats
(b) (4) 94/SPM028/0891-tox **(b) (4)** 94/SPM028/0891-tk	SDZ SID 791: Toxicity Study by Subcutaneous Administration to Beagle Dogs for 4 Weeks followed by a 2 Week Reversibility Period
Twice-daily regimen	
(b) (4) 1663*	AMD 3100: A 14 Day Dosing Schedule Dependency (Once vs. Twice Daily) Study in Female Sprague-Dawley Rats
(b) (4) 89342*	A Range Finding Subcutaneous Injection (Twice Daily) Toxicity Study of AMD-3100 Free Base in the Albino Rat
(b) (4) 89349*	A Range Finding Subcutaneous Injection (Twice Daily) Toxicity Study of AMD3100 Free Base in the Beagle Dog
(b) (4) 89289*	A 28-Day Twice Daily Subcutaneous Injection Toxicity Study of AMD3100 Free Base in the Albino Rat with a 14- Day Recovery Period
(b) (4) 89290*	A 28-Day Twice Daily Subcutaneous Injection Toxicity Study of AMD-3100 Free Base in the Beagle Dog with a 14-Day Recovery
Genotoxicity	
In vitro studies	
Mut.Bakt.15/94	SDZ SID 791: Mutagenicity Test Using S*almonella Typhimurium*
Z48	SDZ SID 791: Chromosomal Aberration Test with V79 Chinese Hamster Cells
In vivo study	
(b) (4) 960379	AMD 3100 Free Base Rat Micronucleus Test
Reproductive and developmental toxicity	
Embryo-fetal toxicity	
(b) (4) 900519	AMD 3100: A Subcutaneous Injection Teratology Study in the Rat
Local tolerance	
(b) (4) 89679	A Comparative Intracutaneous (Intradermal) Irritation Study with Two Formulations of AMD 3100 in the New Zealand White Rabbit
Other toxicity	

Study Number	Study Title
107-025	Effect of SDZ 282-791 on in vivo Antibody Formation (Rat)
(b) (4) 60101	*In Vitro* Evaluation of the Influence of AMD3100 on Human Whole Blood Hemolysis

*** Studies reviewed by Dr. Guodong Fang (Appendix)**

Studies not reviewed within this submission:

Study Number	Study Title
Pharmacology Primary Pharmacodynamics	
AOM0064	The Effects of AMD3100 on MIP-1α Stimulated Calcium Flux
AOM0070	Structural Analogues of AMD3100 Mobilize Progenitor Cells from Bone Marrow *In Vivo* According to their Ability to Inhibit CXCL12 Binding to CXCR4 *In Vitro*
AOM0075	Investigation of the Cross-reactivity of AMD3100 with CXCR7
Secondary Pharmacodynamics	
AOM0047	Direct Injection of AMD3100 Improves Blood Flow to the Diabetic Mouse Hind Limb after Induction of Hindlimb Ischemia
AOM0048	AMD3100, a CXCR4 Antagonist, Augments Mobilization and Incorporation of Bone Marrow-Derived Endothelial Progenitor Cells into Sites of Myocardial Neovascularization
AOM0072	AMD 3100 Administration in a Porcine Model of Myocardial Ischemia Reperfusion Injury
GT-249-EF-1	AMD3100 Rapidly Mobilized Stem Cells after Myocardial Infarction in Nonhuman Primates but Does Not Improve Left Ventricular Remodeling or Function
Safety Pharmacology	
AOM0071	Affinity of SDZ 282 791 (SDZ SID 791) for 5-HT Receptor Binding Sites
Pharmacokinetics	
Methods of analysis	
282-791	Liquid Chromatography-reverse Isotope Dilution Method (LC-RID) for the Determination of [^{14}C]SDZ SID 791 in Biological Samples
ADME	
DMPK08-R001	*In Vitro* Assessment of the Induction Potential of Plerixafor in Primary Cultures of Human Hepatocytes
GT-249-PK-2	*In Vitro* Metabolic Stability of Plerixafor in Rat, Dog and Human Hepatocytes
Toxicology	
Single dose toxicology	
0835RA69.008	Hematology Study in Rats Administered A Single Subcutaneous Dose
AOM0032	AMD 3100: A Single and Repeated-Dose Range Finding Study in Male Yorkshire Pigs
GT-249-TX-3	Correlation of Plasma Electrolyte Changes and Clinical Signs Following a Single Subcutaneous Administration of Plerixafor in Rats
Repeat dose toxicology	
189DFR-tox 189DFR-tk	SDZ SID 791 (SDZ 282-791): A 2-Week Subcutaneous Dose-range-finding Study in Rats
189DFR-tox 189DFR-tk	SDZ SID 791 (SDZ 282-791): A 2-Week Subcutaneous Dose-range-finding Study in Dogs
GT-249-TX-4	Study of Metal Homeostasis upon Daily Dosing with SID 791
(b) (4) 94/SPM030/0883	SDZ SID 791: Dose Range Finding-Study by Subcutaneous Administration to Beagle Dogs
Reproductive and developmental toxicity	
Embryo-fetal toxicity	
6045K	SDZ SID 791: Subcutaneous Embryo–Fetal Development Dose Range Finding Study in Rabbits with Toxicokinetics and Placental Transfer

(b) (4) 900518	AMD 3100: A Subcutaneous Injection Range-finding Teratology Study in the Rat

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Plerixafor (AMD3100) is an antagonist of the CXCR4 chemokine receptor and blocks binding of its cognate ligand, stromal-derived factor-1 (SDF-1). SDF-1 and CXCR4 are involved in the trafficking and homing of human CD34+ cells to the marrow compartment. Inhibition of chemoattractant effect of locally produced SDF-1 in the bone marrow has been attributed to plerixafor's effect in leukocytosis induction and elevation of circulating hematopoietic progenitor cell levels. Plerixafor has been shown to exert an additive effect on the number of circulating stem and progenitor cells when administered in combination with granulocyte-colony stimulating factor (G-CSF).

In cells naturally expressing the chemokine receptors or in transfected cell lines, receptor selectivity of plerixafor toward CXCR4 was investigated. Plerixafor did not have antagonistic effects on several chemokine receptors tested, as supported by the lack of ligand binding to the receptor and/or inhibition of ligand-induced calcium flux. These receptors included CXCR1, 2, 3, 7, and CCR1, 2b, 3, 4, 5, 6, 7, 8 and 9. Plerixafor inhibited the binding of SDF-1 to CXCR4 (IC_{50} = 651 nM), as well as SDF-1 stimulated calcium flux, chemotaxis and GTPγS binding (with IC_{50} values in the nanomolar ranges).

In the *in vivo* studies, plerixafor treatment induced leukocytosis in a dose-dependent fashion. Plerixafor enhanced G-CSF-induced mobilization of hematopoietic progenitor cells (including colony forming unit-granulocyte macrophage (CFU-GM) and burst forming units-erythroid (BFU-E), as well as colony forming units-granulocyte, erythroid, megakaryocyte, macrophage (CFU-GEMM)) in various strains of mice. Plerixafor also mobilized murine stem cells/long-term repopulating (LTR) cells that engrafted primary and secondary lethally-irradiated mice. G-CSF enhanced plerixafor's mobilization and engraftment capacity. In dogs, plerixafor increased G-CSF-induced PBMC mobilization and engraftment (up to 1 year follow-up) in both autologous and allogeneic transplantation models.

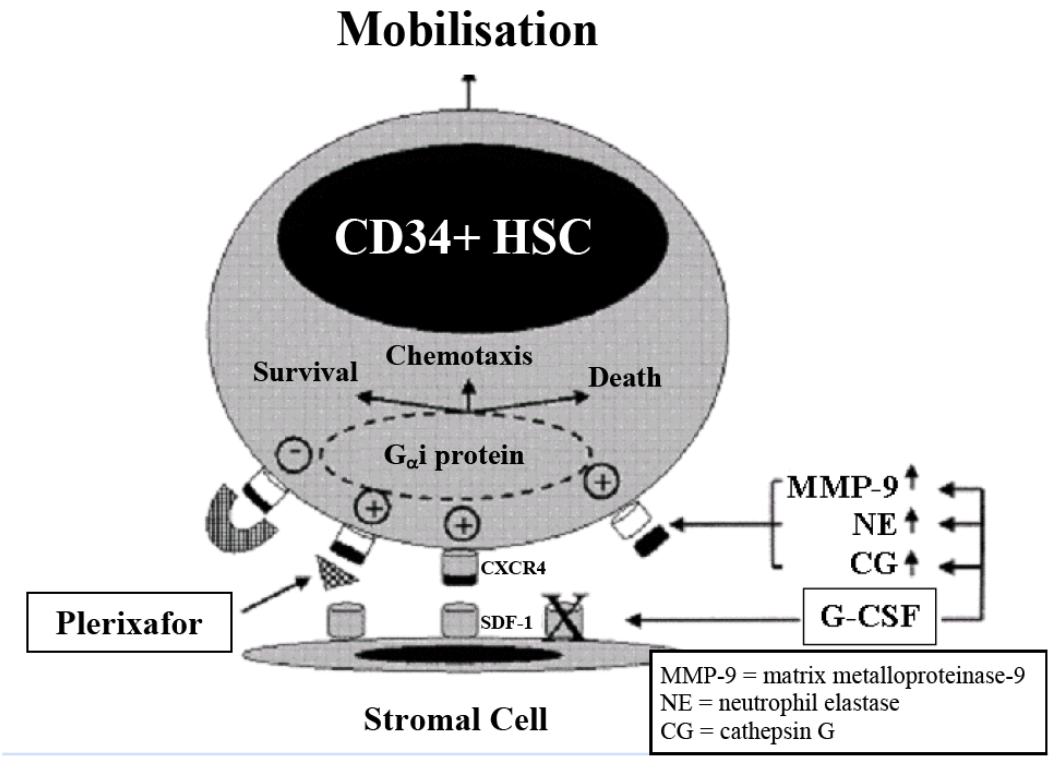
In vitro data indicted that plerixafor bound to calf brain adrenergic alpha (α_1 and α_2) and dopamine (D_2) receptors, exhibited agonism to neuropeptides Y_2 and Y_3 (rat vas deferens and distal colon, respectively) and inhibited angiotensin converting enzyme activity (rabbit lung). Plerixafor demonstrated effects on functions of the nervous and respiratory systems in mice and rats. The CNS suppressive effects were manifested as decreased in-place and locomotor activity, alertness, and startle response, flattened postures, ptosis, and pupil dilatation. These effects may be also the underlining mechanism of the observed decrease in tidal volume and respiratory rate. However, it was noted that plerixafor induced CNS stimulant-like response if the animals were handled. Plerixafor did not inhibit hERG currents in HEK293 cells. In rats, plerixafor treatment (via intravenous infusion or subcutaneous injection) resulted in

lethal cardiodepression effects, i.e., decreased heart rate, blood pressure and myocardial contractility. In contrast, plerixafor increased heart rates and blood pressures in telemetered conscious beagle dogs, but had no remarkable effects on the ECGs. Similar tachycardiac and hypertensive effects were observed in the repeated dose toxicology studies in dogs. Plerixafor may be vasodilative, because it inhibited angiotensin II-induced vasoconstriction in cultured rat aortic smooth muscle cells. The vasodilation effect may be attributed to plerixafor-induced depletion of calcium necessary for contraction. Treatment with plerixafor resulted in reduced tidal volume and respiratory rate in rats.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:
Plerixafor (AMD3100) is a bicyclam antagonist of the G-protein coupled CXCR4 receptor. The essential roles of the interaction between CXCR and its natural ligand SDF-1 (a cytokine, also known as CXCL-12) in stem cell homing and retention (via activation of adhesion mechanism) in bone marrow, embryonic development (including cerebellar development), hematopoiesis, and vascularization were demonstrated in knock-out experiments in mice. Murine embryos that lack SDF-1 or CXCR4 do not survive or display multiple defects, such as impaired B cell hematopoiesis in fetal liver with a severe reduction on pro- and pre-B cell numbers, and impaired bone marrow myelopoiesis, primarily due to the lack of migration of progenitors from fetal liver to the bone marrow (see the review by Lapidot and Petit, Exp Hematology 10: 973-981, 2002). SDF-1/CXCR4 interactions are also important in hematopoietic stem cell (HSC) release and mobilization. Reduction of bone marrow SDF-1 level (e.g., via G-CSF induced activation of proteolytic enzymes, such as matrix metalloproteinase-9 [MMP-9], neutrophil elastase [NE] and cathepsin-G [CG]) was reported to mediate G-CSF-induced mobilization of human and murine stem cells. The finding that neutralizing anti-CXCR4 and anti-SDF-1 antibodies reduced stem cell mobilization also supported the role of SDF-1/CXCR4 signaling in cell egress. The interaction between SDF-1 and CXCR4 was also demonstrated to mediate immature human CD34+-enriched cells homing and repopulation in transplanted NOD/SCID mice. Disruption of SDF-1/CXCR4 interaction by plerixafor is prone to enhance the release of hematopoietic stem cells (HSC) and hematopoietic progenitor cells (HPC) from bone marrow into the peripheral circulation. The proposed mechanism is depicted in the figure below (from the sponsor):

Figure 2.6.2-1: Mechanism of action of plerixafor



Drug activity related to proposed indication:
In vitro studies:
The following studies were reviewed by Dr. Guodong Fang (IND 55851, N-065, May 16, 2002) (see Appendix):
Study AOM0051: Cross screening AMD3100 for CCR4 and CCR7
Study AOM0052: Inhibition of LTB_4 ligand binding to the receptor by AMD3100
Study AOM0053: Inhibition of SDF-1 ligand binding to CRCX4 by AMD3100
Study AOM0054: Inhibition of SDF-1 stimulated calcium flux by AMD3100
Study AOM0055: Inhibition of SDF-1 stimulated chemotaxis by AMD3100
Study AOM0056: Inhibition of SDF-1 stimulated GTPγS binding by AMD3100
Study AOM0057: The effect of AMD3100 on IP10 stimulated calcium flux
Study AOM0058: The effect of AMD3100 on MCP-1 stimulated calcium flux
Study AOM0059: AMD3100 has no significant effect on CCR5 mediated calcium flux

"Chemokine receptor inhibition by AMD3100 is strictly confined to CXCR4": Haste *et al.*, FESB Letters 527: 255-262, 2002.

In this article, the authors demonstrated that AMD3100 specifically bond to CXCR4, with insignificant affinities to other chemokine receptors tested. AMD3100 inhibited CXCR4-mediated calcium signaling and chemotaxis in a concentration-dependent manner in different cell types.

Table 1
Overview of calcium flux experiments performed to demonstrate the CXCR4-specific antagonism by AMD3100

Chemokine receptor	Ligand	Concentration (ng/ml)	Cell type	IC_{50} of AMD3100 (μg/ml)	Figure
CXCR1	IL-8	500	freshly isolated PBMCs	>25	3
CXCR2	IL-8	500	freshly isolated PBMCs	>25	not shown
	GROα	500	freshly isolated PBMCs	>25	3
CXCR3	IP-10	50	U87.CXCR3	>25	not shown
		200	PHA/IL-2-stimulated PBMCs	>25	3
CXCR4	SDF-1	10	U87.CD4.CXCR4	0.13	2
		100	freshly isolated PBMCs	0.03	2
		20	SupT1	0.04	not shown
		10	THP-1	0.01	not shown
		20	HSB-2	0.10	2
		100	Molt-4	0.02	not shown
		2.5	L1210	0.08	2
CCR1	MIP-1α	50	U87.CD4.CCR1	>25	3
		50	purified monocytes	>25	not shown
CCR2	MCP-1	50	purified monocytes	>25	3
CCR3	eotaxin	50	U87.CD4.CCR3	>25	3
CCR4	MDC	20	HOS.CD4.CCR4	>25	3
	TARC	20	HOS.CD4.CCR4	>25	not shown
CCR5	RANTES	5	U87.CD4.CCR5	>25	not shown
		50	purified monocytes	>25	not shown
	MIP-1β	10	U87.CD4.CCR5	>25	3
		50	purified monocytes	>25	not shown
CCR6	MIP-3α	200	PHA/IL-2-stimulated PBMCs	>25	3
CCR7	MIP-3β	200	HSB-2	>25	3
CCR8	I309	500	freshly isolated PBMCs	>25	3
CCR9	TECK	100	Molt-4	>25	3

The authors also demonstrated that AMD3100 was not a CXCR4 agonist, because AMD3100 by itself was unable to elicit intracelluar calcium fluxes, to induce chemotaxis, or to trigger CXCR4 internalization.

"Mutation of Asp^{171} and Asp^{262} of the chemokine receptor CXCR4 impairs its co-receptor function for Human Immunodeficiency Virus-1 entry and abrogates the antagonistic activity of AMD3100": Haste *et al.*, Molecular Pharmacology 60: 164-173, 2001

The interaction of AMD3100 and CXCR4 at the molecular level was investigated by mutational analysis in a set of stably transfected U87.CD4 cell lines, that expressed different forms of CXCR4, wild type and mutants (i.e., CXCR[WT], CXCR4[D171N], CXCR4[D262N], and CXCR4[D171N, D262N]).

It was identified that Asp^{171} and Asp^{262} on CXCR4 (a 7-transmembrane receptor) may be the crucial sites for the interaction between AMD3100 and CRCX4. These negative charged nucleotides located in the transmembrane domains 4 and 6, respectively, were essential for electrostatic interaction of the positive charged bicyclams, such as AMD3100; and mutations at these sites resulted in the loss of AMD3100's affinity as well as antagonistic action.

In vivo studies:
Studies in mice:
The following study was reviewed by Dr. Guodong Fang (IND 55851, N-065, May 16, 2002) (see Appendix):

Study AOM0033: Hematological effects of AMD11070 in mice

"Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist": Broxmeyer *et al.*, J Exp Med, 201: 1307-1318, 2005.

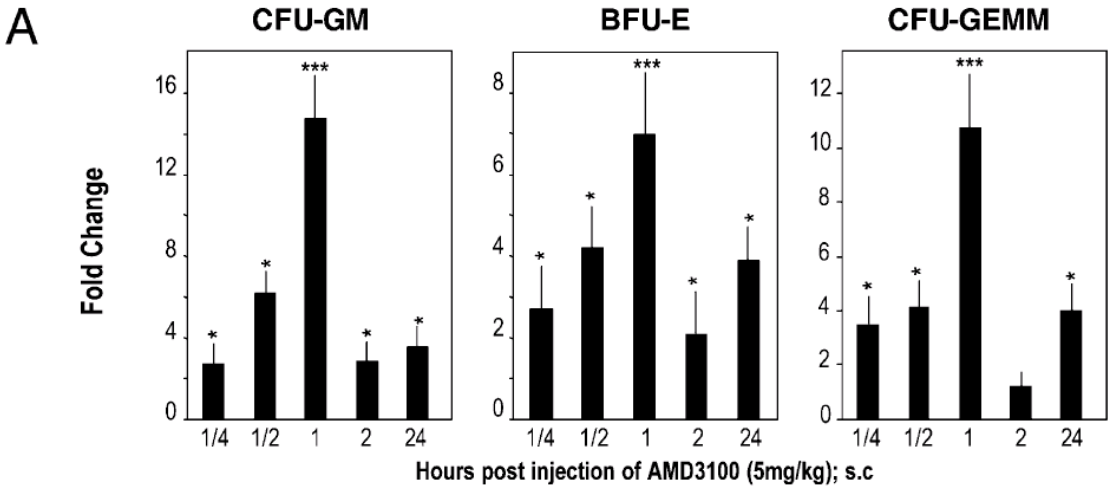
Key study findings: AMD3100 enhanced G-CSF induced mobilization of hematopoietic progenitor cells in various strains of mice.

The small pool of hematopoietic stem cells in bone marrow give rise to blood forming cells through intermediate cells, termed hematopoietic progenitor cells (HPC), including linage-restricted progenitors, such as colony forming unit-granulocyte macrophage (CFU-GM) and burst forming units-erythroid (BFU-E), as well as common myeloid, such as colony forming units-granulocyte, erythroid, megakaryocyte, macrophage (CFU-GEMM). HPCs can be isolated in culture (standard media with cytokines for 7 days), and assayed and quantified by colony forming assay.

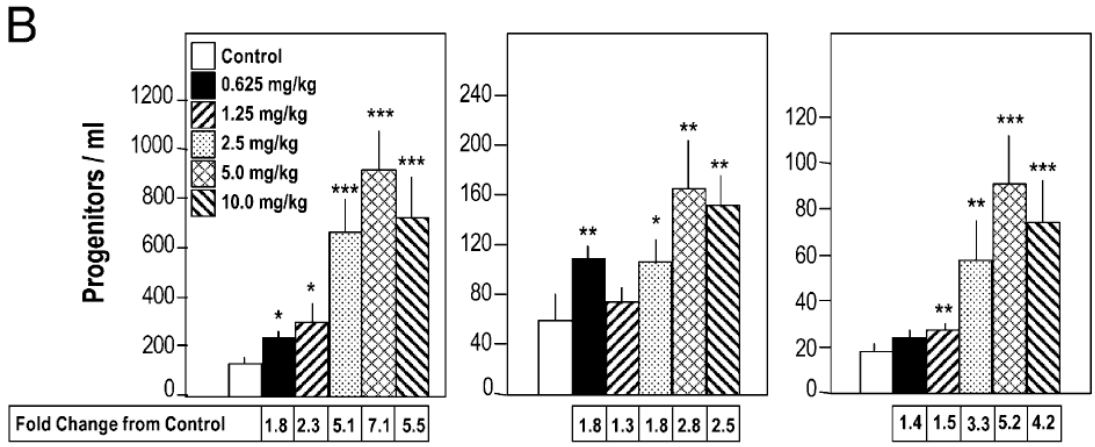
Results:

- Time and dose response effects of AMD3100 in mobilizing hematopoietic progenitor cells (HPC) to the blood of C3H/HeJ mice (figures from the article):

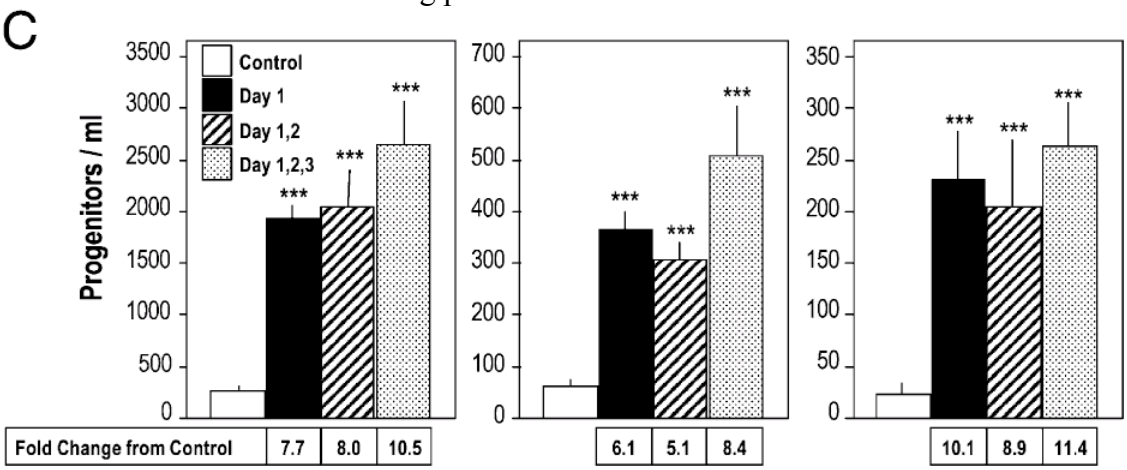
➢ Subcutaneous administration of AMD3100 (5 mg/kg) to mice (n=5) resulted in a peak mobilization of HPC at 1 hr post dose (panel A). The CFU-GM, BFU-E and CFU-GEMM counts were approximately 14-, 7- and 10-fold higher than the respective control counts (time 0 control numbers of CFU-GM, BFU-E and CFU-GEMM per mL of blood were 78 ± 18, 17 ± 2, and 10 ± 2, respectively).



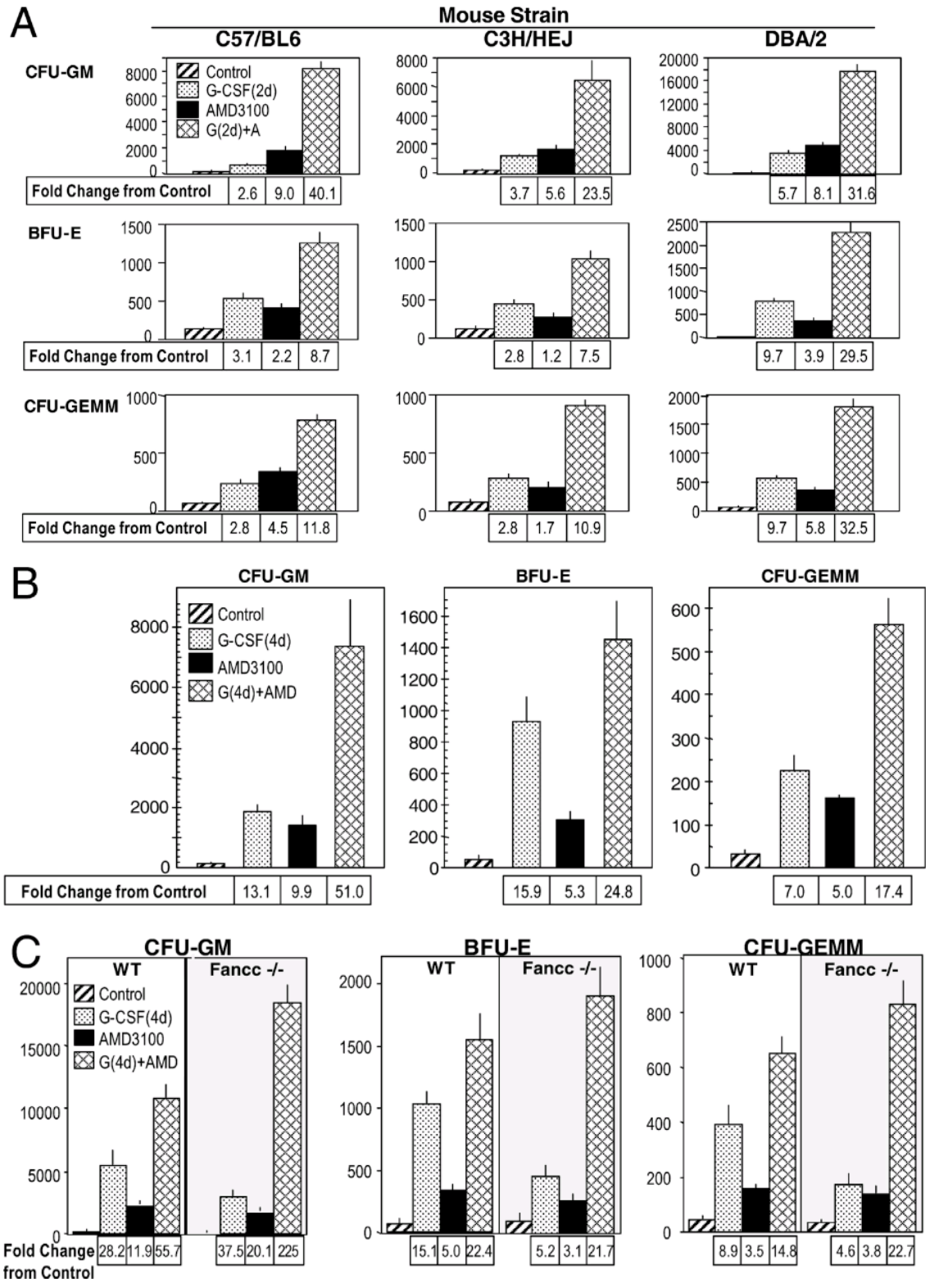
➢ The dose effect on mobilization was investigated by SC injection of control (saline) or AMD3100 (0.625-10 mg/kg) to the mice (n=7). Dose response was analyzed 1 hr after injection. Maximmal mobilization of HPC at 1 hr was noted with 2.5-10 mg/kg AMD3100 (panel B).



- The mobilization response of multiple treatment of AMD3100 (5 mg/kg) on Day 1, Days 1 and 2, or Days 1, 2 and 3 was analyzed 1 hr after the last injection to the mice (control n=14, AMD3100 n=8/group). It was noted that HPC mobilization was similar each day (panel C), indicating the HPC-mobilizing capacity of AMD3100 was not desensitized within the testing period.



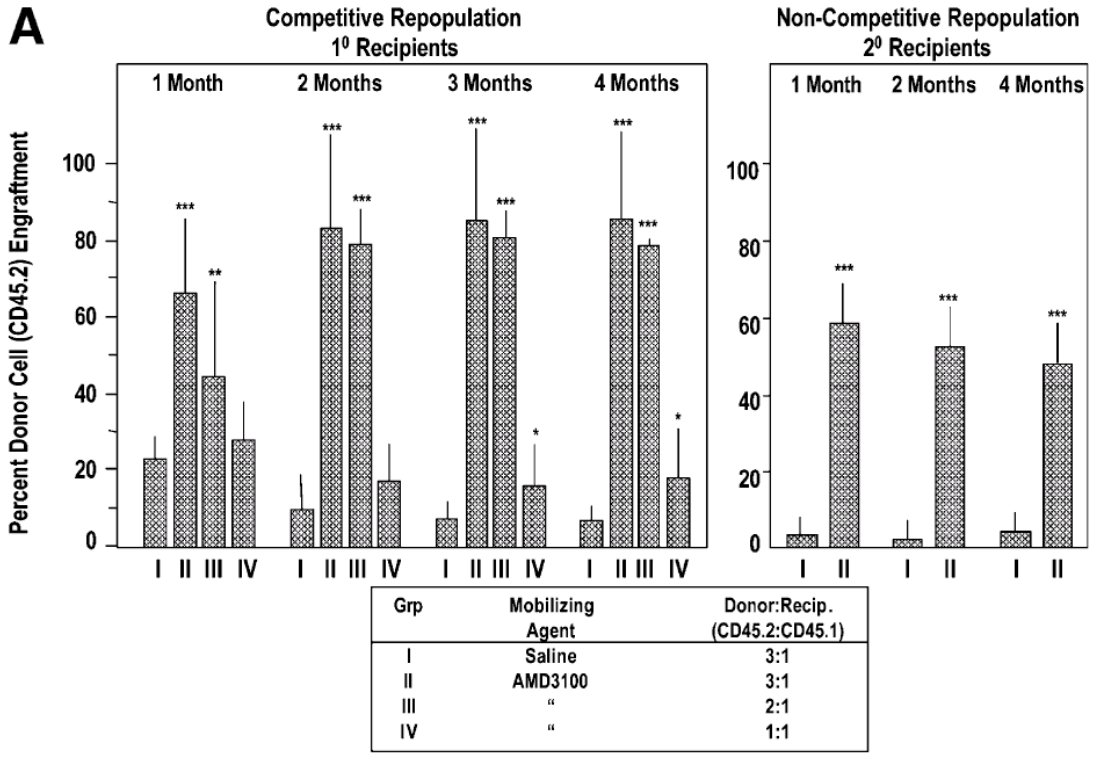
- <u>HPC mobilization response to AMD3100 with or without G-CSF:</u> AMD3100 enhanced G-CSF-induced mobilization of HPCs in various mouse strains that were reported to differ in responsiveness to G-CSF (e.g., response in C57BL/6 was less than that in DBA/2 mice). The data was expressed as HPC/mL of blood. Groups of C57BL/6 (n=15), C3HeJ (n=15) and DBA/2 (n=11) mice were injected with saline or 2.5 μg G-CSF, twice a day SC for 2 (Panel A) or 4 days (Panel B and C). Eighteen hr after 2-4 daily G-CSF administration, mice were treated with saline or a single SC dose of AMD3100 at 5 mg/kg. Panel B is the result in C57BL/6 mice (n=5), while Panel C shows the enhancement of AMD3100 in mice carrying Fanconi's anemia complementation C group gene ($Fancc^{-/-}$, n=8) and in $Fancc^{+/+}$ wild-type (WT, n=15) mice. Clinically, patients who have Fanconi's anemia are reported to be poor responders to HPC mobilization with G-CSF.



- The effects of AMD3100 on long-term repopulation: mobilizing and repopulating hematopoietic stem cells (HSC) in CD45 congenic mice

Mobilization of HPC is of use for short-term repopulation in a transplant setting (see above), while HSCs are required for long-term repopulation. The ability of AMD3100 to mobilize murine HSCs was assessed in a competitive repopulating assay. In this assay, dilutions of donor blood cells ($CD45.2^+$ cells from C57BL/6 mice) that contain LTRs (HSCs with **l**ong-**t**erm **r**epopulating activity) compete with recipient marrow cells ($CD45.1^+$ cells, competitor cells from non-irradiated B6.BoyJ mice) for engraftment in lethally-irradiated recipients (B6.BoyJ mice). Cells collected from both strains of mice were mixed, and the ratio of donor (CD45.2) cells to competitor (CD45.1) marrow cells was set to be 3:1, 2:1 or 1:1. The cell mixtures were injected into the irradiated recipients (n=6) via IV infusion. Results are shown as % CD45.2 donor chimerism in CD 45.1 recipients. As shown in Panel A (left), AMD3100-mobilized cells exhibited higher % donor cell (CD45.2) chimerism in the recipeints engrafted with 3:1, 2:1 and 1:1 ratios of AMD3100-mobilized donor/recipient competitor cells. At 2-4 mo posttransplant, engraftments with 3:1 and 2:1 ratios of AMD3100 treated donor cells sustained a > 8-fold higher chimerism, compared with cells that were mobilized from control medium-treated donor mice.

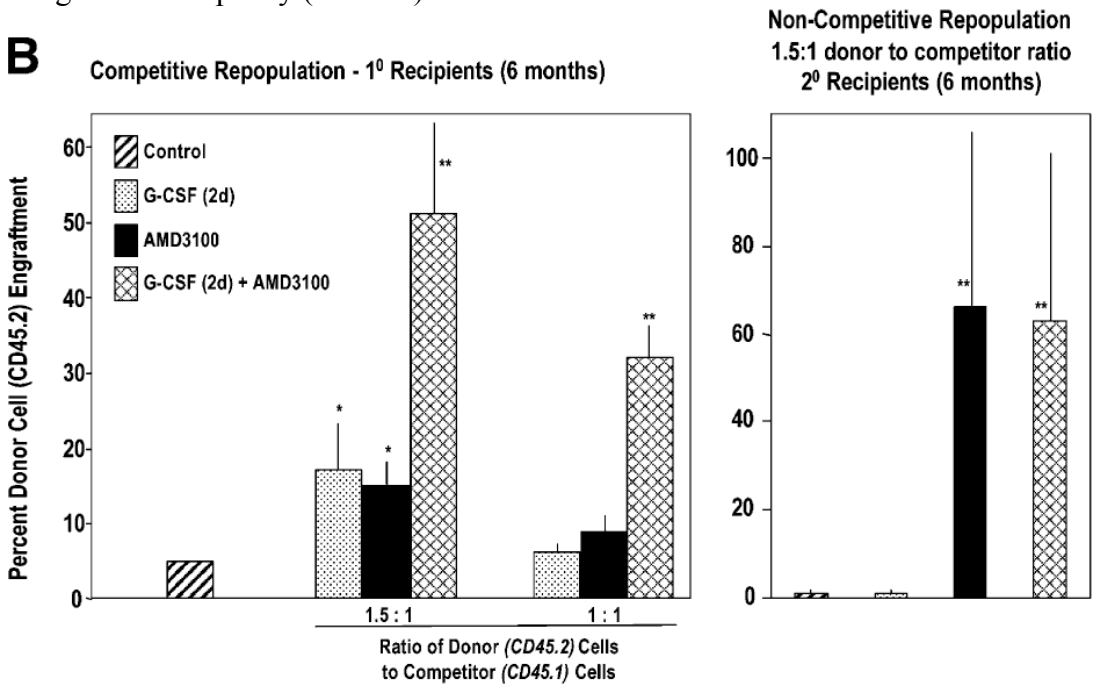
In a non-competitive assay, at 4 mo posttransplant, marrow cells obtained from primary mice (that were competitively engrafted with a 3:1 ratio of donor/recipient cells, n=3) were injected into lethally-irradiated secondary mice (n=3), and the self-renewal capacity of AMD3100-mobilized LTR cells was tested (Panel A, right). In the secondary recipient group where donor cells were from AMD3100-treated mice, all mice survived and ≥ 50% of hematopoietic cells that engrafted the secondary mice were of donor origin.



Grp	Mobilizing Agent	Donor:Recip. (CD45.2:CD45.1)
I	Saline	3:1
II	AMD3100	3:1
III	"	2:1
IV	"	1:1

Thus, the authors demonstrated that AMD3100 mobilized murine long-term repopulating (LTR) cells that engrafted primary and secondary lethally-irradiated mice.

Treatment of donor mice with G-CSF (twice daily x 2d) enhanced AMD3100's mobilization and engraftment capacity (Panel B):



AMD3100 also synergized with G-CSF on mobilization of severe combined immunodeficiency (SCID) repopulating cells (SRC) from normal human volunteers (apheresis samples). These human $CD34^{+}$ $CD38^{-}$ cells expressed a phenotype that was characteristic of highly engrafting mouse hematopoietic stem cells (HSC), and can repopulate the marrow of nonobese diabetic-severe combined immunodeficiency (NOD-SCID) mice (data not shown).

Studies in dogs:

Key findings: AMD3100-mobilized PBMCs, which expressed CXCR4 and responded to SDF-1 stimulation, led to prompt and durable engraftment up to 1 year follow-up in both autologous and allogeneic transplantation models in dogs.

The following studies and one published article described effects of plerixafor for hematopoietic stem cell mobilization in autologous and allogeneic transplantation models in dogs.

Study #AOM0045: AMD3100 for hematopoietic stem cell mobilization in a canine autologous transplantation model

Study #AOM0046: AMD3100 for hematopoietic stem cell mobilization in a canine allogeneic transplantation model: follow-up report

Study #AOM0049: AMD3100 antagonism of CXCR4 expressed on cultured canine PBMCs

"Durable engraftment of AMD3100 mobilized autologous and allogeneic peripheral-blood mononuclear cells in a canine transplantatin model": Burroughs *et al.*, Blood, 106: 4002-4008, 2005.

Four dogs were given a single SC dose of AMD3100 (4 mg/kg) and the plasma pharmacokinetic parameters were obtained (see the table; data expressed as mean ± SEM).

C_{max} (μg/mL)	AUC (hr•μg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)
8.29 ± 0.61	8.29 ± 3.59	1 ± 0	2.98 ± 0.18

AMD3100 administration induced a general leukocytosis with peak increases in total white blood cell (WBC) and absolute neutrophil counts observed 8-10 hr after the injection. Lymphocyte and monocyte counts were increased (1.5 and 4 fold, respectively), while no changes in hematocrits or platelet counts were noted. In addition, the numbers of circulating $CD34^+$ cells and colonogenic progenitor cells (CFU) were increased (3 to10-fold and 2- to 4-fold, respectively), with the peak increase at 8-10 hr post dose. (Figure from Burroughs *et al.*).

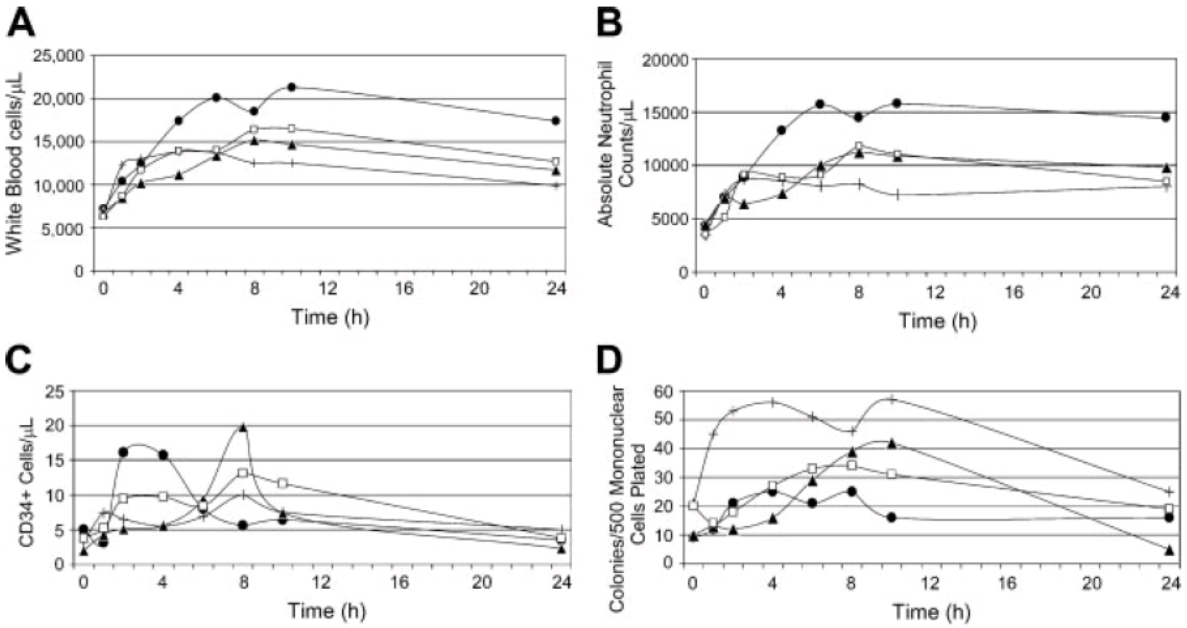


Figure 2. Peripheral blood cell counts of dogs (n = 4) following a single subcutaneous dose of AMD3100 (4 mg/kg). (A) White blood cell counts; (B) absolute neutrophil counts; (C) $CD34^+$ cell counts; (D) colony-forming unit (CFU) counts. E343 (●), G035 (▲), G272 (+), and G105 (□) depict dog identification numbers.

In the autologous transplantation model, 6-7 hr after AMD3100 administration the dogs (n=4, see above) underwent 3-4 hr leukapheresis. After completion of leukapheresis, the dogs received 920 cGy total body irradiation (TBI) delivered at 7cGy/min, which was followed by infusion of autologous AMD3100-mobilized peripheral-blood mononuclear cells (PBMCs) (median $CD34^+$ cell count, 3.9 x 10^6/kg). The result of engraftment is shown in the table below (from the sponsor):

Table 1. Cellular composition of leukapheresis products and engraftment data from dogs given 920 cGy TBI followed by transplantation of either autologous or allogeneic AMD3100-mobilized PBMCs

Dog no.	TNC, $\times 10^8$/kg	CD34, $\times 10^6$/kg	CD3, $\times 10^8$/kg	CD4, $\times 10^8$/kg	CD8, $\times 10^8$/kg	CD14, $\times 10^8$/kg	Engraftment	Clinical GVHD	Survival, d	Cause of death
Autologous HCT										
G275	15.6	3.3	2.6	1.6	0.6	4.3	Yes	NA	365	End of study
G248	13.2	4.5	2.7	1.6	0.8	2.5	Yes	NA	365	End of study
G294	23.0	8.7	0.6	0.4	0.1	4.2	Yes	NA	365	End of study
G315	4.0	2.0	NE	NE	NE	0.3	Yes	NA	365	End of study
DLA-identical HCT										
G383	8.0	2.1	1.4	0.7	0.5	0.6	Yes*	None	430†	Alive
G378	10.7	6.1	4.4	2.2	1.3	2.6	Yes*	None	414†	Alive
G422	12.1	4.7	3.6	2.1	1.3	2.7	Yes*	? Acute S, G	18‡	Pancreatitis, canine HSV
G375	4.5	1.5	2.3	1.2	0.6	1.0	Yes*	None	324†	Alive
G455	31.4	8.2	0.5	0.4	0.1	2.5	Yes*	Chronic S	233†	Alive

TNC indicates total nucleated cell; NA, not applicable; NE, not evaluable; ?, possible; S, skin GVHD; G, gut GVHD; and HSV, herpes simplex virus.
*All animals tested had engraftment of donor origin demonstrated by VNTR.
†Alive.
‡Dead.

Neutrophil and platelet recoveries occurred at median of 9 and 25 days, respectively, after TBI, and all dogs had normal marrow function at 1 year post-transplantation (figures from Module 2, Section 2.6.2.2.2.4).

Figure 2.6.2-4: Engraftment of Neutrophils after Autologous Transplantation of Plerixafor-mobilised Cells in Dogs (n = 4)

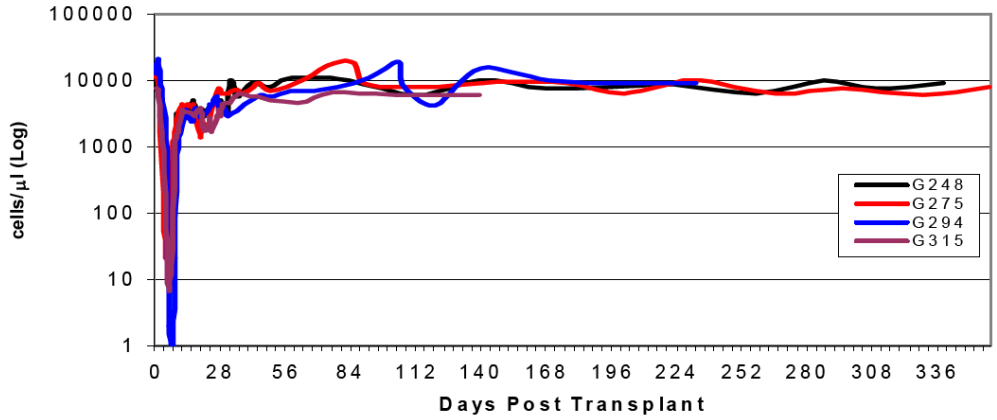
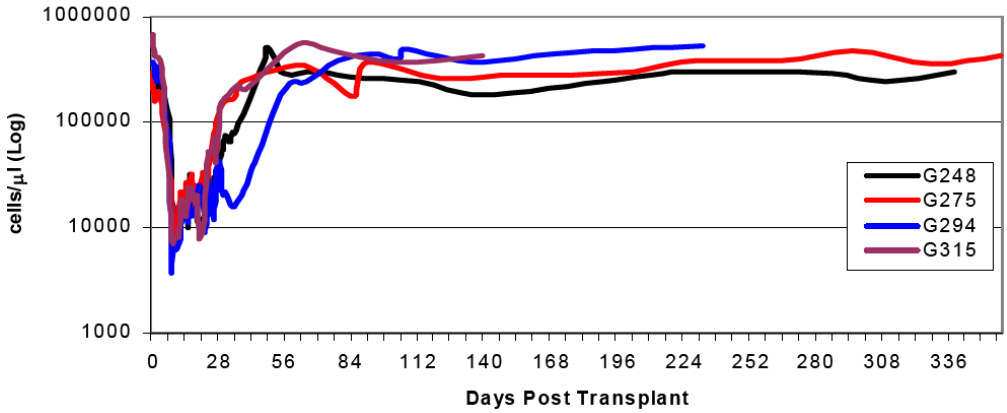


Figure 2.6.2-5: Engraftment of Platelets after Autologous Transplantation of Plerixafor-mobilised Cells in Dogs (n = 4)



In an accompanied *in vitro* study (#AOM 0049), it was demonstrated that cultured AMD3100-mobilized PBMCs expressed CXCR4 (as verified by flow cytometry and homologous competition with radiolabeled ligand). These cells demonstrated human SDF-1α-induced calcium flux (EC_{50}: 1.5-3.3 nM), and AMD3100 inhibited the stimulatory effect (IC_{50}: 649 nm and 248.7 nm, for two different preparations).

In a separate experiment, long term allogeneic engraftment potential of AMD3100-mobilized PBMCs was investigated. Five dogs were irradiated (se above) followed by infusion of AMD3100-mobilized PBMCs (median CD34 cell dose, 4.7 x 10^6/kg) from their dog leukocyte antigen (DLA)-identical littermates. Neutrophil and platelet recoveries occurred at median of 8 and 28 days, respectively, after TBI. One dog, G422, was euthanized on Day 18 after transplantation, due to canine herpes simplex virus infection and pancreatitis. Its neutrophil counts were normal (14.72 x 10^9/L), but platelet counts were below 20 x 10^9/L. For remaining 4 dogs, with a median follow-up period of 53 weeks, recipients' marrow function was normal, and blood-donor chimerism levels were 97% to 100%. (Figure and table from #AOM 0046):

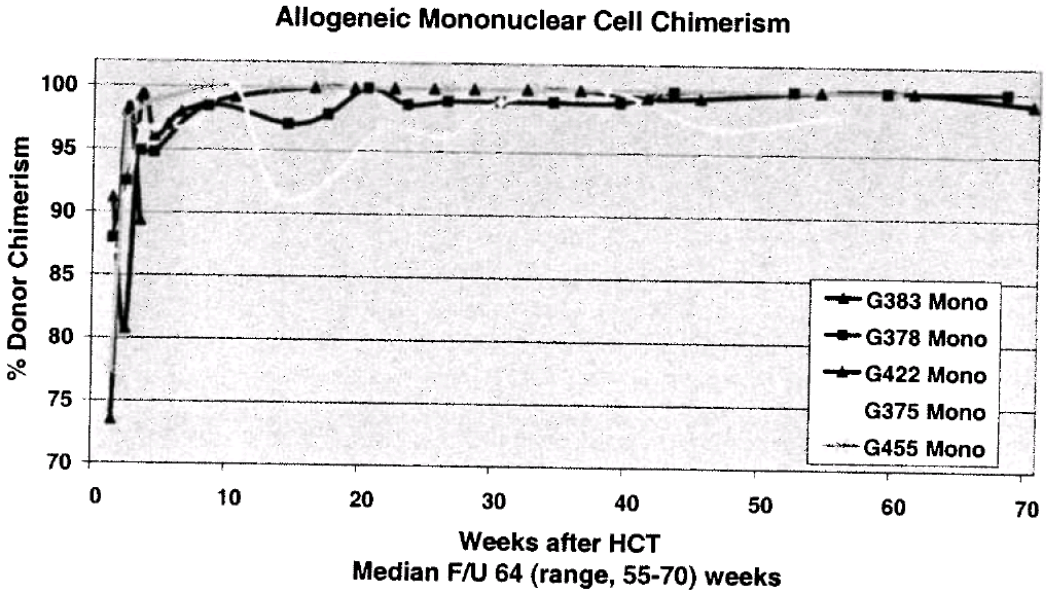


Table 1: Bone marrow evaluation of dogs at necropsy after allogeneic transplantaion of AMD3100 mobilized cells

Dog #	Peripheral blood counts (neutrophil and platelets) at last follow up	Bone marrow evaluation at necropsy	% donor peripheral-blood granulocyte chimerism at last follow up	% donor peripheral blood mononuclear cell chimerism at last follow up	Follow up (weeks)	Cause of Death
G383	Normal	Normocellular with trilineage engraftment	100	99.1	70	End of Study
G378	Normal	Slightly hypocellular with normal trilineage engraftment	100	100	68	End of Study
G422[1]	Neutrophils normal	Markedly hypocellular, few erythrocytes and megakaryocytes	99	89	Day 18	Euthanasia due to pancreatitis
G375	Normal	Normocellular	100	98.1	55	End of Study
G455	Normal	Moderately hypocellular	100	100	60	End of Study

[1] See Reference (1) for further details.

Study in humans:

"Leukocytosis and mobilization of CD34+ hematopoietic progenitor cells by AMD3100, a CXCR4 antagonist": Hubel *et al.*, Supportive Cancer Therapy, 1: 165-172, 2004.

In a Phase 1 study (32 healthy volunteers), the hematological effects (including the ability to mobilize $CD34^+$ hematopoietic progenitor cells), pharmacokinetics and safety of subcutaneous administration of AMD3100 were investigated. The salient results are as the following:

- AMD3100 at a single SC dose of 80 μg/kg induced generalized leukocytosis, with a 3 fold increase in total white blood cell (WBC) counts, and with an increase in neutrophils, lymphcytes, monocytes, eosinophils and basophils. The peak response occurred between 6-9 hr post dose.
- The increase in circulating CD34+ cells followed an AMD3100 dose-dependent manner, i.e., 5 fold and 15.5 fold increases at 80 μg/kg and 240 μg/kg, respectively. The increase peaked at 9 hr post dose at both doses.
- Myeloid progenitor cells, e.g., CFU-GM, CFU-GEMM and BFU-E, showed similar increase in mobilization to the blood with increasing doses of AMD3100.

"Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist": Broxmeyer *et al.*, J Exp Med, 201: 1307-1318, 2005.

The studies in mice by the authors are discussed in a previous section (see above). Studies in humans (healthy volunteers) are summarized below:

- Intravenous administration of AMD3100 (two successive doses at 80 μg/kg, 24 hr apart), alone and with G-CSF, increased circulating hematopoietic progenitor cells (CFU-GM, BFU-E and CFU-GEMM) in healthy volunteers (n=3) within 6 hr post-dosing. Pre-treatment of G-CSF (10 μg/kg SC, 4 or 5 days) enhanced the mobilizing effects.
- Pre-treatment of G-CSF followed by a single IV dose of AMD3100 significantly enhanced numbers of HPCs in apheresed samples from donors, in compared to the samples from donors mobilized with G-CSF or AMD3100 alone. These effects were greater than additive.
- Similar enhancement in mobilizing human HSCs was also observed in healthy volunteers treated with combination of G-CSF and AMD3100.

2.6.2.3 Secondary pharmacodynamics

The following studies were not reviewed, because the proposed pharmacodynamics is not pertinent to the mechanism of action for the indicated treatment.

Study #AOM0048: AMD3100, a CXCR4 antagonist, augments mobilization and incorporation of bone marrow derived endothelial progenitor cells into sites of myocardial neovascularization

Study #AOM0072: The effects of AMD3100 in a pig model of myocardial infarction.

Study #GT-249-EF-1: AMD3100 rapidly mobilized stem cells after myocardial infarction in nonhuman primates but does not improve left ventricular remodeling of function.

Study #AOM0047: Direct injection of AMD3100 improves blood flow to the diabetic mouse hind limb after inductionof hindlimb ischemia.

2.6.2.4 Safety pharmacology

Neurological effects:

✧ *In vitro* studies

Study #107-031 (Sandoz): Receptor affinities (binding) of SDZ SID 791

Key study findings: SDZ SID 791, in the micromolar ranges, showed affinity to alpha1 and 2 adrenergic, as well as dopamine D2 receptors on membranes from calf brain tissues.

Methods: The ability of SDZ SID 791 to compete for the binding of radio-labeled ligands to CNS receptors was assessed by incubation of SDZ SID 791 with ^{3}H-labeled ligands, and membranes from fresh calf brain cortex (for alpha 1 and 2 receptors) or striatal tissues (for dopamine D1 and D2 receptors), or from rat forebrain (for opiate receptors). The assay was corrected with non-specific bindings. The results were expressed as:

$pIC_{50} = -\log IC_{50}$, $pK_i = -\log K_i$.

The summary of receptors tested, the respective ligands and the pK_i values is tabulated below (table from the sponsor):

[^{3}H]ligand	receptor	species	pIC_{50}	pK_i	SEM	N
Prazosin	Alpha 1	Calf		6.2	0.15	2
Clonidine	Alpha 2	Calf		5.9	0.08	2
ADTN	Dopamine D1	Calf	< 5			2
SCH-23390	Dopamine D1	Calf	< 5			2
205-501	Dopamine D2	Calf		5.5	0.13	2
Spiperone	Dopamine D2	Calf	< 5			2
Naloxone	Opiate	Rat	< 5			2

Study #1009304 (AnorMed)

Reviewer's note: The study was comprised of three studies: receptor bindings for adrenergic and GABA receptors, angiotensin converting enzyme (ACE) and neutral endopetidase enzyme assays, and neuropeptide Y receptor functional assays (agonist and antagonist activity). These studies are reviewed and summarized together.

Study designs and results:

Methods were adapted from the scientific literature, according to the investigator of the laboratory conducting the studies. The laboratory also corrected the concentrations of AME-2 (AMD3100, plerixafor) (Lot# 11408) used, due to an error in the assumed molecular weight of the test drug.

The ability of AME-2 (6 μM) to compete for the binding of radio-labeled ligands to central and peripheral nervous receptors was assessed. The receptors assessed included:

- Rat brain/cerebral cortex:
  Adrenergic α_1, α_2, β; DABA transporter, $DABA_A$ agonist site, $GABA_A$ benzodiazepine, $GABA_A$ chloride channel, TBOB and $GABA_B$ (cerebellum)
- Rat heart: benzodiazepine (peripheral)

The results indicated that AME-2 at 6 μM inhibited 40% and 41% binding of prazosin and yohimbine to α_1 and α_2 receptors, respectively. .

AME-2 (concentrations from 0.06 μM to18 μM):

- Inhibited ACE activation (rabbit lung), with IC_{50} values of 4.41 μM, 4.98 μM and 3.31 μM in three separate tests (captopril (10 nM) as the control).
- At 18 μM, exhibited possible agonism with neuropeptides Y_2 (from rat vas deferens) and Y_3 (from rat distal colon).

✧ *In vivo* studies: primary observation test (POT) and Irwin test

Study POT/M/92/10 (Sandoz): The effects of SDZ 282-791 in the primary observation test in mice

Study POT-R-94/5 (Sandoz): The investigation of potential CNS effects of subcutaneously administered SID 791 using the rat primary observation test

Study GT-249-TX-1 (Genzyme): Effects of plerixafor in the Irwin test in rats

Key study findings: Single subcutaneous doses of plerixafor, induced CNS depressant (motoric and bodily inhibition) or CNS stimulant effects in mice and rats.

Methods and results:

OF-1 mice and Sprague Dawley rats:

Study system	Treatment (single dose) Subcutaneous injection	Dose (mg/kg)	Dosage volume (mL/kg)	Animal allocation	Results
POT in male mice During the 1st hr, and 1, 7, 23 hr post dose (PD) (#pot-m-92-10)	Vehicle (a) Plerixafor*	0 0.1 1 10	Unknown	n=3/group	0-1 hr PD: ↓ in-place activity and locomotion, ↓ rearing, flattened body posture at 10 mg/kg; recovered by 1 h PD.
POT in rats 1, 5 and 23 hr PD (#pot-r-94-5)	Vehicle (b) Plerixafor*	0 5 10 20	2 0.5 1 2	n=6/group	0-1 hr PD: dose-dependent ➢ ↓ Local activity, rearing, and locomotion at 5 mg/kg. ➢ Flattened body posture at 5 mg/kg ➢ Labored and/or irregular respiration at ≥ 10 mg/kg ➢ Ptosis at ≥ 10 mg/kg ➢ Slightly ↑ social behavior at 5 and 10 mg/kg 1 hr PD: dose-dependent (see table below for summary), including ↓ abdominal muscle tone, pupil dilatation, impaired

					gait,↓ rectal temperatures, labored respiration 5 hr PD: findings at 20 mg/kg ➢ Slightly ↓ abdominal muscle tone ➢ Dilated pupil (79% of control) ➢ Rectal temperature + 0.4 °C 23 hr PD: Slightly ↓ abdominal muscle tone.
Irwin test in male rats Pretest, 0.5, 2 and 4 hr PD (#gt-249-tx-1)	Vehicle (c) Plerixafor** Chlorproma-zine: Positive control (CNS depressant)	0 2 10 20 5	1	n=6/group	➢ Passivity and dispersion in cage (≥ 2 mg/kg), dose-dependent in incidence and duration of findings The followings were found at 20 mg/kg only: exophthalmos, apathy, ↓ locomotor activity, ↓ alertness and startle response, twitching, abnormal carriage, chromodasryorrhea, and following interaction with the animals, the followings were seen: fast respiration, aggression, ↑ touch response and fearfulness.

(a): 0.5% (w/v) hydroxylpropylmethylcellulose (HPMC), (b): phosphate/chloride buffer; (c): 0.9% w/v NaCl, * plerixafor (SDZ SID-791•8HCl• $2H_2O$), **plerixafor (base, batch # PD05044); PD: post-dose

Thus, in the Irwin test, the rats demonstrated signs of a central nervous system (CNS) depressant if the animals were not handled. However, signs of CNS stimulation were observed following interaction with the animals. The effects were transient and occurred within 2 hr post dose.

The table below is the summary of one hour post-dose findings in POT in rats. Note that the dose was expressed differently in the table (μM/kg, instead of mg/kg).

POT 1 (after 1 hr)
282-791ch, MW = 503, Sf = 1.562, Species = Rat, Route = s.c.
Formulation: 282-791 ch (charge Y021 0294), supplied in portions giving 50 mg of the base form, 5 ml vehicle (charge Y029 0194) added, colourless solution, pH ~7. Doses obtained by variation of injection volume. Control group treated with placebo (charge Y022 0194), 2 ml/kg.

RESULT	DOSE 9.9 μM/kg	DOSE 19.9 μM/kg	DOSE 40 μM/kg
DOSE mg/kg	5.0	10.0	20.0
N	6	6	6
MOT.ACT	0	0	0
LOCOM	0	0	0
BEHAV.STIM	0	0	0
BEHAV.DEPR	0	0	0
MUSCLE TON	0	0	0
NEUR.SYMP	0	0	0
AUTON.SYMP	0	0	0
PUPIL DIAM			
Median (8.0)*	5.0	5.5	5.0
Range (5-10)*	4-8	3-10	4-7
PD REL.%	63	69	63
RECT.TEMP			
Median (37.6)*	38.1	37.7	36.2
Range (37.2-38.0)*	37.8-38.5	37.6-37.8	35.7-36.5
RT CHANGE	0.5	0.1	-1.4
LETHALITY	0	0	0

TABLE 1: SUMMARY OF EFFECTS OF 282-791ch ON SYMPTOM CATEGORIES, 1 HOUR AFTER SUBCUTANEOUS TREATMENT

Cardiovascular effects:
Study #051128-BOP: Effects of AMD3100 on cloned hERG potassium channels expressed in mammalian cells

Key study findings: AMD3100 did not inhibit hERG current up to 50 μg/mL (approximately 0.1 mM); thus an IC_{50} was not determined.

Study designs and results:
The vehicle was HEPES-buffered physiological saline (HB-PS) +DMSO (at a final concentration of 0.3%). Terfenadine ((b) (4), 60 nM which blocks hERG current by approximately 70-80%) served as the positive control and E-4031 ((b) (4), inhibition of hERG currect with IC_{50} = 12 nM) as a reference substance. Two concentrations of AMD3100 were tested. The test was conducted at 35 ± 2 °C. The results are summarized in the following table.

Concentration (μg/mL, n=3)	0	5	50	Terfenadine (60 nM, n=2)
% inhibition of I_{kr}	0.2 ± 0.1	1.8 ± 0.7	0.9 ± 0.3	75.7 ± 3.2

Studies in rat:
Study #9608-0228-01: Dilatation of rat aortic smooth muscle cells, possible implication of free calcium

Key study findings:

- SDZ SID 791 at concentrations 2-20 μg/mL inhibited angiotensin II-induced vasoconstriction of rat aortic smooth muscle cells (SMC). The effect was concentration-dependent and was reverted by the supplement of calcium (4 mM).
- The vasodilatation effect of SDZ SID 791 may be attributed to the depletion of available amount of calcium in the SMC, which was required for vasoconstriction.

Study designs and results:

The direct action of SDZ SID 791 on rat aortic smooth muscle cells was investigated to determine the underlining mechanism of the hypotensive effect of the drug when given *in vivo*. Primary cultures of rat aorta smooth muscle cells (ASMC) were incubated with angiotensin II (AT II, 1 μM) to contract the cells. The inhibition of vasoconstriction was determined by pre-incubation of SDZ SID 791 (0.2, 2, 10 and 20 μg/mL) for 10 min, alone or together with other agents ($CaCl_2$, $MgCl_2$, EDTA, verapamil and diltiazem), before the addition of AT II. The ASMC-contractions were measured by an image analysis system (Leitz MIAS) connected to a phase contract microscope. The planar cross-sectional areas were measured before and 30 min after incubation with the test compound. The initial area of each ASMC before incubation of the test compound served as its own control (100%). Contraction was expressed as the decrease of the corss-section area in comparison to the control.

Results:

Planar cross sectional area (%) (Group mean ± SD, n=10):

Control 96.8 ± 1.6	Control + Ca^{2+} (1.5 mM) 99.1± 0.8	Control + Mg^{2+} (1.5 mM) 96.8± 1.6
AT II 87.6 ± 2.9*	AT II + Ca^{2+} (1.5 mM) 84.5± 2.8 a	AT II + Mg^{2+} (1.5 mM) 887.6± 2.9*
AT II + SZD SID 791 (μg/mL) SZD 0.2 83.8 ± 4.5 SZD 2 93.4 ± 3.7** SZD 10 91.2 ± 2.9** SZD 20 99.6 ± 2.1**	AT II + SZD SID 791 (20 μg/mL) Ca 1.5 mM 99.9 ± 4.8 Ca 2.0 mM 95.5 ± 1.8 a Ca 3.0 mM 95.5 ± 2.5 a Ca 4.0 mM 87.6 ± 1.9 a	AT II + SZD SID 791 (20 μg/mL) Mg 1.5 mM 99.6 ± 2.1 b Mg 4.0 mM 94.7 ± 3.3 c

***: significant compared to the control**
****: significant compared to AT II alone**
a: significant compared to AT II + SDZ SID 791 + calcium 1.5 mM
b: significant compared to AT II + magnesium 1.5 mM
c: significant compared to AT II + SZD SID 791 + magnesium 1.5 mM

Angiotensin II (1 μM) induced vasoconstriction of ASMC was calcium dependent, because preincubation of EDTA (≥ 0.2 mM), a calcium chelator, inhibited the effect. Preincubation of verapamil (10 μM) and diltiazem (10 μM) also inhibited angiotensin II's vasoconstriction.

Study #107-028: Cardiovascular effects of SDZ SID 791 (282-791) in anesthetized rats after intravenous and subcutaneous administration

Key study findings:

- Intravenously administered SDZ SID 791 at 1-10 mg/kg induced mortality in all five rats tested, due to progressive cardiodepression (between 2-5 min post dose) . Treatment related cardiovascular effects included: decreased arterial blood pressure, heart rate, myocardial contractility (+ dP/dt) and cardiac output.
- A similar but nonlethal cardiodepression effect (weak to moderate in severity) was observed following subcutaneous administration of 20 mg/kg SDZ SID 791.

Study designs:

The cardiovascular effects of SDZ SID 791 (Batch Y021 0294, 50 mg/vial) were investigated in male Wistar rats (n=5). The compound was administered via continual intravenous (IV) infusion (1 mL/kg over 10 minutes) at increasing doses of 0.1, 1 and 10 mg/kg with a 20 min wash-out period in between each dose. The control group (n=5) received the placebo solution for SZD SID 791 (batch Y022 0194, 5 mL/vial). Three continual infusions of 1 mL/kg/10 min were administered; the placebo was diluted with PBS corresponding to the concentrations used for the SDZ SID 791 group. SDZ SID 791 was also given to the rats (n=5) subcutaneously (SC) at 20 mg/kg (at 2 mL/kg). The control group (n=5) was treated with the placebo solution (n=5). The parameters (see result) were measured -30, -20, -10 min, and immediately before drug/placebo IV administration and 0, 10 and 20 min post dose. In the case of SC treatment, parameters were collected 3, 10, 30, 60, 90, 120, 180 and 240 min post dose.

Results: (Tables from the sponsor)

IV administration:

Substance: n=5 (a) SDZ SID 791		MAP		HR		+dP/dt		-dP/dt		CO		SVR	
		M	SEM	M	SEM	M	SEM	M	SEM	M	SEM	M	SEM
Baseline value		118	7	350	13	9090	550	-8461	473	257	11	0.46	0.02
Tt (min)	Ta (min)	Percent change from baseline											
0.1 mg/kg i.v.													
10	10	1	1	3	1	5	1	2	3	4	2	-2	3
20	20	-1	1	2	2	4	2	-2	3	* 1	3	-1	3
30	30	-2	1	5	2	5	2	-2	3	* 3	2	-5	2
1 mg/kg i.v.													
40	10	* -13	3	-3	4	* -8	4	-10	5	* -1	3	-13	2
50	20	* -14	3	-1	4	-8	4	-13	4	* -1	3	-14	2
60	30	* -14	1	* -1	2	* -7	2	-12	2	* -1	4	-13	3
10 mg/kg i.v.													
70	10	* -72	4	* -50	5	* -84	2	* -90	2	* -79	4	62	39

*** Statistically significant**

MAP: mean arterial pressure (mmHg); HR: heart rate (beats/min); +dP/dt, –dP/dt: rate of rise and fall of left ventricular pressure (mmHg/s); CO: cardiac output (mL/min/kg); SVR: systemic vascular resistance (mmHg•min/mL/kg); M: mean; SEM: standard error mean; Ta: time after start of infusion (min); Tt: total time (min).

SC administration:

Substance: SDZ SID 791 n=5 20 mg/kg s.c.	MAP		HR		+dP/dt		-dP/dt		CO		SVR	
	M	SEM	M	SEM	M	SEM	M	SEM	M	SEM	M	SEM
Baseline value	111	3	349	13	9975	186	-8880	455	278	12	0.40	0.03
Ta (min)	Percent change from baseline											
3	-1	2	-4	2	-3	2	1	4	-4	2	3	4
10	* -12	3	* -5	2	* -14	3	-12	4	* -10	1	-2	4
30	* -22	2	* -10	3	* -25	3	* -23	2	* -19	2	-4	3
60	* -22	1	* -9	3	* -24	2	* -25	2	* -16	3	-6	3
90	-20	2	* -8	2	* -23	3	* -26	2	* -16	3	-4	4
120	* -18	2	-6	2	* -23	2	* -25	3	* -15	4	-2	5
180	* -22	4	-6	3	* -22	2	-28	6	-14	4	-8	6
240	-20	2	-7	3	* -20	1	-26	4	-17	3	-4	5

There were no remarkable findings in the control groups (data not shown).

Studies in dog:

The following studies were reviewed by Dr. Guodong Fang (IND 55851, N-065, May 16, 2002) (see Appendix):

Study #(b) (4) 93226: A pilot cardiovascular profile study following a single intravenous infusion of AMD3100 free base in the conscious restrained beagle dog

Study #(b) (4) 93227: A cardiovascular profile study following an intravenous infusion of AMD3100 free base in the conscious unrestrained beagle dog

Pulmonary effects:

Study #GT-249-TX-2 ((b) (4) Study # MGE5004): Effect of plerixafor on respiration rate and tidal volume in conscious rats

Key study findings:

- A single subcutaneous (SC) dose of plerixafor at ≥ 10 mg/kg induced significant decrease in tidal volume in rats.
- A significant decrease in respiratory rate was observed at 20 mg/kg of SC plerixafor.
- Two animals (2/8) treated with 20 mg/kg of plerixafor were found dead 20-75 min post dose and 4/8 animals were sedate at 15 min post dose. Other clinical signs included piloerection, exopthalmos, cyanosis chromodacryorrhea, and twitching.

Study designs and results:

The pulmonary effects of plerixafor were inestigated by subcutaneous administration of plerixafor (2, 10 and 20 mg/kg, at 1 mL/kg) to male Sprague Dawley rats (n=8/dose). 0.9% NaCl and morphine (intravenous, 10 mg/kg, at 2 mL/kg) were served as the vehicle control and the positive control, respectively.

The result is summarized as the following (table from the sponsor):

Group	Treatment	Respiration rate (breaths/min) at time (min) post-dose		
		Pre-dose	30 min	120 min
A	Vehicle (1 mL/kg)	159.93 ± 8.75	138.28 ± 6.78	141.96 ± 13.60
D	Plerixafor (2 mg/kg)	185.10 ± 21.99	145.33 ± 12.82	141.11 ± 6.37
B	Plerixafor (10 mg/kg)	189.99 ± 22.19	115.53 ± 7.31	110.81 ± 6.82
C	Plerixafor (20 mg/kg)	173.64 ± 21.38	89.72 ± 7.11 # (4)	96.98 ± 3.89 * (6)
E	Morphine (10 mg/kg)	222.10 ± 23.58	102.37 ± 6.89 †† (7)	99.06 ± 2.68 ††

Vehicle for Plerixafor was 0.9 % w/v sodium chloride.
n = 8 animals per group, unless otherwise stated in parenthesis.
Data are expressed as mean ± s.e. mean.
†† $P \leq 0.01$ when compared to pre-dose values (paired, Student's t-test).
# $P \leq 0.05$ when compared to vehicle data (ANOVA and Dunnett's).
* $P \leq 0.05$ when compared to vehicle data (Kruskal-Wallis and Dunn's test).

Renal effects: No studies conducted.

Gastrointestinal effects: No studies conducted.

Abuse liability: No studies conducted.

Other:
Study #107-029: Effect of SDZ 282-791 on different endocrine parameters in male rats

Key study findings: SDZ282-791 at 10 mg/kg induced the following changes: ↓ growth hormone levels (not significant), stimulating the release of prolactin (significant) and corticosterone (not significant according to analysis of variance, but dose-dependent).

Study designs and results:
The effect of SDZ 282-791 on endocrine parameters, e.g., the release of hormones of the pituitary, adrenal and testes, and the regulation of serum levels of glucose and calcium, were investigated by subcutaneous administration of SDZ 282-791 (0.01 to 10 mg/kg, at 5 mL/kg) to conscious male Sprague Dawley rats (n=4/dose/experiment, control (water): n=8/experiment). The blood levels of hormones, glucose and calcium were measured 1 hour post dose.

The results were summarized in the table below (pool of two studies): the data were expressed as group means and % of the control in the parenthesis.

	GH (ng/mL)	Prolactin (ng/mL)	LH (ng/mL)	Testosterone (ng/mL)	Corticosterone (ng/mL)	Glucose (mg%)	Clacium (mg%)
Vehicle	121.8	14.1	98.8	0.79	25.2	137.6	11.06
0.01 mg/kg	116.2 (95)	24.3 (172)	92.1 (93)	1.23 (156)	55.2 (219)	144.1 (105)	11.2 (102)
0.1 mg/kg	60.9 (50)	15.8 (112)	96.3 (97)	0.98 (124)	73.9 (293)	145.3 (106)	10.6 (95)
1 mg/kg	48.3 (40)	20.3 (145)	126.7 (128)	1.06 (134)	128 (507)	142.7 (104)	10.6 (96)
10 mg/kg	61.8 (51)	46.8 (**333**)	133.8 (135)	1.24 (157)	240 (**951**)	138 (100)	10.4 (94)

2.6.2.5 Pharmacodynamic drug interactions

Not reviewed.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

- Pharmacodynamics:

Summary of the inhibitory effects of AMD3100 (plerixafor) as an antagonist of SDF-1/CXCR4 interaction (*in vitro* system) (Table from the sponsor, Module 2, Section 2.6.2. Pharmacology written summary):

Table 2.6.2-2: Plerixafor Inhibits SDF-1/CXCR4 Interactions

Assay	IC_{50} (nM)	Study Number
^{125}I-SDF-1 ligand binding	651 ± 37 nM (n = 3)	AOM0053
SDF-1 stimulated calcium flux	572 ± 190 nM (n = 5)	AOM0054
SDF-1 stimulated chemotaxis	51 ± 17 nM (n = 4)	AOM0055
SDF-1 stimulated ^{35}S-GTPγS binding	15.4 ± 4.4 nM (n = 4)[a]	AOM0056

[a] In the study AOM0056, EC_{50} is used in place of IC_{50}. They have equivalent meanings for the purposes of this study.

Receptor selectivity of plerixafor toward CXCR in PBMCs or in cell lines transfected with CXCR4:

Table 2.6.2-3: Calcium Flux Experiments Demonstrating CXCR4-selective Antagonism of Plerixafor

Ligand	Concentration (ng/ml)	Cell Type	IC_{50} of Plerixafor (μg/ml)	IC_{50} of Plerixafor[1] (nM)
SDF-1	10	U87.CD4.CXCR4	0.13	164
	100	Freshly isolated PBMC	0.03	38
	20	SupT1	0.04	50
	10	THP-1	0.01	12.5
	20	HSB-2	0.10	126
	100	Molt-4	0.02	252
	2.5	L1210	0.08	100.7

[1] Calculated based on a molecular weight of 794.48 (plerixafor octaydrochloride).

- Safety pharmacology

In Vitro Studies			
Study #	System	Concentrations	Results
107-031	Receptor affinity (membranes from calf brain tissues)	SZD SID 791 (not reported)	Affinities to alph1 and 2 and dopamine receptors (ki =$10^{-6.2}$M, $10^{-5.9}$M and $10^{-5.5}$M, respectively).
051128-BOP	hERG currents	AMD3100: 5 and 50 μg/mL (or 0.01-0.1 mM)	No inhibitory effects
9608-0228-01	Rat aortic smooth muscle cells	SZD SID 791: 0.2, 2, 10 and 20 μg/mL	➢ ↓angiotensin II induced vasoconstriction at ≥ 2 μg/mL ➢ ↑Calcium depletion
In Vivo Study			
Study #	System	Doses (mg/kg)	Results
POT-M-92-10	Nervous system (POT) in mice	Single SC injection 0.1, 1 and 10 mg/kg	0-1 hr: ↓ in-place and locomotor activity, rearing and flattened posture
POT-R-94-5	Nervous system (POT) in rats	Single SC injection 5, 10 and 20 mg/kg	0-1 hr: ↓ in-place and locomotor activity, rearing and flattened posture,↓ abdominal muscle tone, ptosis, dilated pupil, labored respiration, changes in rectal temperature,
GT-249-TX-1	Irwin test in rats	Single SC injection 2, 10 and 20 mg/kg	Mainly CNS depression at 20 mg/kg: exophthalmos, ↓ locomotor activity, alertness & startle response, twitching. CNS stimulation following interaction with the animals.
107-028	Cardiovascular in rats	➢ Continual IV (10 min/dose): 0.1, 1, and 10 mg/kg (1 mL/kg) ➢ Single SC injection: 20 mg/kg	➢ Mortality (all doses, due to cardiodepression): ↓ arterial BP, HR and myocardial contractility (+ dP/dt) ➢ Similar, non-lethal cardio-

			depression effect (see above)
GT-249-TX-2	Pulmonary in rats	Single SC injection 2, 10 and 20 mg/kg	➢ ↓ tidal volume (≥ 10 mg/kg) ➢ ↓ respiratory rate (20 mg/kg) ➢ Mortality (2/8) and sedation (4/8)

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

Note: the following names/codes are used in the PK/TK reports

- SDZ SID-791 (or SZD 282-791) is AMD3100 or plerixafor (free base)
- SDZ SID-791-ch (or SDZ 282-791-ch) is plerixafor octahydrochloride dihydrate.

Mol.formula/weight: -b: $C_{28}H_{54}N_8$ / 502.79 (unlabelled)
-ch: $C_{28}H_{54}N_8 * 8\ HCl * 2\ H_2O$ / 830.51 (unlabelled)

Plerixafor (base or salt) and related compounds used in the PK/ADME studies are tabulated below:

Study Number	Plerixafor (salt or free base) used in the study
3167/DrCH	Unlabeled SDZ 282-791• 8HCl • $2H_2O$ and [^{14}C]SDZ 282-791-ch
9608.0258	[^{14}C]SDZ SID 791-ch
9608.026	Unlabeled SDZ 282-791• 8HCl • $2H_2O$ and [^{14}C]SDZ 282-791-ch
9608.0256	[^{14}C]SDZ SID 791-ch
GT-249-PK-3	Plerixafor (base)
GT-249-PK-4	^{14}C-plerixafor (base)
AOM0036	AMD3100 (base)
7686-108 CMS81280A	^{14}C-AMD3100 (base)
9608.0251	[^{14}C]SDZ SID 791-ch
AOM0038	AMD3100 (base)
GT-249-PK-1	Plerixafor (base)
GT-249-PK-5	Unlabeled plerixafor (base) and [^{14}C]-plerixafor
AOM0067	AMD3100 (base)
XT055036	AMD3100 (base)
DMPK08-R001	Plerixafor (base)
AOM0069	AMD3100 (base)
AOM0073	See Studies 3167/DrCH, 9608.0258, and 9608.026.
AOM0074	See Study 9608.0256

See section 2.6.4.9 for the summary of PK/ADME studies. Briefly, subcutaneously administered plerixafor was absorbed (t_{max} of ~ 0.5-1 hr) and distributed rapidly. High and/or sustained levels of radioactivity were detected in liver, kidney, spleen, injection site, epiphyseal plate and cartilage. Multiple-dose SC administration of plerixafor resulted in the accumulation of the drug or drug-derived compounds. Plerixafor has the potential to cross the blood-brain barrier. No significant levels of metabolites were detected in *in vitro* studies (using liver microsomes of mouse, rat, dog, or human) or in urine and plasma samples collected following SC administration of plerixafor in rats. Three non-parent components detected in plasma and urine appeared to be Cu^{2+} complexes of plerixafor. Excretion of subcutaneously administered plerixafor was mainly urinary; the major component in the urine or plasma was the parent drug. Under the conditions tested, AMD3100 up to 100 μM,

was not a direct or a metabolism-dependent inhibitor of 1A2, 2C9, 2C19, 2D6 and 3A4/5 CYP enzymes. The plasma protein binding of AMD3100 (1-10 μg/mL) was moderate (33-54% for rat, 34-46% for dog, and 37-58% in human). In the SC repeat-dose toxicology studies of up to 4 weeks, conducted in rats and dogs, exposure to plerixafor was mostly dose proportional at doses tested.

2.6.4.2 Methods of Analysis

[see under individual study reviewed]

2.6.4.3 Absorption

Study No. 3167/DrCH (Genzyme)
Title: SDZ 282-791: Absorption, distribution, metabolism, and excretion following single 20 mg/kg subcutaneous doses of [^{14}C]SDZ 282-791-ch in rat

Key study findings:

- Subcutaneously administered SDZ 282-791 (AMD3100) was absorbed rapidly (t_{max} was ~ 1 hr) and distributed widely to various tissues/organs.
- Radioactivity in tissues/organs was detected at all time-points evaluated, i.e. 0.5 hr to 144 hr (6 days) post-dose.
- The injection site, liver, kidney and red pulp of spleen were tissues/organs that had high and sustained radioactivity concentration, up to 144 hr post-dose. Cartilage had high levels of radioactivity up to 4 hr post-dose
- The main excretion route of SDZ 282-791 was via urine.
- The major circulating component in the plasma was the parent drug (60% of radioactivity).

Study system:	Male HanIbrn:WIST rats
Treatment:	[^{14}C] SDZ 282-791-ch (1.65 mg AMD3100 8 HCl•2 H_2O = 1 mg/kg AMD3100). Test article was given subcutaneously at a dose of 20 mg/kg (injection volume 1.2 mL/kg). Specific activity: 6.1 μCi/mg for absorption, metabolism, and excretion studies (AME) studies and 24.4 μCi/mg for the distribution study. Radioactivity dose: 120 μci/kg for absorption/excretion study and 480 μci/kg for the distribution study
Parameters:	SDZ 282-791-ch levels in plasma, urine and feces (for AME), quantitative whole-body autoradiography (QWBAR) and SDZ 282-791-ch levels in some tissues (for distribution), metabolite profile, and radioactivity recovery
Schedule:	Absorption, excretion, and material balance: blood samples were collected at 0.5, 1, 2, 4, 7, 24, 48, 72, and 144 hr post dose (n=4/time point). Urine samples were collected for 0-7, 7-24, 24-48, 48-72, and

72-144 hr intervals; and feces collected at 24, 48, 72 and 144 hr post dose.

Distribution and metabolism: quantitative whole-body autoradiography (QWBAR): in organs/tissues was determined by sacrificing rats (n=1/time point) at 0.5, 2, 4, 24, 72, and 144 hr post dose.

Blood samples were collected prior to sacrifice via retroorbital plexus puncture. Radioactivity was determined by liquid scintillation counting (LSC). After completion of sectioning, liver, lung, kidney, site of injection and cardiac blood were removed from the residual part of the animals sacrificed at 0.5, 2, 72, and 144 (no lung at this time-point) hr post-dose, and processed for metabolite profiling.

Analysis: SDZ 282-791-ch plasma levels and metabolites (in plasma and urine at 24 hr and in tissues at 0.5, 2, 72 and 144 hr) were determined by HPLC analysis (identified by the retention times and α-values [relative retention times] and quantified by integration of radioactive peaks). The radioactivity in plasma, urine and feces (at ~144 hr) was determined by liquid scintillation for total radioactivity. Tissues and carcass were dissolved in 30% methanolic KOH, and the radio-activity in tissues was analyzed by HPLC. The limits of determination were 9 ngEq/mL for plasma and 5 ngEq/g for tissues in QWBAR.

- PK parameters: The PK profile after a single 20 mg/kg subcutaneous dose was summarized:

	Plasma SDZ 282-791	Radioactivity	
		Blood (a)	Plasma (a)
AUC_{0-14h}	Not applicable	68.7	46.8
AUC_{0-24h} *	38.9 µg•h/mL	81.2	48.5
C_{max}	14.1 µg/mL	0.3	15.5
T_{max} (hr)	1	1.3	0.9
$t_{1/2}$ Absorption (hr)	0.25	0.2	0.2
$t_{1/2}\lambda 1$ (hr)	1	1.2	1.1
$t_{1/2}\lambda 2$ (hr)	Not available	53	57
Bioavailability (%)	87**	----	----

*** $AUC_{0-\infty}$ in blood and plasma radioactivity.**

**** According to the sponsor, based on the ratio of the mean urinary excretion of radioactivity after subcutaneous and intravenous dosing (data of Study No. 9608-026).**

(a): AUC and C_{max}: µgEq•h/mL and µgEq/mL, respectively.

Up to 4 hr post dose, most of the radioactivity in whole blood was found in plasma compartment; however, from 24 hr on, the concentration of radioactivity in plasma was lower than in the blood cells.

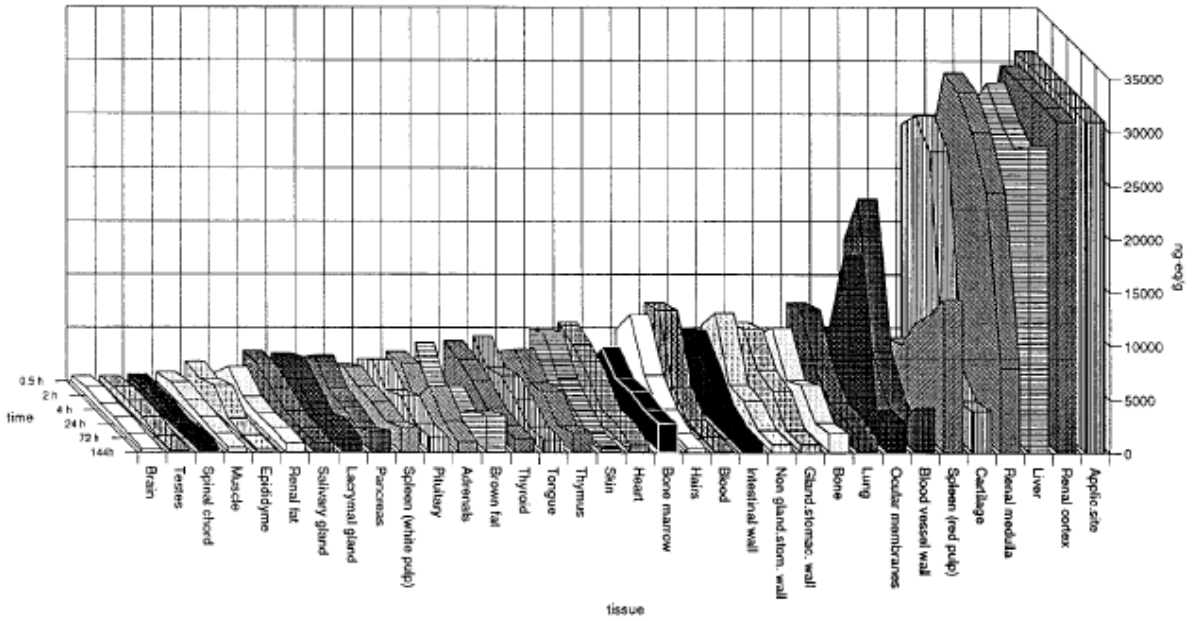
- Tissue distribution: from QWBAR measurement.

The tissue radioactivity concentrations and relative concentrations (expressed as the ratio tissue/blood) in fluids, tissues, and organs, at 0.5-144 hours after the subcutaneous dose, are (see below; tables from the sponsor). The corresponding figures (from the sponsor) are also included.

Concentration of Radioactivity (ngEq/g)

Tissues	0.5 h	2 h	4 h	24 h	72 h	144h
Adrenals	2761	2950	981	3589	1607	994
Applic.site	≥31000[1)]	≥31000[1)]	≥31000[1)]	≥31000[1)]	≥31000[1)]	≥31000[1)]
Blood	7379	7972	2129	413	181	116
Blood vessel wall	13098	18428	5363	2143	1246	4305
Bone	4332	6349	2770	3727	1472	1834
Bone marrow	1380	4576	2968	3136	2715	2793
Brain	489	809	632	675	469	311
Brown fat	3637	[2)]	2309	1114	2576	3504
Cartilage	24069	26245	24202	5174	3510	3936
Epididyme	1858	1602	2331	580	297	258
Gland.stomac. wall	5514	6211	3645	3119	787	774
Hairs	4826	7579	3255	1428	544	468
Heart	5490	4733	3441	1421	1534	755
Intestinal wall	4856	6074	4130	1828	973	973
Lacrymal gland	2639	3577	2317	1014	1807	1133
Liver	8914	27180	30599	28386	27083	28748
Lung	7353	8063	3932	2039	1904	1331
Muscle	835	1266	938	743	544	468
Non gland.stom. wall	5241	7720	2356	2232	787	774
Ocular membranes	5048	6487	14446	[2)]	2695	2902
Pancreas	2227	3736	1996	1601	1013	2001
Pituitary	2063	3365	1881	2694	1406	1456
Renal cortex	22252	≥31000[1)]	≥31000[1)]	≥31000[1)]	≥31000[1)]	≥31000[1)]
Renal fat	770	2690	1260	1006	1063	838
Renal medulla	21568	30238	29722	27284	22992	7889
Salivary gland	2831	2943	2105	1278	956	906
Skin	4932	5902	2922	1562	544	468
Spinal chord	635	637	683	314	802	614
Spleen (red pulp)	3199	5445	6104	9438	11551	14329
Spleen (white pulp)	1640	2685	2217	2070	1605	2434
Testes	529	864	877	716	219	194
Thymus	2813	4336	2566	3011	1214	1945
Thyroid	3780	3362	2719	1541	1284	1323
Tongue	4290	2679	3222	2353	1145	1203

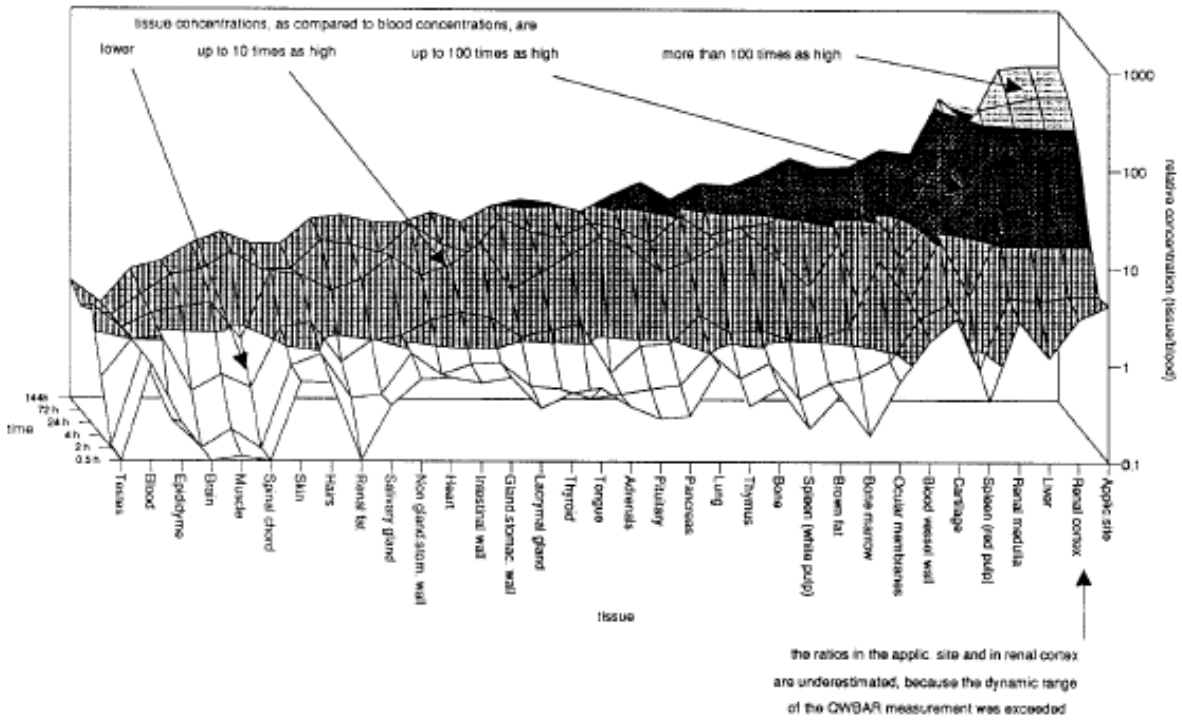
1) upper limit of the dynamic range exceeded
2) not measurable
determination limit 5 ng-eq/g


0.5 h
2 h
4 h
24 h
72 h
144h
time
Brain
Testes
Spinal chord
Muscle
Epididyme
Renal fat
Salivary gland
Lacrymal gland
Pancreas
Spleen (white pulp)
Pituitary
Adrenals
Brown fat
Thyroid
Tongue
Thymus
Skin
Heart
Bone marrow
Hairs
Blood
Intestinal wall
Non gland.stom. wall
Gland.stomach. wall
Bone
Lung
Ocular membranes
Blood vessel wall
Spleen (red pulp)
Cartilage
Renal medulla
Liver
Renal cortex
Applic.site
tissue
35000
30000
25000
20000
15000
10000
5000
0
ng/cm

Relative concentrations of radioactivity (ratio tissue/blood):

Tissues	0.5 h	2 h	4 h	24 h	72 h	144h
Adrenals	0.37	0.37	0.46	8.69	8.88	8.57
Applic.site	≥4.20[1)]	≥3.89[1)]	≥14.56[1)]	≥75.06[1)]	≥171.27[1)]	≥267.24[1)]
Blood	1.00	1.00	1.00	1.00	1.00	1.00
Blood vessel wall	1.78	2.31	2.52	5.19	6.88	37.11
Bone	0.59	0.80	1.30	9.02	8.13	15.81
Bone marrow	0.19	0.57	1.39	7.59	15.00	24.08
Brain	0.07	0.10	0.30	1.63	2.59	2.68
Brown fat	0.49	2)	1.08	2.70	14.23	30.21
Cartilage	3.26	3.29	11.37	12.53	19.39	33.93
Epididyme	0.25	0.20	1.09	1.40	1.64	2.22
Gland.stomac. wall	0.75	0.78	1.71	7.55	4.35	6.67
Hairs	0.65	0.95	1.53	3.46	3.01	4.03
Heart	0.74	0.59	1.62	3.44	8.48	6.51
Intestinal wall	0.66	0.76	1.94	4.43	5.38	8.39
Lacrymal gland	0.36	0.45	1.09	2.46	9.98	9.77
Liver	1.21	3.41	14.37	68.73	149.63	247.83
Lung	1.00	1.01	1.85	4.94	10.52	11.47
Muscle	0.11	0.16	0.44	1.80	3.01	4.03
Non gland.stom. wall	0.71	0.97	1.11	5.40	4.35	6.67
Ocular membranes	0.68	0.81	6.79	[2)]	14.89	25.02
Pancreas	0.30	0.47	0.94	3.88	5.60	17.25
Pituitary	0.28	0.42	0.88	6.52	7.77	12.55
Renal cortex	3.02	≥3.89[1)]	≥14.56[1)]	≥75.06[1)]	≥171.27[1)]	≥267.24[1)]
Renal fat	0.10	0.34	0.59	2.44	5.87	7.22
Renal medulla	2.92	3.79	13.96	66.06	127.03	68.01
Salivary gland	0.38	0.37	0.99	3.09	5.28	7.81
Skin	0.67	0.74	1.37	3.78	3.01	4.03
Spinal chord	0.09	0.08	0.32	0.76	4.43	5.29
Spleen (red pulp)	0.43	0.68	2.87	22.85	63.82	123.53
Spleen (white pulp)	0.22	0.34	1.04	5.01	8.87	20.98
Testes	0.07	0.11	0.41	1.73	1.21	1.67
Thymus	0.38	0.54	1.21	7.29	6.71	16.77
Thyroid	0.51	0.42	1.28	3.73	7.09	11.41
Tongue	0.58	0.34	1.51	5.70	6.33	10.37

1) upper limit of the dynamic range exceeded
2) not measurable



Radioactivity in organs/tissues was detected at all time-points evaluated, i.e. 0.5 hr to 144 hr (6 days) post-dose. Between 2 and 144 hr, the radioactivity decreased slowly, except at the site of injection and in renal cortex and liver where constant concentrations remained. Slight increases in radioactivity were observed in several tissues over time (see the table). In general, the tissue/blood ratio was greater than one at 4 hr post-dose, except in tissues/organs such as brain/spinal cord, muscle, pancreas, pituitary, renal fat, salivary gland and testes, where the ratio was less than 1 up to 4 hr post dose. At 144 hr post dose, the ratio was over 200 at the injection site, and in liver and renal cortex. The ratio was high in spleen red pulp as well.

- Metabolism:

Parent drug represented the major part of radioactivity in all samples examined: plasma (≥60%), urine (>46%) and tissue (≥50%, except for the 144 hr kidney). Other than parent drug (corresponding to peak 32), additional peaks (e.g. peaks 25, 34, and 37) were notable. The structure and biological characteristics of these metabolites were not described in this study.

Proportion of peaks in target tissues/organs, expressed as % of sample radioactivity, is tabulated; see below (table from the sponsor):

sample	time [h]	front	peak 25	peak 26+30	SDZ 282-791 [1)]	peak 34	peak 37	others
applic. site	0.5	0.4	- [2)]	- [2)]	89.8	- [2)]	- [2)]	9.8
blood	0.5	- [2)]	- [2)]	- [2)]	93.6	- [2)]	- [2)]	6.4
kidney	0.5	- [2)]	- [2)]	4.9	75.5	- [2)]	- [2)]	19.6
liver	0.5	- [2)]	- [2)]	- [2)]	87.7	- [2)]	- [2)]	12.3
applic. site	2	1.2	- [2)]	- [2)]	78.8	- [2)]	- [2)]	20.0
blood	2	0.5	- [2)]	- [2)]	92.7	- [2)]	- [2)]	6.8
kidney	2	- [2)]	- [2)]	19.5	67.1	- [2)]	- [2)]	13.4
liver	2	- [2)]	- [2)]	- [2)]	92.7	- [2)]	- [2)]	7.3
applic. site	72	6.1	0.8	- [2)]	65.2	1.4	1.6	24.9
blood	72	1.8	- [2)]	- [2)]	59.6	2.4	2.2	34.0
kidney	72	- [2)]	- [2)]	23.8	60.6	- [2)]	- [2)]	15.6
liver	72	- [2)]	- [2)]	- [2)]	85.0	- [2)]	- [2)]	15.0
applic. site	144	2.3	1.6	- [2)]	49.9	7.6	3.7	34.9
blood	144	1.4	- [2)]	- [2)]	77.7	1.6	1.5	17.8
kidney	144	7.0	- [2)]	29.6	40.0	- [2)]	- [2)]	23.4
liver	144	0.9	- [2)]	- [2)]	79.0	- [2)]	- [2)]	20.1

1): SDZ 282-791 corresponds to peak 32
2): not quantified (because absent, of very low intensity, or ambiguous)

Concentrations of parent drug (ng/mL) and metabolites (ngEq/mL) in blood and liver are summarized in the table below (from the sponsor). These values were derived from the QWBAR measurement (see table above) where the total radioactivity of respective animal was used as 100%.

sample	time [h]	front	peak 25	peak 26+30	SDZ 282-791 [1)]	peak 34	peak 37	others
blood	0.5	- [2)]	- [2)]	- [2)]	6907	- [2)]	- [2)]	472
liver	0.5	- [2)]	- [2)]	- [2)]	7818	- [2)]	- [2)]	1096
blood	2	40	- [2)]	- [2)]	7390	- [2)]	- [2)]	542
liver	2	- [2)]	- [2)]	- [2)]	25196	- [2)]	- [2)]	1984
blood	72	3.2	- [2)]	- [2)]	108	4.3	4.0	62
liver	72	- [2)]	- [2)]	- [2)]	23021	- [2)]	- [2)]	4062
blood	144	1.6	- [2)]	- [2)]	90	1.8	1.7	21
liver	144	259	- [2)]	- [2)]	22711	- [2)]	- [2)]	5778

1): SDZ 282-791 corresponds to peak 32
2): not quantified (because absent, of very low intensity, or ambiguous)

- Excretion and radioactivity recovery: the drug was excreted rapidly via urine (~65% of dose), but a significant portion of the dose (~16%) was retained in the tissues after 144 hours post dose. Approximately 90% of radioactivity was recovered in all 4 rats tested, at 144 hr post-dose.

Excretion (SDZ 282-791, % dose)	
In urine (0-144 h)	63.9
In feces (0-144 h)	7.7
Excretion (% dose)	
Total radioactivity recovery	89.6
In urine	
0-24 h	57.3
0-48 h	60.6
0-72 h	61.1
0-144 h	63.5
Cage wash	0.4
In feces	
0-24 h	0.7
0-48 h	3.6
0-72 h	5.0
0-144 h	7.3
Intestine content	0.4
Carcass (144 h)	18.1

The radioactivity (as % of dose) excreted in urine at 0-24 h was ~60%; therefore, the bulk of urinary excretion occurred within 24 hr after s.c. administration of the drug.

Report No. 9608-0258 (Sandoz): Absorption and disposition in rats following single and multiple subcutaneous doses of [^{14}C]SDZ 282-791-ch

Key study findings:

- Recovery of radioactivity was complete after single-dose administration of the drug (>100% in urine + feces). The amount of radioactivity recovered after multiple-dosing was ~90%; this may be due to the retention of radioactivity (drug and/or metabolite accumulation) after repeat-dosing.
- Subcutaneously administered SDZ 282 791 was absorbed rapidly and reached peak plasma radioactivity concentrations (t_{max}) in 0.5 hr post dose. In the first 7 hours after administration (AUC_{0-7}), the main radioactivity was attributable to the parent drug.
- Higher AUC of radioactivity in plasma and longer elimination $t_{1/2}$ of parent drug after repeated dosing were probably due to retaining of a significant fraction of the dose in tissues.
- The main excretion route of SDZ 282-791 was via urine (~70% and 60% for single and multiple dosing, respectively).
- The major component in the urine was the parent drug (65% of radioactivity).

Study system: Male Wistar Han rats (HsdRccHan:WIST)

Treatment: [^{14}C] SDZ SID 791-ch subcutaneous administration as a single dose (n=4) at 1 mg/kg (or 1.9 µmol/kg) (82 µCi/mg), or multiple doses (7 doses) (n=4) at 1 mg/kg/day (or 2 µmol/kg/day) (~ 105 µCi/mg/day).

Study design: Blood samples (~0.6 mL) were collected at 0.5, 1, 2, 4, 7, 24, 31, 48, 55, 72, and 168 hr after single and multiple dosing. Urine was collected in 0-24, 24-48, 48-72, and 72-168 hr intervals; and feces

collected at 24, 48, 72 and 168 hr post-dose. The intestinal contents and carcasses were analyzed for residual radioactivity. The cages were rinsed with 150-200 mL of water and the radioactivity was measured.

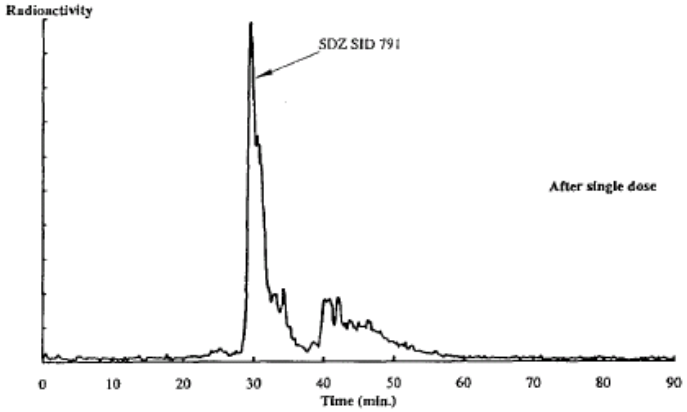
Analysis: Radioactivity in the drug solution was measured by LSC. The plasma concentrations of SDZ SID 791 were determined with a specific liquid chromatography-reverse isotope dilution (LC-RID) method; metabolite profiles were obtained in urine by means of HPLC and radioactivity detection (quantification of parent drug and its metabolites was based on the integrated areas of radioactive peaks). The limit of quantification of radioactivity was 1.4 and 1.1 pmol of [^{14}C] SDZ SID 791-ch/mL of plasma for single and multiple dose experiment, respectively. The quantification limit of SDZ SID 791 in plasma was 13 and 10 pmol/mL for single and multiple dose experiment, respectively. The limit of detection of parent drug and metabolites in urine was 9 and 7 pmol/mL after single and multiple dosing, respectively.

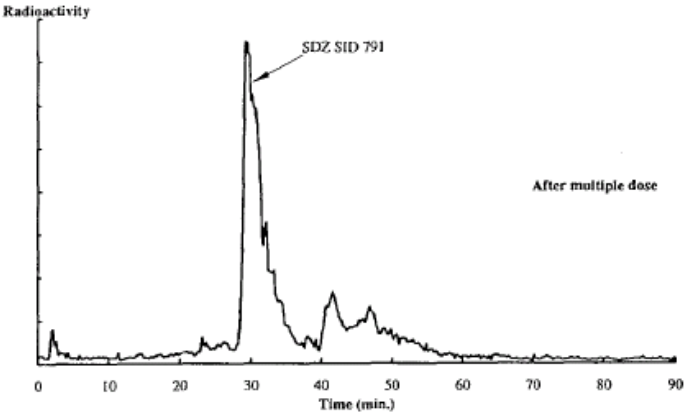
- PK parameters: The PK profiles after a single and multiple subcutaneous dose are summarized:

	Single dose		Multiple dose	
	Parent drug (a)	Radioactivity* (b)	Parent drug	Radioactivity*
$AUC_{0\text{-}7h}$ (pmol•h/mL)	5410	5332	5426	5744
$AUC_{0\text{-}168h}$ (pmol•h/mL)	— (c)	6891	— (d)	14207
AUC (pmol•h/mL)	5444	7757	7241	18099
C_{max} (pmol/mL)	3082	3010	3149	3036
T_{max} (hr)	0.5	0.5	0.5	0.5
$t_{1/2\ absorption}$ (h)	0.1	0.2	0.1	0.2
$t_{1/2}\lambda_1$ (h)	0.9	0.9	0.8	0.7
$t_{1/2}\lambda_2$ (h)	---	107	6.5	94

***Note: the radioactivity was also converted as pmol/mL.**
(a): unchanged drug, (b) total radioactivity in plasma, (c) below quantification limit at collection time ≥ 24 hrs, (d) below quantification limit at 168 hrs.

- Metabolism: Metabolite patterns in 0-72 and 0-168 hr in urine samples following a single and the last of seven (2 μmol/kg/day) SC doses of [^{14}C]SDZ SID 791-ch are shown below (figures from the sponsor):





Approximately 70% of the radioactivity was associated with the main peak (with retention time (Rt) = 29 min) corresponding to the parent drug, and 25-28% with 3-4 peaks not completely separated (Rt = 41-52 min). The remainder of radioactivity (3-6%) was associated with 3-5 minor peaks (less than 2% each). No apparent differences in metabolite patterns were noted following single or multiple dosing. It was later found that some of the non-parent compounds (referred to as metabolites in this report) are likely complexes of Cu^{2+} with plerixafor; see review of Report # GT-249-PK5 for additional information.

- Excretion: The radioactivity in excreta (% of total dose) and radioactivity recovery

	Single dose	Multiple dose (within 168 hr)
Total radioactivity recovery	108.5	86.7
In urine		
0-24 h	65.9	
0-48 h	67.8	
0-72 h	68.9	
0-168 h	71.7	58.5
In feces		
0-24 h	2.7	
0-48 h	4.6	
0-72 h	6.0	
0-168 h	8.9	13.1
Intestine content	0.2	0.2
Cage wash	0.3	0.2
Carcass	27.5	14.7

The majority of radioactivity was excreted via urine, and the elimination was rapid, i.e., ~65% of radioactivity in urine being recovered within the first day following the single dose.

Study No. 9608-026 (Sandoz): Absorption, distribution, metabolism and excretion following single 20 mg/kg oral and 2 mg/kg intravenous doses of [^{14}C]SDZ SID 791-ch in rat

Key study findings:

- Orally administered SDZ 282 791 was absorbed poorly and characterized with high inter-animal variability (1-10%). The radioactivity was below detection limit 4 hr post dose.
- Following the IV dose, the radioactivity was widely distributed; main target organs of distribution were liver, kidney, lung, thyroid, and cartilage. Liver was the main organ of radioactivity distribution in animals dosed orally.
- Concentration of radioactivity in pituitary was elevated at 2 hr post-dose; radioactivity was still detectable at Hr 72, the last time-point examined. This indicates that plerixafor can cross the blood-brain barrier.
- The main excretion route of SDZ 282-791 was via urine following the intravenous dose, while the main excretion route was fecal when the drug was administrated orally.
- Parent drug was the major component in plasma, urine and in feces.

Study system:	Male HanIbrn:WIST rats (n=4/administration route)
Treatment:	[^{14}C] SDZ 282-791-ch (1.65 mg AMD3100 8 HCl•2 H_2O = 1 mg/kg AMD3100) orally at 20 mg/kg or intravenously at 2 mg/kg (injection volume 1.2 mL/kg). The radioactive dose was 120.4 μCi/kg.
Parameters:	SDZ 282-791-ch levels in plasma, urine and feces, quantitative whole-body autoradiography (QWBAR) and SDZ 282-791-ch levels in some tissues (for distribution), metabolite profile, and radioactivity recovery
Schedule:	For AME: blood samples were collected at 0.25 (IV dose), 0.5, 1, 2, 4, 7, 24, 48, and 72 hr post dose (n=4/time point); urine samples collected for 0-7, 7-24, 24-48, and 48-72 hr intervals; and feces was collected at 24, 48, and 72 hr post dose. The intestinal contents and carcasses were analyzed for residual radioactivity. The cages were rinsed with 50-150 mL of water and the radioactivity was measured. For distribution: QWBAR: radioactivity in tissues was determined by sacrificing the rats (n=1/time point) at 2 and 24 hr post dose in the oral study, and at 0.08 (~5 min), 0.5, 2, 4 and 72 hr post dose in IV study. Urine was collected for 0-24, 24-48, and 48-72 hr intervals; and feces collected at 72 hr post dose.
Analysis:	SDZ 282-791-ch plasma levels by HPLC, and metabolite profiling were determined by HPLC analysis (identified by the retention times and α-values and quantified by integration of radioactive peaks) with off-line radioactivity. Concentrations of parent drug and its metabolites were reported as ngEq/mL, and these values can be converted to nmol/L by multiplication with a factor of 1.989. The radioactivity in plasma, urine, feces, intestinal content or rinsings was

determined by liquid scintillation for total radioactivity. Carcasses were dissolved in 30% methanolic KOH, and the radio-activity in tissues was analyzed by HPLC. The QWBA followed the conventional methodology. The absorbance values of the regions of interest were captured by a video camera, measured using a MCID/BRS2 analyzer, and converted into radioactivity concentrations using a calibration curve obtained from a radioactive blood scale processed under the same conditions as the samples. The determination limit of radioactivity corresponded to 10 and 1 ngEq/mL of plasma after oral and I.V. administration, respectively. The determination limit of radioactivity corresponded to 476 and 48 ngEq/g of tissue in QWBAR after oral and IV administration, respectively.

- PK parameters: The PK profile after a single 20 mg/kg oral dose and a 2 mg/kg IV dose of [^{14}C] SDZ 282-791-ch is summarized:

	Oral (n=3)*	IV (n=4)
Radioactivity (mean) (ngEq/mL)		
Time (hr, post dose)		
0.25		2658
0.5	874 ± 1262	1885
1	600 ± 831	1104
2	279 ± 402	444
4	78 ± 116	81
7	56 (n=1)	18
24	---	4
48	---	4
72	---	3
T_{max} (hr)	0.7	0.25
AUC_{0-4h} (ngEq•h/mL)	1384 ± 1971	2508
AUC_{0-7h} (ngEq•h/mL)	4062 (n=1)	3095
AUC_{0-24h} (ngEq•h/mL)		2947
AUC_{0-72h} (ngEq•h/mL)		3127

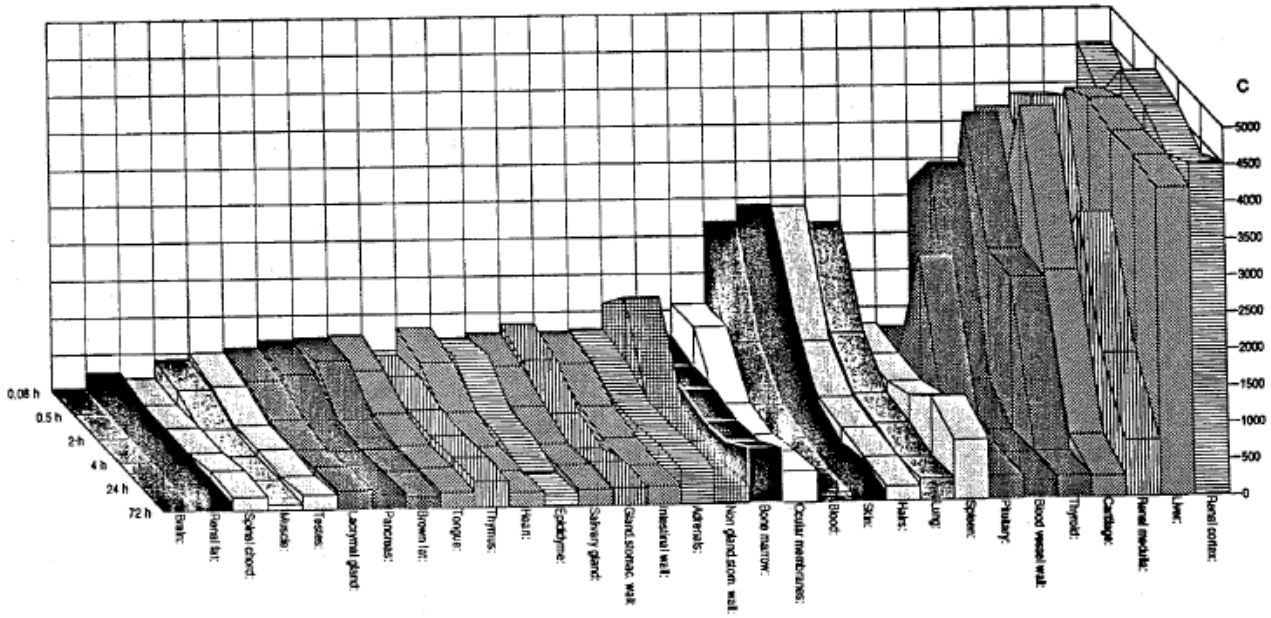
--- Plasma radioactivity concentrations after 4 hr post oral dose were below determination limit
*** The mean was calculated based on data from 3 animals (rats # 2, 3, and 4). Rat #1 died between 7-24 hr. The cause of death was not reported: rat #4 had very high levels of radioactivity at all time-points and a very high AUC, compared to other rats.**

- Tissue distribution:

The mean tissue levels of radioactivity at various time points (n=2/time point) following a single IV dose are tabulated below (table from the sponsor) and the corresponding figure is followed.

Tissues	0.08 h	0.5 h	2 h	4 h	24 h	72 h
Adrenals:	762	667	433	426	490	478
Blood:	2180	875	352	215	107	126
Blood vessel wall:	2674	3282	637	281	295	257
Bone marrow:	669	568	582	480	526	763
Brain:	49*	27*	14*	18*	13*	10*
Brown fat:	698	699	227	149	181	154
Cartilage:	3691	2538	4351	2450	532	292
Epididyme:	673	761	320	291	130	166
Gland.stomac. wall:	845	680	278	126	276	241
Hairs:	2390	880	446	348	281	202
Heart:	809	660	312	171	168	197
Intestinal wall:	733	620	375	265	218	259
Lacrymal gland:	569	448	241	149	247	252
Liver:	3007	4255	4443	4320	4326	4205
Lung:	2154	986	488	410	274	297
Muscle:	417	329	124	81	79	79
Non gland.st. wall:	1103	1515	331	200	152	216
Ocular membranes:	1084	1097	381	277	380	431
Pancreas:	666	528	226	134	191	204
Pituitary:	735	671	2352	439	316	316
Renal cortex:	4533	4148	4815	4650	4317	4519
Renal fat:	259	165	126	114	113	99
Renal medulla:	3869	4148	2100	3228	1637	772
Salivary gland:	730	594	382	198	236	224
Skin:	2416	1220	371	142	234	117
Spinal chord:	174	136	150	138	216	182
Spleen:	774	690	650	780	1089	816
Testes:	509	379	175	174	136	200
Thymus:	447	477	353	322	358	356
Thyroid:	788	3945	2422	2368	320	293
Tongue:	722	565	311	204	226	211

* : analyzed by liquid scintillation counting



The radioactivity was widely distributed; the main target organs of distribution were liver, kidney, lung, thyroid, and cartilage.

Biliary secretion was not significant, since only a small amount of radioactivity was found in feces.

The radioactivity following a single oral dose was detected 2 hr post dose in the liver. At 24 hr post dose, radioactivity was observed in liver, renal cortex and medulla, spleen and skin. The dose-normalized radioactivity was in the range of 88-243 ngEq/g. The low radioactivity concentrations in tissues were in line with the low absorption extent of the drug. See table below (from the sponsor).

Tissues	2 h	24 h
Kidney:	$	3142
Liver:	1880	4857
Renal cortex:	$	4332
Renal medulla:	$	1951
Skin:	$	1933
Spleen:	*	1759

$: traces
*: below determination limit (476 ng-eq/g)

- Metabolite patterns: plasma levels of parent drug and metabolites following IV (n=3) and oral dosing (n=1), peak 32 corresponding to SDZ 282-791 are shown below (table from the sponsor):

Concentrations of parent drug (ng/mL) and metabolites (ngEq/mL)

sample	front peak	peak 32 [1)]	peak 34	others	total RA
rat 11 (i.v.) AUC pool 0-24h	560	2665	198	55	3478
rat 12 (i.v.) AUC pool 0-24h	210	1970	114	175	2469
rat 13 (i.v.) AUC pool 0-24h	346	2195	126	168	2835
rat 14 (i.v.) AUC pool 0-24h	560	2135	166	145	3006
rats 1-3 (p.o.) AUC pools 0-4h	37	135	13	19	204

1) Peak 32 corresponds to SDZ 282-791

Proportions of peaks, expressed as % of sample radioactivity:

sample	front peak	peak 32 [1)]	peak 34	others
rat 11 (*i.v.*) AUC pool 0-24h	16	77	6	1
rat 12 (i.v.) AUC pool 0-24h	9	80	5	6
rat 13 (i.v.) AUC pool 0-24h	12	77	4	7
rat 14 (i.v.) AUC pool 0-24h	19	71	6	4
rats 1-3 (p.o.) AUC pools 0-4h	18	66	6	10

1) Peak 32 corresponds to SDZ 282-791

- Parent drug and metabolites in the excreta (urine and feces samples):

Proportions of peaks, expressed as % of sample radioactivity (table from the sponsor):

sample	front	peak 32 [1)]	peak 34	peak 37	peak 42	others
rat 11 (i.v.) urine 0-24h	- [2)]	59	17	12	- [2)]	12
rat 12 (i.v.) urine 0-24h	- [2)]	67	10	12	- [2)]	10
rat 13 (i.v.) urine 0-24h	- [2)]	70	6	14	- [2)]	10
rat 14 (i.v.) urine 0-24h	- [2)]	73	6	9	- [2)]	11
rat 1 (p.o.) urine 0-24h	15	14	5	14	24	28
rat 2 (p.o.) urine 0-24h	12	33	11	17	11	16
rat 3 (p.o.) urine 0-24h	10	27	13	14	11	24
rat 4 (p.o.) urine 0-24h	- [2)]	68	11	10	2	10
rat 3 (p.o.) feces 0-24h	- [2)]	51	14	6	10	20

1) peak 32 corresponds to SDZ 282-791
2) not quantified (because absent, of very low intensity, or ambigous)

Percentage of the dose excreted in the first 24 hr post dose (table from the sponsor):

sample	front	peak 32 [1]	peak 34	peak 37	peak 42	others	total RA
rat 11 (i.v.) urine 0-24h	- [2]	37	11	12	- [2]	12	63.1
rat 12 (i.v.) urine 0-24h	- [2]	40	6	12	- [2]	10	59.6
rat 13 (i.v.) urine 0-24h	- [2]	54	5	14	- [2]	10	76.6
rat 14 (i.v.) urine 0-24h	- [2]	51	4	9	- [2]	11	69.3
rat 1 (p.o.) urine 0-24h	0.1	0.1	<0.05	0.1	0.1	0.1	0.5
rat 2 (p.o.) urine 0-24h	0.1	0.3	0.1	0.2	0.1	0.1	0.9
rat 3 (p.o.) urine 0-24h	0.1	0.2	0.1	0.1	0.1	0.1	0.7
rat 4 (p.o.) urine 0-24h	- [2]	5.3	0.9	0.8	0.1	0.7	7.8
rat 3 (p.o.) feces 0-24h	- [2]	40	11	5	8	16	80.0

In the plasma, after I.V. or oral dosing, SDZ 282-791 accounted for the main component of the radioactivity (66-80%). After I.V. dosing, SDZ 282-791 was the major compound found in the urine (37%-54% of radioactivity). After oral dosing, SDZ 282-791 was the major compound found in the feces (40% of radioactivity). Moreover, after oral dosing, while urinary excretion was minimal, the parent drug was the main component of radioactivity in the urine.

- Excretion and recovery of radioactivity: the drug was excreted mainly via feces (oral) or urine (IV).

	Oral (n=3)	Intravenous
Excretion (% dose)		
Total radioactivity recovery	87.4	99.4
In urine		
0-7 h	0.7	61.3
0-24 h	3.1	67.2
0-48 h	3.3	68.8
0-72 h	3.6	69.8
Cage wash	0	0.5
Total urine	3.9*	70.4
In feces		
0-24 h	29.2	1.2
0-48 h	54.6	3.1
0-72 h	59.1	4.4
Intestine content	21.8	2
Total feces	80.9	5.3
Carcass	2.6	23.8

***: individual data were 0.7%, 1.3% and 9.6%**

Majority of radioactivity was recovered in most of the animals (recovery of 88% and 99% for oral and IV respectively). Little residual radioactivity remained in carcasses of animals that received the oral dose; ~22% of radioactivity was in the intestinal content.

Study No. 9608-0256 (Sandoz): Absorption and disposition in dogs following single subcutaneous and intravenous doses of [^{14}C]SDZ SID 791-ch

Key study findings:

- SDZ 282 791 was absorbed rapidly (t_{max}= 0.7 hr) and completely. The bioavailability was 99%, after SC administration.
- The main excretion route of SDZ 282-791 was via urine, following either subcutaneous or intravenous doses.
- The major component in the urine was the parent drug (~65% of sample radioactivity), after IV or SC administration. A significant fraction of the radioactive dose (~30%) was retained in the animals, as demonstrated by only ~70% recovery of radioactivity during the 0-168 hr (up to 7 days) assessment time.

Study system: Male Beagle dogs (n=3/treatment group)

Treatment: [^{14}C] SDZ SID 791-ch given subcutaneously or intravenously at 0.5 µmol/kg (0.41 mg/kg of the labeled drug or 0.25 mg -b/kg). The radioactive doses were ~ 27 µCi/kg.

Parameters: SDZ 282-791-ch levels in plasma, urine and feces, metabolite pattern in urine, and other PK parameters: percentage absorption (f_a: the ratio of the mean AUC of radioactivity after SC and IV doses), bioavailability (f: the ratio of the mean AUC of unchanged drug), total body clearance (CL: $dose_{IV}/AUC_{IV}$), renal clearance (CL_R: CL•f_e, where f_e was the fraction of the dose excreted unchanged in urine) and volume of distribution at steady state (V_{ss}: MRT•CL, where MRT [the mean residence time]= AUMC/AUC, AUMC: area under the first moment curve)

Schedule: Blood samples (~ 3 mL) were collected at 0.08 (~ 5 min, IV only), 0.25, 0.5, 1, 2, 3, 5, 7, 10, 24, 32, 48, 72, and 168 hr post dose (n=3/time point); urine and feces samples collected at 24, 48, 72, 96 and 168 hr post dose.

Analysis: Radioactivity (in dosing solution, plasma, urine, and feces) was measured by liquid scintillation counting (LSC).

- PK parameters: The PK profile after a single subcutaneous or intravenous dose (0.5 µmol/kg or 0.41 mg/kg) of SZD SID-791-ch (AMD3100) are summarized:

	SC (0.41 mg/kg)		IV (0.41 mg/kg)	
	Plasma AMD3100 (a)	Radioactivity* (b)	Plasma AMD3100	Radioactivity*
$AUC_{0\text{-}10h}$ (pmol•h/mL)	3456		3504	
$AUC_{0\text{-}168h}$ (pmol•h/mL)		4270		4201
AUC (pmol•h/mL) (c)	3532	5427	3568	4677
AUMC (pmol•h^2/mL)	8343		6609	
MRT (h)	2.3		1.9	
C_{max} (pmol/mL)	1157	1098	2557**	2362**
T_{max} (hr)	0.7	0.7		
$t_{1/2\ absorption}$ (h)	0.2	0.2		

	SC (0.41 mg/kg)		IV (0.41 mg/kg)	
	Plasma AMD3100 (a)	Radioactivity* (b)	Plasma AMD3100	Radioactivity*
$t_{1/2}\lambda_1$ (h)	1.7	1.7	0.7	1.4
$t_{1/2}\lambda_2$ (h)		181	2.0	118
CL (L/h)			1.9	
Vss (L/kg)			0.3	
Absorption (%)		116		
Bioavailability (%)	99			

***Note: the radioactivity was also converted as pmol/mL.**
(a): unchanged drug, (b) total radioactivity in plasma, (c) mean AUC
**** As C_0: concentration at 0 time, calculated by extrapolation using the half-life of the first disposition phase.**

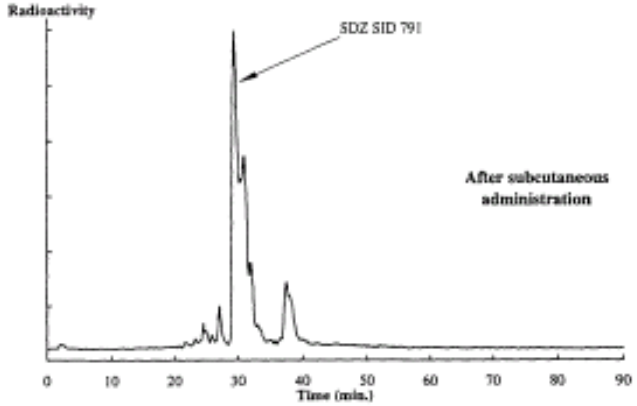
Comments:

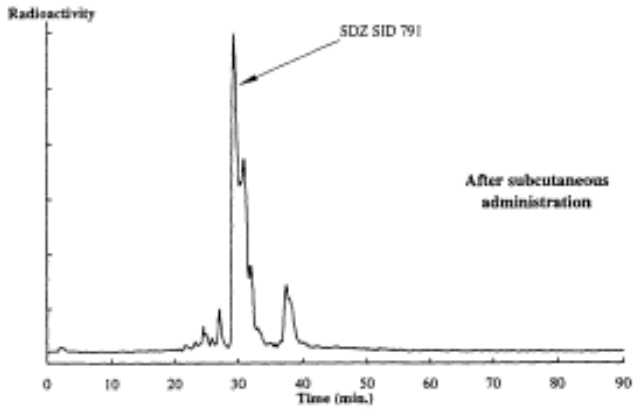
- ✧ The total radioactivity decreased biphasically with two $t_{1/2}$ values ($t_{1/2}\lambda_1$ and $t_{1/2}\lambda_2$); while the decrease of unchanged SDZ 282-791 (AMD3100) was monophasic and only one $t_{1/2}$ value was obtained after the SC dose. A biphasic decline of the parent drug concentrations was observed following IV dose, with two half-lives corresponding to the distribution phase and the elimination phase.
- ✧ The AUCs of radioactivity were slightly higher than those of plasma parent drug.
- ✧ The total body clearance represented < 5% of the hepatic blood flow in dogs (~41 L/h).

- Metabolism:

Parent drug represented the major part of radioactivity in urine samples following both SC and IV injections, with 62-68% of total radioactivity. Other than parent drug (corresponding to peak with retention [Rt] = (b) (4)), two more peaks (b) (4) were notable. Minor compounds were peaks with Rt = (b) (4) and (b) (4) min (together (b) (4)) and the remainder of radioactivity ((b) (4)) was associated with 10 minor peaks amounting each to < (b) (4) . The structure and biological characteristics of these metabolites were not described in this study.

The chromatography pattern depicting the metabolite profile after a single SC (Top) or IV (Bottom) dose are shown in the figures below (figures from the sponsor):





Of note, based on another study (see review of Report # GT-249-PK5), some of the non-parent compounds are likely complexes of CU^{2+} and plerixafor.

- Excretion and radioactivity recovery: the drug was excreted mainly via urine (68% and 63% of the radioactivity, after SC and IV dose, respectively). More than 50% of radioactivity was excreted into urine during the first 48 hrs post-dose.

	Subcutaneous	Intravenous
Excretion (% dose)		
Total radioactivity recovery	72.6	70.3
In urine		
0-24 h	25.3	51.9
0-48 h	52.9	57.9
0-72 h	58.3	59.4
0-96 h	65.0	60.5
0-168 h	67.9	63.0
In feces		
0-24 h	1.6	5.6
0-48 h	2.9	6.4
0-72 h	3.2	6.9
0-96 h	3.9	7.0
0-168 h	4.7	7.3

2.6.4.4 Distribution

GT-249-PK-3 (Genzyme): Stability of plerixafor in human, dog and rat whole blood

Key study findings:

- Plerixafor (AMD3100) was stable in human, dog, and rat plasma after 4 hrs of incubation at 37º C.

Stability of plerixafor (AMD3100, Lot # 46-446-02) and deuterated plerixafor in whole blood was tested in rat (male, Sprague-Dawley), dog (female, Beagle) and human (male and female) fresh whole blood. After incubation with plerixafor (0.1 µM and 1 µM,

n=3/concentration) at 37°C, for 0, 1, and 4 hours. The blood samples were lysed, precipitated and 2000 ng/mL of internal standard was added to the incubates. Percent (%) of parent drug remaining at 1 and 4 hours relative to the 0 hr sample were determined based on peak ratios to the internal standard. The plerixafor standard curve was made by incubating whole blood with plerixafor stock solutions to achieve the final concentrations of 5, 50, 500, 1000 and 5000 ng/mL. Blood samples were assayed using LC/MS/MS, with a detection range from 5 ng/mL (LLOQ) to 5000 ng/mL (ULOQ). Two compounds, enalapril and procaine, were used as controls to monitor the integrity of the assay.

Result are summarized in the tables below (from the sponsor), and indicate that plerixafor was stable in the rat, dog and human whole blood, when incubated for up to 4 hrs at 37°C.

✧ Male rat:

Compound	Time point (hrs)	1		2		3			
		Analyte Area /IS Area	Percent (%) of parent remaining	Analyte Area /IS Area	Percent (%) of parent remaining	Analyte Area /IS Area	Percent (%) of parent remaining	Mean of % parent remaining	SD
Plerixafor (1.0 µM)	0	0.231	100	0.237	100	0.233	100	100	N/A
	1	0.234	101	0.237	100	0.228	97.9	100	1.74
	4	0.228	98.7	0.234	98.7	0.230	98.7	98.7	0.0169
Plerixafor (0.1 µM)	0	0.0197	100	0.0192	100	0.0220	100	100	N/A
	1	0.0198	101	0.0186	96.9	0.0204	92.7	96.7	4.03
	4	0.0191	97.0	0.0185	96.4	0.0203	92.3	95.2	2.68
Enalapril (as control)	0	2.24	100	1.83	100	1.67	100	100	N/A
	1	0.453	20.2	0.428	23.4	0.459	27.5	23.7	3.64
	4	0.00545	0.243	0.00407	0.222	0.00322	0.193	0.220	0.0254
Procaine (as control)	0	17.7	100	14.9	100	12.8	100	100	N/A
	1	17.7	100	14.8	99.3	12.9	101	100	0.727
	4	10.0	56.5	9.84	66.0	8.85	69.1	63.9	6.59

✧ Female dog:

Compound	Time point (h)	1		2		3			
		Analyte Area /IS Area	Percent (%) of parent remaining	Analyte Area /IS Area	Percent (%) of parent remaining	Analyte Area /IS Area	Percent (%) of parent remaining	Mean of % parent remaining	SD
Plerixafor (1.0 µM)	0	0.244	100	0.226	100	0.222	100	100	N/A
	1	0.243	100	0.227	100	0.218	98.2	99.4	1.14
	4	0.237	97.1	0.225	100	0.212	95.5	97.4	2.10
Plerixafor (0.1 µM)	0	0.0215	100	0.0215	100	0.0204	100	100	N/A
	1	0.0213	99.1	0.0208	96.7	0.0203	100	98.4	1.51
	4	0.0203	94.4	0.0197	91.6	0.0185	90.7	92.2	2.10
Enalapril (as control)	0	8.49	100	7.81	100	8.55	100	100	N/A
	1	8.49	100	7.72	98.8	8.44	98.7	99.2	0.707
	4	8.42	99.2	7.73	99.0	7.10	83.0	93.7	9.26
Procaine (as control)	0	32.2	100	33.3	100	34.4	100	100	N/A
	1	33.0	102	33.3	100	32.1	93.3	98.6	4.74
	4	32.5	101	26.6	79.9	27.4	79.7	86.8	12.2

✧ Human:

Male whole blood:

Compound	Time point (h)	1		2		3			
		Analyte Area /IS Area	Percent (%) of parent remaining	Analyte Area /IS Area	Percent (%) of parent remaining	Analyte Area /IS Area	Percent (%) of parent remaining	Mean of % parent remaining	SD
Plerixafor (1.0 µM)	0	0.177	100	0.184	100	0.223	100	100	N/A
	1	0.220	124	0.204	111	0.223	100	112	10.9
	4	0.202	114	0.202	110	0.212	95.1	106	9.39
Plerixafor (0.1 µM)	0	0.0241	100	0.0254	100	0.0268	100	100	N/A
	1	0.0230	95.4	0.0250	98.4	0.0250	93.3	95.7	2.70
	4	0.0215	89.2	0.0247	97.2	0.0251	93.7	93.4	4.31
Enalapril (as control)	0	27.6	100	30.5	100	29.1	100	100	N/A
	1	29.1	105	30.3	99.3	28.6	98.3	101	3.86
	4	20.0	72.5	22.9	75.1	16.2	55.7	67.7	10.5
Procaine (as control)	0	0.122	100	0.104	100	0.156	100	100	N/A
	1	0.0556	45.6	0.0607	58.4	0.0523	33.5	45.8	12.4
	4	0.0343	28.1	0.0261	25.1	0.0298	19.1	24.1	4.59

Female whole blood:

Compound	Time point (h)	1		2		3			
		Analyte Area /IS Area	Percent (%) of parent remaining	Analyte Area /IS Area	Percent (%) of parent remaining	Analyte Area /IS Area	Percent (%) of parent remaining	Mean of % parent remaining	SD
Plerixafor (1.0 µM)	0	0.238	100	0.200	100	0.256	100	100	N/A
	1	0.229	96.4	0.227	114	0.232	90.8	100	11.9
	4	0.236	99.2	0.219	110	0.217	85.0	98.0	12.4
Plerixafor (0.1 µM)	0	0.0209	100	0.0196	100	0.0216	100	100	N/A
	1	0.0213	102	0.0203	104	0.0184	85.0	96.8	10.6
	4	0.0202	96.4	0.0201	103	0.0227	105	101	4.49
Enalapril (as control)	0	18.5	100	25.0	100	24.3	100	100	N/A
	1	18.6	101	14.2	56.8	13.0	53.5	70.3	26.3
	4	8.53	46.1	6.37	25.5	7.64	31.4	34.3	10.6
Procaine (as control)	0	37.4	100	57.9	100	44.9	100	100	N/A
	1	0.0359	0.0960	0.0355	0.0613	0.0435	0.0969	0.0847	0.0203
	4	0.0175	0.0468	0.0149	0.0257	0.0136	0.0303	0.0343	0.0111

GT-249-PK-4 (Genzyme): Determination of red blood cell partitioning of plerixafor in rat, dog and human whole blood

Key study findings:

- Distribution of plerixafor to red blood cells was not significant in rat or dog whole blood, after 2 hrs of incubation at 37º C.
- A small fraction of plerixafor (partitioning coefficient 0.1-0.2) was distributed to human RBC. This finding was independent of concentrations tested (0.1 and 1 µM plerixafor).

^{14}C-Plerixafor (specific activity 80.9 μCi/mg) was incubated with whole blood (male CD rat, female Beagle dog and male and female human) samples at 37°C for 2 hours. The final concentrations in the whole blood incubations of plerixafor were 0.1 μM and 1 μM. Aliquots of incubate (~350 μL) were centrifuged and the radioactivity in the plasma and red blood cells (RBC) were counted in a liquid scintillation counter. The RBC partitioning ratio was calculated according to the following equation:

$K_{RBC/plasma} = [C_{plasma}\,(control)/C_{plasma}\,(plasma\ from\ blood\ incubation) - 1] \bullet 1/(H-0.05) + 1$

H: the hematocrit value, 0.46 for rat, 0.42 for dog, 0.45 for human male, and 0.39 for human female.

No uptake of plerixafor to RBCs was observed in rat or dog whole blood (n=4). In comparison, there was a minor partitioning of plerixafor to RBCs of the human (male and female). Results are tabulated below:

^{14}C-plerixafor	0.1 μM			1 μM		
Radioactivity (dpm)	^{14}C DPM_{ref}	^{14}C DPM_{PL}	$K_{RBC/plasma}$	^{14}C DPM_{ref}	^{14}C DPM_{PL}	$K_{RBC/plasma}$
Rat						
	925 ± 37	1511 ± 38	0.05	8987 ± 354	15689 ± 394	-0.04
Dog						
	831 ± 47	1400 ± 40	-0.10	8224 ± 205	14736 ± 268	-0.19
Human (male)						
	896 ± 34	1318 ± 28	0.2	9104 ± 487	13405 ± 359	0.2
Human (female)						
	900 ± 42	1246 ± 24	0.18	8654 ± 179	12380 ± 354	0.11

^{14}C DPM_{ref}, ^{14}C DPM_{PL}: radioactivity of ^{14}C-plerixafor in reference plasma (control) and harvested plasma for spiked whole blood.

AOM 0036 (AnorMed Inc. report): Interspecies protein binding of AMD3100 using ultrafiltration analysis

Key study findings:

- The plasma protein binding of AMD3100 (1-10 μg/mL) was moderate (33-54% for rat, 34-46% for dog, and 37-58% in human). It was independent of concentrations tested, with no obvious inter-species differences.
- In the 3 species tested, the highest concentration of AMD3100 resulted in reduced plasma protein binding, suggesting saturation of protein binding at a concentration between 3 and 10 μg/mL.

The ability of AMD3100 to bind protein from rat, dog (pooled plasma samples of mixed sex for these two species) and human plasma (one male donor) was investigated. AMD3100 (with final concentrations of 1, 3 and 10 μg/mL plasma) was incubated with pre-equilibrated plasma samples or PBS for 15 min at 37°C. **(b) (4)**

(b) (4)

The amount of bound AMD3100 was corrected for the amount of non-specific binding (NSB, calculated using AMD3100 in PBS). The experiment was carried out in triplicates for rat and dog and duplicated in human samples.

The following is excerpted from the report, for calculation of % protein binding, (3100 refers to AMD3100):

A. PBS Method-Using the amount of AMD3100 NSB in PBS as a correction factor

1. % 3100 free in PBS control ($\%F_{PBS}$) = $\frac{\text{conc 3100 in PBS filtrate}}{\text{Total conc of 3100 in PBS}} \times 100$

2. % 3100 apparently bound in plasma (%Bapp) = $\frac{\text{conc of 3100 in plasma filtrate}}{\text{Total conc of 3100 in plasma}} \times 100$

3. % 3100 Non-specifically bound ($\%NSB_{PBS}$) = 100 - $\%F_{PBS}$

4. % 3100 bound in Plasma (%B) = $100 - \frac{(\%Bapp)}{\%F_{PBS}} \times 100$

Results are summarized in the table below (from the sponsor):

Species	Plerixafor (µg/ml)	Mean % Bound ± SEM (n = 3)
Rat	1.0	54.3 ± 7.0
	3.0	52.0 ± 5.9
	10.0	33.0 ± 4.7
Dog	1.0	38.0 ± 8.2
	3.0	46.0 ± 14.2
	10.0	34.0 ± 15.2
Human	1.0	53.5 ± 2.5[a]
	3.0	58.0 ± 0.0[a]
	10.0	37.0 ± 0.8[a]

[a] n = 2

The results for the low and high binding protein controls gave expected results consistent with published data (data not shown).

Study No. CMS 81280A ((b) (4) 7686-108): Pharmacokinetics, excretion, mass balance, and quantitative whole-body autoradiography following subcutaneous administration of ^{14}C-AMD3100 to rats

Key study findings:

- Subcutaneously administered AMD3100 was absorbed rapidly (blood and plasma T_{max} was 0.5 hr) and was widely distributed. The injection site (0.5 hr), liver (4 hr), renal cortex (4 hr), small intestine contents (4 hr), cartilage (2 hr), and epiphyseal plate (2 hr) were tissues that had high radioactivity concentrations. While radioactivity decreased in most organs/tissues from 0.5 hr to 4 hr after dose administration, in adrenal, bone marrow, kidney, liver, renal cortex, and spleen, radioactivity was still detectable at 336 hr post dose.
- The main excretion route of AMD3100 was via urine; the highest concentration of radioactivity was observed at 2 hr post dose.
- A significant percentage of the dose (19% and 16% for male and female rats, respectively) remained in carcasses at 168 hr post dose.
- Small amounts of radioactivity were observed in the pituitary gland (up to 336 hrs post-dose, last time-point examined). In addition, occasional and small amounts of radioactivity were detected in cerebrum, olfactory lobe, and spinal fluid. These data suggest that AMD3100 has the potential to cross the blood-brain barrier.
- Because concentrations of radioactivity were measureable through 336 hr post dose in uveal tract and skin, AMD3100 or AMD3100-derived compounds may have affinity for melanin.

Study system: Wistar Han rats (HsdRccHan:WIST) and male Long Evans rats (HsdBlu:LE).

Study design: Two groups of rats were used for whole-body autoradiography (WBA) and mass balance studies. See group designation, animal allocation, target dose level and dose volume in the table below (from the sponsor):

Group	Number of Animals Male	Number of Animals Female	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)	Samples Collected
1	3[a]	3[a]	Subcutaneous	1.23	10	Urine, feces, expired air, and carcass
2	8[b]	-	Subcutaneous	1.23	10	Blood and carcass for WBA

WBA Whole-body autoradiography.
Note: The dose was approximately 100 μCi/kg.
a Wistar Han.
b Long Evans.

Treatment: [^{14}C] AMD3100 (specific activity: 80.9 μCi/mg, purity 97.3%) subcutaneously at a dose of 1.23 mg/kg (i.e., ~ 100 μCi/kg) (injection volume 10 mL/kg)

Parameters: Urine and feces, quantitative whole-body autoradiography (QWBA) and radioactivity recovery. In addition, animals were monitored for clinical signs (twice daily) and body weights (on the day of dosing and at sacrifice [Group 1 only]).

Schedule: For excretion and mass balance (Group 1): urine samples were collected for 0-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hr intervals; and feces collected at 24 hr intervals through 168 hr post dose. Expired air was collected at 0-8, 8-24, and at 24 hr intervals through 168 hr post dose. Cage rinse samples were collected at 24 hr intervals through 144 hr post dose. After the last excreta collections, cages were washed and wiped. The cage wash samples and wipe gauze were collected.
For QWBA (Group 2): radioactivity in tissues was determined by sacrificing the rats (n=1/time point) at 0.5, 2, 4, 24, 48, 72, 168 and 336 hr post dose. Blood samples collected prior sacrifice to obtain plasma.

Analysis: The radioactivity in plasma, urine, cage wash/rinse samples and dose wipes (extracted first) was determined by liquid scintillation (LSC) for total radioactivity. Feces samples (homogenized first), blood samples and the corresponding duplicate aliquots were combusted and the resulting $^{14}CO_2$ was trapped and radioanalysis was performed by LSC. All sample combustions were done in a (b) (4) .
The QWBA followed the conventional methodology. Each carcass was cut, homogenized, digested in NaOH, and radioactivity was determined by LSC. The limits of determination were 13.3 ngEq/mL and ULOQ 116,000 ngEq/g of tissue in QWBA.

- Overall dose administered: 1.25 mg/kg, 18.3-18.5 µCi/animal, or 101 µCi/kg.
- Life signs and body weights: Not remarkable
- Concentrations of radioactivity in blood and plasma at specified time points after a single subcutaneous injection of ^{14}C-AMD3100 in male Long Evans rats (n=1/time point):
  The highest concentration of radioactivity was at 0.5 hr post dose. Up to 4 hr post dose, most of the radioactivity in whole blood was found in the plasma; however, from 24 hr on, the concentration of radioactivity in plasma was lower than in the blood cells.

Collection Time Point (hours)	Animal Number	ng Equivalents ^{14}C-AMD3100/g	
		Blood	Plasma
0.5	B00607	798	1490
2	B00608	402	650
4	B00609	92.6	155
24	B00610	11.0	4.92
48	B00611	7.63	3.22
72	B00612	5.38	2.24
168	B00613	3.29	2.04
336	B00614	2.18	1.31

Collection Time Point (hours)	Animal Number	Blood:Plasma Ratio
0.5	B00607	0.536
2	B00608	0.619
4	B00609	0.597
24	B00610	2.24
48	B00611	2.37
72	B00612	2.40
168	B00613	1.62
336	B00614	1.66

- PK parameters: The PK profile in organs/tissues after a single 1.23 mg/kg subcutaneous dose is summarized:

	Radioactivity (via LSC)	
	Blood (a)	Plasma (a)
AUC_{0-4h}	3.9	5.0
$AUC_{0-\infty}$	4.5	5.7
C_{max}	0.8	1.5
T_{max} (hr)	0.5	0.5
$t_{1/2}$ terminal (hr)	209	330

(a): AUC and C_{max}: µgEq•h/mL and µgEq/mL, respectively.

- Tissue distribution: male Long Evans rats

The radioactivity concentrations and relative concentrations (expressed as the ratio tissue/plasma), after the SC administration, are tabulated (see below; tables from the sponsor).

Because concentrations of radioactivity were measureable through 336 hr post dose in uveal tract and skin, ^{14}C-AMD3100-derived radioactivity may have affinity for melanin.

Concentrations of radioactivity (ngEq/g):

	ng Equivalents ^{14}C-AMD3100/g							
	Animal Number (Sacrifice Time)							
Tissue	B00607 (0.5 Hours)	B00608 (2 Hours)	B00609 (4 Hours)	B00610 (24 Hours)	B00611 (48 Hours)	B00612 (72 Hours)	B00613 (168 Hours)	B00614 (336 Hours)
Adrenal gland	431	466	364	361	398	318	259	290
Aorta	1900	778	372	83.9	68.2	24.1	34.2	30.8
Blood	737	421	99.0	BLQ	BLQ	BLQ	BLQ	BLQ
Bone	167	122	59.6	39.5	18.4	BLQ	BLQ	BLQ
Bone marrow	352	609	874	1110	1110	771	764	945
Cartilage	2820	10500	11100	1590	481	284	192	235
Cecum	644	449	185	98.7	77.0	52.8	49.9	27.7
Cecum contents	BLQ	44.6	2650	116	49.3	40.3	17.4	BLQ
Cerebellum	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Cerebrum	18.5	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Cerebrospinal fluid	157	112	61.3	66.6	54.1	34.4	33.7	29.9
Diaphragm	276	210	78.0	53.7	40.9	18.2	17.3	17.3
Dose site	46800	8710	4010	4820	7510	916	344	400
Epididymis	316	409	156	60.8	42.6	38.4	22.3	30.9
Epiphyseal plate	11500	25500	11500	403	305	153	96.9	58.9
Esophageal contents	573	124	858	BLQ	19.8	BLQ	BLQ	BLQ
Esophagus	733	294	334	47.4	91.6	69.3	20.1	21.1
Exorbital lacrimal gland	182	155	88.8	40.4	45.6	33.5	40.0	30.6
Eye	132	81.1	61.3	30.2	48.4	21.8	21.0	51.2
Eye (lens)	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Fat (abdominal)	109	69.6	35.8	13.3	25.9	BLQ	BLQ	BLQ
Fat (brown)	341	350	125	40.8	51.8	27.0	21.9	24.1
Harderian gland	240	159	109	68.8	56.7	40.0	17.9	25.8
Intra-orbital lacrimal gland	214	152	88.8	44.9	39.8	29.6	21.8	28.0

Kidney	3130	4830	4020	4120	3280	2540	1050	1450
Large intestinal contents	NR	14.3	30.4	251	129	68.5	27.1	18.8
Large intestine	610	431	215	88.2	77.8	38.2	37.0	40.5
Liver	1810	5620	8010	4300	4150	2570	1630	1540
Lung	648	469	269	178	228	123	82.5	80.1
Lymph nodes	598	843	1560	1400	1070	919	524	866
Medulla	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Muscle	123	76.6	34.6	19.2	18.7	16.1	BLQ	BLQ
Myocardium	378	236	94.8	45.3	45.4	26.3	18.0	19.3
Nasal turbinates	257	195	115	46.9	61.5	42.2	44.3	48.1
Olfactory lobe	34.6	18.6	BLQ	BLQ	BLQ	BLQ	32.4	24.9
Pancreas	244	173	73.5	40.7	31.1	21.0	19.7	23.5
Periosteum	524	347	158	71.5	73.4	46.2	40.3	46.4
Pituitary gland	321	289	189	119	160	145	67.1	96.6
Preputial gland	468	273	135	115	65.8	55.3	45.5	NR
Prostate	157	158	226	50.9	32.9	20.0	17.7	21.4
Renal cortex	3230	5610	5740	5270	4500	3390	1570	1990
Renal medulla	3110	3780	2850	2390	1220	1200	420	522
Salivary gland	390	268	120	57.1	58.7	38.1	31.9	34.5
Seminal vesicle	94.4	69.7	75.0	16.9	19.5	14.1	BLQ	BLQ
Skin	676	458	184	101	70.1	58.5	24.1	45.5
Small intestinal contents	1230	4840	9590	71.2	35.8	45.3	13.4	BLQ
Small intestine	443	444	322	152	76.2	29.4	37.8	54.2
Spinal cord	13.5	17.6	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Spleen	491	816	916	1240	1260	980	762	1110
Spleen (red pulp)	560	964	1230	1690	1640	1390	1090	1560
Spleen (white pulp)	313	407	370	580	478	383	242	319
Stomach	544	421	155	79.8	67.8	46.3	37.9	44.5
Stomach contents	451	812	879	BLQ	BLQ	BLQ	BLQ	BLQ
Testis	155	103	65.4	35.3	25.5	25.9	19.6	29.9
Thymus	217	214	216	206	252	198	161	162
Thyroid	505	206	120	85.9	80.7	58.5	47.6	53.0
Urinary bladder	608	355	105	259	55.7	33.0	62.6	BLQ
Urine	43100	164000[a]	1860	14.5	BLQ	97.6	82.6	76.0
Uveal tract	725	486	385	261	203	154	151	194

BLQ Below limit of quantitation (<13.3 ng equivalents ^{14}C-AMD3100/g)

[a] One or more samples were above the upper limit of quantitation (ULOQ) (>116,000 ng equivalents ^{14}C-AMD3100/g)

NR Not represented (tissue not present in section).

Tissue:plasma concentration ratio:

	Tissue:Plasma Concentration Ratios							
	Animal Number (Sacrifice Time)							
Tissue	B00607 (0.5 Hours)	B00608 (2 Hours)	B00609 (4 Hours)	B00610 (24 Hours)	B00611 (48 Hours)	B00612 (72 Hours)	B00613 (168 Hours)	B00614 (336 Hours)
Adrenal gland	0.289	0.717	2.35	73.4	124	142	127	221
Aorta	1.28	1.20	2.40	17.1	21.2	10.8	16.8	23.5
Blood	0.495	0.648	0.639	NA	NA	NA	NA	NA
Bone	0.112	0.188	0.385	8.03	5.71	NA	NA	NA
Bone marrow	0.236	0.937	5.64	226	345	344	375	721
Cartilage	1.89	16.2	71.6	323	149	127	94.1	179
Cecum	0.432	0.691	1.19	20.1	23.9	23.6	24.5	21.1
Cecum contents	NA	0.0686	17.1	23.6	15.3	18.0	8.53	NA
Cerebellum	NA	NA	NA	NA	NA	NA	NA	NA
Cerebrum	0.0124	NA	NA	NA	NA	NA	NA	NA
Cerebrospinal Fluid	0.105	0.172	0.395	13.5	16.8	15.4	16.5	22.8
Diaphragm	0.185	0.323	0.503	10.9	12.7	8.13	8.48	13.2
Dose site	31.4	13.4	25.9	980	2330	409	169	305
Epididymis	0.212	0.629	1.01	12.4	13.2	17.1	10.9	23.6
Epiphyseal plate	7.72	39.2	74.2	81.9	94.7	68.3	47.5	45.0
Esophageal contents	0.385	0.191	5.54	NA	6.15	NA	NA	NA
Esophagus	0.492	0.452	2.15	9.63	28.4	30.9	9.85	16.1
Exorbital lacrimal gland	0.122	0.238	0.573	8.21	14.2	15.0	19.6	23.4
Eye	0.0886	0.125	0.395	6.14	15.0	9.73	10.3	39.1
Eye (lens)	NA	NA	NA	NA	NA	NA	NA	NA
Fat (abdominal)	0.0732	0.107	0.231	2.70	8.04	NA	NA	NA
Fat (brown)	0.229	0.538	0.806	8.29	16.1	12.1	10.7	18.4
Harderian gland	0.161	0.245	0.703	14.0	17.6	17.9	8.77	19.7
Intra-orbital lacrimal gland	0.144	0.234	0.573	9.13	12.4	13.2	10.7	21.4
Kidney	2.10	7.43	25.9	837	1020	1130	515	1110
Large intestinal contents	NA	0.0220	0.196	51.0	40.1	30.6	13.3	14.4
Large intestine	0.409	0.663	1.39	17.9	24.2	17.1	18.1	30.9
Liver	1.21	8.65	51.7	874	1290	1150	799	1180
Lung	0.435	0.722	1.74	36.2	70.8	54.9	40.4	61.1
Lymph nodes	0.401	1.30	10.1	285	332	410	257	661
Medulla	NA	NA	NA	NA	NA	NA	NA	NA
Muscle	0.0826	0.118	0.223	3.90	5.81	7.19	NA	NA
Myocardium	0.254	0.363	0.612	9.21	14.1	11.7	8.82	14.7
Nasal turbinates	0.172	0.300	0.742	9.53	19.1	18.8	21.7	36.7
Olfactory lobe	0.0232	0.0286	NA	NA	NA	NA	15.9	19.0
Pancreas	0.164	0.266	0.474	8.27	9.66	9.38	9.66	17.9
Periosteum	0.352	0.534	1.02	14.5	22.8	20.6	19.8	35.4
Pituitary gland	0.215	0.445	1.22	24.2	49.7	64.7	32.9	73.7
Preputial gland	0.314	0.420	0.871	23.4	20.4	24.7	22.3	NA
Prostate	0.105	0.243	1.46	10.3	10.2	8.93	8.68	16.3
Renal cortex	2.17	8.63	37.0	1070	1400	1510	770	1520
Renal medulla	2.09	5.82	18.4	486	379	536	206	398
Salivary gland	0.262	0.412	0.774	11.6	18.2	17.0	15.6	26.3
Seminal vesicle	0.0634	0.107	0.484	3.43	6.06	6.29	NA	NA
Skin	0.454	0.705	1.19	20.5	21.8	26.1	11.8	34.7
Small intestinal contents	0.826	7.45	61.9	14.5	11.1	20.2	6.57	NA
Small intestine	0.297	0.683	2.08	30.9	23.7	13.1	18.5	41.4
Spinal cord	0.00906	0.0271	NA	NA	NA	NA	NA	NA

Spleen	0.330	1.26	5.91	252	391	438	374	847
Spleen (red pulp)	0.376	1.48	7.94	343	509	621	534	1190
Spleen (white pulp)	0.210	0.626	2.39	118	148	171	119	244
Stomach	0.365	0.648	1.00	16.2	21.1	20.7	18.6	34.0
Stomach contents	0.303	1.25	5.67	NA	NA	NA	NA	NA
Testis	0.104	0.158	0.422	7.17	7.92	11.6	9.61	22.8
Thymus	0.146	0.329	1.39	41.9	78.3	88.4	78.9	124
Thyroid	0.339	0.317	0.774	17.5	25.1	26.1	23.3	40.5
Urinary bladder	0.408	0.546	0.677	52.6	17.3	14.7	30.7	NA
Urine	28.9	NA	12.0	2.95	NA	43.6	40.5	58.0
Uveal tract	0.487	0.748	2.48	53.0	63.0	68.8	74.0	148

NA Not applicable

The tissue:plasma ratios were less than 1 between 0.5 to 2 hr post dose in most tissues. Afterwards, the ratios generally increased over time (with the highest ratio [2330] at the injection site, observed 4 hr post dose), indicating retention of ^{14}C-AMD3100 in most tissues. At 336 hr post dose, the highest tissue:plasma ratios were in renal cortex (1520), spleen red pulp (1190), liver (1180), kidney (1110), spleen (847), and bone marrow (721). It was also noted that tissues rich in melanin, e.g., skin and uveal tract, showed notable tissue:plasma ratios of 34.7 and 148, respectively.

- Excretion and radioactivity recovery: the drug was excreted rapidly via urine (~65% of dose at 24 hr post dose); only a smaller fraction of radioactivity was recovered in feces (8-11%, 7 days post dose). At 168 hr post dose, carcasses contained ~19% and 16% of the dose in males and females, respectively. Recovery of radioactivity was nearly complete (99 %) in all six rats tested.

Group 1 (Wistar rats):

	Males (n=3)	Females (N=3)
Excretion (% dose)		
Total radioactivity recovery	99.1 ± 2.3	99.0 ± 0.5
Urine		
0-8 h	50.3	47.9
0-24 h	60.3	64.3
0-48 h	61.9	66.6
0-72 h	63.1	68.1
0-96 h	64.1	69.2
0-120 h	65.1	70.2
0-144 h	65.8	71.0
0-168 h	66.4	71.8
Feces		
0-24 h	5.51	3.67
0-48 h	7.30	5.06
0-72 h	8.46	6.06
0-96 h	9.32	6.71
0-120 h	10.0	7.25
0-144 h	10.7	7.73
0-168 h	11.3	8.12
Cage rinse		
0-24 h	0.97	1.17
0-48 h	1.14	1.48
0-72 h	1.20	1.59

0-96 h	1.27	1.87
0-120 h	1.62	2.02
0-144 h	1.68	2.12
Expired air		
0-8 h	0.00	0.00
0-24 h	0.00	0.00
0-48 h	0.00	0.05
0-72 h	0.02	0.13
0-96 h	0.06	0.21
0-120 h	0.11	0.30
0-144 h	0.18	0.40
0-168 h	0.25	0.50

Study #9608-0251 (Genzyme): Whole-body autoradiography in rats following single and multiple subcutaneous doses of [^{14}C]SDZ SID 791-ch

Key study findings:

- Subcutaneously administered SDZ 282 791 was widely distributed.
- The target organs of drug accumulation/retention after a single-dose administration were adrenal, bone marrow, cartilage, liver, kidney and spleen red pulp.
- Repeated dosing showed that the drug or drug-related compounds may accumulate in organs/tissues: when comparing the same organs/tissues, higher concentrations of radioactivity were observed after repeated dosing. In addition, more tissues had sustained or detectable levels of radioactivity at Hrs 168 or 336 after repeated dosing.
- There were no obvious gender-specific differences in tissue distribution of radioactivity.

Study system:	Wistar Han rats (HsdRccHan:WIST)
Treatment:	[^{14}C] SDZ SID 791-ch subcutaneously at 1.51 mg/kg (82 μCi/mg) in single dose study and 1.65 mg/kg/day (~ 105 μCi/mg/day) in the multiple dose (7 doses) experiment.
Study design:	Quantitative whole body autoradiography (QWBAR): radioactivity in tissues was determined by sacrificing the rats (n=1/sex/time point) at 0.5, 2, 4, 24, 48, 72, 168 and 336 hr after single and multiple dosing.
Analysis:	Radioactivity in the drug solution was measured by LSC. In QWBAR, the absorbance values of the regions of interest were captured by a video camera, measured using a **(b) (4)** analyzer, and converted into radioactivity concentrations using a calibration curve. The limit of quantification of radioactivity was 110 and 79 ngEq/g of tissue for single and multiple dose experiments, respectively.

- Tissue distribution of radioactivity:
  - ✧ Single dose:

Males:

Tissue	RAT 111 (0.5 h)	RAT 112 (2 h)	RAT 113 (4 h)	RAT 114 (24 h)	RAT 115 (72 h)	RAT 116 (168 h)	RAT 117 (336 h)
Adrenals	1'224	1'474	1'266	511	259	411	-
Blood	1'766	663	-	-	-	-	-
Bone marrow	466	1'078	1'231	1'388	1'053	1'278	525
Brain	295	367	-	-	-	-	-
Brown fat	557	410	-	-	-	-	-
Cartilage	8'827	13'261	8'674	6'763	419	823	348
Epididyme	705	422	-	-	-	-	-
Hairs	2'384	968	599	-	-	-	-
Heart	1'153	576	-	-	-	-	-
Intestinal wall	1'230	849	634	172	-	-	-
Lacrimal gland	980	648	-	-	-	-	-
Liver	2'335	4'160	4'836	3'695	3'725	3'996	2'218
Lung	1'937	933	706	349	-	-	-
Muscle	650	341	-	-	-	-	-
Ocular Membrane	2'617	1'657	342	-	-	-	-
Pancreas	766	481	-	-	-	-	-
Pelvis	12'608	2'380	1'251	289	-	-	-
Renal cortex	7'730	7'714	8'426	6'948	4'500	4'032	2'484
Renal fat	440	555	-	-	-	-	-
Renal medulla	5'057	5'625	5'116	2'654	997	699	472
Salivary gland	915	513	442	-	-	-	-
Skin	1'370	778	443	-	-	-	-
Spleen (red pulp)	1'649	2'381	2'307	3'004	1'992	2'516	1'616
Spleen (white pulp)	-	-	-	-	-	-	-
Stomachal wall	2'137	1'000	383	191	-	-	-
Testes	554	266	-	-	-	-	-
Thymus	816	558	421	166	-	-	-
Thyroid	854	341	272	-	-	-	-

- : below quantification limit (110 ng-b-eq/g)

Females:

Region	RAT 118 (0.5 h)	RAT 119 (2 h)	RAT 120 (4 h)	RAT 121 (24 h)	RAT 122 (72 h)	RAT 123 (168 h)	RAT 124 (336 h)
Adrenals	567	266	128	-	-	-	-
Blood	378	238	-	-	-	-	-
Bone marrow	227	292	432	614	989	1'002	1'265
Brain	-	-	-	-	-	-	-
Brown fat	-	-	-	-	-	-	-
Cartilage	3'993	8'171	10'214	2'465	2'040	642	1'244
Hairs	530	391	208	-	-	-	-
Heart	-	-	-	-	-	-	-
Intestinal wall	242	244	-	-	-	-	-
Lacrimal gland	-	-	-	-	-	-	-
Liver	964	2'993	4'476	4'028	4'213	2'925	2'496
Lung	489	399	293	157	-	-	-
Muscle	-	-	-	-	-	-	-
Ovary	603	383	133	-	-	-	-
Ocular membrane	956	903	*	-	-	-	-
Pelvis	2'577	3'284	1'229	730	157	227	241
Renal cortex	5'253	7'999	7'497	6'027	5'783	3'393	3'441
Renal fat	-	-	-	-	-	-	-
Renal medulla	2'853	5'304	4'801	2'587	2'448	1'215	1'169
Salivary gland	238	228	-	-	-	-	-
Skin	395	344	-	-	-	-	-
Spleen (red pulp)	362	896	1'527	2'885	2'627	1'770	1'917
Spleen (white pulp)	-	-	-	-	-	-	-
Stomachal wall	706	170	-	-	-	-	-
Thymus	-	-	-	176	-	-	-
Thyroid	441	573	-	-	-	-	-
Uterus	673	590	-	-	-	-	-

- : below quantification limit (110 ng-b-eq/g)
*: no sample available

The highest concentrations of radioactivity were observed at 2 hr post dose in the following tissues/organs (in a decreasing concentration order): cartilage, kidney (cortex > medulla), and the liver. The distribution pattern was similar in the females, although with lower radioactivity. In the 0.5-4 hr post dose period, the radioactivity decreased rapidly in most tissues, except the liver, kidney (renal cortex), spleen (red pulp), renal medulla, bone marrow, cartilage and adrenal, where a significant radioactivity retention was observed even at 336 hr post dose.

✧ Multiple doses: once daily for 7 days.

Males:

Region	RAT 141 [0.5 h]	RAT 142 [2h]	RAT 143 [4h]	RAT 144 [24h]	RAT 145 [72h]	RAT 146 [168h]	RAT 147 [336h]
Adrenals	2'539	2'487	1'592	1'474	1'809	1'566	1'662
Blood	1'575	634	312	197	-	-	-
Bone marrow	5'602	5'622	6'302	4'670	8'719	3'687	5'226
Brain	169	212	119	118	-	-	-
Brown fat	766	692	455	255	255	272	230
Cartilage	21'528	19'941	17'165	9'961	4'987	2'628	3'481
Epididyme	-	672	265	-	-	-	-
Hairs	2'545	1'399	804	434	195	89	80
Heart	1'069	848	613	406	227	192	-
Intestinal wall	1'724	1'224	1'113	679	711	488	195
Lacrimal gland	474	440	325	312	269	*	214
Liver	20'247	23'272	27'227	20'122	21'806	18'424	12'640
Lung	2'582	2'444	1'416	1'462	1'003	389	314
Muscle	478	425	303	285	-	-	-
Ocular Membrane	1'132	1'322	833	709	659	566	448
Pancreas	949	904	564	464	289	241	121
Pelvis	6'963	3'915	2'012	1'515	1'273	1'193	1'036
Renal cortex	32'292	34'707	29'797	27'070	24'866	12'076	12'083
Renal fat	249	-	-	-	-	-	-
Renal medulla	14'905	15'573	20'221	15'330	13'094	5'933	6'430
Salivary gland	1'283	861	798	757	889	381	221
Skin	2'980	745	701	416	223	94	-
Spleen (red pulp)	10'687	12'096	12'193	11'131	16'816	13'753	7'130
Spleen (white pulp)	-	-	-	-	-	-	-
Stomachal wall	2'081	1'561	816	840	254	261	-
Testes	-	401	276	-	-	-	-
Thymus	1'380	1'488	1'690	1'270	1'328	1'332	354
Thyroid	1'207	*	1'016	367	662	428	96

- : below quantification limit (79 ng-b-eq/g)
*: no sample available

Females:

Region	RAT 148 [0.5h]	RAT 149 [2 h]	RAT 150 [4 h]	RAT 151 [24 h]	RAT 152 [72 h]	RAT 153 [168 h]	RAT 154 [336 h]
Adrenals	3'028	2'354	2'140	1'857	2'460	1'529	1'392
Blood	1'628	365	294	213	-	-	-
Bone marrow	5'784	7'293	5'938	4'856	4'420	3'617	4'304
Brain	-	-	-	-	-	-	-
Brown fat	-	-	-	-	-	-	-
Cartilage	23'817	27'498	23'983	15'195	4'552	3'531	2'923
Epididyme	-	-	-	-	-	-	-
Hairs	1'478	819	490	355	286	217	-
Heart	1'090	355	489	337	205	276	102
Intestinal wall	1'952	956	1'169	306	648	562	200
Lacrimal gland	-	-	-	-	-	-	-
Liver	21'531	23'714	21'244	20'764	24'737	19'771	5'284
Lung	3'012	1'547	2'199	1'449	1'153	641	274
Muscle	220	133	294	126	-	-	-
Ocular Membrane	705	831	659	895	306	377	213
Ovary	2'594	1'392	1'666	1'072	838	718	1'277
Pancreas	958	801	733	354	280	290	246
Pelvis	-	-	-	-	-	-	-
Renal cortex	53'275	93'135	45'604	42'125	31'402	29'861	6'179
Renal fat	886	401	318	-	-	-	-
Renal medulla	21'343	18'783	17'940	16'170	12'989	11'360	3'130
Salivary gland	1'770	959	886	838	579	619	297
Skin	1'437	858	1'086	336	409	186	-
Spleen (red pulp)	13'276	14'213	15'203	13'807	14'723	12'904	4'016
Spleen (white pulp)	-	-	-	-	-	-	-
Stomachal wall	1'427	892	1'049	835	498	305	181
Thymus	2'001	1'272	1'570	1'424	1'636	1'374	285
Thyroid	997	1'173	1'129	629	848	567	90
Uterus	2'762	1'457	2'305	873	818	792	666

- : below quantification limit (79 ng-eq/g)

2.6.4.5 Metabolism

Two *in vitro* studies and one *in vivo* study are reviewed in this section. Additional *in vivo* metabolism data are under Section 2.6.4.3 "Absorption".

Study No. AOM 0038 (AnorMed): *In vitro* interspecies metabolism profile of AMD3100

Key study findings: No significant levels of metabolites were detected in *in vitro* studies using liver microsomes of mouse, rat, dog, or human.

Study design and results

The metabolic pathways and the metabolites formed during incubation of AMD3100 with microsomal fractions from mouse, rat, dog, and human liver were investigated. The microsomal sample (1 mg/mL) was incubated with AMD3100 (10 µM) in a buffer containing 0.8 mM β-nicotinamide adenine dinucleotide phosphate (NADPH, cofactor) for 30 min at 37°C in duplicate. The sample volume was 250 µL. Controls included no-microsome and no-NADPH controls. AMD3100 and its metabolites were analyzed by HPLC coupled to UV detection and quantified as absolute peak area (PA). The change in peak area was calculated relative to the controls:

$$\% \text{ change in peak area} = ((PA_{sample}/PA_{control}) - 1) \times 100$$

The peak area of AMD3100 was not significantly changed after incubation with liver microsomal fractions in the presence of NADPH, when compared to the controls. The largest % change in peak area (loss of ~24%) was found in microsomal samples from the mouse liver (table from the sponsor), and was attributable to the intrinsic error of sample preparation and analysis.

Sample	AMD3100 Peak Area							
	Mouse		Rat		Dog		Human	
	A	B	A	B	A	B	A	B
No microsome control	142.2	139.6	142.2	139.6	142.2	139.6	142.2	139.6
No cofactor control	167.5	147.4	124.2	141.2	125.4	128.3	128.8	130.9
Sample	127.4	133.0	128.5	125.4	103.5	154.6	130.24	126.71
% change in peak area[3] (using no-microsome control)	-10.4	-4.7	-9.6	-10.2	-27.2	10.7	-8.4	-9.2
% change in peak area[3] (using no-cofactor control)	-23.9	-9.8	3.5	-11.2	-17.5	20.5	1.1	-3.2
Average % change in peak area (using no-microsome control)	-7.6		-9.9		-8.2		-8.8	
Average % change in peak area (using no-cofactor control)	-16.9		-3.9		1.5		-1.0	

Report No. GT-249-PK-1 (Genzyme): *In vitro* metabolic stability of plerixafor in rat, dog and human liver microsomes

Key study findings: Plerixafor was stable in the rat, dog, or human microsomes, under the conditions of the study.

Study design and results

The *in vitro* metabolic stability of plerixafor (AMD3100) was investigated by incubation with rat, dog, and human liver microsomes with or without NADPH cofactor. Plerixafor samples, at concentrations of 0.1, 1 and 10 µM, were incubated at 37°C with the liver microsomes (in triplicates at 0.5 mg/mL protein concentration) for 10, 20, 30 and 60 min. Negative controls (no cofactor) were included to account for any non-NADPH dependent reactions. Disappearance of plerixafor was measured by LC/MS/MS to assess metabolic stability. The following reactions were used as the marker substrate reaction for the

respective liver microsomes: 7-ethoxycoumarin O-dealkylation for rat and dog liver microsomes and testosterone 6β-hydroxylation for human liver microsomes.

There was no loss of plerixafor at various concentrations and time points tested, with or without NADPH, suggesting that plerixafor is stable in rat, dog and human microsomes under the condition of the study. The intrinsic clearance of plerixafor (based on time-dependent disappearance of plerixafor) in rat, dog and human microsomes is summarized in the table below:

	Intrinsic clearance (CL_{int}, mL/min/mg protein)		
	Rat	Dog	Human
Plerixafor + cofactor + microsomes	< 7.7	< 5.5	< 4.5
Plerixafor + microsomes	< 7.7	< 5.5	< 4.5

Report No. GT-249-PK-5 (Genzyme): Metabolite profile of plerixafor in plasma and urine following subcutaneous administration to rats

Key study findings:

- Three non-parent components were detected, accounting for 6-9% of total radioactivity in plasma and 16-90% of total radioactivity in urine. These components were not metabolites; they were Cu^{2+} complexes of plerixafor.

Note: This study was related to **Study No. CMS81280A ((b) (4) 7686-108):** Pharmacokinetics, excretion, mass balance, and quantitative whole-body autoradiography following subcutaneous administration of ^{14}C-AMD3100 to rats (under Section 2.6.4.4 "Distribution"). The metabolic profile of plerixafor in plasma and urine samples in the (b) (4) study was investigated. The results were compared with those obtained in **Study #9608-0258 (Sandoz):** Absorption and disposition in rats following single and multiple subcutaneous doses of [^{14}C]SDZ 282-791-ch (under Section 2.6.4.3 "Absorption") and **Study No. 9608-0256 (Sandoz):** Absorption and disposition in dogs following single subcutaneous and intravenous doses of [^{14}C]SDZ SID 791-ch (under Section 2.6.4.3 "Absorption")

Study system: Wistar Han rats (HsdRccHan:WIST) and male Long Evans rats (HsdBlu:LE).

Study design: Two groups of rats were used for whole-body autoradiography (WBA) and mass balance studies. See group designation, animal allocation, target dose level and dose volume in the table below (from the sponsor):

Group	Number of Animals Male	Number of Animals Female	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)	Samples Collected
1	3[a]	3[a]	Subcutaneous	1.23	10	Urine, feces, expired air, and carcass
2	8[b]	-	Subcutaneous	1.23	10	Blood and carcass for WBA

WBA Whole-body autoradiography.
Note: The dose was approximately 100 μCi/kg.
a Wistar Han.
b Long Evans.

Treatment: [^{14}C] AMD3100 (specific activity: 80.9 μCi/mg, purity 97.3%) subcutaneously at a dose of 1.23 mg/kg (i.e., ~ 100 μCi/kg) (injection volume 10 mL/kg)

Parameters: Metabolic profiles in urine (Group 1) and in plasma (Group 2). In an additional experiment, plerixafor was mixed with Cu^{2+} in an aqueous solution and the mixture was subjected to the same analysis for metabolic profile.

Schedule: (Group 1): urine samples collected for 0-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hr intervals. Samples were pooled and analyzed for the 0-8, 8-24 and 24-48 hr time ranges.
(Group 2): rats were sacrificed (n=1/time point) at 0.5, 2, 4, 24, 48, 72, 168 and 336 hr post dose. Blood samples collected prior sacrifice to obtain plasma.

Analysis: Urine samples were pooled based on equal weight percentage. Plasma samples were pooled based on equal volume. Plerixafor and its metabolites in plasma samples were extracted and extraction efficacy was determined by comparison of the total radioactivity in plasma following extraction versus total radioactivity in the same volume of plasma without extraction via HPLC-Radio chromatogram analysis; the extraction efficacy was on average 97%. Plerixafor and its metabolites were detected by HPLC analysis. Non-parent components detected in the HLPC separations of plasma, urine and plerixafor-Cu^{2+} solution were collected and subjected to mass spectrometry (MS) analysis to elucidate molecular structure. The HPLC method used in the Sandoz study (see above) was applied to profile the pooled urine sample from 0 to 48 hr post dose in the current study.

- Metabolic profile:
  - Non-parent components:

For male rat plasma, 3 non-parent components (I, II and III), contributing to approximately 6 to 9% of total radioactivity, were detected in the 0.5 and 2 hr pooled samples. Similarly, these non-parent components were also detected in the pooled urine samples and contributed to approximately 16%-90% of total radioactivity. There was no apparent gender difference of this profile in the urine samples. Plasma samples were collected from male rats only.

Addition of plerixafor to control rat plasma and urine samples, resulted in radioactivity chromatograms similar to those obtained for dosed animals. In addition, mixing plerixafor with Cu^{2+} generated similar chromatograms. Data suggest that the non-parent compounds may be complexes of Cu^{2+} and plerixafor.

- The molecular structure of non-parent components:

With the exception of differences in ion intensities, HPLC and MS analysis indicated all fractions (I, II and III) shared a similar spectrum, which had 7 major plerixafor-related ions (including *m/z* 313, 340, 427, 564, 678, 792 and 967). Further analysis by tandem mass spectrometry (MS/MS) demonstrated that all fractions shared the same fragmentation pattern for ions. These spectra and fragmentation patterns were consistent with complexes of plerixafor with Cu^{2+}. Full scan MS of plerixafor with Cu^{2+} solution is shown in the figure below (from the sponsor). The 1:1 and 2:1 ratios of Cu^{2+} : plerixafor that were observed were consistent with plerixafor's two potential chelating sites, the two (b) (4) rings.

(b) (4)

2.6.4.6 Excretion

See studies reviewed under Section 2.6.4.3 "Absorption" and section 2.6.4.4 "Distribution".

2.6.4.7 Pharmacokinetic drug interactions

Study N0. AOM0067 (AnorMed): Inhibition of CYP450 isoforms by AMD3100 using fluoremetric substrate detection

Key study findings: AMD3100, up to 100 μM, is not considered an inhibitor of CYP enzymes 1A2, 2C9, 2C19, 2D6 or 3A4 (IC_{50}s are greater than 100 μM).

Study design and results

The potential of AMD3100 (6.4 nM to 100 μM) to inhibit human cytochrome P450 (CYP) enzyme activity was assessed using a recombinant source of CYP450s, in conjunction with fluorescent substrates. The baculovirus expression system contained human CYP450 cDNA, human P450 reductase, and human cytochrome b5 (referred to as supersomes). IC_{50} values were determined using the appropriate fluorescent substrates for the CYP supersomes in the presence of a β-NADPH regenerating system necessary for enzyme activity. The control inhibitors were tested along with AMD3100 in the determination of the IC_{50} values. CYP inhibitors and their IC_{50} values, and corresponding fluorescent substrates are listed below.

CYP450	Fluorescent substrate	Control inhibitor	IC_{50} (μM)[1]	Historical IC_{50} (μM)[2]
1A2	CEC	Furafylline	5.64	3.43 ± 0.48
2C9	MFC	Sulphaphenazole	0.22	0.30 ± 0.05
2C19	CEC	Tranylcypromine	2.41	3.31 ± 0.13
2D6	AMMC	Quinidine	0.01	0.01 ± 0.001
3A4	DBF	Ketoconozole	0.05	0.08 ± 0.001

1. Average of 3 control IC_{50} determinations performed side by side with AMD3100 IC_{50} determination.
2. Standard errors are based on n=17
3. The abbreviations of the substrates were: CEC (7-ethoxy-3-cyanocoumarin), MFC (7-methoxy-4-trifluoromethylcoumarin), AMMC (3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methylcoumarin), DBF (dibenzylfluorescin)

Under the conditions of the study, AMD3100, up to 100 μM, showed no or minimal inhibition of the CYP enzymes tested. Results (n=3/contration) are tabulated (table from the sponsor):

P450	AMD3100 IC_{50} (μM)	% Inhibition at 100 μM
3A4	> 100 μM	5
2D6	> 100 μM	15
2C9	> 100 μM	3
2C19	> 100 μM	7
1A2	> 100 μM	9

Report N0. XT055036 (AnorMed): *In vitro* evaluation of AMD3100 as an inhibitor of human cytochrome P450 enzymes

Key study findings: under the conditions tested, AMD3100 up to 100 μM, was not a direct or a metabolism-dependent inhibitor of 1A2, 2C9, 2C19, 2D6 and 3A4/5 CYP enzymes.

Study design and results

The potential of AMD3100 in the induction of human cytochrome P450 (CYP) enzymes, including CYP1A2, 2C9, 2C19 , 2D6 and 3A4/5, was assessed in pooled human liver

microsomes (n=16 donors, mixed gender). Human liver microsomes were incubated with marker substrates (see table below) in the presence or absence of AMD3100 (concentrations ranged from 0.01 to 100 μM). In addition, the ability of AMD3100 to function as a metabolism-dependent CYP inhibitor was assessed by pre-incubation of AMD3100 with human liver microsomes and an NADPH-generating system for 0 min or 30 min to allow for the generation of metabolites or intermediates that might inhibit CYP activity. Known direct and metabolism-dependent inhibitors of CYP enzmes were included as positive controls. All analyses (determination of marker metabolites) were performed via HPLC/MS/MS methods.

The following CYP450 substrate/inhibitors were used in the direct inhibition assays:

CYP450	Substrate	CYP reaction	Control inhibitor	Vehicle	Concentration
1A2	Phenacetin	*O*-deethylation	□-Naphthoflavone	Methanol	0.5 μM
2C9	Diclofenac	4'-Hydroxylation	Sulphaphenazole	Methanol	2.0 μM
2C19	*S*-Mephenytoin	4'-Hydroxylation	Modafinil	DMSO	250 μM
2D6	Dextromethrorphen	*O*-demethylation	Quinidine	High purity H_2O	0.5 μM
3A4/5	Testosterone	6β-Hydroxylation	Ketoconozole	Methanol	0.15 μM
3A4/5	Midazolam	1'-Hydroxylation	Ketoconozole	Methanol	0.075 μM

The following CYP450 inhibitors were used in the metabolism-dependent inhibition assays:

CYP450	Control inhibitor	Vehicle	Concentration
1A2	Furafylline	DMSO	2.0 μM
2C9	Tienilic acid	Tris base	0.25 μM
2C19	Ticlopidine	High purity H_2O	0.75 μM
2D6	Metoclopramide	High purity H_2O	20 μM
3A4/5	Troleandomycin	Acetonitrile	25 μM*
3A4/5	Troleandomycin	Acetonitrile	7.5 μM**

*** In testosterone 6β-hydroxylation, ** in midazolam 1'-hydroxylation**

Under the conditions of studies, AMD3100 did not exert an inhibitory effect, directly or metabolism-dependently, on the activities of CYP enzymes tested, as no or minimal inhibition was observed at the highest concentration examined (100 μM). The result is summarized in the table below (table from the sponsor):

Table 3: Summary of results: *In vitro* evaluation of AMD3100 as an inhibitor of human CYP enzymes

		Direct inhibition		Metabolism-dependent inhibition		
		Zero-minute pre-incubation		30-minute pre-incubation		Potential for metabolism-dependent inhibition
Enzyme	CYP Reaction	IC50 (μM)	Maximum inhibition at 100 μM (%)[a]	IC50 (μM)	Maximum inhibition at 100 μM (%)[a]	
CYP1A2	Phenacetin *O*-deethylation	>100	NA	>100	NA	No
CYP2C9	Diclofenac 4'-hydroxylation-plate 1	>100	NA	>100	NA	No
CYP2C9	Diclofenac 4'-hydroxylation-plate 2	>100	NA	>100	NA	No
CYP2C19	*S*-Mephenytoin 4'-hydroxylation	>100	NA	>100	NA	No
CYP2D6	Dextromethorphan *O*-demethylation	>100	NA	>100	NA	No
CYP3A4/5	Testosterone 6β-hydroxylation	>100	2.6	>100	5.9	No
CYP3A4/5	Midazolam 1'-hydroxylation	>100	NA	>100	NA	No

Notes Values were calculated using the average data obtained from duplicates for each incubation condition. The IC50 values were calculated using XLfit.

a Maximum inhibition (%) is calculated using the following formula and data for the highest concentration of test article for which usable data were collected (results are rounded to two significant figures): Maximum inhibition (%) = 100% – Percent of solvent control activity

NA Inhibition was not observed at the highest concentration of AMD3100 studied (100 μM) as indicated by a "percent of solvent control activity" greater than 100%.

2.6.4.8 Other Pharmacokinetic Studies

Study No. AOM0069 (AnorMed): Pharmacokinetics of AMD3100 following a single 5 mg/kg subcutaneous injection in mice

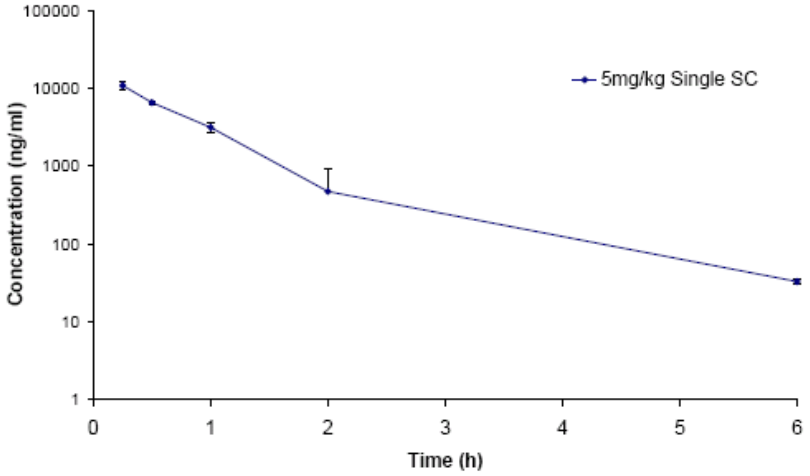
Key study findings:

- The absorption of AMD3100 following a single subcutaneous injection in mice was rapid, with t_{max} at 0.25 hr (C_{max}: 10.95 ng/mL).
- The phase 1 elimination of AMD3100 from plasma was also rapid with $t_{1/2}$ of 0.75 hr and plasma clearance (Cl/F) of 564 mL/h/kg.

Note: This report is part of a **(b) (4)** study report (#AI-A03002), entitled "Blood plasma sample collection following subcutaneous administration of 1.5, 5 and 20 mg/kg AMD11070 and 5 mg/kg AMD3100 to mice" (May 2, 2003). The sponsor has included #AI-A03002 as an appendix. Since AMD11070 is beyond the scope of this NDA, the **(b) (4)** report is not reviewed.

Study system:	Male Swiss Webster mice
Treatment:	AMD3100 (batch # 11408) subcutaneously at a single dose of 5 mg/kg.
Study design:	Blood samples (0.8-1 mL) were collected via cardiac puncture (n=2/time point) at 0.25, 0.5, 1, 2, 6, and 24 hr after dosing.
Analysis:	AMD3100 plasma concentrations were determined by LC-MS, with calibration curves comprised of concentrations ranged from 0.01 µM to 12.5 µM. Mean concentration values (n=2) were used to calculate pharmacokinetic (PK) parameters (see below). Non-compartmental PK analysis was carried out using WinNonlin V.4.0.1. Pharmacokinetic parameters were calculated as follows (table from the sponsor):

The plasma concentration time curve is depicted in the figure below, and the corresponding PK parameters are showing in the table (both figure and table are from the sponsor):



Parameter	Value
C_{max} | C_{max}/D (ng-eq/ml)	10 951 | 2190
t_{max} (h)	0.25
t_{last} (h)	6
$t_{1/2}$ (h)	0.75
AUC_{0-7} | AUC/D (ng-eq·h/ml)	8844 | 1769
AUC_{0-t} | AUC/D (ng-eq·h/ml)	8823 | 1765
$AUC_{0-\infty}$ | AUC/D (ng-eq·h/ml)	8858 | 1772
F	1.0
V_z/F(ml/kg)	612
Cl/F (ml/h/kg)	564

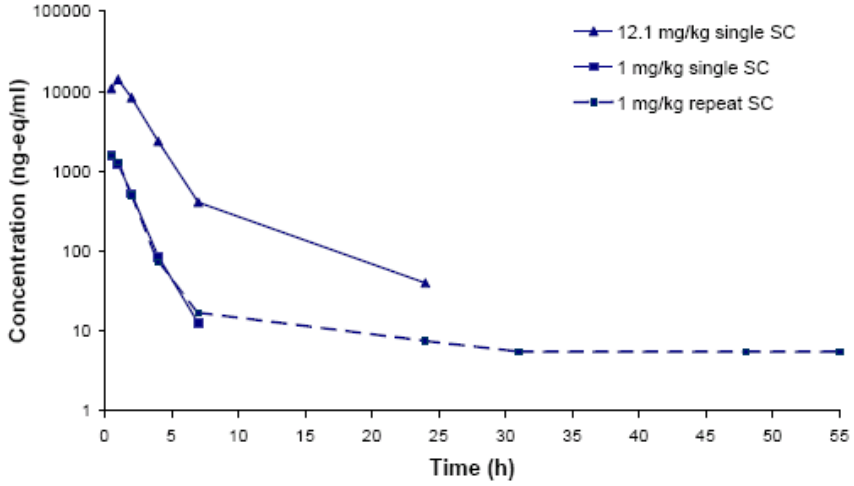
Study N0. AOM0073 (AnorMed): Noncompartmental pharmacokinetic analysis of plasma concentration versus time data for AMD3100 and radioactivity in rats from Sandoz studies 3167/DrCh, 9608.0258, and 96008.026

Key study findings:

- Higher AUC values were observed after repeated SC administration, when compared to single-dose SC administration.
- The phase 1 elimination of AMD3100 from plasma was rapid. Following SC administration of the drug to rats, at a single dose of 12.1 mg/kg, a single dose of 1 mg/kg, or repeat dose of 1 mg/kg/day, elimination $t_{1/2}$ were 1.16 hr, 0.9 hr and 0.96 hr, respectively. Elimination $t_{1/2}$ was calculated using the first phase of elimination (7 hrs) which corresponded to the majority of plasma clearance. After Hr 7, elimination occurred at a different rate, i.e. more slowly.

Note: The sponsor compiled the PK parameters for AMD3100 (^{14}C labeled) obtained from three studies in male rats: Study 3167/DrCh (single subcutaneous injection at 12.1 mg/kg), Study 9608.0258 (single SC dose of 1 mg/kg and seven once-daily SC doses of 1 mg/kg/day), and Study 9608.026 (a single oral dose at 20 mg/kg and a single intravenous dose of 2 mg/kg). The data were processed using Winonlin V.4.0.1 noncompartmental analysis, and radioactivity was expressed as ngEq/mL.

Noncompartmental PK parameters from these three studies are summarized in the table below (table from the sponsor). Log plasma concentration versus time curve for AMD3100 following SC administration to rats are shown in a figure provided by the sponsor.



Parameter	12.1 mg/kg Single SC Dose (n = 4)		1 mg/kg Single (day 1) SC Dose [day 1] (n = 4)		1 mg/kg Multiple (day 7) SC Dose [day 7] (n = 4)		1.2 mg/kg Single IV Dose(n = 4)
	Radioactivity	[^{14}C]AMD3100	Radioactivity	[^{14}C]AMD3100	Radioactivity	[^{14}C]AMD3100	Radioactivity
C_{max} | C_{max}/D (ng-eq/ml)	15403 | 1273	14125 | 1167	1513 | 1513	1550 | 1550	1526 | 1526	1583 | 1583	2658 | 2215
t_{max} (h)	1	1	0.5	0.5	0.5	0.5	0.25
t_{last} (h)	144	24	168	7	168	55	72
$t_{1/2}$ (h)	1.22	1.16	0.99	0.90	1.31	0.96	0.97
AUC_{0-7} | AUC/D (ng-eq·h/ml)	39020 | 3225	35095 | 2900	2681 | 2681	2720 | 2720	2889 | 2889	2728 | 2728	3095 | 2579
AUC_{0-24} | AUC/D (ng-eq·h/ml)	43950 | 3632	38903 | 3215	2895 | 2895	N/A | N/A	3667 | 3667	2938 | 2938	3282 | 2735
AUC_{0-t} | AUC/D (ng-eq·h/ml)	47346 | 3913	38903 | 3215	3444 | 3444	2720 | 2720	7140 | 7140	3116 | 3116	3462 | 2885
$AUC_{0-\infty}$ | AUC/D (ng-eq·h/ml)	47373 | 3915	38970 | 3221	3447 | 3447	2736 | 2736	7166 | 7166	3124 | 3124	3466 | 2888
F	1	1	1	1	1	1	-
V_z/F(ml/kg)	450	521	412	477	264	443	483
Cl/F (ml/h/kg)	255	310	290	365	140	320	346

N/A – Not applicable
ND – Not determineded

Study N0. AOM0074 (AnorMed): Noncompartmental pharmacokinetic analysis of plasma concentration versus time data for AMD3100 and radioactivity in dogs from Sandoz study 96008.0256

Key study findings:

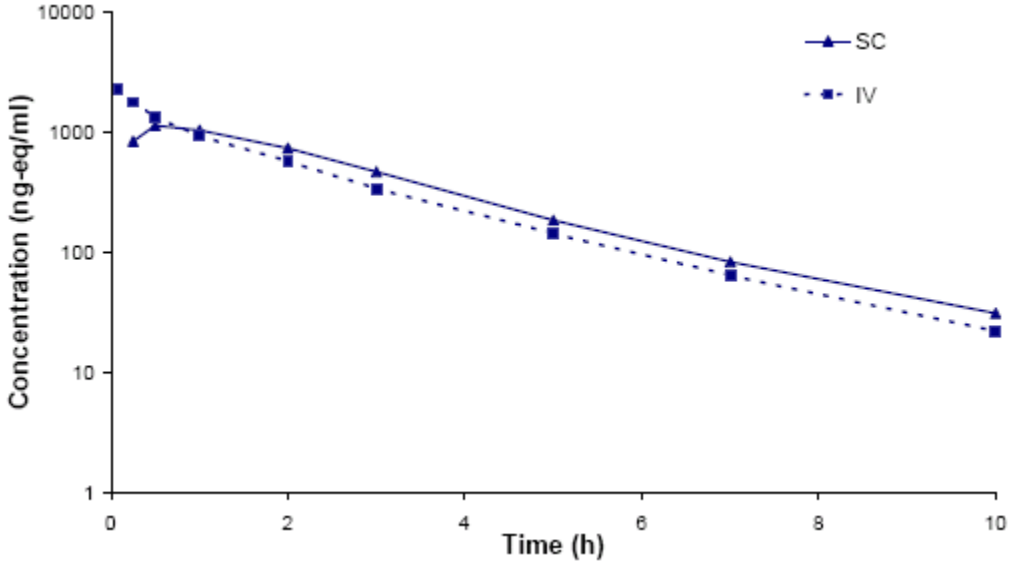
- The PK parameters were generally comparable following a single SC or IV administration, except twice higher C_{max} values were observed after IV dosing.

- The elimination of AMD3100 from plasma was rapid, with elimination $t_{1/2}$ of 1.58 hr and 1.56 hr following SC and IV administration, respectively.

Note: The unit of AUC was converted from pmol•h/mL to ngEq•h/mL.

Noncompartmental PK parameters are summarized in the table below (table from the sponsor). Log plasma concentration versus time curve for AMD3100 following SC administration to rats are shown in a figure provided by the sponsor.

Parameter	0.25 mg/kg Single SC Dose (n = 3)		0.25 mg/kg Single IV Dose (n = 3)	
	Radioactivity	[^{14}C]AMD3100	Radioactivity	[^{14}C]AMD3100
C_{max} | C_{max}/D (ng-eq/ml)	525 | 2100	568 | 2272	1057 | 4228	1142 | 4568
t_{max} (h)	0.5	0.5	0.08	0.08
t_{last} (h)	178	10	168	10
$t_{1/2e}$ (h)	1.93	1.58	1.74	1.56
AUC_{0-7} | AUC/D (ng-eq-h/ml)	1587 | 6348	1652 | 6608	1598 | 6392	1645 | 6580
AUC_{0-24} | AUC/D (ng-eq-h/ml)	1849 | 7396	-	1792 | 7168	-
AUC_{0-t} | AUC/D (ng-eq-h/ml)	2179 | 8716	1738 | 6952	2061 | 8244	1710 | 6840
$AUC_{0-\infty}$ | AUC/D (ng-eq-h/ml)	2184 | 8736	1773 | 7092	2065 | 8260	1735 | 6940
F	1.0	1.0	-	
V_z/F(ml/kg)	318	322	303	324
Cl/F (ml/h/kg)	114	141	121	144



After a single IV administration, AMD3100 elimination from plasma followed a bi-exponential manner. The first phase of elimination following a SC dose was obscured by the absorption phase, as a result only a single phase of elimination can be observed.

2.6.4.9 Discussion and Conclusions

ADME

- Considering the route of administration (i.e. SC), absorption of radioactivity is expected to be complete. This was confirmed in the studies.
- Subcutaneously administered AMD3100 was absorbed rapidly (t_{max} was ~ 0.5-1 hr) after single- or repeat-dose administration.
- Subcutaneously administered AMD3100 was distributed widely to various tissues. Radioactivity was detectable in most tissues/organs up to 144 hr (6 days) post-dose. The following tissues/organs had high and/or sustained radioactivity concentrations: injection site, liver, kidney, and spleen (high and sustained radioactivity); adrenal, epiphyseal plate and cartilage had high levels of radioactivity mostly up to 4 hr post-dose. While radioactivity decreased in most organs/tissues from 0.5 hr to 4 hr after dose administration, in bone marrow, kidney, liver, and spleen, radioactivity was still detectable at 336 hr post dose.
- Because small concentrations of radioactivity were measureable through 336 hr post dose in uveal tract and skin, AMD3100 or AMD3100-derived compounds may have affinity for melanin.
- Multiple-dose SC administration of AMD3100 can cause the drug or drug-derived compounds to accumulate in tissues/organs.
- AMD3100 has the potential to cross the blood-brain barrier: small amounts of radioactivity were detected in the pituitary gland (up to 336 hrs post-dose, last time-point examined). In addition, occasional and small amounts of radioactivity were detected in cerebrum, olfactory lobe, and spinal fluid.
- Plerixafor (AMD3100) distribution to red blood cells was not significant in rat and dog whole blood, after 2 hrs of incubation at 37º C. A small fraction of plerixafor was distributed to human RBC, independent of concentrations tested (0.1 and 1 μM of AMD3100).
- No significant levels of metabolites were detected in *in vitro* studies (using liver microsomes of mouse, rat, dog, or human) or in urine and plasma samples collected following subcutaneously administered plerixafor in rats.
- Three non-parent components were detected, accounting for 6-9% of total radioactivity in plasma and 16-90% of total radioactivity in urine. These components were likely to be Cu^{2+} complexes with plerixafor.
- The major component in the plasma or urine was the parent drug (more than 60% of radioactivity in most SC or IV studies reviewed). The major component in the feces was also the parent drug.
- Plerixafor (AMD3100) was stable in human, dog, and rat plasma after 4 hrs of incubation at 37º C.
- Under the conditions tested, AMD3100 up to 100 μM, was not a direct or a metabolism-dependent inhibitor of 1A2, 2C9, 2C19, 2D6 and 3A4/5 CYP enzymes.
- The plasma protein binding of AMD3100 (1-10 μg/mL) was moderate (33-54% for rat, 34-46% for dog, and 37-58% in human). In the 3 species tested, the highest concentration of AMD3100 resulted in reduced plasma protein binding, suggesting saturation of protein binding at a concentration between 3 and 10 μg/mL.

- The elimination half-life of AMD3100 after SC administration was ~1 hr in rats. The elimination half-life was ~1.5 hr in dogs (data not reviewed) and ~ 4.8 hrs in humans (based on summary of data provided by the sponsor.
- The main excretion route of SDZ 282-791 was via urine after SC or IV administration (e.g., ~70% and 60% after single- and repeat-dose SC administration, respectively). Fecal excretion was small after SC or IV dosing; up to ~10%. Some of the radioactivity was retained in tissues/organs for an extended period of time, as shown by detectable levels of radioactivity after 6 or 7 days post-dose.

PK/TK data from the repeat-dose (up to 4 weeks) toxicology studies

- Exposure to plerixafor following SC administration to rats and dogs was mostly dose proportional at 1-12 mg/kg or 18-24 mg/kg for rats and 0.25-4 mg/kg for dogs.
- Slightly increased exposures during Week 4, more evident in dogs than rats, suggest the potential for AMD3100 accumulation after repeated dosing.
- There were no obvious differences in the PK parameters in males versus females.
- T_{max} was approximately 1 hr in dogs.

2.6.4.10 Tables and figures to include comparative TK summary

The following data are from studies reviewed in the Toxicology section (i.e., repeat-dose toxicity studies, Section 2.6.6.3). These studies are: in rats: Study #428R (A 4-week subcutaneous toxicity study in rats), #432R (An additional 4-week subcutaneous toxicity study in rats), and #(b) (4) 900519 (A subcutaneous injection teratology study in the rats); in dogs: (b) (4) (b) (4) No. 94 (study by subcutaneous administration to Beagle dogs for 4 weeks followed by a 2-week reversibility period). See respective sections for details.

Species (Duration)	Dose		Sex	C_{max} (ng/mL)		AUC (ng•h/mL)		Dose normalized (b) C_{max}		Dose normalized (b) AUC	
	Salt (mg/kg) (a)	Base (mg/kg) (a)		Day 1	End of Study	Day 1	End of Study	Day 1	End of Study	Day 1	End of Study
Rat (4-week)	1	0.6	M F	2450 1390	2730 1870	8760 3310	8190 6510	4083 2317	4550 3117	14600 5517	13650 10850
	3	1.9	M F	6080 4890	7550 6250	21900 19600	30360 22390	3200 2574	3974 3289	11526 10316	15979 11784
	12	7.6	M F	17130 16980	26610 27070	57000 67450	63500 62660	2254 2234	3501 3562	7500 8875	8355 8245
Rat (4-week)	18	11.4	M F	36900 28400	47800 47000	125600 111800	134800 110900	3237 2491	4193 4123	11018 9807	11825 9728
	24	15.2	M F	33400 32100	NS 54600	139900 129400	NS 161700	2197 2112	---- 3592	9204 8513	---- 10638
Rat (GD6-17)	NA	0.5	F	843*	924**	3154	2102	1686	1848	6307	4204
	NA	3	F	4574	5951	14056	16751	1525	1984	4685	5584
	NA	15	F	13369	23047	63473	81296	891	1536	4232	5420
Dog (4-week)	0.25	0.16	M F	272 220	289 190	2056 1590	2549 1500	1700 1375	1806 1188	12850 9938	15931 9375
	1	0.6	M F	869 1090	1236 1300	4578 6260	6915 7610	1448 1817	2060 2167	7630 10433	11525 12683
	4	2.5	M F	4565 6880	6188 12140	22678 31290	25803 39140	1826 2752	2475 4856	9071 12516	10321 15656

NS: no samples due to mortality
a: Plerixafor (SDZ SID 791) salt and free base, (b) (4)
b: Dose normalized C_{max} and AUC values were normalized by free base dose levels.
***: on GD 6 and **: on GD 17.**

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

✧ Summary of plasma pharmacokinetic parameters in male rats following SC and IV administration (from the sponsor: Module 2, Section 2.6.4, Table 2.6.4-5):

Table 2.6.4-5: Mean ± SD Plasma Pharmacokinetic Parameters of Radioactivity and [^{14}C]-Plerixafor in Male Rat Following SC and IV Administration

Parameter	12.1 mg/kg Single SC Dose (n = 4)		1.0 mg/kg Single SC Dose [Day 1] (n = 4)		1.0 mg/kg Multiple SC Dose [Day 7] (n = 4)		1.2 mg/kg Single IV Dose (n = 4)	
	Total Radioactivity	[^{14}C]-Plerixafor	Total Radioactivity	[^{14}C]-Plerixafor	Total Radioactivity	[^{14}C]-Plerixafor	Total Radioactivity	[^{14}C]-Plerixafor
C_{max} (ng-eq/ml)	15,403	14,125	1513	1550	1526	1583	2658	ND
C_{max}/D (ng-eq/ml)	1273	1167	1513	1550	1526	1583	2215	ND
t_{max} (h)	1	1	0.5	0.5	0.5	0.5 ± 0	0.25	ND
t_{last} (h)	144	24	168	7	168	55	72	ND
$t_{1/2}$ (h)	1.22	1.16	0.99	0.90	1.31	0.96	0.97	ND
AUC_{0-7} (ng-eq·h/ml)	39,020	35,095	2681	2720	2889	2728 ± 223	3095	ND
AUC_{0-7}/D (ng-eq·h/ml)	3225	2900	2681	2720	2889	2728	2579	ND
AUC_{0-24} (ng-eq·h/ml)	43,950	38,903	2895	N/A	3667	2938	3282	2947[a]
AUC_{0-24}/D (ng-eq·h/ml)	3632	3215	2895	N/A	3667	2938	2735	2456
V_z (ml/kg)	450	521	412	477	264	443	483	ND
Cl (ml/h/kg)	255	310	290	365	140	320	346	ND

[a] pooled plasma AUC_{0-24} from Study No. 9608.026.
N/A – not applicable, ND – not determined

Note: the above data are from Study 3167 DrCH (single SC dose), Study 9608.0258 (single and multiple SC doe), and Study 9608.026 (single IV dose). Some of the data regarding Study 3167 DrCH and Study 9608.0258 are slightly different from those in the table below, which are based on Study AOM0073.

✧ Non-compartmental PK parameters following a single dose of pleroxafor (SDZ SID 791, or AMD3100) via oral, subcutaneous or intravenous administration to mouse, rat, dog and human are summarized in the following table (from the sponsor: Module 2, Section 2.6.5, Table 2.6.5-3)

Table 2.6.5-3: Pharmacokinetics: Absorption After a Single Dose

Species	Mouse	Rat[a]		Rat[a]		Rat[a]	Rat	Dog[b]		Dog[b]		Human
Study No.	AOM0069	3167/DrCH		9608.0258		9608.026	9608.026	9608.0256		9608.0256		010237
Number of Animals/Subjects per timepoint – Gender (M/F)	2M	4M		4M		4M	4M	3M		3M		3M, 2F
Feeding Condition	Fasted	Fasted		Fasted		Fasted	Fasted	Fasted		Fasted		Fed
Vehicle/Formulation	Plerixafor in Water	[^{14}C]Plerixafor Octahydrochloride Dihydrate in Water		[^{14}C]Plerixafor Octahydrochloride Dihydrate in Water		[^{14}C]Plerixafor Octahydrochloride Dihydrate in Water	[^{14}C]Plerixafor Octahydrochloride Dihydrate in Water	[^{14}C]Plerixafor Octahydrochloride Dihydrate in Water		[^{14}C]Plerixafor Octahydrochloride Dihydrate in Water		Plerixafor in Water
Route	SC	SC		SC		IV	PO	SC		IV		SC
Dose (mg/kg)[c]	5	12.1		1.0		1.2	12.1	0.25		0.25		0.24
Sample	Plasma	Plasma		Plasma		Plasma	Plasma	Plasma		Plasma		Plasma
Analyte	plerixafor	TRA	RAP	TRA	RAP	TRA	TRA	TRA	RAP	TRA	RAP	plerixafor
Assay	HPLC-MS	LSC	HPLC-LSC	LSC	LC-RID	LSC	LSC	LSC	LC-RID	LSC	LC-RID	HPLC-ECD
C_{max} (ng/ml or ng-eq/ml)	10,951	15,403	14,125	1517	1550	2658	149[d]	525	568	1057	1142	847
t_{max} (h)	0.25	1	1	0.5	0.5	0.25	0.7	0.5	0.5	0.08	0.08	0.65
t_{last} (h)	6	144	24	168	7	72	4	178	10	168	10	24
$t_{1/2}$ (h)	0.75	1.22	1.16	0.99	0.90	0.97	ND	1.93	1.58	1.74	1.56	4.83
$AUC_{0-\infty}$ (ng·h/ml or ng-eq·h/ml)	8858	47373	38,970	3447	2736	3466	ND	2184	1773	2065	1735	3159

Abbreviations: TRA – Total Radioactivity, RAP – Radioactive Parent

[a] PK parameters calculated in report AOM0073

[b] PK parameters calculated in report AOM0074

[c] Doses are expressed as plerixafor free base

[d] Calculated using animals 1, 2 and 3

✧ Summary of excretion of radioactivity in male rats following SC, oral and IV administration (from the sponsor: Module 2, Section 2.6.4, Table 2.6.4-9):

Table 2.6.4-9: Excretion of Radioactivity in Male Rats Following 1 mg/kg SC, 12.1 mg/kg SC, 12.1 mg/kg PO and 1.2 mg/kg IV Doses

Matrix	Time (h)	Mean % of Administered Radioactivity ± SD			
		1 mg/kg SC Dose (n = 4)	12.1 mg/kg SC Dose (n = 4)	12.1 mg/kg PO Dose (n = 4)	1.2 mg/kg IV Dose (n = 4)
Urine	0–7	ND	ND	0.7 ± 0.0[a]	61.3 ± 6.7
	0–24	65.9 ± 2.0	57.3 ± 3.4	3.1 ± 4.1	67.2 ± 7.5
	0–48	67.8 ± 1.9	60.6 ± 3.7	3.3 ± 4.8[a]	68.8 ± 7.4
	0–72	68.9 ± 1.9	61.1 ± 4.2	3.6 ± 5.1[a]	69.8 ± 7.2
	0–144	ND	63.5 ± 3.4	ND	ND
	0–168	71.7 ± 2.5	ND	ND	ND
	Cage wash (0–168)	0.3 ± 0.1	0.4 ± 0.3	0.0 ± 0.0[a]	0.5 ± 0.7
	Total urine (0–168)	72	63.9 ± 3.2	3.9 ± 5.0	70.4 ± 6.8
Faeces	0–24	2.7 ± 0.5	0.7 ± 0.5	29.2 ± 44.0	1.2 ± 1.3
	0–48	4.6 ± 0.9	3.6 ± 0.9	54.6 ± 37.5[a]	3.1 ± 2.7[a]
	0–72	6.0 ± 1.2	5.0 ± 1.7	59.1 ± 35.4[a]	4.4 ± 1.4[a]
	0–144	ND	7.3 ± 3.5	ND	ND
	0–168	8.9 ± 2.1	ND	ND	ND
	Intestinal content (0–168)	0.2 ± 0.1	0.4 ± 0.2[a]	21.8 ± 35.6	2.0 ± 1.0
	Total faeces (0–168)	9.1	7.7 ± 3.7	80.9 ± 7.3[a]	5.3 ± 1.6
Carcass	Carcass	27.5 ± 1.3	18.1 ± 2.9	2.6 ± 1.6	23.8 ± 1.0
Mass Balance	Recovery (0–168)	108.5 ± 1.3	89.6 ± 3.6	87.4 ± 1.4	99.4 ± 6.3

[a] n = 3

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

The toxicity of subcutaneous administration of plerixafor (AMD3100) was investigated in mice (single dose), rats (up to 4 weeks), and dogs (15 days and 4 weeks). Single dosing via subcutaneous (SC) or intravenous (IV) injections in mice and rats induced mainly neurological findings, including sedation, spasms, dyspnea and ventral recumbency. LD_{50} values were 16 and > 50 mg/kg for SC administration in mice and rats, respectively. LD_{50} value was 5.2 mg/kg for IV administration in both mice and rats. Daily treatment with plerixafor up to 18 mg/kg/d (rats) or 4 mg/kg/d (dogs) for 4 weeks was tolerated. The common findings included: GI (diarrhea, emesis, increased defication, salivation in dog) and nervous system (sedation, tremors, spasms, twitching, recumbency and ataxia and mydriasis in dog) related clinical signs, decreased body weight gain/food consumption, increased white counts (total and differential), changes in seral and urine levels of calcium and magnesium. Increased urinary calcium and magnesium, indicating loss of electrolytes, was accompanied by reduction in bone mineral content and bone volume in at least one study. Increased calcium levels in cerebro-spinal fluid was reported in dogs in a non-GLP dose range finding study (this study was not reviewed). The target organs were liver (hematopoiesis), spleen (increased hematopoiesis and lymphoid atrophy), thymus (congestion and lymphoid atrophy), and injection sites (subcutaneous hemorrhage and inflammation). Although there was no histopathological evidence of lesions in the heart, cardiovascular effects were seen in animals (see below). Prolonged duration in treatment (single dose vs. 2 weeks vs. 4 weeks) of plerixafor did not introduce different toxicology findings. There were no ECG changes in the dog. Hypertensive and tachycardic effects of plerixafor observed in dogs may be secondary to CNS effects or may be due to exaggerated pharmacodynamic effects, i.e., manipulation of signaling pathways of PI3K/AKT/eNOS (and VEGF/bEGF) or altered/dyscfunctional vasoconstriction via inhibition of CXCR4/SDF-1α interaction.

Genetic toxicology:

Plerixafor was not mutagenic in bacterial Ames test (*Salmonella typhimurium* TA98, TA97a, TA100, TA102 and TA1535), and was not clastogenic in the chromosome aberration test in V79 Chinese hamster cells (CHO), in the presence or absence of rat liver S9-mix. Subcutaneously administered plerixafor up to 25 mg/kg did not induce bone marrow toxicity and did not induce micronuleus formation.

Carcinogenicity:

Not conducted.

Reproductive toxicology:

The reproductive and developmental toxicology (embryofetal development) of plerixafor was investigated in both rodent (pivotal study in rats) and non-rodent (pilot study in rabbits) species. The study in rabbits was not reviewed; any information presented in this review is based on summary information provided by the sponsor. In rats, embryofetal toxicities were

seen in the presence of maternal toxicity (deficits in corrected gestation weight gain and decreased food consumption). Similarly, maternal toxicities, including mortality, body weight loss, decreased food consumption and clinical signs, were accompanied by embryofetal toxicities in rabbits. Plerixafor caused dose-dependent embryotoxicity (increased resorption and post-implantation loss in rats and rabbits, decreased fetal weight in rats, and decreased litter size in rabbits), as well as fetal toxicity (external, visceral and head malformations and/or variations in rats, and external malformation in rabbits). The data indicated that pleixafor was teratogenic under the conditions of the studies.

Special toxicology:
Plerixafor administered intracutaneously to New Zealand white rabbits demonstrated a concentration-related and formulation-dependent local irritation effect with severity more noticeable in the HCl-based formulation (i.e., the clinical formulation) when compared to the formulation containing citric acid. Subcutaneous administration of plerixafor to rats did not inhibit antibody formation to sheep red blood cells under the conditions tested. Plerixafor was not hemolytic in the *in vitro* assay in human whole blood.

2.6.6.2 Single-dose toxicity
Single dose studies via subcutaneous or intravenous administration in mouse, and rat are reviewed and the findings are briefly summarized below.

Key study findings:
- Single doses of SDZ 282 791, administered subcutaneously (SC) or intravenously (IV) in rodents (mice and rats), induced mortality. LD_{50} values were as follows: SC: 16.3 mg/kg and >50 mg/kg, for mice and rats, respectively, and IV: 5.2 mg/kg for both mice and rats. Clinical signs of toxicity included sedation, spasm, dyspenia, and ventral recumbency.
- The clinical signs were transient and mostly dose-related.

Studies in mice: studies followed the OECD-GLP regulation
Study RCC Project 380586: Acute subcutaneous toxicity study in mice
Study RCC Project 380564: Acute intravenous toxicity study in mice

Objective:
The tolerability of SDZ SID 791 was investigated in mice after a single SC or IV injection, followed by a 14-day observation period.

Methods and results:
- ✧ Animals: HanIbm:NMRI (SPF) mice
- ✧ Parameters

Mortality and clinical signs	Four times during Day 1 and once daily Days 2-15
Body weight	On Days 1, 5 and 8
Mean lethal dose	LOG-LOGIT-model (COX, Analysis of Binary Data)

Results:

✧ Treatments and results are as follows:

Test System	Subcutaneous	Intravenous
Dose (mg/kg)	0 (placebo*), 2, 14 and 20 (G1, 2, 3, 4), at 2 mL/kg	0 (placebo), 2, 5 and 8 (G1, 2, 3, 4), at 2 mL/kg
Dose (mg/m^2)	6, 42 and 60 mg/m^2/day, for G2, 3 and 4, respectively	6, 15 and 24 mg/m^2/day, for G2, 3 and 4 respectively
N (n/sex/group)	N= 5	N=5
Mortality	30% in G3 (M: 1/5, F: 2/5), 70% in G4 (M: 3/5, F: 4/5), all on Day 1.	40% in G3 (M: 2/5, F: 2/5), 90% in G4 (M: 4/5, F: 5/5), all on Day 1.
Clinical sign	CNS signs in G3 and G4: • Sedation, during 1 and 2 hr post dose (PD): ➢ G3: M 1-2/5, 1-2/5 (severity**/# animals) ➢ G4: M 1-2/5, F 1/5 • Spasm, during 1st hr PD ➢ G3: F 1-2/4 ➢ G4: M 2/5, F 2/5 • Dyspenia, during 1 and 2 hr PD ➢ G3: M 1/5, F 1/5 ➢ G4: M 1/2, F 1/1 • Ventral recumbency, during 1st hr PD ➢ G3: F 1/2 ➢ G4: M 1/5, F 1/5 • Abnormal posture, during 1st hr PD ➢ G3: M 1/1	CNS signs in all mice , except G1: • Sedation, within 1st hr post dose (PD): ➢ G2: M 1/5, F 1/5 ➢ G3: M 1-2/5, 1-2/5 ➢ G4: M 1-3/5, F 3/5 • Spasm, within 1st hr PD ➢ G3: M 2/5, F 1-2/4 ➢ G4: M 1-2/5, F 2/5 • Dyspenia, within 1st hr PD ➢ G3: M 1/5, F 1/5 • Ventral recumbency, within 1st hr PD ➢ G4: M 1/5, F 1/5
Body Weights	Not remarkable	Not remarkable
Necropsy findings	Not remarkable	Not remarkable
LD_{50} (mg/kg)	M: 22, F: 12.7, pooled: 16.3	M: 5.4, F: 4.7, pooled: 5.2

*** The composition of the placebo was not reported.**
**** Severity: maximum grade: 3**
PD: Post-dose
G1, G2, G3, and G4: Group 1 (control), Groups 2, 3 and 4, respectively.

Studies in rats: both studies followed the OECD-GLP regulation
Study RCC Project 380575: Acute subcutaneous toxicity study in rats
Study RCC Project 379787: Acute intravenous toxicity study in rats

Objective:

The tolerability of SDZ SID 791 was investigated in rats after a single SC or IV injection, followed by a 14-day observation period

Methods and results:

✧ Animals: HanIbm:WIST (SPF) rats

✧ Parameters

Mortality and clinical signs	Four or five times during Day 1 and once daily Days 2-15
Body weight	On Days 1, 5 and 8
Mean lethal dose	LOG-LOGIT-model (COX, Analysis of Binary Data)

Results:

✧ Treatments and results are as follows:

Test System	Subcutaneous	Intravenous
Dose (mg/kg)	0 (placebo*), 2, 20, 30, 40 and 50 (G1, 2, 3, 4, 5 and 6), at 2-5 mL/kg	0 (placebo), 2, 5 and 8 (G1, 2, 3, 4), at 2 mL/kg
Dose (mg/m^2)	12, 120, 180, 240 and 300 mg/m^2/day, for G2, 3, 4, 5 and 6, respectively	12, 30 and 48 mg/m^2/day, for G2, 3 and 4 respectively
N (n/sex/group)	N= 5	N=5
Mortality	30% in G5 (F: 3/5), 30% in G6 (M: 1/5, F: 2/5), all on Day 1 (1-2 hr post dose).	40% in G3 (M: 1/5, F: 3/5), 90% in G4 (M: 4/5, F: 5/5), all on Day 1.
Clinical sign	CNS signs in G3-G6, including sedation, spasm, dyspenia, uncontrolled movements, ventral or lateral recumbency, hunched posture. The signs were transient and most obvious in the first 2-3 hours PD. Severity was dose-dependent in most findings. In addition, ruffled fur was observed in G4-G6 and the finding lasted up to Day 6or 7 PD.	CNS signs in all mice , except G1: • Sedation, up to 1st hr post dose (PD): ➢ G2: M 1/5, F 1/5 ➢ G3: M 2-3/5, F 1-3/5 ➢ G4: M 1/1 • Spasm, within 1st hr PD ➢ G3: M 2/5, F 2-3/4 ➢ G4: M 2/5, F 2/5 • Dyspenia, within 1st hr PD ➢ G3: M 1/5, F 1/5 ➢ G4: M 1-2/5, F 2/5 • Ventral recumbency, within 1st hr PD ➢ G3: M 1/5, F 1/5
Body Weights	Reduced weight gains in G4-6 (see table below)	Not remarkable
Necropsy findings	Not remarkable	Not remarkable
LD_{50} (mg/kg)	> 50 mg/kg (estimated, the LOGIT model could not be applied to these data)	M: 6., F: 4.3, pooled: 5.2

*** The composition of the placebo was not reported.**
**** Severity: maximum grade: 3**
PD: Post-dose
G1, G2, G3, and G4: Group 1 (control), Groups 2, 3 and 4, respectively.

Summary of weight gains in SDZ SID 791-treated (subcutaneously) rats: data are expressed as (positive) weight gain (g)/percent change from Day 1 (%)

	Males				females			
	Group 1	Group 4	Group 5	Group 6	Group 1	Group 4	Group 5	Group 6
Day 1-Day 8	36 /15	22.7/9	28.8/13	20.1/9	9.7/4	2.3/1	5.3/3	8.9/5
Day 1-Day 15	58.9/24	41.5/17	48.7/22	40.5/19	19.2/10	10.4/5	14.4/7	16.2/8

Comment:
The decrease in weight gains in SDZ SID 791-treated rats was not dose-dependent. This may be partly due to changes in the number of rats in higher dose groups, because of unscheduled deaths.

2.6.6.3 Repeat-dose toxicity

■ Once daily doses:

Study title:

A 4-week subcutaneous toxicity study in rats

Key study findings:

- AMD3100 related toxicities, included: decreased body weight gains/food intake, hematological effects (↑ total and differentiated white blood cell counts), decreased serum magnesium, and increased urinary levels of calcium and magnesium.
- The main target organs were liver (hematopoiesis) and injection sites (subcutaneous hemorrhage and inflammation).

Study no.: (b) (4) Study 428R

Volume #, and page #: Electronic, module 4 (428r-tox.pdf)

Conducting laboratory and location: (b) (4)

Date of study initiation: April 6, 1994

GLP compliance: Yes

QA report: yes (X) no ( )

Drug, lot #, and % purity: AMD3100 (SDZ SID 791-ch), Batch # Y021 0294, purity: 100.3% (RP-HPLC); placebo (for Group 1, control): 5 mL ampoule consisting dinatriumhydrogen-phosphate (5 mg), NaCl (6 mg), HCl 25%, and water for injection up to 1 mL (batch # Y022 0194)

Reviewer's note: the compound used in this study was the hydrochloride salt of SDZ SID 791, and the dose levels refer to the salt. (b) (4)

Methods

Doses: 0 (control), 1, 3 and 12 mg/kg (free base, as Groups 1, 2, 3 and 4) [or, 0.6, 1.9 and 7.6 mg free base/kg, respectively]

Species/strain: Rats (SPF, HanIbm: WIST; BRL)

Number/sex/group or time point:

Main study (428R): n=10/sex/group

Recovery animals (428RR): n=6/sex/group for Groups 1 and 4

Satellite groups used for toxicokinetics study (428KR): 4/sex/group

Route, formulation, volume: subcutaneous injection at a dose volume of 2 mL/kg

- Formulation: AMD3100 lyophilisate was first reconstituted with the vehicle, and further diluted with 0.9% NaCl at dose concentrations of 0.5, 1.5 and 6 mg/mL (0.05%, 0.15% and 0.6%) for Groups 2, 3 and 4, respectively.
- Vehicle for AMD3100 lyophilisate: vehicle injection solution 5 mL ampoule (batch # Y029 0194), consisting of dinatriumhydrogen-phosphate (5 mg/mL), NaOH (2.84 mg/mL) and water for injection up to 1 mL
- The control animals were injected with placebo (see above) at volume of administration corresponding to the high dose (Group 4).

Age:	~8-9 weeks
Weight:	143-334 g
Schedule:	Once daily for 4 consecutive weeks (31/32 days in main study animals, and 28 days for recovery animals). The main study was followed by a 5-week (35-day) recovery period.

Dose justification: Dose selection was based on a dose range-finding study (Study # 189DFR, not reviewed), in which subcutaneous injection of AMD3100 at doses of 5, 10, 15 and 20 mg/kg (daily, for 4 weeks) resulted in mortality (one at 15 mg/kg, 3 at 20 mg/kg, cause of death was undetermined and the administration of 20 mg/kg was terminated earlier). The following toxicities were observed: clinical signs such as sedation and piloerection (≥ 5 mg/kg), ventral recumbency, twitch and labored respiration (≥ 10 mg/kg), elevated body temperature (1-2 °C, ≥ 15 mg/kg), decreased body weight gains, hematological changes (≥ 15 mg/kg, ↓ RBC and HGB, ↑ reticulocytes , white blood cell and platelet counts), ↓ serum calcium and magnesium, and ↑ urine calcium levels. The histological findings identified the target organs as liver and spleen (increased extramedullary hematopoiesis and megakaryocytes, dose-dependent at all doses) and thymus (histiocyte aggregates and focal lymphoid hyperplasia). The TK data indicated a T_{max} values at 1-2 hours, and higher exposures, thus higher toxicities, in the males.

Observation and Times:

Clinical signs:	Once daily for mortality, moribundity and gross abnormality. The highest grade of severity of the symptom in individual animals was recorded daily.
Body weights:	Weekly for weight gain. Individual body weight was determined daily.
Food consumption:	Once weekly during the acclimation, dosing and recovery periods.
Body temperature:	Rectal temperature was measured on treatment Day 25 before dosing and 0.5, 2 and 6 hr after dosing in all TK animals.
Ophthalmoscopy:	Once prior to the start of treatment in all main and recovery animals and on surviving Groups 1 and 4 main study animals on Day 23.
EKG:	Not performed.
Hematology:	Blood samples were taken (from right retroorbital plexus) at pretest and 24 hr after the last treatment (Day 29) in main study animals, as well as on Days 28, 44 and 58 in recovery animals.
Clinical chemistry:	Blood samples were taken (from right retroorbital plexus) at pretest and 24 hr after the second (Day 3) and the last treatment (Day 29) in main study animals, as well as on Days 28 and 44 in recovery animals.
Urinalysis:	At pretest and on Day 29 (all groups) and on Days 28 and 44 (recovery groups), urine was collected for ~ 24 hours.
Bone marrow smears:	From the primary and recovery necropsies. Samples were only examined microscopically for Groups 1 and 4.
Gross pathology:	Scheduled sacrifice: Day 31/32 or Day 63.
Organ weights:	At scheduled sacrifice. Adrenal, brain, heart, kidney, liver, ovaries, pancreas, pituitary, prostate, spleen, testis (including epididymides), thymus, thyroid, and uterus. In case of mortality, organs were not to be weighed from animals died or euthanized as moribund.

Histopathology: Day 31/32 or Day 63 (main study and recovery groups, respectively). All tissues collected from all animal. For tissues identified microscopically as potential targets (i.e., liver and injection site), the H&E stained slides were examined in male or female animals in Groups 2 and 3 of the main study and recovery groups (Groups 1 and 4). Other organs were only examined in the control and Group 4 of the main study. In case of mortality, all organs were examined in all unscheduled deaths. See inventory list for organs examined.

Toxicokinetics: Trough level and C_{max}: Blood samples (0.5 mL) were collected from main study animals on Days 30/31 (C_{max}:1 hour after dosing) and 24 hr after dosing (trough level, Days 31/32). Blood samples were collected from TK animals 1 hr (C_{max}) and 24 hr (trough) after dosing on Day 15. TK study: Blood samples were collected from TK animals on Days 1 and 30 , at 0.5, 1, 2, 4, 7, and 24 hours post-dose (n=4/ time point). LLOQ was 50 ng/mL plasma (via HPLC analysis).

Results:

Mortality: No mortality occurred.

Clinical signs:

AMD3100-related clinical signs were mainly in Group 4, including increased incidence of ventral recumbency, rales, twitching, and/or necrosis near injection sites in all AMD3100-treated males and females. The findings resolved. The incidence during Day 1 and Day 30 are summarized in the table below: data are indicated as incidence/animals affected.

Study Group	428R			428RR	428KR	
Sex	M	F		M	M	F
Dose Group	G4	G1	G4	G4	G4	G4
Number of animals	10	10	10	6	4	4
Ventral recombency	3/3		2/1	2/2	4/4	4/4
Twitching	3/3		2/1	2/2	4/4	9/4
Rales	3/3		2/1	2/2	4/4	4/4
Necrosis (injection site)	6/1	8/1	13/2			

Study Groups: 428R (main study group), 428RR (recovery group), and 428KR (toxicokinetic [TK] group).
Dose Group: G1 and G4 for Groups 1 (control) and 4, respectively.

Comments:

Except for necrosis around the injection sites, most of the clinical signs occurred 10-30 min after dosing, starting on Day 17. Necrosis occurred between Days 11-18.

Body weights and weight gains:

There were no significant changes in group mean body weights in comparison to the control (the most reduction 4-5%). However, reductions of weekly mean body weight gains in treated animals, in comparison to the concurrent control, were observed through the recovery period. Reductions of weight gains were more apparent in females than in males, and changes reached statistical significance mainly in females. The TK animals had much greater individual variations, thus weight gain changes in these animals did not reach statistical significance. The results (% reduction from the control) are summarized in the table below:

Study Group	Main study (428R)				Recovery animals (428RR)			
	Males		Females		Males		Females	
Dose Group	G1 (g)	G4 (%)	G1 (g)	G4 (%)	G1 (g)	G4 (%)	G1 (g)	G4 (%)
No. of animals	10	10	10	10	6	6	6	6
Week 1	32.6	NC	17.8	19	34.7	7	16	↑ 23**
Week 2	27.2	17	15.1	4	26.3	23	15.8	15
Week 3	21.5	15	14.8	24*	18.8	10	10.8	↑ 11**
Week 4	15.6	8	8.3	24	13	NC	8	9
Week 7					8.7	↑ 21**	6.8	**81**
Week 9					3.7	19	4.3	60

Numbers in bolded prints represent statistically significant changes
NC: no changes, **: more weight gains than the control
*** Groups 2 and 3 also showed 24% reduction in weight gain compared to the control during this week.**

Food consumption:
Decreased food consumption occurred mainly in female recovery animals. Occasionally significant decreases in food consumption were observed in male rats in the main study group, with no dose dependence in severity. Percent decreases from the control (g/week) are summarized in the table below:

Study Group	Main study (428R)			Recovery animals (428RR)	
	Males			Females	
Dose Group	G1 (g/wk)	G2 (%)	G4 (%)	G1 (g/wk)	G4 (%)
No. of animals	10	10	10	6	6
Week 1	163	3	NC	131	4
Week 2	169	5	**8**	145	**21**
Week 3	169	**11**	7	148	11
Week 4	179	5	3	149	15
Week 5				138	**8**
Week 6				139	**12**
Week 7				127	**16**
Week 8				138	**9**
Week 9				123	**14**

Numbers in bolded prints represent statistically significant changes

Body temperature: Not remarkable

Ophthalmoscopy: Not remarkable

EKG: Not performed

Hematology:
Percent changes from the vehicle control (Group 1) are summarized in the table below:
Main study/428R (Day 29):

	Males			Females		
Dose Group	G2	G3	G4	G2	G3	G4
Number of animals	10	10	10	10	10	10
Retic % ↓						17 NS
Platelet ↓				12		9 NS
WBC ↑			89	42	50	85
Seg band cells (Ab) ↑			226			104

	Males			Females		
Dose Group	G2	G3	G4	G2	G3	G4
Seg band cells (%) ↑			66	↓ 26		11 NS
Lympho (Ab) ↑			37	54	54	70
Lympho (%) ↓		6	23			6 NS
Mono (Ab) ↑		126	402		94	285
Mono (%) ↑	29 NS	88	159			106
Luc (%) ↑	29 NS	62	88			
Baso (Ab) ↑			279			190
Baso (%) ↑			100			75 NS
Eosino (Ab) ↑			155			138
Eosino (%) ↑			32 NS			35 NS

Recovery animals/428RR:

	Day 28		Day 44		Day 58
	M	F	M	F	M
Dose Group	G4	G4	G4	G4	G4
Number of animals	6	6	6	6	6
Retic % ↓		22			
MCV ↑					4
MCH ↑	4				
MCHC ↑	3	3			
WBC ↑	114	65	28		
Seg band cells (Ab) ↑	285	130	55		49
Seg band cells (%) ↑	77	34 NS			
Lympho (Ab) ↑	60	41			
Lympho (%) ↓	25	14	10		
Mono (Ab) ↑	602	283	191	42	112
Mono (%) ↑	229	140	143		77
Luc (%) ↑	33	17 NS			35
Baso (Ab) ↑	278	167			71
Baso (%) ↑	80	50			40
Eosino (Ab) ↑	175	131			
Eosino (%) ↑	27 NS	44 NS			

NS: not statistically significant

Comment:

- ✧ The main hematological effects of AMD3100 were increased white blood cell counts, total and differential (leukocytosis). This finding supported the reports in the literature (see "Pharmacology").

There were no remarkable findings in bone marrow examination.

Clinical chemistry:

The only notable effect of AMD3100 treatment was decreased serum magnesium levels. The decrease was dose-dependent, and reached statistical significance in treated males (7%, 7% and 15% for Groups 2, 3 and 4, respectively) and females (7% in Group 4) in main study groups. The finding resolved at the end of the recovery period.

Urinalysis:

Decreased urinary pH and increased urine calcium and magnesium were the main findings at the end of AMD3100 treatment. Findings were reversible.

Study Group	428R (Day 29)				428RR (Day 28)				428RR (Day 44)	
	Males		Females		Males		Females		Females	
Dose Group	G1	G4	G1	G4	G1	G4	G1	G4	G1	G4
N	10	10	10	10	6	6	6	6	6	6
pH	6.4	**6.1**	6.3	**5.8**	6.8	**6.4**	6.6	**5.8**	6.4	**6.0**

Numbers in bolded prints represent statistically significant changes

The % changes from the control are summarized:

Study Group	428R (Day 29)				428RR (Day 28)	
	Males		Females		M	F
Dose Group	G3	G4	G3	G4	G4	G4
Number of animals	10	10	10	10	6	6
Calcium ↑	**116**	**321**	**28**	**82**	**212**	**145**
Magnesium ↑		**37**		26	38	16

Comment:
Increased urine calcium and magnesium indicated loss of electrolytes, but the mechanism was not clear. The sponsor suggested that it may be due to gelate binding effect of AMD3100. The bone mineral content or bone density of the tibia and humerus did not change (data not shown).

Gross pathology:
The gross pathological findings were summarized in the table below.
Main study groups (Day 31/32) and recovery animals (Day 63):

	Males			Females			
Dose Group	G1	G2	G4	G1	G2	G3	G4
No. of animals	16	10	16	16	10	10	16
Injection sites							
Hemorrhage	1	1	5		1		3
Lymph node							
Discoloration			1				
Spleen							
Enlarged			1			1	1

Organ weights:
Organ weight changes (absolute or relative body weight, % change from the control) are summarized in the following table:

	Males				Females			
Dose Group	G3	G4	G3	G4	G3	G4	G3	G4
Parameter	g	g	% BW	% BW	g	g	% BW	% BW
Number of animals	10	10	10	10	10	10	10	10
Spleen ↑	20	32	16	38	23	29	26	33
Thymus ↓		23		18		11NS		8NS

g: absolute weight, % BW: relative to body weight, NS: not statistically significant

Comments:
- ✧ The findings of increased hemopoiesis and congestion were consistent with increased splenic weights. However, there was no histopathological evidence for reduction of thymic weight.
- ✧ All Findings resolved.

Histopathological findings:

The following table is the summary of incidence and severity (average of the group) of salient histological findings. (Table from the sponsor)

Main study/428R: (Severity: Grade 1: minimal, Grade 2: slight, Grade 3: moderate)

	DOSE GROUP:	K		A		B		C	
	SEX:	M	F	M	F	M	F	M	F
ORGAN/FINDING	NO.ANIMALS:	10	10	10	10	10	10	10	10
LIVER	NO. EXAM.:	10	10	10	10	10	10	10	10
- HEMATOPOIESIS	GRADE 1 :	4	6	4	9	9	8	6	7
	GRADE 2 :			1	1		2	3	2
	TOTAL AFFECTED:	4	6	5	10	9	10	9	9
	MEAN GRADING :	0.4	0.6	0.6	1.1	0.9	1.2	1.2	1.1
INJECTION SITE	NO. EXAM.:	10	10	10	10	10	9	10	10
- INFLAMMATION	GRADE 1 :	1	2	2	3	1	4	1	2
	GRADE 2 :		2		3	2	2	2	5
	GRADE 3 :			2				4	1
	TOTAL AFFECTED:	1	4	4	6	3	6	7	8
	MEAN GRADING :	0.1	0.6	0.8	0.9	0.5	0.9	1.7	1.5
- HAEMORRHAGE	GRADE 2 :			1	1	1		3	2
	GRADE 3 :	1						1	1
	TOTAL AFFECTED:	1		1	1	1		4	3
	MEAN GRADING :	0.3		0.2	0.2	0.2		0.9	0.7

k, a, b, c:Groups 1 (control), 2, 3, and 4, respectively.

Also, to a less degree of severity, hematopoiesis was observed in spleen (all in Group 4): 1/10 male with slight congestion and slight hematopoiesis, 1/10 female with moderate congestion and 1/10 female with slight hematopoiesis (data not shown in the table above).

Recovery animals/428RR:

ORGAN/FINDING	DOSE GROUP:	K		A		B		C	
	SEX:	M	F	M	F	M	F	M	F
	NO.ANIMALS:	6	6					6	6
LIVER	NO. EXAM.:	6	6					6	6
- HEMATOPOIESIS	GRADE 1 :	2							
	TOTAL AFFECTED:	2							
	MEAN GRADING :	0.3							
INJECTION SITE	NO. EXAM.:	6	6					6	6
- INFLAMMATION	GRADE 1 :	1							1
	GRADE 2 :							1	
	TOTAL AFFECTED:	1						1	1
	MEAN GRADING :	0.2						0.3	0.2

The findings in spleen resolved at the end of the recovery period.

Comments:
The histopathological effects of AMD3100 at doses up to 12 mg/kg were minimal to slight hematopoiesis in liver and minimal to moderate subcutaneous lesions (hemorrhage and inflammation) at injection sites. These findings were also seen in the control and were partially recovered. The underlining mechanisms of AMD3100-related toxicities may be due to its leukocytotic effects.

Toxicokinetics:

✧ C_{max} and trough levels (μg/mL, Mean ± SD) after the last dose (Day 30/31) in main study/428R animals:

	Males			Females		
Dose Group	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Dose (mg/kg/d)	1	3	12	1	3	12
N	8	10	10	10	10	10
C_{max}* (1h) Day 30/31	2.42 ± 0.77	4.77 ± 1.75	20.3 ± 8.59	2.17 ± 0.54	4.75 ± 1.33	25.88 ± 8.64
Mean C_{max} (1h)/dose	2.42	1.59	1.69	1.61	1.58	2.16
Trough (24h) Day 31/32	0.064 ± 0.046	0.205 ± 0.136	0.728 ± 0.421	0.076 ± 0.08	0.123 ± 0.105	0.82 ± 0.317
Mean trough (24h)/dose	0.064	0.068	0.061	0.076	0.041	0.068

✧ C_{max} and Trough levels (μg/mL, Mean ± SD) on Day 15/16 in TK/428KR animals:

	Males			Females		
Dose Group	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
N	4	4	4	4	4	4
Dose (mg/kg/d)	1	3	12	1	3	12
C_{max} (1h) Day 15	1.72 ± 0.40	5.76 ± 0.43	18.37 ± 4.67	1.02 ± 0.44	4.59 ± 1.08	17.45 ± 2.21
Mean C_{max} (1h)/dose	1.72	1.92	1.53	1.02	1.53	1.45
Trough (24h) Day 16	0.026 ± 0.052	0.158 ± 0.079	0.451 ± 0.0.168	0.06 ± 0.046	0.1 ± 0.091	0.457 ± 0.183
Mean trough (24h)/dose	0.026	0.053	0.038	0.06	0.033	0.038

*** The sponsor referred to these values as C_{max} values on the respective days of analysis, on an assumption that T_{max} was 1 hr. The reviewer cautions that T_{max} was not 1 hr (see table below); thus these values should more appropriately be referred as "approximate C_{max}".**

The C_{max}, $AUC_{0\text{-}24hr}$, and dose-normalized parameters are summarized in the tables below: in TK/428KR animals (n=4/sex/group)

Males:

	Day 1			Day 30		
Dose Group	G2	G3	G4	G2	G3	G4
Dose (mg/kg/d)	1	3	12	1	3	12
C_{max} (μg/mL)	2.45	6.08	17.13	2.73	7.55	26.61
C_{max}/dose	2.45	2.03	1.43	2.73	2.52	2.22
$AUC_{0\text{-}24h}$ (μg•h/mL)	8.76	21.90	57.00	18.19	30.36	63.50
AUC/dose	8.76	7.30	4.75	8.19	10.12	5.29
T_{max} (hr)	0.5	0.62	0.75	0.5	0.5	0.5

Females:

	Day 1			Day 30		
Dose Group	G2	G3	G4	G2	G3	G4
Dose (mg/kg/d)	1	3	12	1	3	12
C_{max} (μg/mL)	1.39	4.89	16.98	1.87	6.25	27.07
C_{max}/dose	1.39	1.69	1.41	1.87	2.08	2.26
AUC (μg•h/mL)	3.31	19.60	67.45	6.51	22.39	62.66
AUC/dose	3.31	6.53	5.62	6.51	7.46	5.22
T_{max} (hr)	0.5	1	1	0.5	0.625	0.625

Summary:

✧ The C_{max} and trough levels of AMD3100 were mostly dose-proportional and did not show gender difference.

✧ The AUC values were mostly dose proportional in both males and females. Repeated administrations of AMD3100 did not exhibit evidence of accumulation, since the AUC values were comparable on Day 1 and Day 30.

Study title:

An additional 4-week subcutaneous toxicity study in rats

Key study findings:

- The AMD3100 related toxicities, included: mortality (23/40 at 24 mg/kg), decreased body weight gains/food intake, hematological effects (↓reticulocyte counts and ↑ total and

differential white blood cell counts), decreased serum magnesium, and increased urine levels of calcium and magnesium, as well as increased spleen and decreased thymus weights.

- The main target organs on microscopic examinations were injection sites, spleen and thymus.

Study no.: (b) (4) Study 432R
Volume #, and page #: Electronic, module 4 (432r.pdf)
Conducting laboratory and location: (b) (4)

Date of study initiation: May 26, 1994
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: AMD3100 (SDZ SID 791), Batch # Y021 0294, purity: 100.3% (RP-HPLC); placebo (for Group 1, the control): 5 mL ampoule consisting dinatriumhydrogen-phosphate (5 mg), NaCl (6 mg), HCl 25%, and water for injection up to 1 mL (batch # Y022 0194)
Reviewer's note: the compound used in this study was the hydrochloride salt of SDZ SID 791, and the dose levels refer to the salt. (b) (4)

Methods

Doses: 0 (control), 18 and 24 mg/kg (free base, as Groups 1, 2, and 3) [or, 11.4 and 15.2 mg free base/kg, respectively]
Species/strain: Rats (SPF, HanIbm: WIST; BRL)
Number/sex/group or time point:
Main study (432R): n=10/sex/group
Recovery animals (432RR): n=6/sex/group
Satellite groups used for toxicokinetics study (432KR): 4/sex/group

Route, formulation, volume: subcutaneous injection at dose volume of 3 mL/kg

- Formulation: AMD3100 lyophilisate was first reconstituted with the vehicle, and further diluted with 0.9% NaCl at dose concentrations of 6 and 8 mg/mL (0.6% and 0.8%) for Groups 2, and 3, respectively.
- Vehicle for AMD3100 lyophilisate: vehicle injection solution 5 mL ampoule (batch # Y029 0194), consisting of dinatriumhydrogen-phosphate (5 mg/mL), NaOH (2.84 mg/mL) and water for injection up to 1 mL
- The control animals were injected with placebo at a volume corresponding to the high dose (Group 3).

Age: ~7-9 weeks
Weight: 130-316 g
Schedule: Once daily for 4 consecutive weeks (31/32 days in main study animals, and 28 days for recovery animals). The main study was followed by a 7-week (49-day) recovery period.

Dose justification: Dose selection was based on Study # 428R (see above), in which subcutaneous injection of AMD3100 at doses up to 12 mg/kg (daily, for 4 weeks) resulted in elevated urine calcium levels, increased spleen and liver weights, and histological findings included increased extramedullary hematopoiesis in the liver and subcutaneous hemorrhage and inflammation at the injection site. The doses were extended in the present study. The maximum tolerance dose (MTD) was reached in the present study.

Observation and Times:

Clinical signs:	Once daily for mortality, moribundity and gross abnormality. The highest grade of severity of the symptom in individual animals was recorded daily.
Body weights:	For weight gain, weekly from pretest, during treatment until end of treatment or recovery period. Individual body weight was determined daily.
Food consumption:	Once weekly during the acclimation, dosing and recovery periods. Food consumption was reported weekly for individual animals.
Body temperature:	On treatment Day 26 before dosing and 0.5, 2 and 6 hr after dosing in surviving TK animals.
Ophthalmoscopy:	Once prior to the start of treatment in all main and recovery animals and on surviving Groups 1 and 3 main study animals on Day 24.
EKG:	Not performed.
Hematology:	Blood samples were taken (from right retroorbital plexus) at pretest and 24 hr after the last treatment (Day 29) in main study animals, as well as on Days 28, 44, 57 and 71 in recovery animals.
Clinical chemistry:	Blood samples were taken (from right retroorbital plexus) at pretest and 24 hr after the second (Day 3) and the last treatment (Day 29) in main study animals, as well as on Days 28, 44, 57 and 71 in recovery animals. Additional blood samples were taken from TK animals 1 and 4 hr after dosing on Day 29 to determine serum magnesium and calcium.
Urinalysis:	At pretest and on Day 29 (all groups) and on Days 28, 44 and 57 (recovery groups), urine was collected for ~ 24 hours.
Bone marrow smears:	From the primary and recovery necropsies. Samples were only examined microscopically for those from Groups 1 and 3.
Special bone examination:	The tibia and the humorus were processed and stained for assessment of mineralization and general pathology. Humorus length and bone volume measurements were performed using the Bioquant Image Analysis system and by Archimedes principle using analytical balance attachment, respectively. Microradiographs of humeri were produced on SO-343 high resolution film and the mean radiograph density was obtained.
Gross pathology:	Scheduled sacrifice: Day 31/32 or Day 77.
Organ weights:	At scheduled sacrifice. Adrenal, brain, heart, kidney, liver, ovaries, pancreas, pituitary, prostate, spleen, testis (including epididymides),

thymus, thyroid, and uterus. Organs were not weighed from animals that died or were euthanized as moribund.

Histopathology: Day 31/32 or Day 77 (main study and recovery groups, respectively). All tissues collected from all animal. For tissues identified microscopically as potential targets (i.e., liver, thymus, spleen, injection site and adrenal), the H&E stained slides were examined in male or female animals in Group 2 of the main study and recovery groups. The rest of organs were only examined in the control and Group 3 of the main study and recovery groups. All organs were examined in all unscheduled deaths. See inventory list for organs examined.

Spermatogenesis; Additional 3-4 μm sections of testes (n=5, Groups 1 and 3 only) were examinined and stained with PAS/Hematoxylin for staging of spermatogenesis.

Toxicokinetics: Trough level and C_{max}: Blood samples (0.5 mL) were collected from main study animals on Days 30/31 (C_{max}:1 hour after dosing) and 24 hr after dosing (trough level, Days 31/32). Blood samples were collected from TK animals 1 hr (C_{max}) and 24 hr (trough) after dosing on Day 15. TK study: Blood samples were collected from TK animals on Days 1 and 29 , at 0.5, 1, 2, 4, 7, and 24 hours post-dose (n=4/ time point). LLOQ was 18 ng/mL plasma. (*Reviewer's note: the LLOQ value in this study was different from that in Study 428, where the LLOQ was 50 ng/mL*)

Results:

Mortality:

Totally 23/40 treatment-related deaths were reported. The deaths occurred at 24 mg/kg and took place mostly during Week 1 to Week 4. There were no obvious clinical signs prior to death, nor could the cause of death be determined, since there were no drug-related pathological findings. The data are summarized in the table below:

	Males	Females
Main study animals (432R)	5/10 (Wk 1: 2, Wk 2: 1, Wk 3: 1 Wk 4: 1)	4/10 (Wk 3: 1, Wk 4: 2)*
Recovery animals (432RR)	4/6 (Wk 2: 1, Wk 3: 2, Wk 4:1)	4/6 (Wk 2: 1, Wk 3: 1, Wk 4: 1 Wk 5 (D8): 1)
TK animals (432KR)	4/4 (Wk 2:3, Wk 4: 1)	2/4 (Wk 3: 1)*
Total	13/20	10/20

***: one more female in this group was found dead; however, the time of the finding was not recorded.**

Clinical signs:

AMD3100-related clinical signs were mainly increased incidence of recumbency, twitching, and excitation, labored breathing and/or necrosis near injection sites in all AMD3100-treated males and females. The findings resolved. The incidence during Week 1 and Week 4 are summarized in the table below: data are indicated as incidence/animals affected.

Males:

Study Group	432R			432RR			432KR		
Dose Group	G1	G2	G3	G1	G2	G3	G1	G2	G3
Number of animals	10	10	10	6	6	6	4	4	4
Ventral recombency		250/10	199/10		126/6	108/6		84/4	55/4
Hyper excitation		30/10	151/8		18/6	78/6			27/4
Twitching			12/6			8/4			4/3
Labored respiration		40/10	151/8		24/6	78/6			27/4
Rales								4/4	
Necrosis (injection site)		21/3	16/2	8/1		4/1			

Females:

Study Group	432R			432RR			432KR		
Dose Group	G1	G2	G3	G1	G2	G3	G1	G2	G3
Number of animals	10	10	10	6	6	6	4	4	4
Ventral recombency		150/10	270/10		66/6	121/6		84/4	99/4
Hyper excitation		30/10	220/10		18/6	91/6			56/4
Twitching			9/3			5/3			2/1
Labored respiration		40/10	220/10		24/6	91/6			71/4
Rales								4/4	3/3
Necrosis (injection site)	4/1		14/2					4/1	

Body weights and weight gains:

There were no significant changes in group mean body weights in comparison to the control. However, reductions of group mean body weight gains in treated animals, in comparison with the control, were observed through the treatment period. Reductions of weight gains were more apparent in males than in females, and changes reached statistical significance mainly in males. Findings resolved in the recovery period. The results (% reduction from the control) are summarized in the tables below:

Males:

	Main study			Recovery animals			TK study		
Group	G1 (g)	G2 (%)	G3 (%)	G1 (g)	G2 (%)	G3 (%)	G1 (g)	G2 (%)	G3 (%)
No. of animals§	10	10	10	6	6	6	4	4	4
Week 1	37	**22**	**30**	34.8	**27**	24	8.3	52	90
Week 2	31.9	**32**	**31**	25.5	**25**	**33**	13.5	46	107*
Week 3	21.4	25		22.8	**30**	**39**	5.8	86	186*
Week 4	15.9	**43**	**55**	14.2	49	58	16.5	70	N=0
Week 5				9		50			

§ Number of animals at the beginning of the study.
Numbers in bolded prints represent statistically significant changes
Changes less than 10% from the control are not shown.
*** Negative weight gain (i.e., net weight loss)**

Females:

	Main study			Recovery animals			TK study		
Group	G1 (g)	G2 (%)	G3 (%)	G1 (g)	G2 (%)	G3 (%)	G1 (g)	G2 (%)	G3 (%)
No. of animals§	10	10	10	6	6	6	4	4	4
Week 1	17.5	29	**45**	14		13	9	28	80
Week 2	12.8	20	12	13.5		30	9.8	20	59
Week 3	9.8			10.2		26	6.3	16	25
Week 4	10.9	35	30	9.7	15	45	5		20
Week 5				8	13	13			

Changes in body weight gain of pooled data (male + female) are depicted in the following figure (from the sponsor):

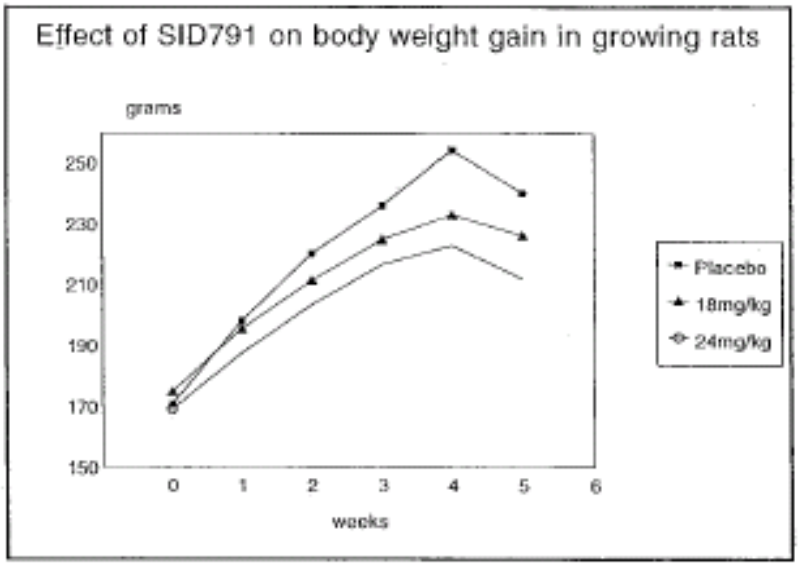


Figure 2: Body weight gain of rats during s.c.-treatment with 18 or 24mg/kg of SID-791

Food consumption:

Decreased food consumption occurred mainly in males. There was no dose dependence in severity. The finding resolved. The % decreases from the control (g/week) are summarized in the tables below:

Males:

Study Group	432R			432RR			432KR		
Dose Group	G1 (g/wk)	G2 (%)	G3 (%)	G1 (g/wk)	G2 (%)	G3 (%)	G1 (g/wk)	G2 (%)	G3 (%)
No.§	10	10	10	6	6	6	4	4	4
Week 1	158			163			139		
Week 2	161	7	10	162	29	15	147		12NS
Week 3	152	8		157	29	21	132		
Week 4	158	11	16 NS	161	14NS	16NS	148		N=0
Week 5				168	31NS	10NS			

Females:

Study Group	432R			432RR			432KR		
Dose Group	G1 (g/wk)	G2 (%)	G3 (%)	G1 (g/wk)	G2 (%)	G3 (%)	G1 (g/wk)	G2 (%)	G3 (%)
No.§	10	10	10	6	6	6	4	4	4
Week 1	113			111			126	10NS	
Week 2	112			120			123		
Week 3	116		8	119	13NS		114		12NS
Week 4	116	7	14	130			133		
Week 5				120					

NS: not statistically significant

According to the sponsor, the lack of findings in the TK/432KR animals could be attributed to two possible reasons:

1. Animals in this study were caged individually, while paired animals (2 males or 2 females) were housed in the other two studies.
2. The data may be unreliable because of smaller number of animals in this study (n=4/sex/group); the number dropped further to 0, 1 or 2/sex/group toward the end of the study.

Changes in food consumption of pooled data (male + female) are depicted in the following figure (from the sponsor):

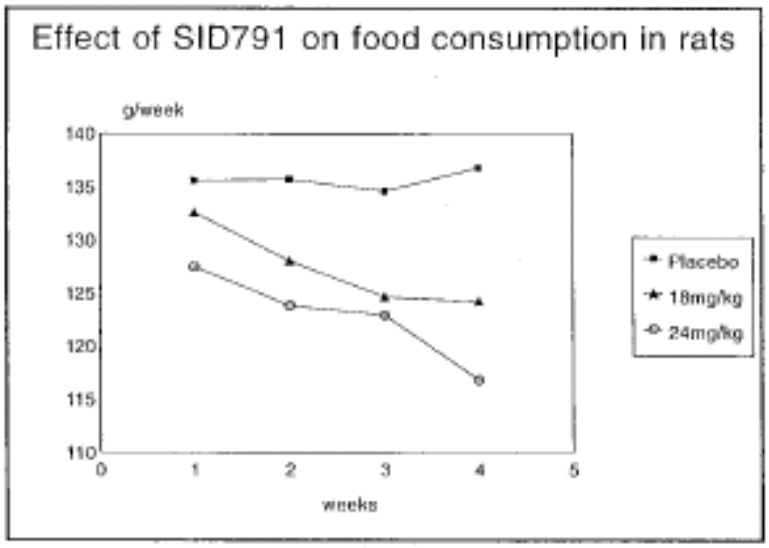


Figure 1: Food consumption during s.c.-treatment of rats with 18 or 24mg/kg of SID-791

Body temperature: Not remarkable

Ophthalmoscopy: Not remarkable

EKG: Not performed

Hematology:

The % changes from the vehicle control (Group 1) were summarized in the table below:

Study Group	Day 29 (432R)				Day 28 (432RR)			
Sex	Males		Females		Males		Females	
Dose Group	G2	G3	G2	G3	G2	G3	G2	G3
Number of animals	10	5	10	6	6	2	6	2
Retic % ↓			17	21	29	37		15
WBC ↑	67	80	72	136	67	66	96	118
Seg band cells (Ab) ↑	203	196	116	263	224	246	286	371
Seg band cells (%) ↑	72	55			100	101	94	111
Lympho (Ab) ↑		43	49	83	27	16	54	65
Lympho (%) ↓	24	19	13	18	24	29	21	24
Mono (Ab) ↑	265	246	306	577	219	326	280	321

Study Group	Day 29 (432R)				Day 28 (432RR)			
Sex	Males		Females		Males		Females	
Dose Group	G2	G3	G2	G3	G2	G3	G2	G3
Mono (%) ↑	120	90	145	169	79	164	97	97
Luc (%) ↑	57	50	60	50	36	21		17
Baso (Ab) ↑	148	232	171	293	228	78	173	319
Baso (%) ↑	100	75			100		33	100
Eosino (Ab) ↑	115	140	190	279	138	170	148	173
Eosino (%) ↑	24	29 NS	73	67	43	57	32	32

Study Group	Day 44 (432RR)			
Sex	Males		Females	
Dose Group	G2	G3	G2	G3
Number of animals	6	2	6	2
Retic % ↓		19		
WBC ↑		11	22	17
Seg band cells (Ab) ↑	17	41	91	80
Seg band cells (%) ↑	30	28	55	55
Lympho (Ab) ↑		10		
Lympho (%) ↓	10	10		10
Mono (Ab) ↑	71	90	76	84
Mono (%) ↑	81	69	43	57
Luc (%) ↑	12	47		
Baso (Ab) ↑		61	11	22
Baso (%) ↑		40		
Eosino (Ab) ↑			17	29
Eosino (%) ↑	31			14

Comment:

✧ The findings of decreased reticulocyte count and increased white blood counts (total and differentiated), that were dose-dependent, resolved by Day 57.

There were no remarkable findings in bone marrow examination.

Clinical chemistry:

No remarkable findings, except decreased serum magnesium levels. The decrease was minor, without evidence of dose-dependence, and only reached statistical significance in treated males in main study groups (9% and 18% for Groups 2 and 3, respectively). The finding resolved. Sporadic and slight increases in serum calcium levels were also observed, and the changes (2-7%) were occasionally significant (data not shown). Increased serum calcium levels were observed as early as in the first week of the treatment. Findings resolved.

Urinalysis:

Decreased urinary pH and increased urine calcium and magnesium were the main findings at the end of AMD3100 treatment. Findings resolved.

	Day 29 (432R)						Day 28 (432RR)					
	Males			Females			Males			Females		
Group	G1	G2	G3	G1	G2	G3	G1	G2	G3	G1	G2	G3
N	10	10	5	10	10	6	6	6	2	6	6	2
pH	6.6	**6.1**	**6**	6.5	**6**	**5.8**	6.7	6	5.8	6.3	5.9	6

The % changes from the control are summarized:

	Day 29 (432R)				Day 28 (432RR)			
	Males		Females		Males		Females	
Group	G2	G3	G2	G3	G2	G3	G2	G3
Number of animals	10	5	10	6	6	2	6	2
Calcium ↑	**157**	**300**	**25**	**56**	292	353	86	34
Magnesium ↑	**43**	57	**44**	**44**	61	67	44	38

Data in bolded prints represented statistically significant changes.

Comment:
Urinary and seral calcium were increased. On the other hand, serum magnesium levels were decreased while the urine magnesium levels were increased. Increased electrolyte loss to urine supported dose-dependent reduction in bone mineral content of the tibia and humerus (see below).

Special bone examinations:
There was a dose-related reduction in bone mineral content (BMC) of tibia and humerus (↓ 9-14% and 17-18% compared to the control at 18 and 24 mg/kg, respectively). However, the reduction in bone mineral density (BMD) was insignificant (~2-5%). It was also found that treatment of AMD310, although did not affect longitudinal bone grow (as indicated by humerus length), was associated with growth-driven modeling drifts on the periosteal surface which resulted in reduced bone volume. The findings resolved. As indicated in the histopathological report, no evidence of increased resorptive activity (bone remodeling) or mineralization defects were observed in cancellous or cortical bone tissue. Increased mineral loss (calcium and magnesium) from bone was reflected by increased urine mineral levels.

The result of bone examination is tabulated below (values as group means and % changes from the control):

	Tibia		Humerus				
	BMC (mg)	BMD (mg/cm^2)	BMC (mg)	BMD (mg/cm^2)	Length (mm)	Volume (μL)	Radiograph density
Group 1 (n=20)	163.3	112.9	97	106.2	25.2	175	1105
Group 2 (n=20)	148.2 90.8%	110.6 98%	83.6 **86.2%**	104.8 98.7%	24.8 98.4%	159 **90.9%**	1011 **91.5%**
Group 3 (n=11)*	135.8 83.2%	107.2 **95%**	79.6 **82.1%**	102.4 96.4%	25 99.2%	155 88.6%	98.5 **89.1%**

*** Data from surviving animals.**
Numbers in bolded prints indicate statistically significant changes.

Gross pathology:
The gross pathological findings were mainly lesions at the injection sites (subcutaneous edema and hemorrhage). The findings were more often in Groups 2 and 3 males as well as Group 3 females.

Findings resolved in recovery animals.

Organ weights:
Organ weight changes (absolute or relative body weight, % change from the control) are summarized in the following table:

	Males				Females			
Group	G2	G3	G2	G3	G2	G3	G2	G3
Parameter	gm	gm	% BW	% BW	gm	gm	% BW	% BW
Number of animals	10	5	10	5	10	6	10	6
Heart ↓		19						
Liver ↑	14	23	↓ 6	↓ 12			6	9
Spleen ↑	15 NS	12 NS	25	29	17 NS	32	18	38
Thymus ↓	40	29	35	21		23	13 NS	16 NS
Thyroid ↑	33 NS	7 NS	51	29 NS				

gm: absolute weight, % BW: relative to body weight
NS: not statistically significant

Comments:
- ✧ The findings in heart, and thyroid were likely incidental, because of lack of supportive histopathological evidence.
- ✧ Increased liver weights may be correlated with hematopoiesis and inflammation in the liver. However, the increases only occurred in males, despite similar histopathological findings in both sexes.
- ✧ The findings of lymphoid atrophy and increased hemopoiesis were consistent with decreased thymic weights and increased splenic weights, respectively.
- ✧ All Findings resolved.

Histopathological findings:
The following table is the summary of incidence and severity (expressed as incidence/group mean of severity) of drug-related histological findings.
Main study (432R) groups (Day 31/32):

Sex	Males			Females		
Group	1	2	3	1	2	3
No. of animals	10	a	5	10	a	6
Adrenal, cortex					(10)	
Hematopoiesis					1/1	2/1
Vacuolation	1/1					
Eyes						
Periorbital hemorrhage/inflammation	5/1.6			8/1.1		6/1.5
Harderian gland						
Inflammation/hemorrhage	5/1.6		2/1.5	6/1.5		2/2
Heart						
Myocarditis	1/1		1/1			
Injection sites		(10)			(10)	
Acanth/hyperkerat			1/2			
Hair shaft granuloma		1/1		1/1		
Subcutaneous hemorrhage	3/2	6/2	4/2.5	6/1.2	4/1.5	3/2.7
Subcutaneous inflammation	5/1	10/1.7	2/3	5/1.2	9/1.6	6/2
Ulcer/scab		1/2	1/1	1/1	1/1	2/2
Vasculitis		1/1				

Sex	Males			Females		
Group	1	2	3	1	2	3
No. of animals	10	a	5	10	a	6
Kidney						
Focal mineralization				10/1.2		6/2.2
Interstitial inflammation						1/3
Tubular baso/dilatation	6/1		2/1	7/1.6		4/1.3
Liver		(10)			(10)	
Apoptosis			1/1			
Hematopoiesis		9/1	5/1	3/1	10/1.5	6/1.7
Hepatocyte vacuolation			1/1			
Inflammatory cell focus	6/1.3	1/1	1/1	6/1	2/1	
Lung						
Alveolar macrophages	1/1			1/1		2/1.5
Congestion/hemorrhage	3/1			2/1		
Edema						1/1
Lymph node, mandibular						
Granuloma			1/2			
Hemorrhage			4/1.8	2/1		5/1.6
Lymph node, tracheobronchial						
Hemorrhage			1/1	2/1		3/1
Pancreas						
Acinar atrophy			1/1	2/1		
Preputial gland						
Inflammation			1/1			
Sciatic nerve						
Axonal degeneration	2/1			1/1		
Spleen		(10)			(10)	
Increased hemopoiesis	3/1	5/1	2/1.5	6/1	10/1.7	6/1.7
Thymus		(10)			(10)	
Congestion	7/1.3		3/1	5/1		1/1
Lymphoid atrophy		10/1	5/1	1/1	8/1	4/1

a: not microscopically examined, unless otherwise indicated by numbers in the parenthesis.
Severity: 1: minimal, 2:, slight, 3: moderate, 4: marked

Day 77 (recovery animals/432RR):

Sex	Males			Females		
Group	1	2	3	1	2	3
No. of animals	a	a	2	a	a	2
Adrenal, cortex				(6)	(5)	
Hematopoiesis				1/1		
Injection sites	(6)	(6)		(6)	(6)	
Acanth/hyperkerat						
Hair shaft granuloma		1/1			1/1	
Subcutaneous hemorrhage					1/1	4/3
Subcutaneous inflammation		4/1			1/1	1/1
Liver	(6)	(6)		(6)	(6)	
Hematopoiesis	3/1	2/1		1/1		1/1
Hepatocyte vacuolation	1/1	2/1				
Inflammatory cell focus	1/1	1/1		1/1	1/1	1/1
Lung						
Congestion/hemorrhage						1/1
Spleen	(6)	(6)		(6)	(6)	
Increased hemopoiesis	4/1	4/1		5/1.4	2/1.5	1/1
Lymphoid atrophy						1/1

Sex	Males			Females		
Group	1	2	3	1	2	3
No. of animals	a	a	2	a	a	2
Thymus		(6)			(6)	
Congestion	5/1.2	6/1.5	1/2			2/1

Histopathological findings in the unscheduled deaths (all Group 3 animals):

Study group	Main		Recover	
Sex	M	F	M	F
No. of animals	5	4	4	4
Autolysis	4/1.3	2/2.5	1/1	
Adrenal, cortex				
Hematopoiesis				1/1
Vacuolation		1/1		1/2
Bone (femur, tibia)				
Myelofibrosis	1/1			
Harderian gland				
Inflammation/hemorrhage	1/1			
Heart				
Myocarditis			2/2	
Injection sites				
Subcutaneous hemorrhage	2/3	4/2.5	4/2	
Subcutaneous inflammation	4/2.8	4/2.8	4/2.8	4/3
Kidney				
Focal mineralization		4/1.8		
Tubular baso/dilatation		2/1.5		
Liver				
Hematopoiesis	5/1		3/1	1/1
Hepatocyte vacuolation	2/1			
Lung				
Alveolar macrophages	1/1			
Congestion/hemorrhage	4/1.3	4/1.5	4/1.3	
Lymph node, tracheobronchial				
Hemorrhage		3/1.3	1/1	
Pancreas				
Acinar atrophy	2/1			
Preputial gland				
Inflammation	2/1		1/1	
Sciatic nerve				
Axonal degeneration		1/1	1/1	1/1
Skin				
Focal mineralization	1/2			
Myositis			1/3	
Spleen				
Increased hemopoiesis	2/2		3/1.3	2/1.5
Lymphoid atrophy	1/1		1/3	
Thymus				
Congestion	4/1	2/1.5	3/1	3/1
Lymphoid atrophy	2/1			
Thyroid gland				
Vacuolar folicular cells	1/2			

Adequate Battery: yes (x), no ( )

Peer review: yes (x), no ( )

Comments:

- ✧ Subcutaneous injection of AMD3100 in rats identified the target organs as injection site, spleen and thymus, with minor lesions in liver, kidney, lymph nodes, and glands, such as adrenal, pancreas, and thyroid. The incidence and severity of these lesions exhibited a dose-dependent trend.
- ✧ The lesions around the eyes (eyes and Hardrian gland) may be caused by blood samplings from the periorbital plexus, while the findings of the skin could be due to frequent injection of the drug.
- ✧ There were no changes in the staging of spermatogenesis; there were no findings in male or female reproductive organs.
- ✧ Most of the findings were recovered or partially recovered at the end of the 7-week recovery period.

Toxicokinetics:

- ✧ C_{max} and trough levels (μg/mL, Mean ± SD) after the last dose (Day 30/31) in main study/432R animals:

	Males		Females	
Dose Group	Group 2 (n=10)	Group 3 (n=5)	Group 2 (n=10)	Group 3 (n=6)
Dose (mg/kg/d)	18	24	18	24
C_{max}* (1h) Day 30/31	30.1 ± 3.3	41.9 ± 10.5	29.1 ± 6	38.7 ± 4.4
Mean C_{max} (1h)/dose	1.67	1.74	1.62	1.61
Trough (24h) Day 31/32	0.66 ± 0.11	0.9 ± 0.3	0.71 ± 0.07	1.01 ± 0.05
Mean trough (24h)/dose	0.0367	0.0375	0.0394	0.042

- ✧ C_{max} and Trough levels (μg/mL, Mean ± SD) on Day 15/16 in TK/432KR animals:

	Males		Females	
Dose Group	Group 2 (n=4)	Group 3 (n=4)	Group 2 (n=4)	Group 3 (n=4)
Dose (mg/kg/d)	18	24	18	24
C_{max} (1h) Day 15	36.3 ± 4.8	No samples	32.9 ± 8.5	50.3 ± 19.3
Mean C_{max} (1h)/dose	2.02	---	1.83	2.10
Trough (24h) Day 16	0.81 ± 0.49	No samples	0.83 ± 0.3	1.06 ± 0.22
Mean trough (24h)/dose	0.045	---	0.046	0.044

*** The sponsor referred to these values as C_{max} values on the respective days of analysis, on an assumption that T_{max} was 1 hr. The reviewer cautions that T_{max} was not 1 hr (see table below); thus these values should more appropriately be referred as "approximate C_{max}".**

The C_{max}, $AUC_{0\text{-}24hr}$, and dose-normalized parameters are summarized in the tables below, in TK/432KR animals (n=4/sex/group):

	Day 1				Day 30			
	Males		Females		Males		Females	
Group	G2	G3	G2	G3	G2	G3	G2	G3
Dose (mg/kg/d)	18	24	18	24	18	24	18	24
C_{max} (μg/mL)	36.9	33.4	28.4	32.1	47.8	NS	47	54.6*
C_{max}/dose	2.1	1.4	1.6	1.3	2.7	---	2.6	2.3
AUC (μg•h/mL)	125.6	139.9	111.8	129.4	134.8	NS	110.9	161.7*
AUC/dose	7.0	5.8	6.2	5.4	7.5	---	6.2	6.7
T_{max} (hr)	0.9	1.5	1	1.3	0.6	---	0.6	0.8*

NS; No samples due to mortality, * n=2

Comment:

- ✧ The C_{max} and trough levels of AMD3100 followed a dose dependent fashion and did not show gender specific differences in this dose range.
- ✧ The AUC values were dose-proportional in both males and females. There were no obvious gender differences in systemic exposures. Repeated administrations of AMD3100 did not exhibit evidence of accumulation, since the AUC levels were comparable on Day 1 and Day 30.

Study summary and discussion of repeated dose toxicology studies in rats:

See Section 2.6.7 "Toxicology tabulated summary".

- Studies #428R and 432R followed a similar study protocol and the doses were continual from #428R to #432R without dose overlap. These two studies will be summarized together.
- Treatment of AMD3100 in rats, dosing from 1 to 24 mg/kg/day for 4 weeks, induced the following findings: mortality (23/40 at 24 mg/kg/day), reduced body weight gain/food consumption, hematological parameters (decreased reticulocytes, increased white cell counts), decreased serum magnesium and/or calcium, and increased urinary levels of magnesium and calcium, and changed organ weights (↑ spleen, ↓ thymus). The main target tissues/organs, based on histopathological examinations, were injection site (subcutaneous hemorrhage and inflammation), liver (hematopoiesis), spleen (hemopoiesis, lymphoid atrophy) and thymus (congestion).
- The cause of death at 24 mg/kg/day was not determined. However, the findings occurred in all three groups (i.e., main study, recovery and TK animals) with high incidence, and should be considered AMD3100-related.
- Clinical signs, including ventral recumbency, decreased activity, twitching and labored breathing, may suggest CNS effects of AMD3100.
- Increased urinary calcium and magnesium may indicate loss of electrolytes. At higher doses (≥ 18 mg/kg/d), this finding was associated with dose-dependent reduction in bone mineral content of the tibia and humerus of the treated animals.
- Treatment of AMD3100 did not influence longitudinal bone growth or bone remodeling, and did not cause mineralization defects, but affected growth-driven modeling drifts on the periosteal surface, as indicated by reduced bone volumes of humerus. Experimental evidences in mice indicated that disruption of CXCR4 enhanced osteoclastogenesis, increased bone loss and elevated markers of bone resorption, as well as promoted bone tumor growth (Hirbe *et al.*, "Disruption of CXCR4 enhances osteoclastogenesis and tumor growth in bone", PNAS 104: 14062-14067, 2007). These researchers also found G-CSF treatment was associated with increased marker of osteoclasts activity and decreased bone mineral density. According to them, short-term administration of AMD3100 (5 mg/kg subcutaneously every 12 hr for 3 days) did not lead to increased tumor growth (Hirbe *et al.*, Blood 109: 3424-3431, 2007).
- Toxicokinetics:
  - ✧ The C_{max} and trough levels of AMD3100 were mostly dose-proportional and did not show significant gender differences in the dose range tested.
  - ✧ The AUC values were dose proportional in both males and females.
  - ✧ Repeated administrations of AMD3100 did not exhibit clear evidence of accumulation, since the AUC values were comparable on Day 1 and Day 30.

Study title: ((b) (4) Study 189DFD)
A 2-week subcutaneous dose-range-finding study

This non-GLP range-finding study was not reviewed. The result of the study is excerpted from the package.

Subcutaneously administered AMD3100 (SZD SID 791) in dogs (n=1/sex/group) at doses of 0 (control), 2, 4 or 6 mg/kg/day for 15 days induced mortality (moribund sacrifices at 6 mg/kg), clinical signs (at ≥ 2 mg/kg) that involved GI (diarrhea, emesis, increased defecation, salivation) and nervous (tremor, ataxia, impaired balance, sedation and mydriasis) systems, reduced body weight gain and food consumption (at ≥ 4 mg/kg), transient decrease in body temperature (1.1-1.7°C, at 6 mg/kg), transient increase in heart rate (~50-100% at 1 hr post-dose at ≥ 2 mg/kg), increased lymphoctes, decreased serum magnesium and a 2-3 fold increase in cerebro-spinal fluid (CSF) calcium. Pathological findings included, increased heart and kidney weights, moderate fibroid necrosis of the myocardial blood vessel wall (possibly related to moribund clinical signs), and minimal extramedullary hematopoiesis in the liver. The toxicokinetic data indicated a linear dose proportionality for the C_{max} and $AUC_{0\text{-}24h}$.

Study title:
Toxicity study by subcutaneous administration to beagle dogs for 4 weeks followed by a 2-week reversibility period

Key study findings:
- The AMD3100 related toxicity, included: GI clinical signs (diarrhea), decreased body weight gains/food intake (especially in females), increased white blood cell counts (total and differential), increased heart rate and blood pressure, and urinalysis findings (↑ volume and ↓ specific gravities, and increased urine levels of calcium and magnesium).
- These findings were mainly associated with high dose groups (4 mg/kg/day).
- Occasionally, low levels of AMD3100 were detected in cerebro-spinal fluid, indicating that the drug can cross the blood-brain barrier.

Study no.: (b) (4) (b) (4) No. 94/SPM028/0891
Volume #, and page #: Electronic module 4 ((b) (4) 94-spm028-0891-tox.pdf)
Conducting laboratory and location: (b) (4) (b) (4)

Date of study initiation: May 31, 1994
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: AMD3100 (SDZ SID 791), Batch # Y021 0294, purity: 100.3% (RP-HPLC); placebo (for Group 1, the control,): 5 mL ampoule consisting dinatriumhydrogen-phosphate (5 mg, NaCl (6 mg), HCl 25%, and water for injection up to 1 mL (batch # Y022 0194)

Reviewer's note: the compound used in this study was the hydrochloride salt of SDZ SID 791, and the dose levels refer to the salt. (b) (4)

Methods

Doses: 0 (control), 0.25, 1 and 4 mg/kg (free base, as Groups 1, 2, 3 and 4)
Species/strain: Beagle dogs
Number/sex/group or time point:
Main study: n=3/sex/group
Recovery animals: n=2/sex/group of Groups 1 and 4
There were no separate satellite groups, all AMD3100-treated animals (Groups 2-4) were used for toxicokinetic study: 3/sex/group

Route, formulation, volume: subcutaneous injection at dose volume of 1 mL/kg

- Formulation: AMD3100 lyophilisate (50 mg/ampoule) was first reconstituted with the vehicle, and further diluted with 0.9% NaCl at dose concentrations which provided the required dosages at a constant volume-dosage if 1 mL/kg.
- Vehicle for AMD3100 lyophilisate: vehicle injection solution 5 mL ampoule (batch # Y029 0194), consisting dinatriumhydrogen-phosphate (5 mg/mL), NaOH (2.84 mg/mL) and water for injection up to 1 mL
- The control animals were injected with placebo at a volume corresponding to the high dose (Group 4).

Age: ~16-18 weeks
Weight: 6.6-10.1 kg
Schedule: Once daily for 4 consecutive weeks (31/32 days in main study animals, and 28 days for recovery animals). The main study was followed by a 2-week recovery period.

Dose justification: Dose selection is based on a dose range-finding study ((b) (4) (b) (4) Schedule No. SPM/030), in which a single dose at 6 mg/kg was associated with increased heart rate, ataxia, prostration, emesis, hunched posture and muscle tremors. Similar but less severe signs were observed at 5 mg/kg. Also according another dose range-finding study (Study # 189DFD), subcutaneous injection of AMD3100 at dose of 6 mg/kg/day caused moribund sacrifice after 10 administrations. The dose range-finding study also indicated that 4 mg/kg approximated the MTD, and thus 4 mg/kg was selected as the high dose in the current study.

Observation and Times:

Clinical signs: Once daily for mortality, moribundity and gross abnormality. The highest grade of severity of the symptom in individual animals was recorded daily.
Body weights: Individual body weight was determined weekly from pretest, during treatment until end of treatment or recovery period.
Food consumption: Daily during the acclimation, dosing and recovery periods. Food consumption was reported weekly for individual animals.

Water consumption:	Twice during the acclimation, then weekly (over a 3-day period on each occasion) during dosing and recovery periods.
Veterinary exam	Once before dosing and once before necropsy.
Neurological exam	Once during the acclimation, before dosing and in Weeks 1 and 4, to examine reflexes, reactions and functions of cranial and spinal nerves, postural and general observations (such as behavioral changes).
Body temperature:	Rectal temperature was measured on treatment Day 1 and on one occasion during Week 4, data were recorded before dosing and 0.5, 2 and 2 hr after dosing all animals.
Blood pressure:	Once on all animals prior to the start of treatment, on Day 1 and during Week 4, and before necropsy.
Ophthalmoscopy:	Once on all animals prior to the start of treatment and during Week 4.
EKG:	On Day 1 and during Week 1, tracings were obtained before dosing, 0.5, 2 and 4 hours after dosing.
Hematology:	Blood samples were taken (from right jugular vein) prior to the start of treatment and during Week 4 (before dosing).
Clinical chemistry:	Blood samples were taken (from right jugular vein) prior to the start of treatment and during Week 4 (before dosing) of main study period and Week 2 of recovery period.
Urinalysis:	Overnight urine samples were collected from all animals prior to the start of treatment and during Week 4 of main study period and Week 2 of recovery period. The urine samples obtained during Week 4 were also tested for calcium and magnesium concentrations.
Bone marrow smears:	From the main study and recovery necropsies. Samples were prepared and fixed for all animals. The samples form the main study animals were examined by counting 100 nucleated cells and computing the myeloid : erythroid ratio.
Cerebral-spine fluid:	Immediately before necropsy a sample (1-2 mL) of CSF was obtained from all main study animals. The samples were deep frozen and kept for analysis.
Gross pathology:	Scheduled sacrifice: Day 31/32 or Day 43.
Organ weights:	At scheduled sacrifice. Adrenal, brain, heart, kidney, liver, lung, ovaries, spleen, testis (including epididymides), thymus, thyroid (with parathyroid), and uterus (with cervix).
Histopathology:	Day 31/32 or Day 43 (main study and recovery groups, respectively). All tissues collected from all animal. The H&E stained slides were examined for all animals. See inventory list for organs examined..
Toxicokinetics:	Blood samples (2 mL) were collected from all main study animals of Groups 2, 3 and 4 on Day 2 and during Week 4, before dosing and 0.5, 1, 2, 4, 7 and 24 hours post-dose. The blood samples from Group 1 were collected only before dosing on each toxicokinetic blood sampleing occasion. Additionally, trough level blood samples (pre-treatment) were collected from the treated animals immediately before dosing on one occasion in the second half of Week 2. Immediately before necropsy a sample (1-2 mL) of cerebro-spinal fluid (CSF) was obtained from each main study animal. In addition, approximately 3 g

of tissue samples from brain (hemispherical white matter) and kidney (cortex and medulla) were taken for the measurement of the tissue levels of AMD3100.
LLOQ was 22.4 ng/mL plasma, or 6.6 ng/mL for CSF (via HPLC analysis).

Results:
Mortality: No mortality occurred.

Clinical signs:
AMD3100-related clinical signs were mainly in Groups 3 and 4, including diarrhea and/or lesions near injection sites. Occasionally diarrhea was also seen in Groups 1 and 2. The findings resolved. The incidence and findings are summarized in the table below:

Sex	Males				Females			
Group	G1	G2	G3	G4	G1	G2	G3	G4
Number of animals	5	3	3	5	5	3	3	5
Diarrhea								
Week 1	1		2	4	2		2	3
Week 2	1	2	2	4		1	2	2
Week 3	2	1	3	4	1	1	2	3
Week 4	3	2	3	5	1	1	3	3
R-Week 1 (n=2)	2			1	1			
Injection site								
Thickening								
Week 1							1	
Week 2				2				2
Week 3				4				3
Week 4	2	1	2	5				4
R-Week 1				1				1
Swelling								
Week 1								
Week 2	1			1				
Week 3								
Week 4	1							1
R-Week 1								

G1, G2, G3 and G4: Groups 1, 2, 3 and 4. R: recovery

Body weights and weight gains:
There were no significant changes in group mean body weights in comparison to the control. Statistically significant reduction of mean body weight gain in Group 4 females, in comparison with the concurrent control, was observed. Weight gains (kg) and % reductions from the control (in parenthesis) are summarized in the table below:

	Males				Females			
Group	G1	G2	G3	G4	G1	G2	G3	G4
No. of animals	3	3	3	3	3	3	3	3
Week 1- Week 4	1.2	0.8 (↓ 33%)	1.1 (↓ 8%)	0.9 (↓ 25%)	1.1	1 (↓ 9%)	0.7 (↓ 36%)	**0.4 (↓ 64%)**
R-Week 0-2 (n=2)	0.4			0.9	0.3			0.4

Numbers in bolded prints represent statistically significant changes

Food consumption:
Decreased food consumption occurred mainly in female animals. The reduction was not statistically significant, 15% and 24% reduction from the control for Groups 3 and 4, respectively. A slight reduction (7%) was also observed in Group 4 males. For animals (both in Group 4: male #5702 and female #5649) had particularly lower food intake, canned food or diet moistened with water was offered and food intake was improved. The finding resolved.

Water consumption: Not remarkable.

Veterinary examination: Not remarkable

Neurological examination: Not remarkable

Body temperature: Not remarkable

Ophthalmoscopy: Not remarkable

EKG and cardiovascular parameters:
The findings were mainly on Day 1 of the treatment (30 min, 2 and 4 hours post-dose) and most of the parameters of AMD3100- treated dogs were comparable to the control during Week 4.

- ✧ Group 2 females: the deviations from the control were attributable to animal #5669 that had lower heart rates before dosing and during Day 1 post-dose. The group means of Group 1 (actual values) and Group 2 (actual values and % changes from the control [in parenthesis]), as well as the individual data of #5669 (actual values) are summarized:

	HR (b/min)	P (msec)	QRS (msec)	ST (msec)	QT (msec)	QTc (msec)	P (mV)
Before dosing on Day 1							
Mean G1	146	37	38	129	167	260	0.27
Mean G2	**113 (↓ 23%)**	35	41	139	180	246	**0.19 (↓ 30%)**
#5669	96	32	44	144	188	238	0.16
30 min after dosing on Day 1							
Mean G1	149	35	38	132	169	264	0.28
Mean G2	**89 (↓ 40%)**	**43 (↑ 23%)**	**46 (↑ 21%)**	**155 (↑ 17%)**	**201 (↑ 19%)**	**244 (↓ 8%)**	0.28
#5669	76	42	46	168	214	241	0.24
2 hours after dosing on Day 1							
Mean G1	162	38	38	123	161	260	0.32
Mean G2	**122 (↓25%)**	35	42	139	**181 (↑ 12%)**	257	0.26
#5669	116	32	42	150	192	267	0.23
Before dosing during Week 4							
Mean G1	148	36	39	126	164	257	0.27
Mean G2	127	37	39	**146**	**185**	268	0.25
#5669	121	36	44	146	190	270	0.25

HR; Heart rate, EKG parameters: intervals (millisecond, msce) and amplitude (mV)
Data in bolded prints indicate statistically significant changes in comparison to the control.

✧ Group 4 animals: the main findings were increased heart rates and correspondingly shortened QT values (hence no changes in QTc).

Males:

	HR (b/min)	P (msec)	PR (msec)	ST (msec)	QT (msec)	QTc (msec)
30 min after dosing on Day 1						
Mean G1	131	36	102	150	186	274
Mean G4	159 (↑ 21%)	34	**81 (↓ 21%)**	132	168	269
#5730	211	32	74	110	142	266
2hr after dosing on Day 1						
Mean G1	134	36	97	144	181	269
Mean G4	161 (↑ 20%)	35	81	128	165	269
#5730	198	38	74	114	148	269
4 hr after dosing on Day 1						
Mean G1	140	36	92	140	176	267
Mean G4	156 (↑ 11%)	34	80	130	166	264
#5730	200	42	80	116	146	267
30 min after doing during Week 4						
Mean G1	129	39	104	147	186	270
Mean G4	**163 (↑ 26%)**	34	**82 (↓ 21%)**	**128 (↓ 13%)**	**166 (↓ 11%)**	272

Females:

	HR (b/min)	P (msec)	QT (msec)	QTc (msec)	P (mV)
2 hr after dosing on Day1					
Mean G1	162	38	161	260	0.32
Mean G4	172	**34 (↓ 11%)**	156	263	0.34
4 hr after dosing on Day1					
Mean G1	144	37	166	254	0.28
Mean G4	**163 (↑ 27%)**	35	134	**268 (↑ 6%)**	**0.39 (↑ 39%)**

Data in bolded prints indicate statistically significant changes in comparison to the control.

Comment:

- Decreased heart rates in Group 2 females, mainly associated with animal #5669, may incidental, because of lack of dose-relationship. The finding resolved during Week 4. The bradycardia effect in #5669 may result in prolonged QT interval in this animal. The QTc interval (QT intervals corrected for heart rate) decreased.
- Increased heart rates in Group 4 males and females may be drug-related. The tachycardia effect was transient, and the findings resolved during Week 4 (i.e., no findings 2 and 4 hours after dosing during Week 4),.

✧ Blood pressure: AMD3100 induced increases in blood pressure and pulse rate in Group 4 males and females. BP (mmHg) and pulse rate (beat/min) are as following:

Males:

	Systolic		Diastolic		Mean arterial		Pulse		Pulse rate	
	G1	G4	G1	G4	G1	G4	G1	G4	G1	G4
Day 1										
30 min	138	**151**	93	**105**	108	**122**	45	46	140	160
2 hr	130	**148**	86	**103**	101	**119**	44	45	140	170
4 hr	139	145	88	**99**	105	114	51	46	144	162

	Systolic		Diastolic		Mean arterial		Pulse		Pulse rate	
	G1	G4	G1	G4	G1	G4	G1	G4	G1	G4
Week 4										
30 min	142	157	95	107	110	123	45	50	132	**160**
2 hr	144	160	98	**116**	113	**131**	46	44	132	158
4 hr	132	149	90	**106**	103	**121**	43	43	132	150

Females:

	Systolic		Diastolic		Mean arterial		Pulse		Pulse rate	
	G1	G4	G1	G4	G1	G4	G1	G4	G1	G4
Day 1										
30 min	142	147	96	100	111	115	46	47	150	150
2 hr	139	138	91	98	107	111	48	**40**	152	174
4 hr	136	139	90	**101**	105	114	46	**38**	154	166
Week 4										
30 min	146	156	100	108	115	123	46	48	146	164
2 hr	135	**151**	90	**104**	105	**119**	45	47	136	160
4 hr	148	151	98	102	116	118	50	49	148	150

Data in bolded prints indicated statistically significant changes in comparison to the control.

Comment:

- ✧ The pre-dosing values of blood pressure and pulse rate were comparable for Groups 1 and 4.
- ✧ AMD3100 caused hypertension and tachycardia.

Hematology:
Not remarkable, except significant increases of WBC counts (total ↑ 27% and lymphocyte ↑ 35%) in Group 4 females during Week 4. The increase in lymphocyte counts (28%) in Group 4 males was not statistically significant. The reversibility of the finding was uncertain, because hematology examination was not performed in recovery animals.

Bone marrow smear examination indicated normal cellularity with decreases in erythoid series in Group 3 (1/3 M and 1/3 F) and Group 4 (1/3 M and 2/3F). The myeloid: erythoid ratio was not affected in AMD3100-treated dogs.

Clinical chemistry:
Not remarkable.

Urinalysis:
Increased urine volume, decreased specific gravity (SG) and increased urine calcium and magnesium were the main findings at the end of AMD3100 treatment in Group 4 males. Females were not affected. Since there were no remarkable findings in water intake, increased urine volume was not due to changes in water intake. Findings resolved.

The actual values and % changes from the control were summarized:

Group	G1	G4
Number of animals	3	3
Volume (mL)	81	**105 (↑ 104%)**
SG	1059	**1035 (↓ 2%)**

Group	G1	G4
Calcium (mmol)	0.11	0.25 (↑ 127%)
Magnesium (mmol) ↑	0.27	0.43 (↑ 59%)

Data in bolded prints indicate statistically significant changes.

Gross pathology: Not remarkable

Organ weights: Not remarkable

Histopathological findings: Not remarkable.
Findings at the injection sites, such as cellulitis, subcutaneous edema and/or fibrosis, fibroid necrosis, hemorrhage and scab, were found in all animals, including the controls. Since no clear evidence of dose-related incidence and severity was related to AMD3100 treatment, these lesions were most likely due to repeated needle penetrations. However, the incidence of thickening of the skin were increased in Group 4 (see "Clinical signs"), AMD3100 caused local irritation to the skin (see below).

Toxicokinetics:

- The C_{max}, $AUC_{0\text{-}24hr}$, dose-normalized parameters and trough levels are summarized in the tables below: (n=3/sex/group)

Males:

	Day 2			Week 4		
Group	G2	G3	G4	G2	G3	G4
Dose (mg/kg/d)	0.25	1	4	0.25	1	4
C_{max} (ng/mL)	271.8	868.9	4565.4	289.1	1236.1	6188.3
C_{max}/dose	1087.2	868.9	1141.3	1156.4	1236.1	1522.1
$AUC_{0\text{-}24h}$ (ng•h/mL)	2056	4578.3	22678.2	2549	6915.3	25803.2
AUC/dose	8224	4578.3	5669.5	10196	6915.3	6450.8
T_{max} (hr)	2	1.2	1	2	1	0.8

Females:

	Day 1			Week 4		
Group	G2	G3	G4	G2	G3	G4
Dose (mg/kg/d)	0.25	1	4	0.25	1	4
C_{max} (ng/mL)	218.7	1090.2	6880.1	188.1	1296.6	12135.6
C_{max}/dose	874.8	1090.2	1720	752.4	1296.6	3033.9
$AUC_{0\text{-}24h}$ (ng•h/mL)	1586.5	6264.6	31292.5	1503.8	7610.6	39139.2
AUC/dose	6346	6264.6	7823.1	6015.2	7610.6	9784.8
T_{max} (hr)	1	0.8	0.8	1	0.8	0.7

- Trough levels of AMD3100 (ng/mL; mean ± SE) in main study groups:

Day 2 (24 hour postdose):

		Males (n=3)		Females (n=3)	
	Dose (mg/kg)	Mean ± SE	Mean/dose	Mean ± SE	Mean/dose
Group 2	0.25	26.9 ± 16.7	107.6	32.6 ± 1.9	130.4
Group 3	1	17.5 ± 7.7	17.5	97.2 ± 32.1	97.2
Group 4	4	51.5 ± 22.4	12.9	121.7 ± 56.0	30.4

Week 2 (in second half of Week 2):

		Males (n=3)		Females (n=3)	
	Dose (mg/kg)	Mean ± SE	Mean/dose	Mean ± SE	Mean/dose
Group 2	0.25	18.5 ± 9.3	74	33.1 ± 5.6	132.4
Group 3	1	65.4 ± 38.6	65.4	95.5 ± 16.2	95.5
Group 4	4	103.8 ± 23.1	26.0	121.6 ± 55.5	30.4

Week 4 (24 hur postdose):

		Males (n=3)		Females (n=3)	
	Dose (mg/kg)	Mean ± SE	Mean/dose	Mean ± SE	Mean/dose
Group 2	0.25	30.1 ± 13.3	120.4	35.4 ± 2.0	141.6
Group 3	1	78.0 ± 50.2	78.0	130.9 ± 37.8	130.9
Group 4	4	166.6 ± 46.7	41.7	139.6 ± 29.7	34.9

- Concentration (ng/mL) of AMD3100 in cerebro-spinal fluid (CSF) after treatment for 4 weeks (tables from the sponsor):

Dose mg/kg/day	Animal ->	5698/1M	5720/1M	5728/1M
0.00		0.0*	0.0*	0.0*

Dose mg/kg/day	Animal ->	5714/3M	5716/3M	5726/3M
1.00		0.7*	2.4*	1.3*

Dose mg/kg/day	Animal ->	5645/1F	5659/1F	5665/1F
0.00		0.7*	0.0*	0.1*

Dose mg/kg/day	Animal ->	5655/3F	5663/3F	5673/3F
1.00		2.4*	2.5*	1.6*

Dose mg/kg/day	Animal ->	5706/2M	5718/2M	5722/2M
0.25		0.0*	0.0*	1.0*

Dose mg/kg/day	Animal ->	5702/4M	5704/4M	5730/4M
4.00		3.3*	14.7	1.7*

Dose mg/kg/day	Animal ->	5647/2F	5661/2F	5669/2F
0.25		7.8	0.0*	1.2*

Dose mg/kg/day	Animal ->	5651/4F	5653/4F	5667/4F
4.00		5.2*	14.6	6.8

*** Values below the LOQ (6.6 ng/mL for 0.3 mL CSF injected.**

Comment:

- The C_{max} and AUC values were mostly dose-proportional. In Group 4, females showed a higher exposure than males.
- In Week 4, slightly increased C_{max} and AUC values in Group 4 females suggested that repeated administration of AMD3100 may be accumulative.
- The trough levels (24-hour values on Day 2 and during Week 4, and the pretreatment concentrations in Week 2) were decreasing with increased dose. These values were higher in females too.
- The AMD3100 levels in CSF were mostly under LLOQ (6.6 ng/mL); occasionally AMD3100 was detected (Group 2: #5714/F:7.8 ng/mL, Group 4: #5704/M: 14.7 ng/mL, and #5653/F: 14.6 ng/mL).

Summary of the study:
Treatment of AMD3100 at doses of 0.5, 1 and 4 mg/kg/day for 4 weeks was tolerated in dogs. The clinical signs indicated the GI and neurological effects of AMD3100. Decreased body weight gains, especially in Group 4 females, were associated with decreased food intake in certain animals. Inappetence also indicated GI disturbing effects of AMD3100. AMD3100 treatment caused increases in heart rate and blood pressure, mainly at 4 mg/kg/day.

The findings of increased WBC counts and increased urinary calcium and magnesium were consistent with findings in rats. As noted that AMD3100-induced leukocytosis was less remarkable in comparison with the finding in rats, i.e., ↑ 27% at 4 mg/kg (80 mg/m^2) in female dogs versus ↑ 85-89% at 12 mg/kg (72 mg/m^2) in rats in Week4.

- Twice daily dose:

The following studies were reviewed by Dr. Guodong Fang (IND 55851, N-065, May 16, 2002) (see Appendix):

Study (b) (4) 1663: A 14-day dosing schedule dependency (once vs. twice daily) study in rats
Study (b) (4) 89342: A range finding subcutaneous injection (twice daily) toxicity study of AMD3100 free base in the albino rat
Study (b) (4) 89349: A range finding subcutaneous injection (twice daily) toxicity study of AMD3100 free base in the beagle dog
Study (b) (4) 89289: A 28-day twice daily subcutaneous injection toxicity study of AMD3100 free base in the albino rat with a 14-day recovery period
Study (b) (4) 89290: A 28-day twice daily subcutaneous injection toxicity study of AMD3100 free base in the beagle dog with a 14-day recovery period

Histopathology inventory (optional) Summary of once daily toxicity studies only.

Study (Duration)	(b) (4) 428R (4-wk)	(b) (4) 432R (4-wk)	(b) (4) (b) (4) 94 (4-wk)
Species	Rat	Rat	Dog
Adrenals	x*, §	x (female), §	x, §
Aorta	x*	x*	x
Bone Marrow smear	x*	x*	x
Bone (femur)	x*	x*	x
Brain	x*, §	x*, §	x, §
Cecum	x*	x*	x
Cervix			
Colon	x*	x*	x
Duodenum	x*	x*	x
Epididymis	x*, §	x*, §	x, §
Esophagus	x*	x*	x
Eye	x*	x*	x
Fallopian tube			

Study (Duration)	(b) (4) 428R (4-wk)	(b) (4) 432R (4-wk)	(b) (4) (b) (4) 94 (4-wk)
Species	Rat	Rat	Dog
Gall bladder			x
Gross lesions	x*	x*	x
Harderian gland	x*	x*	
Heart	x*, §	x*, §	x, §
Ileum	x*	x*	x
Injection site	x	x	x
Jejunum	x*	x*	x
Kidneys	x, §	x*, §	x, §
Lacrimal gland	x*	x*	x
Larynx			
Liver	x, §	x, §	x, §
Lungs	x*	x*	x, §
Lymph nodes, tracheobronchial	x*		x
Lymph nodes mandibular	x*	x	x
Lymph nodes, mesenteric	x*	x	x
Mammary Gland	x*		x
Nasal cavity			
Optic nerves	x*	x*	x
Ovaries	x, §	x*, §	x, §
Pancreas	x*		x
Parathyroid	x*	x*, §	x, §
Peripheral nerve			
Pharynx			
Pituitary	x*, §	x*, §	x
Prostate	x*, §	x*, §	x
Rectum	x*	x*	x
Salivary gland	x*	x*	x
Sciatic nerve	x*	x*	x
Seminal vesicles		x*	
Skeletal muscle	x*	x*	x
Skin	x*	x*	x
Spinal cord	x*	x*	x
Spleen	x*, §	x, §	x, §
Sternum	x*	x*	x
Stomach	x*	x*	x
Testes	x*, §	x*, §	x, §
Thymus	x*, §	x, §	x, §
Thyroid	x, §	x*, §	x, §

Study (Duration)	(b) (4) 428R (4-wk)	(b) (4) 432R (4-wk)	(b) (4) (b) (4) 94 (4-wk)
Species	Rat	Rat	Dog
Tongue	x*	x*	x
Trachea	x*	x*	x
Urinary bladder	x*	x*	x
Uterus	x,* §	x	x, §
Vagina	x*	x*	x
Zymbal gland			

x, histopathology performed;
*** performed only in Groups 1 and 4 (or Group 2 in Study 34232R)) non-recovery animals.**
§ organ weight obtained

2.6.6.4 Genetic toxicology

Study title: Mutagenicity test using *Salmonella typhimurium*

Key study findings:
- AMD3100 at concentrations of 8-5000 μg/plate, with or without S-9 mix, was not mutagenic in *Salmonella typhimurium* TA98, TA97a, TA100, TA102 and TA1535 strains, under the conditions tested.

Study no.: #Mut.Bakt. 15/94
Volume #, and page #: Electronic submission, Module 4 (mb-15-94.pdf)
Conducting laboratory and location: Genetic Toxicology, (b) (4) (b) (4)
Date of study initiation: March 23, 1994
GLP compliance: Yes (OECD)
QA reports: yes (x) no ( )
Drug, lot #, and % purity: AMD3100 (SDZ SID 791), Batch # 93802, Purity: 96.4 %

Formulation/vehicle: Distilled water
Methods:
Strains: *Salmonella typhimurium* TA97a, TA98, TA100, TA1535 and TA102
Concentration selection criteria

Basis of concentration selection: based on previous experiments; data were not available in this submission. The sponsor followed OEDC guideline 471 (May 26, 1983) and employed concentrations up to 5 mg/plate as the highest concentration in the study. AMD3100 did not precipitate on the test plates at ≥ 5000 μg/plate, but was toxic to TA100 and TA102 in the presence of S9-mix. In the absence of S9-mix, the bacteriotoxicity of AMD3100 was observed at 5000 μg/plate (for all strains except TA97a), at 2500 μg/plate (for TA98 and TA100), and at 1250 μg/plate (for TA100).

Test agent stability: Stable
Metabolic activation system: Aroclor 1254 induced rat liver microsome S-9 mix
Controls:
Vehicle: Distilled water (100 μL/plate for plate incorporation test)
Negative controls: vehicle control
Positive controls: all dissolved in DMSO
With S-9: TA97a, TA98, TA100, TA102 and TA1535: 2-aminoanthracene (3 μg/plate), TA98: Benzo[a]pyrene (3 μg/plate)
Without S-9: TA98: 2-nitrofluorene (2 μg/plate), TA 97a: 9-aminoacridine (100 μg/plate), TA100 and TA1535: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (3.0 μg/plate), TA102: mitomycin C (0.5 μg/plate).
Exposure conditions:
Incubation and sampling times:
- Plate incorporation: 2-4 days

Concentrations used:
- Experiment 1: 8, 40, 200, 1000 and 5000 μg/plate.
- Experiments 2, 3 and 4: 312.5, 625, 1250, 2500 and 5000 μg/plate

Study design: Plate incorporation for initial test (Experiment 1); plate incubation for two confirmatory studies (Experiment 2; Experiment 3 and 4 repeated to make up the missing data in Experiment 2, due to bacteriotoxicity)
Analysis:
No. of replicates: 3 plates for each test compound concentration
Counting method: automated colony counter (image analyzer).

Result:
Study validity:
The study is considered valid, because:
- Tester strain integrity was documented in the report.
- Both negative (vehicle) and positive control data were within the laboratory historical range with the exception of Experiment 2 (see below).
- The mean positive control value (± S9-mix) exhibited at least three fold increase over the respective mean vehicle control value for each tester strain, except for TA102 (1.5 fold is acceptable).
- There was a minimum of three nontoxic concentrations (≤ 50% reduction in mean number of revertants/plate relative to the mean vehicle control value) in each tester strain, both in the absence and presence of S9-mix.

The following experiment was considered invalid:
- in Experiment 2, the performance in the absence of S9-mix in strains TA1535, TA100 and TA102: because the mean solvent control values were too low and/or the mean values of most test groups were below the normal values. Experiments 3 and 4 were repeated, in the absence of S9-mix, for the test in TA1535 and TA100 (Experiment 3), and in TA102 (Experiment 4). (see table below for results)

All tester strain culture titers (10^8cells/mL) were less than conventionally recommended titers (3 x 10^8 cells/mL).

Study outcome:

- Experiment 1:

The numbers of revertant colonies/plate at each test compound concentration, and vehicle and positive controls are summarized in the table below:

Treatment	Concentration (μg/plate)	Revertant colonies/plate (mean ± SD, n=3)				
Without S-9						
		TA1535	TA97a	TA98	TA100	TA102
Dist. H_2O	100 μL/plate	19± 1	152 ± 11	30 ± 10	85 ± 5	309 ± 12
AMD3100	8	21 ± 3	181 ± 10	33 ± 1	86 ± 7	295 ± 11
	40	17 ± 9	169 ± 9	27 ± 3	86 ± 3	307 ± 12
	200	18 ± 5	167 ± 10	25 ± 4	91 ± 10	296 ± 15
	1000	24 ± 2	175 ± 3	25 ± 6	81 ± 8	318 ± 12
	5000	15 ± 1	157 ± 1	27 ± 5	97 ± 1*	183 ± 18*
Positive control	(see above for + controls)	441 ± 80	1281 ± 197	138 ± 11	846 ± 33	1097 ± 171
With S-9						
Dist. H_2O	100 μL/plate	16± 3	167 ± 6	33 ± 6	98 ± 11	343 ± 13
AMD3100	4	17 ± 3	169 ± 5	31 ± 7	99 ± 21	353 ± 35
	20	15 ± 1	158 ± 14	35 ± 6	101 ± 10	390 ± 3
	100	18 ± 4	167 ± 10	27 ± 1	102 ± 9	364 ± 13
	500	12 ± 3	161 ± 1	36 ± 2	100 ± 12	133 ± 6
	2500*	15 ± 8	153 ± 8	29 ± 11	86 ± 2	374 ± 21
Positive control	(see above for + controls)	191 ± 18	782 ± 37	1918 ± 76 478 ± 33	1311 ± 48	1120 ± 91

***: Bacteriotoxic: reduced background bacterial lawn or no bacteria background**
Two positive controls for TA98 (with S9): the upper value is 2-aminoanthracene (3 μg/plate) and lower value is benzo[a]pyrene (3 μg/plate). This was similar to Experiment 2 and Experiment 3.

Experiment 2:

Treatment	Concentration (μg/plate)	Revertant colonies/plate (mean ± SD, n=3)				
Without S-9						
		TA1535	TA97a	TA98	TA100	TA102
Dist. H_2O	100 μL/plate	13 ± 4 (a)	141 ± 6	32 ± 4	48 ± 22 **(a)**	217 ± 27
AMD3100	312.5	12 ± 3	181 ± 19	30 ± 7	66 ± 18*	238 ± 12
	625	15 ± 2	175 ± 8	33 ± 8	71 ± 4*	168 ± 11
	1250	17 ± 3	185 ± 15	28 ± 2	55 ± 7*	162 ± 28
	2500	18 ± 4	119 ± 27	21 ± 4*	32 ± 28*	107 ± 25
	5000	16 ± 4*	175 ± 8	18 ± 3*	**	150 ± 19*
Positive control	(see above for + controls)	1103 ± 36	739 ± 171	186 ± 13	54 ± 9**(a)**	1255 ± 44
With S-9						
Dist. H_2O	100 μL/plate	16 ± 1	175 ± 3	39 ± 4	110 ± 2	345 ± 14
AMD3100	312.5	12 ± 2	198 ± 16	34 ± 4	138 ± 18	349 ± 30
	625	19 ± 1	204 ± 11	28 ± 2	129 ± 3	372 ± 30
	1250	17 ± 9	195 ± 29	36 ± 6	133 ± 8	349 ± 29
	2500	14 ± 2	209 ± 9	30 ± 4	111 ± 8	302 ± 25
	5000	13 ± 4	192 ± 8	33 ± 5	78 ± 13*	148 ± 27
Positive control	(see above for + controls)	237 ± 16	816 ± 57	1842 ± 60 460 ± 37	1013 ± 56	802 ± 58

***: Bacteriotoxic: reduced background bacterial lawn; ** small colonies or no bacteria background**
(a): lower than the laboratory's historical range (range 80-140)

Experiment 3 (TA 1535 & TA 100) and Experiment 4 (TA 102):

Treatment	Concentration (μg/plate)	Revertant colonies/plate (mean ± SD, n=3)		
Without S-9				
		TA1535	TA100	TA102
Dist. H_2O	100 μL/plate	24 ± 10	121 ± 5	325 ± 6
AMD3100	312.5	34 ± 3	121 ± 16	344 ± 13
	625	20 ± 5	115 ± 7	350 ± 21
	1250	17 ± 5	106 ± 4*	368 ± 29
	2500	20 ± 1	82 ± 3*	359 ± 5
	5000	34 ± 3*	90 ± 13*	276 ± 23*
Positive control	(see above for + controls)	1306 ± 109	1258 ± 36	1253 ± 25
With S-9				
Positive control	(see above for + controls)	266 ± 12	1224 ± 321	840 ± 36

Study title: Chromosome aberration test with V79 Chinese hamster cells

Key study findings:
- AMD3100 did not show clastogenic potential with or without S9-mix under the conditions of the study.

Study no.: #Z48
Volume #, and page #: Electronic submission, Module 4 (z48.pdf)
Conducting laboratory and location: (b) (4) .
Date of study initiation: March 25, 1994
GLP compliance: Yes (OECD)
QA reports: yes (x) no ( )
Drug, lot #, and % purity: AMD3100 (SDZ SID 791), Batch # 93802, Purity: 96.4 %

Formulation/vehicle: Distilled water
Methods:
Cells: V79 Chinese hamster cells
Concentration selection criteria
Based on reduced cell growth (cytotoxicity studies) and on the depression of mitotic index during the chromosomal aberration test. A total of three experiments were performed.

Range finding studies: none.
Test agent stability: Stable
Metabolic activation system: Aroclor 1254 induced rat liver microsome S-9 mix
Controls:
Vehicle: Distilled water
Negative controls: In the absence of S9-mix: the culture medium (b) (4) minimal essential medium (MEM).

In the presence of S9-mix: only S9-mix (10%) was added.

Positive controls:

With S-9: Cyclophosphamide (CP: 17.5 μM)

Without S-9: Ethyl methanesulfonate (EMS, Experiment CA2: 12.5 mM; Experiment CA1 and CA3: 15 mM)

Historical control data (n=16): 1.91% abnormal cells (0.75%-4.25%) in the medium control and 2.97% (0.75%-6%) in10% S9 control.

Assessment of cell growth: cell density estimated relative to the control:

+++: normal cell density, ++: 100-75% cell density from the control, +: 75-50%, –: 50-25%, and – –: <25%.

Exposure conditions:

Incubation and sampling times:

- Pulse treatment 3 hr and recovery time 17-21 hr without (Exp. 1, 2 and 3) or with S9 (Exp. 1 and 2)
- Continuous treatment 20 hr without S9 (Experiment 1, 2 and 3)

Concentrations used in the Experiments:

- Cytotoxicity test (all three experiments): 50, 71, 102, 145, 206, 294, 419, 597, 851, 121, 1728, 2462, 3509 and 5000 μg/mL.
- Cell count assessment from the cytotoxicity test (Data not shown):
  - ✧ Treatment for 3 hr without S9-mix: 96-98% of MEM control at AMD3100 concentrations of 1212-5000 μg/mL, except 3509 μg/mL (56.4%).
  - ✧ Treatment for 20 hr without S9-mix: 100-108% of the MEM control at AMD3100 concentrations 1728-5000 μg/mL.
  - ✧ Treatment for 3 hr with S9-mix: 81-96% of MEM control at AMD3100 concentrations 851-5000 μg/mL.
- Cell morphology assessment from the cytotoxicity test (Data not shown):
  - ✧ Treatment for 3 hr without S9-mix: no effects on cell morphology up to 5000 μg/mL.
  - ✧ Treatment for 20 hr without S9-mix: treatment-related morphological changes (with increasing severity) at concentrations >851 μg/mL.
  - ✧ Treatment for 3 hr with S9-mix: treatment-related morphological changes (with increasing severity) at concentrations >419 μg/mL.
  - ✧ In the absence of metabolic activation, SDZ SID 791 produced a decrease in the mitotic index which was not reproducibly dose-dependent. In the presence of S9, there was no decrease in the mitotic index.
- Based on reduction of cell growth and depression of mitotic index, the following concentrations were analyzed in the chromosomal aberration assay designated as Experiment CA1, CA2 and CA3 (SDZ SID 791 concentrations as μg/mL):

	First Experiment (CA1) μg/mL	Second Experiment (CA2) μg/mL	Third Experiment (CA3) μg/mL
Without S9 Treatment time 20 h	1000, 3000, 5000	250, 1000, 3000, 5000	250, 1000, 3000, 5000
With S9 Treatment time 3 h	1000, 3000, 5000	1000, 3000, 5000	

Study design: Counting the % cells with chromosome aberration in metaphase

- Only structural aberrations were counted. Numerical aberrations were not determined by this protocol.
- Cytotoxicity was based on mitotic index (% of mitotic cells within the total population of mitotic and non-mitotic cells)
- Statistics: A statistical analysis was not performed, because aberration values did not exceed the historical control range.

Analysis:

No. of replicates: Duplicate cultures for each test compound concentration, vehicle and positive controls.

Counting method:

- Observation under the microscope. The following structural aberrations were recorded: chromatid breaks (deletions), isolocus breaks, chromosome breaks, all forms of chromatid exchanges, decentrics, tricentrics, ring chromosomes and interstitial deletions; but did not include cells with only gaps (i.e., chromatid gaps and isolocus gaps).
- Cells with more than five aberrations were recorded as multiple aberrant cells.
- The mitotic index was determined by counting 1000 cells originally from one Petri dish (2000-4000 cells per concentration) and recording the numbers of metaphases among them. The mitotic index was expressed as a percentage of the associated control value.

Result:

Study validity:

The study is considered valid, because:

- There was an apparent increase (no statistical analysis) in percent aberrant cells in the positive control relative to the solvent control in each assay, with or without S9.
- The vehicle control data were within the laboratory historical range.

Study outcome: for chromosome aberration assay only (data for cytotoxicity test or morphology assessment are not shown. (Tables from the sponsor)

- Experiment CA1, CA2 and CA3 (without S9): 20 hour-incubation

	Experiment CA1				Experiment CA2			
	Cell Growth	Mitotic Index %	% Abn. Cells	% Cells Exch.	Cell Growth	Mitotic Index %	% Abn. Cells	% Cells Exch.
Control								
MEM	+ + +	8.5*	1.0	0.0	+ + +	7.7*	1.0	0.0
SDZ SID 791 (µg/ml)								
5000	+ +	60**	#14.1	#8.4	+ +	32.5**	2.0	0.5
4000	+ + +	76.5	ND	ND	+ +	36.4	ND	ND
3000	+ + +	69.4	1.5	0.0	+ + +	40.3	2.5	0.0
2000	+ + +	77.6	ND	ND	+ + +	36.4	ND	ND
1000	+ + +	55.3	0.5	0.0	+ + +	63.6	0.0	0.0
500	+ + +	43.5	ND	ND	+ + +	85.7	ND	ND
250	+ + +	64.7	ND	ND	+ + +	105.2	0.5	0.0
Positive Control, EMS (mM)								
15.0	ND	ND	5.0	1.0				
12.5					ND	ND	13.0	0.0

ND not determined
* Mitotic indices as % of mitotic cells within the total population of mitotic and non-mitotic cells.
** Mitotic indices as % of the controls.
# 191 cells analyzed

	Experiment CA3			
	Cell Growth	Mitotic Index %	% Abn. Cells	% Cells Exch.
Control				
MEM	+ + +	6.8*	1.5	0.0
SDZ SID 791 (µg/ml)				
5000	+ +	57.4**	3.5	0.0
4000	+ +	72.1	ND	ND
3000	+ + +	73.5	1.5	0.0
2000	+ + +	83.8	ND	ND
1000	+ + +	72.1	0.0	0.0
500	+ + +	92.4	ND	ND
250	+ + +	82.4	1.5	0.0
Positive Control, EMS (mM)				
15.0	ND	ND	14.0	1.0

- Experiment CA1 and CA2 (with S9): 3 hour-incubation and recovery for 17 hr.

	Experiment CA1				Experiment CA2			
	Cell Growth	Mitotic Index %	% Abn. Cells	% Cells Exch.	Cell Growth	Mitotic Index %	% Abn. Cells	% Cells Exch.
Control								
S9	+ + +	9.6*	1.5	0.0	+ + +	11.0*	0.5	0.0
SDZ SID 791 (µg/ml)								
5000	+ + +	107.3	1.5	0.0	+ + +	86.3**	0.5	0.0
4000	+ + +	117.7	ND	ND	+ + +	104.5	ND	ND
3000	+ + +	96.9	1.0	0.5	+ + +	107.3	1.0	0.0
2000	+ + +	105.2	ND	ND	+ + +	111.8	ND	ND
1000	+ + +	101.0	0.5	0.0	+ + +	102.7	1.0	0.0
500	+ + +	89.9	ND	ND	+ + +	112.7	ND	ND
250	+ + +	96.9	ND	ND	+ + +	100.0	ND	ND
Positive Control, CP (µM)								
#17.5	ND	ND	42.0	14.0	ND	ND	38.0	4.0

ND not determined
* Mitotic indices as % of mitotic cells within the total population of mitotic and non-mitotic cells.
** Mitotic indices as % of the controls.
# 50 cells analyzed

Study title: Rat micronucleus test

Key study findings:
- AMD3100 was negative in the rat bone marrow micronucleus assay under the conditions tested.

Study no.: (b) (4) Project #960379
Volume #, and page #: Electronic submission, Module 4 ((b) (4) 960379.pdf)
Conducting laboratory and location: (b) (4)

Date of study initiation: September 22, 2004
GLP compliance: Yes (OECD)
QA reports: yes (x) no ( )

Drug, lot #, and % purity: AMD3100 (20 mg/mL, injection solution, also known as P104), Batch # PD04084, Purity: 99.2 % by HPLC

Formulation/vehicle: 0.9% (w/v) sodium chloride USP

Methods:

Species: Rat/ Hsd:SD®

Dose selection criteria:

The dose selection was based on the dose range-finding study performed in the (b) (4) Study #89289 ("A 28-day twice daily subcutaneous injection toxicity study of AMD3100 free base in the albino rat with a 14-day recovery period") and Study #89342, ("A range finding subcutaneous injection, twice daily, toxicity study of AMD3100 free base in the albino rat" [non-GLP]). According to the study data (not reviewed) the MTD was estimated at 25 mg/kg.

Dose range finding studies: see above.

Test agent stability: Stable

Metabolic activation system: not applicable

Controls: (not used in the dose range-finding study)

Vehicle: 0.9% Sodium chloride

Negative controls: Vehicle control

Positive controls:

Cyclophosphamide monohydrate (2 mg/mL): single oral gavage administration at 10 mL/kg.

Study design:

- Rats:
  ~7-8 weeks, weight: 161-231 g. Micronucleus analysis: n=5/sex/dose; positive control: n=3
- Dose schedule: single subcutaneous injections at 0, 6.25, 12.5 and 25 mg/kg (as Groups 1, 2, 3 and 4), two doses 24 hour apart. Dosing volume: 4 mL/kg.
- Micronucleus assay:
  - Bone marrow harvest time points: Twice samplings in Groups 1 and 4 at 24 and 48 hours, respectively, after dosing; and for Groups 2 and 3 and positive controls, only one sampling at 24 hours post-dosing.
  - Slide analysis: The bone marrow cells collected from both femurs of each rat were used to prepare smears on slides. Eight smears were prepared from each animal. Cells on slides were stained, and scored for micronuclei and the MIE (micronucleated immature erythrocytes) and MME (micrnucleated mature erythrocytes).
  - The extent of treatment-induced chromosome damage, indicated by the number of MIE, was determined by analyzing 2000 immature erythrocytes (IE) per animals for the presence of micronuclei.
  - The number of MME, was the mean value expressed per 2000 mature erythrocytes (ME) examined.
  - In addition, the proportion of immature erythrocytes (IE), expressed as percent IE/(IE + ME), was assessed by examination of a total of ≥1000 erythrocytes per animal.
  - Historical background frequency of micronuclei was: individual animal mean MIE 3.02 (i.e., 0.15%) and the mean group MIE 2.99 (i.e., 0.15%).

- In-life observation: All animals were examined twice daily for mortality, clinical signs and reaction to treatment. In addition, detailed examination was performed once daily and cage-side observation hourly for the first four hours following treatment. Individual body weights were measured prior to randomization, prior to treatment and at termination.

Assay acceptance criteria:

- Levene's test (SAS 1999) for homogeneity of variance was performed on absolute deviations. An appropriate minimal transformation was applied to achieve homoscedasticity (Steel and Terrie 1960, Sokal and Rohlf 1980). When this approached failed, subsequent statistical analyses were conducted on rank transformed data.
- The data were analyzed using SAS Proc MultTest. Since no sex interaction was found ($P>0.05$), the contrasts of interest (pairwise group comparison or one-sided trend as indicated in the table footnote, see below) were average across both sexes.
- A positive response was the detection of a statistically significant dose-related increase in the incidence of micronucleated immature erythrocytes (MIE) above the control level ($p\leq 0.01$). Individual and/or group mean values should also exceed the laboratory historical control range.
- Bone marrow toxicity was indicated by a substantial and statistically significant decrease in the proportion of immature erythrocytes (IE/IE+ME, see above) ($p\leq 0.01$). This decrease would normally be evident at the second sampling time (48 hr). Because of the long transition time of erythroid cells [late normoblast → immature erythrocyte (~ 6 hr) → mature erythrocyte in bone marrow (~ 30 hr)], it was possible to see a decrease in immature erythrocytes at the first sampling time (24 hr). In case of severe bone marrow depression (proportion of immature erythrocytes <20% of that for the concurrent control group), interpretation of increase in the incidence of MIE should be with caution.

Result:

Study validity:

The study is considered valid, because of:

- Acceptable controls: the incidence of MIE for the vehicle control was close to or within the laboratory historical vehicle/negative control range, while the positive control had a statistically significantly higher number of MIE's than the vehicle control ($p \leq 0.01$).
- Acceptable high dose: the high dose used 25 mg/kg, was the MTD of dose range-finding study.

Study outcome:

- ✧ There were no remarkable findings in mortality or clinical signs.
- ✧ Micronucleus analysis (mean ± SD) at 24 and 48 hr sampling time: table from the sponsor.

	Dose (mg/kg)	IE/(IE+ME) %	MIE (♂)	MIE (♀)	MIE (♂+♀)	MME (♂+♀)
24 hour sampling time						
Vehicle	0	46	1.6	3.0	2.3	0.0
AMD3100	6.25	48	2.6	2.6	2.6	0.0
	12.5	45	2.6	2.6	2.6	0.0
	25	46	2.2	1.4	1.8	0.0
CP	20	41	47.7	39	43.3*	0.0
48 hour sampling time						
Vehicle	0	45	2.2	1.6	1.9	0.0
AMD3100	25	45	1.8	1.4	1.0	0.0

***: statistically significant (p<0.001)**
%IE/(IE+ME): proportion of immature erythrocytes
MIE: number of micronucleated cells observed per 2000 IE examined
MME: number of micronucleated ME observed (mean value expressed per 2000 ME examined)

- ✧ AMD3100 did not increase the incidence of micronuleated immature erythrocytes (MIE) at either sampling time. Individual and group mean values for treated animals were within the range of historical control values (data not shown).
- ✧ The incidence of MME for all groups was uniformly low, confirming the absence of micronucleus-like artifacts.
- ✧ AMD3100 treatment did not reduce the proportion of immature erythrocytes, indicating a lack of bone marrow toxicity.

2.6.6.5 Carcinogenicity

No studies performed.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

No studies performed.

Embryofetal development

Study title: A subcutaneous injection teratology study in the rats

Key study findings:
- AMD3100 was teratogenic under the conditions tested.
- Dose dependent embryonic toxicity (fetal death, increased resorption and post-implantation loss, and decreased fetal weights) and fetal toxicity (external, visceral and skeletal malformations and/or variations) were mainly observed at 15 mg/kg of AMD3100. These toxicities were observed in the presence of maternal toxicities under the conditions of the study.

- Treatment of dams at 15 mg/kg were associated with maternal toxicities, based on deficits in corrected gestation weight gain from GD 6 (41%) and total body weight gain from GD 6 (23%), and decrease in food consumption (23% to 27%).
- The estimated NOAEL for maternal toxicities was 3 mg/kg/day.

Study no.: #900519
Volume #, and page #: Electronic submission, Module 4 ((b) (4) 900519.pdf)
Conducting laboratory and location: (b) (4)

Date of study initiation: November 29, 2004
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: AMD3100 (drug product P104, 20 mg/mL injection), Batch # PD04084, Purity: 99.2%

Methods
Doses: 0 (control), 0.5, 3 and 15 mg/kg (as Groups 1, 2, 3 and 4)
Species/strain: Sprague-Dawley CD rats [(b) (4) :CD®(SD) IGS BR]
Number/sex/group or time point: 22/group
Satellite groups used for TK study: n=6 (Groups 2, 3, and 4).

Route, formulation, volume: subcutaneous injection at dose volume of 2 mL/kg/day
- Formulation: AMD3100 20mg/mL
- Vehicle: 0.9% sodium chloride for injection, USP

Study design: Pregnant females (76-83 days, 223-312 g) were treated by once daily injection during gestation days (GDs) 6-17. The animals in the main study were sacrificed on GD21, while the TK animals on GD 17.

Dose justification: dose selection was based on the result of a dose range-finding study in pregnant rats (Study #900518). AMD3100 at 1, 5, 15 and 30 mg/kg was administered via subcutaneous injection during GD 6-17. Maternal toxicity (mortality) occurred at 30 mg/kg. Increased incidence of resorption and decreased fetal weight were observed at 15 mg/kg and ≥ 5 mg/kg, respectively. No dose justification is necessary, since maternal toxicity and embryo-fetal toxicity were reached in the present study.

Parameters and endpoints evaluated:
Clinical signs: Mortality, moribundity and clinical signs (twice daily), detailed examination (all main study animals on the gestation days of body weight assessment).
Body weights: All animals on GDs 0, 3, 6, 9, 12, 15, 18 and 21
Food consumption: Main study animals on GDs 3→6, 6→9, 9→12, 12→15, 15→18, and 18→21; not for TK animals.

Gross pathology:	At scheduled necropsy: major viscera of all main study animals including gross evaluation of placenta
Histopathology:	All organs/tissues were considered normal unless otherwise indicated
Toxicokinetics:	On GDs 6 and 17 at 0.5, 2, 6, 12 and 24 hr postdose; n=3/sex/group (Groups 2, 3, and 4).
Cesarean section:	GD 21
Reproductive parameters:	Dams: gravid uterine weight, uterine site description (live fetus, early, middle or late resorption), corpora lutea (main study animals) Non-pregnant uterine: implantation sites Fetal examination (live fetuses): weights, sexes, external findings, visceral examination at ~0.5 hr on approximately 50% of the fetuses from each litter, skeletal examination on the rest of 50% fetuses)

Statistical analyses: group variances were compared using Levene's test at the 0.05 significance level; when differences between group variances were not significant, the following methods were employed:

- ANOVA (parametric) followed by Duncan's t-Test to compare the treated groups against controls: body weights, food consumption and reproductive parameters.

However, if the Levene's test indicated heterogeneous variance ($p \leq 0.05$), the following tests would be used:

- Kruskal-Wallis test (non-parametric) followed by Duncan's t-Test to compare the treated groups against controls.

In addition, the overall incidence data (including all groups) were analyzed, first overall test, then pairwise group comparison of each treated group with the control:

- Fisher's Exact Test: fetal and maternal examination data and reproductive parameters.

Calculations:

Preimplantation loss (%) = [(No. of corpora lutea – No. of implant)/No. of corpora lutea] x 100

Postimplantation loss (%) = [(No. of implants – No. of live fetuses)/No of implants] x 100

Results

Mortality (dams): No AMD3100-related mortality. Female #3515, pregnant with 15 live fetuses and one early resorption, was euthanized on GD 19. The cause of moribundity was not certain, but gross examination did not show AMD3100-related evidence. The clinical signs of the female included decreased activity, piloerection, dehydration, decreased fecal output, pale eyes and skin, decreased muscle tone, abnormal gait, uncoordination, decreased respiratory rate, labored breathing and abnormal breathing sounds, cold to touch and weakness.

Clinical signs (dams): Not remarkable

Body weights (dams) and gravid uterine weights:

- ✧ Treatment-related reduction of group mean body weights and weight gains occurred at 15 mg/kg. Statistically significant changes in % deviations from the control group mean weights were 6%, 8% and 7%, on GD 15, 18 and 21, respectively (data for GD 15 and 18 are not shown in the table below), while significant deficits of weight gains were seen between GD 9-12, GD 12-15 and GD 15-18.
- ✧ Significant deficits in weight gain from GD6 (i.e., gestation weight gain), before (-23%) and after (-41%) correction with gravid uterine weight, were observed in the same group.
- ✧ Data of mean body weights and weight gains of dams, gravid uterine weights, corrected total body weights (GD 0-21) and gestation weight gains (GD 6-21) are summarized in the table below:

	Control	0.5 mg/kg	3 mg/kg	15 mg/kg
N (number of gravid females)	22	22	22	21*
Mean body weight on GD0 (g)	222.5	220.1	222.1	223.8
Mean body weight on GD6 (g)	265.5	261.1	262.1	266.7
Mean body weight on GD21 (g)	398.8	391	390	**369.9** (– 7%)
Weight gain (g)				
GD0-GD3	25	24	22	27
GD3-GD6	18	17	18	16
GD6-GD9	10	13	11	6
GD9-GD12	19	17	13	**11**
GD12-GD15	21	17	17	**11**
GD15-GD18	38	38	35	**30**
GD18-GD21	46	45	51 (n=21)§	45
Mean total weight gain (g, $BW_{GD21} - BW_{GD0}$) **(**without correction with gravid urine weight)	176.3	170.9	167.9	146.1
Gestation weight gain (g, $BW_{GD21} - BW_{GD6}$) **(**without correction with gravid urine weight)	133.4	129.9	129.4 (n=21)	**103.2** (– 23%)
Gravid uterine weights (g)	100.5	93.9	96.8	83.9 (–17%)
Corrected body weights (g) on GD21	298.3	297.1	293.2 (n=21)	286.0 (–4%)
Corrected gestation weight gain (GD 6-21) (g)	32.9	36	32.6 (n=21)	**19.3** (-41%)

*** All females were pregnant except #4514.**

§ Female #3515 was euthanized on GD 19, thus n=21 in Group 3.

Bolded numbers indicate statistically significant changes compared to the control. The numbers in the parentheses represent % reduction from the control (group mean).

Corrected body weight: Mean body weight on GD21 minus gravid uterine weight

Food consumption (dams)

Decreased food consumption occurred in Group 4 during GD 12 and GD18; statistically significant decrease was also seen in Group 3 on GD 15-18. The finding resolved after cessation of AMD3100 treatment on GD 17. Mean food consumption (g) is summarized in the table below.

	Control	0.5 mg/kg	3 mg/kg	15 mg/kg
GD 3-6	24	24	24	25
GD 6-9	24	24	24	23
GD 9-12	25	25	24	23
GD 12-15	28	26	25	**23** (–18%)
GD 15-18	31	29	**27** (–13%)	**27** (–13%)
GD 18-21	29	29	28	29

Numbers in the parentheses represent % deviation from the control.

Toxicokinetics:
Blood samples collected on GD 6 and GD 17 were analyzed using LC-MS/MS system, with the lower limit of quantification (LLOQ) at 1 ng/mL and the upper limit of quantification (ULOQ) at 200 ng/mL.
The means of C_{max}, AUC and T_{max} are summarized in the table below:

	C_{max} (μg/mL)	AUC_{0-24h} (μg·hr/mL)	C_{max}/ dose	AUC_{0-24} /dose	T_{max} (hr)
GD 6:					
0.5 mg/kg (n=6)	0.84	3.15	1.69	6.31	0.5
3 mg/kg (n=6)	4.57	14.06	1.53	4.69	0.5
15 mg/kg (n=6)	13.40	63.48	0.89	4.23	2
GD 17:					
0.5 mg/kg (n=6)	0.92	2.1	1.85	4.2	0.5
3 mg/kg (n=6)	5.95	16.75	1.98	5.58	0.5
15 mg/kg (n=6)	23.05	81.3	1.54	5.42	0.5

- The C_{max} and AUC increased dose-dependently, but the increase of dose normalized C_{max} and dose-normalized AUC was less than dose-proportional on GD 6. These parameters followed an approximately linear fashion on GD 17.
- C_{max} and AUC values increased slightly in Group 4 on GD17 when compared to those values on GD6.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):
There were no AMD3100-related necropsy findings in dams, and thus no warrant for histopathological examination.
The AMD3100-related reproductive effects were seen mainly at the dose of 15 mg/kg wherein post-implantation loss (%) and number of early/total resorption were increased. Although there were no changes in the number of viable fetuses, the fetal weights were reduced. There was one dead fetus in this group (dam #4501). The caesarian and fetal parameters for gravid rats are summarized in the table below:

	Control	0.5 mg/kg	3 mg/kg	15 mg/kg
Females mated	22	22	22	22
Number of females pregnant (%)	22 (100)	22 (100)	22 (100)	21 (95)
Aborted	0	0	0	0
Premature birth	0	0	0	0
Pregnant at C-section	22	22	21	21
Dams with viable fetuses	22	22	21	21
Dams with all resorption	0	0	0	0
Corpora lutea				
Total (approximated)	364	361	357	370
Average/animal (mean)	16.6	16.4	17	17.6
Implantation sites				
Total (approximated)	312	297	300	298
Average/animal (mean)	14.2	13.5	14.3	14.2
Preimplantation loss (%)	13.7	17.1	13.6	18.5
Postimplantation loss (%)	3.8	6.1	7.1	**13.5***
Dead fetuses (average/litter, %)	0	0	0	0.05

	Control	0.5 mg/kg	3 mg/kg	15 mg/kg
Total Resorptions				
Total	12	19	21	39
% (resorptions/implantation sites x 100%)	3.8	6.3	7	13
Average/animal (mean)	0.5	0.86	1	**1.86***
Early resorptions				
Total	11	19	21	29
% (resorptions/implantation sites x 100%)	5.4	6.3	7	9.7
Average/animal (mean)	0.4	0.86	1	**1.4***
Middle resorptions				
Total	1	0	0	5
% (resorptions/implantation sites x 100%)	0.3	0	0	1.7
Average/animal (mean)	0.05	0	0	0.24
Late resorptions				
Total	0	0	0	5
% (resorptions/implantation sites x 100%)	0	0	0	1.7
Average/animal (mean)	0	0	0	0.24
Viable fetuses				
Total (approximated)	301	277	279	258
% (viable/implantation sites x 100%)	94.6	94.7	90.9	86.6
Average/animal (mean)	13.7	12.6	13.3	12.3
Viable male fetuses (approximated) (%)	145 (48.8)	143 (51)	139 (48.7)	134 (48.5)
Group litter fetal body weight (g) (mean)	5.5	5.6	5.5	**4.9***
Mean male fetal weight (g)	5.7	5.7	5.6	**5.1***
Mean female fetal weight (g)	5.3	5.4	5.3	**4.7***

***: Statistically significant**

Comment:

- Treatment of AMD3100 up to 15 mg/kg in dams from GD 6 to GD 17 did not seem to affect pregnancy rate (95%, 21/ 22 rats were pregnant at 15 mg/kg). Whether this rate was within historical control ranges of the laboratory was not certain.
- Decreased gravid uterine weight in Group 4 (15 mg/kg) was supported by increased total resorption and decreased fetal weights at this dose level.
- Embryolethality of AMD3100 at 15 mg/kg was evidenced by one dead fetus (in #4501) and increased resorption.

Offspring (malformations, variations, etc.):

The incidence of fetal external/visceral and skeletal malformations and variations is shown in the tables below.

- Summary of the findings (total events of malformation and anomalies): Numbers of fetuses and litters affected are summarized.

	Fetus				Litter			
Group (mg/kg)	0	0.5	3	15	0	0.5	3	15
Number evaluated	301	278	280	259	22	22	21	21
Major malformation (Total): head, external, visceral and skeletal								
Affected	3	2	1	**20***	3	1	1	**13***
Total incidence (%)	1	0.7	0.4	**8***	13.6	4.5	4.8	**62***
Minor external/visceral anomalies (Total)								
Affected	2	4	5	**13***	2	2	4	**9***
Total incidence (%)	0.7	1.4	1.8	**5***	9	9	19	**43***
Minor skeletal anomalies (Total)								
Affected	40	27	**18***	38	15	15	10	19
Total incidence (%)	13	9.7	**6.4***	14.7	68	68	47.6	90

✧ External malformations and anomalies: data are indicated as "incidence (%)"

	Fetus				Litter			
Group (mg/kg)	0	0.5	3	15	0	0.5	3	15
Number evaluated	301	278	280	259	22	22	21	21
Major malformations								
Cranium								
Cyst at parietal/frontal bones	0	0	0	1 (0.4)	0	0	0	1 (4.8)
Eyes								
Absent (anophthalmia)	1	0	0	1 (0.4)	1	0	0	1 (4.8)
Reduced (microphthalmis)	0	0	0	1 (0.4)	0	0	0	1 (4.8)
Face								
Lower jaw reduced (mandibular micrognathia)	0	2 (0.7)	0	0	0	1 (4.5)	0	0
Upper jaw reduced (maxillary micrognathia)	0	2 (0.7)	0	0	0	1 (4.5)	0	0
Abdomen								
Intestine protruding at umbilicus (omphalocele)	0	0	1 (0.4)	1 (0.4)	0	0	1 (4.8)	1 (4.8)
Tail								
Absent (acaudia)	0	0	0	1 (0.4)	0	0	0	1 (4.8)
Shortened (microcaudia)	1 (0.3)	0	1 (0.4)	1 (0.4)	1 (4.5)	0	1 (4.8)	1 (4.8)
Limbs								
Digits of hindpaw shortened (brachydactyly)	0	0	0	1 (0.4)	0	0	0	1 (4.8)
Minor anomalies								
Abdomen								
Genital papilla (tubercule) reduced	0	2 (0.7)	0	0	0	1 (4.5)	0	0
Tail								
Kinked	0	2 (0.7)	0	0	0	1 (4.5)	0	0
Limbs								
Abnormal flexure of hindlimbs	0	0	1 (0.4)	0	0	0	1 (4.8)	0

✧ Visceral malformation and anomalies: data are indicated as "incidence (%)"

	Fetus				Litter			
Group (mg/kg)	0	0.5	3	15	0	0.5	3	15
Number evaluated	152	141	141	133	22	22	21	21
Major malformations								
Heart								
Globular heart	0	0	0	1 (0.8)	0	0	0	1 (4.8)
Dilatation of ascending aorta	0	0	0	1 (0.8)	0	0	0	1 (4.8)
Ringed aorta	0	0	0	2 (1.5)	0	0	0	2 (9.5)
Interventricular septal defect	0	0	0	3 (2.3)	0	0	0	3 (14)
Dilatation of pulmonary truncus	0	0	0	2 (1.5)	0	0	0	2 (9.5)
Stenosis of descending aorta	0	0	0	1 (0.8)	0	0	0	1 (4.8)
Abdominal cavity								
Intestine: stenosis	1 (0.7)	0	0	1 (0.8)	1 (4.5)	0	0	1 (4.8)
Kidneys								
Fused kidneys	0	0	1 (0.7)	0	0	0	1 (4.8)	0
Minor anormalies								
Adrenal glands								
Discoloration dark	0	0	0	**5* (3.8)**	0	0	0	**5* (23.8)**
Heart								
Innominate artery absent	0	0	0	3 (2.2)	0	0	0	2 (9.5)
Lungs and Thymus								
Supernumerary lung lobes	0	0	0	1 (0.8)	0	0	0	1 (4.8)
Kidneys								
Reduction of the renal papilla	0	0	1 (0.7)	0	0	0	1 (4.8)	0
Renal papilla absent	0	0	1 (0.7)	1 (0.8)	0	0	1 (4.8)	1 (4.8)
Ureters								
Dilated	1	0	4 (2.8)	4 (3)	1 (4.5)	0	3 (14)	4 (19)

✧ Skeletal malformations and anomalies: data are indicated as "incidence (%)"

	Fetus				Litter			
Group (mg/kg)	0	0.5	3	15	0	0.5	3	15
Number evaluated	149	139	140	130	22	22	21	21
Major malformations								
Vertebral column								
Multiple fusions & anomalies in thoracic vertebrae	0	0	1 (0.7)	0	0	0	1 (4.8)	0
Lumbar vertebrae (centra and arches) all absent	0	0	1 (0.7)	0	0	0	1 (4.8)	0
Minor anomalies								
See table below (from sponsor)								

	Group 1 Vehicle Control		Group 2 AMD3100 0.5 mg/kg/day		Group 3 AMD3100 3 mg/kg/day		Group 4 AMD3100 15 mg/kg/day	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
Skeletal (SKE)	22	149	22	139	21	140	21	130
Minor Skeletal Anomalies (Cont'd)	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
Minor Skeletal Anomalies (Total)	15	40	15	27	10	18 **	19	38
Skull								
Hyoid bone incomplete ossification	13	33	7	11 ***	4 *	4 ***	3 **	4 ***
Vertebral Column								
Reduced number of caudal vertebrae	0	0	0	0	1	1	1	1
Caudal vertebrae incomplete ossification	0	0	0	0	0	0	1	2
25 presacral vertebrae	0	0	0	0	0	0	1	1
Extra presacral vertebrae	0	0	0	0	0	0	1	1
Sacral vertebral centrum absent	0	0	0	0	1	1	0	0
Sacral vertebral arches incomplete ossification	0	0	0	0	0	0	2	2
Sacral vertebral arches unossified	0	0	0	0	0	0	4 *	4 *
Sacral vertebral arches absent	0	0	0	0	1	1	1	1
Sacral vertebral centrum fused	0	0	0	0	0	0	1	2
Sacral vertebral centrum incomplete ossification	0	0	0	0	0	0	1	1
Lumbar centrum bipartite	0	0	0	0	0	0	1	1
Lumbar centrum displaced to left of midline	0	0	0	0	0	0	1	1
Lumbar centrum fused	0	0	0	0	0	0	1	1
Lumbar vertebral arches incomplete ossification	0	0	0	0	0	0	1	1
Thoracic vertebral arches fused	0	0	0	0	1	1	0	0
Thoracic vertebral arches absent	0	0	0	0	1	1	0	0
Thoracic vertebral centrum absent	0	0	0	0	1	1	0	0
Thoracic vertebral centrum displaced from midline	0	0	0	0	0	0	14 ***	21 ***

Ribs								
Fused rib(s)	0	0	0	0	1	1	1	1
Notched rib(s)	0	0	0	0	1	1	0	0
Rib(s) absent	0	0	0	0	1	1	0	0
Rudimentary 14th rib(s)	0	0	2	3	1	1	0	0
Ossification center(s) on 7th cervical vertebrae	0	0	0	0	2	2	2	2
Bifurcated (branched) rib(s)	0	0	0	0	0	0	1	1

L/E = Litters examined L/A = Litters affected
F/E = Fetuses examined F/A = Fetuses affected
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

✧ Technique of Wilson (examination of the head) malformations and anomalies: data are indicated as "incidence (%)"

	Fetus				Litter			
Group (mg/kg)	0	0.5	3	15	0	0.5	3	15
Number evaluated	152	141	140	129	22	22	21	21
Major malformations								
Severe/moderate to severe dilatation of the lateral ventricles (hydrocephaly)	2	0	0	**11* (8.5)**	2	0	0	**9* (42.8)**
Severe dilatation of olfactory ventricles	0	0	0	**6* (4.7)**	0	0	0	3 (14)
Nasal septum: reduction in nasal turbinate formation	0	2 (1.4)	0	0	0	1 (4.5)	0	0
Minor anomalies								
Moderate dilatation of lateral ventricles	0	0	0	3 (2.3)	0	0	0	3 (14)
Moderate dilatation of olfactory ventricle	0	0	0	2 (1.6)	0	0	0	2 (9.5)
Protruding tongue	0	1 (0.7)	0	0	0	1 (4.5)	0	0

Comments:

- Fetal developmental toxicities, such as major external, visceral and head malformations were significantly increased (in the numbers of affected litters as well as affected fetuses) in the dams treated with 15 mg/kg of AMD3100. Significantly increased incidence in minor external and visceral anomalies was also observed in this group.
- Some fetal effects (e.g., minor skeletal anomalies in ribs) were noted in the group that received 3 mg/kg of AMD3100.
- All treated groups had a statistically significantly lower incidence of incomplete ossification of the hyoid bone. According to the sponsor, the incidence was within historical control ranges.

● Common skeletal variations (table adapted from sponsor's report)

Group (mg/kg)	0	0.5	3	15
Affected fetuses/litter (Mean % ± SD)				
Thoracic centrum variants (unossified incomplete, semi-bipartite, bipartite)	9.4 ± 10.5	17.4 ± 20.6	34.9 ± 27.6*	87.3 ± 18.7*
Sternebrae 1 to 4 (unossified, incomplete, semi-bipartite, bipartite)	0 ± 0	0 ± 0	0.7 ± 3.1	1.5 ± 4.7
Sternebrae 5 and xiphisternum (unossified, incomplete, semi-bipartite, bipartite)	9 ± 15.2	5.5 ± 11.7	8.8 ± 13.9	39.6 ± 20.3*

***: statistically different from the control**

Summary of individual study findings:

- AMD3100 treatment induced decreased mean body weight (on GD 21) and body weight gains, gestation weight gains (before and after correction with gravid uterine weight), and decreased food consumption in Group 4 (15 mg/kg) dams. These changes were significantly different from the control. However, the gravid uterine weights were not

different in comparison to the control. Only transient decreases in food consumption (GD15-18) were seen in this group. The NOAEL of maternal toxicities was 3 mg/kg.

- Embryonic toxicities were observed mainly in Group 4 (15 mg/kg). AMD3100 induced fetal death (one dead fetus in dam #4501), increased number of total resorption (↑ early resorption), increased post implantation loss and decreased fetal weights. However, it did not induce obvious uterine toxicities (e.g., pregnancy rate, corpora lutea or implantation sites), nor it affected total number of viable fetuses as well as litter size (viable fetuses/dam).
- Fetal toxicities included external, visceral, skeletal and head malformations and anomalies. Although the major malformation findings and variations were seen in Group 4, percentage of fetus/litter with common skeletal variations was increased significantly in Group 3.
- In Group 4, the embryo-fetal toxicities were accompanied by maternal toxicity.
- The exposure to AMD3100 at a dose causing teratogenic toxicities, i.e., the $AUC_{0\text{-}24}$ values of 15 mg/kg, was ~60-80 μg•hr/mL. In a clinical PK study (#1101), the $AUC_{0\text{-}24}$ value following a single subcutaneous dose at 240 μg/kg (the recommended clinical dose) in cohort with normal renal function was 5.1 μg•hr/mL. Although the subjects were mixed in this cohort, e.g., some subjects received a single dose of plerixafor while cancer patients received plerixafor plus G-CSF, the PK parameters were comparable. The AUC at which teratogenic effects were observed is approximately 10 fold the AUC in subjects administered the recommended human dose of 240 μg/kg/day.

Study title: A subcutaneous embryo-fetal development dose-range finding study in rabbits with toxicokinetics and placental transfer

This study was not reviewed. The dose-finding study was a non-GLP compliant study. In brief, inseminated female rabbits were treated with AMD3100 (0, 1, 3 or 10 mg/kg) via subcutaneous injection from Day 6 to Day 18 post insemination (p.i.). Mortality occurred at 3 mg/kg (1/6 dams) and at 10 mg/kg (3/6 dams). Maternal toxicities were observed at doses ≥ 1 mg/kg, as evidenced by body weight loss, decreased food consumption, clinical signs (weak limbs, sedation, miosis, exophthalmus, negative cornea reflex, cyanosis, and slow or shallow breathing). Increased pre-implantation loss was seen in one dam at 10 mg/kg. In this group, mean post-implantation loss was increased and the mean litter size was reduced. The pregnancy rate or mean numbers of corpora lutea were unaffected. Fetal toxicities (increased incidence of external malformations) were found at doses ≥ 3 mg/kg. These findings included: aplasia of toes, and head malformations (tel- and or mesencephalon flattened, rhombencephalon bulged, flattened face with vesicular evagination, aplasia of eye anlagen, jaw dyplasia). According to the sponsor, the NOAEL of embryo-fetal toxicities was 1 mg/kg, corresponding to a maternal $AUC_{0\text{-}6\ h}$ of 4882.1 ng•h/mL.

Prenatal and postnatal development

No studies performed.

2.6.6.7 Local tolerance

Study title: A comparative intracutaneous (intradermal) irritation study with two formulations of AMD3100 in the New Zealand white rabbit

Key study findings:

- The HCl preparation of AMD3100 (Formulation A) had more noticeable local irritation effect than the citric acid formulation (Formulation B).
- The degree of irritation was AMD3100 concentration-related, regardless of the formulation.
- CNS-related signs of toxicity were observed in animals treated with citric acid-based formulation.

Study no.: #(b) (4) 89679 (AnorMED)
Volume #, and page #: Electronic module 4 ((b) 89679.pdf)
Conducting laboratory and location: (b) (4)
Date of study initiation: November 8, 2000
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: AMD3100, Batch #B99P122
Formulation
Formulation A: AMD3100 50.1 mg/mL in HCl base, purity 50.1 mg/mL (100%)
Formulation B: AMD3100 124.7 mg/mL in citric acid base, purity 124.7 mg/mL (100%)
Vehicle: 0.9% Sodium chloride (served as negative reference control)
Positive reference control: Cottonseed oil N.F. (lot # PM0229)

Methods:
Doses:
Formulations A and B were prepared to make test solutions at various concentrations (table from the sponsor):

Number	Formulation and Concentration (mg/mL)	
	A	B
1	50.1	124.7
2	25	50
3	12	25
4	6	12
5	3	6
6	1.5	3
7	-	1.5

Study design:

- Test system: New Zealand white rabbits (5-6 months)
- The local irritation effects of two AMD3100 formulations were assessed in two groups of rabbits (Groups 1 and 2 for Formulations A and B, respectively).
  - Group 1: one male (#102), two females (#151 and #153)
  - Group 2: two males (#202 and #203), two females (#251*→#203 and #253)
    * replaced by #203 due to mortality
- Intracutaneous injections at an injection volume of 0.2 mL/site; up to 19 sites on each rabbit.
- Mortality and clinical signs were examined twice daily and complete detailed physical examination was performed weekly during pretest period and daily during treatment period.
- Body weights were measured pretest and on Days 1 and 4 of treatment.
- Assessment of local reactions: The appearance of each injection site was recorded immediately after injection and again at 24, 48 and 72 hr after injection. Sites were graded for erythema and edema, according to the following criteria (table from the sponsor):

Reaction	Numerical Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond the exposure area)	4
Total possible score for irritation	8
NOTE – Other adverse changes at the injection sites were recorded and reported.	

- The primary Irritation Index (PII): The combined score of erythema and edema over 72 hr time course for each animal, separately for each test formulation concentration, was divided by the number of observations (= the number of treatment x the number of time point). The mean PII score was ranked as response category of: negligible: 0-0.4 (PII score), slight: 0.5-1.9, moderate: 2.0-4.9, and severe: 5.0-8.0. (Based on the ISO 10993 Part 10 Test Guidance)

Results:

Mortality:

One Group 2 (i.e., Formulation B) female (#251) was euthanized approximately 1 hr post dosing, due to severe clinical signs. These signs included CNS signs (severe incoordination, tremors and convulsion), lying on side, labored breathing, and weakness.

Clinical signs:

Findings were mainly associated with Formulation B (or only in Group 2) and occurred on Day 1, including uncoordination (severe in #202), lying on side, weakness, and decreased activity.

Body weight: not remarkable

Dermal reactions:

The negative and positive controls: table from the sponsor:

Group 1	PII	Classification
Saline	0.24	Negligible irritant
Cottonseed oil	1.24	Slight irritant
Group 2	**PII**	**Classification**
Saline	0.07	Negligible irritant
Cottonseed oil	0.26	Negligible irritant

The AMD3100-treated result: table from the sponsor.

Formulation A (Group 1):

HCl Formulation	PII	Classification
AMD 3100 50.1 mg/mL	1.78	Slight irritant
AMD 3100 25 mg/mL	1.78	Slight irritant
AMD 3100 12 mg/mL	1.11	Slight irritant
AMD 3100 6 mg/mL	0.89	Slight irritant
AMD 3100 3 mg/mL	0.78	Slight irritant
AMD 3100 1.5 mg/mL	0.44	Negligible irritant

Formulation B (Group2):

Citric Acid Formulation	PII	Classification
AMD 3100 124.7 mg/mL	1.33	Slight irritant
AMD 3100 50 mg/mL	1.55	Slight irritant
AMD 3100 25 mg/mL	0.78	Slight irritant
AMD 3100 12 mg/mL	0.44	Negligible irritant
AMD 3100 6 mg/mL	0.22	Negligible irritant
AMD 3100 3 mg/mL	0.11	Negligible irritant
AMD 3100 1.5 mg/mL	0.00	Negligible irritant

Comment:

- Injection site reactions to Formulation A were more pronounced than those to Formulation B, because AMD3100 at ≥3 mg/mL in Formulation A was slightly irritable, while Formulation B at the concentrations of ≥25 mg/mL exerted similar irritation effects.

- CNS-related signs of toxicity (mortality and clinical signs) were more evident in animals treated with Formulation B (containing citric acid).

2.6.6.8 Special toxicology studies

Study title: Effect of SDZ 282-791 on *in vivo* antibody formation (rat)

Key study findings: Subcutaneous administration of SZD 282-791 to rats, at doses up to 20 mg/kg/day x 4 days, did not inhibit *in vivo* antibody formation to sheep red blood cells, as assessed by splenic plaque formation capacity.

Study no.: #107-025 (b) (4)
Volume #, and page #: Electronic module 4 (107-025.pdf)
Conducting laboratory and location: (b) (4)
Date of study initiation: January 23, 1996
GLP compliance: Not reported
QA reports: Not reported
Drug, lot #, and % purity: SZD SID 791, no information on batch number or purity

Formulation/vehicle: Ethanol + Tween 80 (pH adjusted to 7.3 with KOH)

Methods:
Dose: Subcutaneous injection of SZD 282-791 (same as SZD SID 791) at 8 or 20 mg/kg on Days 0, 1, 2 and 3. Cyclosporin (SIM, in solvent G* + corn oil), as positive control, was administered at 3 mg/kg subcutaneously, following the same dosing schedule.
*** No information was provided on the formulation of solvent G.**

Study design:
- Test system: female OFA rats (n=5/group).
- Splenic plaque forming capacity test: On Day 0, rats were immunized intravenously with 1 x 10^6 sheep erythrocytes. On Day 6, spleen was harvested and processed to cell suspensions. Cells (1.5 x 10^6) were plated to soft agar with sheep red blood cells (RBC, 2 x 10^8) and complement (fresh guinea pig serum). Plaques were counted after 2 hr-incubation.
- Animals were weighed on Days 0 and 6.

Results:
The result of splenic plaque formation on sheep RBC-soft agar plates is summarized in the table below (from the sponsor):

Table 1. *In vivo* antibody formation (rat)

Compound	Dose and route	Weight body (gram) day 0	day 6	Weight spleen (mgram)	Cells per spleen (10^6)	Plaques per spleen (10^3)
Control	-	157	190	582	717	114±65
SIM	3 mg/kg sc	150	180	474	574	5.7±4.5
Control	-	164	187	575	721	210±44
282-791	8 mg/kg sc	167	192	516	637	188±32
	20 mg/kg sc	164	190	587	729	219±65

Treatment of cyclosporin inhibited *in vivo* antibody formation of the splenic cells, and hence less cell lysis on sheep RBC soft agar plates (less plaque formation) was observed. Under the same condition of study, treatment of SZD SID-791 for 4 consecutive days did not inhibit *in vivo* antibody formation.

Study title: *In vitro* evaluation of the influence of AMD3100 on human whole blood hemolysis

Key study findings: Under conditions tested, AMD3100 (0.2 μg/mL) or AMD3100 placebo did not show in vitro hemolytic or flocculating effects when incubated with human whole blood samples for 1 hr.

Study no.: #(b) (4) 60101 (AnorMed)
Volume #, and page #: Electronic, module 4 ((b) 60101.pdf)
Conducting laboratory and location: (b) (4)

Date of study initiation: June 4, 1998
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: AMD3100 injection (10 mg/mL, 1 mL vial), Batch #31288, purity: 99.5%

Formulation/vehicle: AMD3100 placebo (buffered normal saline, 1 mL vial), Batch #31278

Methods:
Dose: AMD3100 at the final concentration of 0.2 μg/mL and diluted placebo were prepared by mixing 100 μL of the test compound or the placebo with 10 mL of saline, and the resultant solutions were further diluted with saline to reach the target concentration.

Study design:

- Test system: Human whole blood samples (4 samples: 2 males and 2 females).
- The control: negative control: 0.9 % NaCl, positive control: 5% saponin.
- 0.2 mL each of the test compound, placebo, negative and positive controls were mixed with 1.8 mL of human whole blood, incubated at 37 °C for 1 hr. Plasma was separated, and the level of hemolysis and flocculation were visually observed. The absorption at 660 nm (A_{660}) of the plasma (via (b) (4) plasma hemoglobin (Hb) (via (b) (4)) and hemolytic index (via the (b) (4)) were determined.
- The qualifying criteria:
  - Negative control: Hb < 0.4 g/dL, visual hemolysis: H° (no observed reaction) or H^{+} (weak positive reaction)
  - Positive control: Hb > 4 g/dL, H^{+++} (strong positive reaction)
  - AMD3100 or placebo to be considered to have a significant *in vitro* lymolytic and/or flocculating effect on whole blood if all the following observations were obtained:
    - Hemolytic effect: visual hemolysis ≥ H^{++} (moderate positive reaction), Hb ≥ 0.4 g/dL, and hemolytic index > 3
    - Flocculating effect: visual flocculation ≥ F^{++} (moderate positive reaction), net A_{660} > 0.2

Results:

The *in vitro* hemolytic and flocculating effects of AMD3100, placebo and controls are summarized in the table below (from the sponsor):

Tube No	Parameter	Identification	Human #1 (female)	Human #2 (female)	Human #3 (male)	Human #4 (male)
1	Hemoglobin (g/dL)	Negative Control	0.2	0.1	0.1	0.2
2		Positive control	12.2	11.9	14.0	15.0
3		Buffered normal saline	0.2	0.2	0.2	0.3
4		AMD-3100	0.1	0.1	0.2	0.2
1	Plasma Hemolysis	Negative Control	H°	H°	H+	H+
2		Positive control	H+++	H+++	H+++	H+++
3		Buffered normal saline	H°	H°	H+	H++
4		AMD-3100	H°	H°	H+	H++
1	Hemolytic index	Negative Control	2	2	3	2
2		Positive control	440	390	460	520
3		Buffered normal saline	2	2	2	3
4		AMD-3100	2	2	3	4
1	Visual Flocculation	Negative Control	F°	F°	F°	F°
2		Positive control	F°	F°	F°	F°
3		Buffered normal saline	F°	F°	F°	F°
4		AMD-3100	F°	F°	F°	F°
1	Net A660	Negative Control	N/A	N/A	N/A	N/A
2		Positive control	1.534	1.067	1.488	1.320
3		Buffered normal saline	0.000	0.000	0.008	0.000
4		AMD-3100	0.000	0.000	0.005	0.013

H°F°: No observed reaction
H+, F+: Weak positive reaction
H++, F++: Moderate positive reaction
H+++, F+++: Strong positive reaction
N/A: Not Applicable

2.6.6.9 Discussion and Conclusions

Pharmacology, safety pharmacology, pharmacokinetic/ADME, and toxicology studies supporting the marketing application of plerixafor for the poposed indication were conducted in *in vitro* systems as well as in rats, rabbits, and dogs. The general toxicology studies were conducted in appropriate animal species, using the administration route, dosing schedule/duration that adequately addressed safety concerns for the indicated patient population and the intended duration of administration. The target organs of plerixafor are bone, liver, spleen, and cardiovascular and central nervous system.

The rodent and nonrodent species in general did not demonstrate significant differences in susceptibility to plerixafor treatment. Toxicities attributable to the pharmacological effects of plerixafor included leukocytosis, i.e., increased white blood cell counts (total and differential). Literature reports, including gene knock-out data, have confirmed the association between targeting SDF-1-CXCR4 axis and toxicity in the hematopoietic system (Nagasawa *et al.*, Nature 635-638, 1996; Zou *et al.*, Nature 393: 595-599, 1998; Broxmeyer *et al.*, J Exp Med, 201: 1307-1318, 2005; Burroughs *et al.*, Blood, 106:4002-4008, 2005). In these reports, myelopoiesis was the main linage affected; in general erythroid parameters or platelet counts were not affected. Increased circulating white counts could likely lead to

inflammation. The hematological findings in dogs were less remarkable in comparison with those in rats, based on comparable systemic exposure to plerixafor.

Increased hematopoiesis was observed in liver and spleen of rats. The finding may be associated with mobilization of marrow hematopoietic stem cells and progenitor cells by plerixafor. It was not certain, however, whether lymphoid atrophy in spleen and thymus was a direct consequence of plerixafor treatment, or a secondary reaction to decreased weight gain and food intake.

Plerixafor treatment induced changes in bone mineral content of tibia and humerus (↓ 9-18%) and growth-driven modeling drifts on the peristeal surface of humerus in rats. According to Hirbe *et al.* (PNAS 104: 14062-14067, 2007), disruption of CXCR4 enhanced osteoclastogenesis, bone loss and increased markers of bone resorption in mice. Loss of bone mineral contents was coincident with increased urine calcium and magnesium levels.

Another common finding in rats and dogs was the CNS clinical signs. Although there was no histopathological evidence of lesions in brain or spine, CNS depressant-like signs were observed. These signs included: ventral recumbency, decreased activity, twitching and labored breathing in rats, and tremor, ataxia, impaired balance, sedation and mydriasis in dogs. These signs were also found in mice and rats in the POT test and the Irwin test (See Safety Pharmacology). According to the PK data, the distribution of plerixafor radioactivity in brain, spinal cord or cerebro-spinal fluid (CSF) was minimal, however, detectable radioactivity in the CNS increased with respect to blood levels 4 days postdose. In addition, small but measurable levels of plerixafor were detected in the CSF of dogs in one study, further indicating that the drug can cross the blood-brain barrier. In mice lacking the expression of CXCR4, Zou and colleagues demonstrated the role of CXCR4 in cerebellar development (such as neuronal cell migration, axon guidance in the developing nervous system, and cerebellar neuronal layer formation) (Zou *et al.*, Nature 393: 595-599). Plerixafor, in the micromolar ranges, showed affinity to α_1 and α_2 adrenergic, and dopamine D_2 receptors on membranes from calf brain tissues (Study 107-031). Another mechanism of plerixafor's CNS effects, is perhaps via changing the calcium and magnesium levels in the plasma and in the CSF. In a 15-day study in dogs, plerixafor was found to increase CSF calcium levels 2-3 fold higher than the control. In rats and dogs, serum levels of these minerals were changed. Serum magnesium levels were dereased in all studies, but changes in calcium levels were not consistent.

Treatment with plerixafor resulted in diverted cardiovascular effects in rats and in dogs. Plerixafor administered through IV infusion to rats under anesthesia induced lethal cardiodepression, i.e., ↓ BP, HR and myocardial contractility. In telemetered conscious dogs, plerixafor induced tachycardia and hypertension. Together with depressed respiratory signs (decreased tidal volumes and respiratory rates), these findings may be attributed to plerixafor's CNS effects. The differences (i.e., suppression in rats versus stimulation in dogs) may be due to different experimental settings (i.e., anesthetized rats versus conscious dogs). Plerixafor was also demonstrated to have direct vascular effects, such as vasodilation in rat aortic smooth muscle cells and fibroid necrosis of the myocardial blood vessel wall in dogs. Based on a published article, mice lacking CXCR4 died *in utero* and were defective in

vascular development, hematopoiesis and cardiogenesis (Tachibana *et al.*, Nature 393: 591-594, 1998). As scientific data link the SDF-1-CXCR4 axis to neovascularization (Salcedo *et al.*, Am J Path, 154: 1125-1135, 1999), it is likely that agents disrupting the SDF-1-CXCR4 axis, such as plerixafor, directly affect the cadiovascular system.

Repeated dose toxicity studies which are not reviewed, are summarized in a table provided by the sponsor; see Section 2.6.7. There were no additional histopathological findings in these studies. Furthermore, twice daily subcutaneous toxicology studies (reviewed by Dr. Guodong Fang) showed key findings similar to those seen in once daily subcutaneous studies reviewed in this NDA.

Plerixafor was negative in bacterial Ames test, *in vitro* chromosomal aberration test in V79 Chinese hamster cells, and *in vivo* micronucleus test in rats. The effect of plerixafor on male or female fertility was not studied in designated reproductive toxicology studies. However, based on results of the repeated dose toxicity study in rats and in dogs (up to 4 weeks followed by 4 week-observation), there were no indication of plerixafor effects in the reproductive organs in male or female animals. At a SC dose of 90 mg/m^2, plerixafor increased post-implantation loss and early (and/or total) resoprtion. Dose-dependent embryo-fetal toxicities included external (reduced or absent eyes, reduced lower or upper jaw, protruding intestine, and absent or shortened tail) , visceral (globular heart, dilated or ring aorta, interventricular septal defect, stenosis of intestine, and fused kidneys), and head (severe to moderate hydrocephaly, severe dilatation of olfactory ventricle, and reduction in nasal turbinate formation) malformtions and variations, as well as retarded skeletal development. The embryofetal development study in rabbits was a non-GLP study and was not reviewed. Based on summary data provided by the sponsor, malformations in rabbits were observed at doses ≥ 36 mg/m^2 and included the following: external (aplasia of toes), head (tel- and/or mesence-phalon flattened, flattened face with vesicular evagination, aplasia of eye anlagen, jaw dysplasia). Therefore, plerixafor is considered teratogenic in rats and rabbits.

Treatment of plerixafor in rats did not affect *in vivo* antibody formation to sheep red blood cells as assessed by splenic plaque formation capacity. Plerixafor did not induce hemolysis in human whole blood samples. Local irritation (slight in severity) was observed when plerixafor was administered via intracutaneous injection to New Zealand white rabbits. Measurable levels of radioactivity were detected in skin and uveal tract of rats at 336 hours (14 days) following a single subcutaneous administration of plerixafor; however phototoxicity of plerixafor has not been investigated (it is not known whether plerixafor absorbs the light).

2.6.6.10 Tables and Figures

See text of review for pertinent tables and figures.

TOXICOLOGY TABULATED SUMMARY

General toxicology

Single Dose Toxicity Studies					
Species	**Route**	**N/sex/ dose**	**mg/kg**	**mg/m^2**	**Significant findings**
Mouse	SC	5	2	6	Not remarkable
			14	42	Mortality (30%), clinical signs (sedation, spasm, dyspenia, ventral recumbency)
			20	60	Mortality (70%), clinical signs (see above)
					LD_{50}: 16.3 mg/kg
Mouse	IV	5	2	6	Clinical signs (sedation)
			5	15	Mortality (40%), clinical signs (sedation, spasm, dyspenia)
			8	24	Mortality (90%), clinical signs (sedation, spasm, dyspenia, ventral recumbency)
					LD_{50}: 5.2 mg/kg
Rat	SC	5	2	12	Not remarkable
			20	120	Not remarkable
			30	180	Clinical signs (sedation, spasm, dyspenia, uncontrolled movement, ventral or lateral recumbency, hunched posture)
			40	240	Mortality (30%), CNS signs (see above, more severe)
			50	300	Mortality (30%), CNS signs (see above, more severe)
					LD_{50} > 50 mg/kg
Rat	IV	5	2	12	CNS signs (sedation)
			5	30	Morality (40%), CNS signs (sedation, spasm, dyspenia, ventral recumbency)
			8	48	Morality (90%), CNS signs (sedation, spasm, dyspenia)
					LD_{50}: 5.2 mg/kg
Repeat Dose Toxicity Studies					
Species	**Route**	**N/sex/ dose**	**mg/kg/ day**	**mg/m^2 /day**	**Significant findings**
Rat	SC Daily 4 week	10 (a) 6 (b) 4 (c)	12 3 1	72 18 6	72 mg/m^2/d: ventral recumbency, twitching, rales, necrosis at injection site, ↓ weekly BW gains: up to –24% from control, not recovered in ♀ (–81% in week 7), not recoverable ↓ weekly food consumption (♀): up to –21% from control, ↑white counts (total and differential), ↓ reticulocytes (♀), ↓ serum Mg^{2+}, urinalysis findings (↑ Ca^{2+}, Mg^{2+}, ↓ pH), ↓ thymus weights, ↑ spleen weights, histopathological findings: liver (hematopoiesis), injection site (inflammation and hemorrhage) 18 mg/m^2/d: ↓ weekly BW gains: up to –24% from control, ↓ serum Mg^{2+} (♂), ↑ spleen weights, histopathological findings: liver (hematopoiesis), injection site (inflammation and hemorrhage) 6 mg/m^2/d: ↓ weekly BW gains: up to –24% from control, ↓ serum Mg^{2+} (♂), histopathological findings: liver (hematopoiesis), injection site (inflammation and hemorrhage))
Rat	SC Daily 4 week	10 (a) 6 (b) 4 (c)	24 18	144 108	144 mg/m^2/d: Mortality (♂13/20, ♀12/20), hyper excitation, ventral recumbency, twitching, labored respiration, rales, necrosis at injection site, ↓ BW gains (–12 to –186%, ♂>♀), ↓food intake (♂: –10 to –16%), ↓ reticulocytes, ↑white counts (total and differential), ↓ serum Mg^{2+} (♂), ↑ serum Ca^{2+}, urinalysis findings (↑ Ca^{2+}, Mg^{2+}, ↓ pH), ↑ liver and spleen weights, ↓ thymus weights, Histopathological findings: injection site (hemorrhage, inflammation), spleen (lymphoid atrophy, ↑ hemopoiesis), thymus (congestion, lymphoid

					atrophy), also minor lesions in liver, kidney, lymph nodesadrenal, pancreas and thyroid. 108 mg/m^2/d: hyper excitation, ventral recumbency, labored respiration, necrosis at injection site and/or rales, ↓ BW gains (–13 to –86%, ♂>♀), ↓food intake (♂: –14 to –31%), ↓ reticulocytes, ↑white counts (total and differentiated), ↓ serum Mg^{2+} (♂), ↑ serum Ca^{2+}, urinalysis findings (↑ Ca^{2+}, Mg^{2+}, ↓ pH), ↑ liver and spleen weights, ↓ thymus weights, similar but less severe histopathological findings as 144 mg/m^2/d.
Dog	SC Daily 4 week	3 (a) 2 (b)	4 1 0.25	80 20 5	80 mg/m^2/d: diarrhea, lesions near injection sites (thickness, swelling), ↓ BW gains (♂ –25%, ♀ –64%), ↓food intake (♀): –24%, ↑ HR (up to 26-27% in Week 4), ↑ BP (systolic, diastolic and mean arterial pressures), ↑white counts (total and differential), BM: normal cellularity but ↓ erythroid series, urinalysis findings (♂ only: ↑ urine volume, ↓ specific gravity, ↑ Ca^{2+}, Mg^{2+}), no remarkable histopathological findings. 20 mg/m^2/d: diarrhea, ↓ BW gains (♂ –8%, ♀ –36%) ↓food intake (♀): –15% 5 mg/m^2/d: diarrhea, ↓ BW gains (♂ –33%, ♀ –9%)

Route of administration: SC (subcutaneous), IV (intravenous)
Study groups: main (a), recovery (b, only the control and the high dose groups) and toxicokinetics/TK (c)

Summary of mean plasma TK parameters in general toxicology studies: following once daily or twice daily dosing regimen in rats and dogs (tables from the sponsor: Section 2.6.6 "Toxicology written summary")
Note: See Appendix for the review of studies following twice daily dosing regimen.

Table 2.6.6-6: Mean Plasma TK Parameters of Plerixafor in Male and Female Rats following once- or twice-daily dosing for 29 or 30 Consecutive SC doses (428R-tk and 432R; (b) (4) 89289)

Dose (mg/kg)	Inter-val (Day)	Male					Female				
		$AUC_{(0-24h)}$ (µg·h/ml)	C_{max} (µg/ml)	% Day 1 C_{max}	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{(0-24h)}$ (µg·h/ml)	C_{max} (µg/ml)	% Day 1 C_{max}	t_{max} (h)	$t_{1/2}$ (h)
Once-daily dosing regimen											
0.6	1	8.76	2.45	----	0.5	n/a[a]	3.31	1.39	----	0.5	n/a
	30	18.19	2.73	+11	0.5	n/a	6.51	1.87	+34	0.5	n/a
1.9	1	21.90	6.08	----	0.6	n/a	19.60	4.89	----	1.0	n/a
	30	30.36	7.55	+24	0.5	n/a	22.39	6.25	+28	0.6	n/a
7.6	1	57.00	17.13	----	0.8	n/a	67.45	16.98	----	1.0	n/a
	30	63.50	26.61	+55	0.5	n/a	62.66	27.07	+59	0.6	n/a
11.4	1	125.60	36.90	----	0.9	n/a	111.80	28.40	----	1.0	n/a
	29	134.80	47.80	+30	0.6	n/a	110.90	47.00	+65	0.6	n/a
15.2	1	139.90	33.40	----	1.5	n/a	129.40	32.10	----	1.3	n/a
	29[b]	----	----	----	----	----	161.70	54.60	+70	0.8	n/a
Twice-daily dosing regimen											
0.3 BID	1	0.46	0.39	----	0.5	c	0.49	0.44	----	0.5	c
	28	0.82	0.42	+6	0.5	c	1.06	0.50	+14	0.5	c
12 BID	1	29.28	11.07	----	0.5	3.8	36.22	13.58	----	0.6	3.1
	28	45.54	23.97	+117	0.5	8.1	39.36	19.81	+46	0.5	7.6

[a] n/a = Not available; elimination half-life not calculated for rats treated on once-daily dosing regimen
[b] All males were dead and half the females were dead by this sampling point
c Plasma concentrations insufficient to calculate elimination half-life

Table 2.6.6-7: Mean Plasma TK Parameters of Plerixafor in Male and Female Dogs following once- or twice-daily dosing for 4 weeks ((b) (4) 94/SPM028/0891-tk; (b) (4) 89290)

Dose (mg/kg)	Interval	Male					Female				
		$AUC_{(0-24h)}$ (µg·h/ml)	C_{max} (µg/ml)	% Day 1 or 2 C_{max}	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{(0-24h)}$ (µg·h/ml)	C_{max} (µg/ml)	% Day 1 or 2 C_{max}	t_{max} (h)	$t_{1/2}$ (h)
Once-daily dosing regimen											
0.25	Day 2	2.06	0.27	----	2.0	n/a[a]	1.59	0.22	----	1.0	n/a
	Week 4	2.55	0.29	+6	2.0	n/a	1.50	0.19	-14	1.0	n/a
1	Day 2	4.58	0.87	----	1.2	n/a	6.27	1.09	----	0.83	n/a
	Week 4	6.92	1.24	+42	1.0	n/a	7.61	1.30	+19	0.83	n/a
4	Day 2	22.68	4.57	----	1.0	n/a	31.29	6.88	----	0.83	n/a
	Week 4	25.80	6.09	+33	0.8	n/a	39.14	12.14	+76	0.66	n/a
Twice-daily dosing regimen											
0.15 BID	Day 1	0.58[b]	0.19	----	0.7	1.6	0.60[2]	0.19	----	1.3	1.6
	Day 28	0.58	0.17	-9	1.0	2.1	0.65	0.25	+29	1.3	2.0
0.75 BID	Day 1	3.95[b]	1.13	----	0.5	2.3	4.62[2]	1.45	----	0.5	2.2
	Day 28	4.47	1.07	-5	0.7	5.6	4.93	1.30	-10	0.7	5.6
4 BID	Day 1	29.92[b]	14.09	----	0.5	3.0	34.45[2]	13.24	---	0.5	3.1
	Day 28	25.71	8.26	-41	0.5	5.7	31.81	9.26	-30	0.5	5.6

[a] n/a = Not available; elimination half-life not calculated for dogs treated on once-daily dosing regimen
[b] The $AUC_{(0-17h)}$ value included the afternoon dose only.

Reviewer's note: the doses indicated in the once-daily dosing regimen refer to the salt.
(b) (4)

Non-pivotal general toxicology studies: Table from the sponsor (Section 2.6 Nonclinical summary, 2.6.7 toxicology tabulated summary). These studies were not reviewed in the NDA, except ITR 1663, (b) (4) 89342 and (b) (4) 89349 (reviewed by Dr. GuoDong Feng).

Table 2.6.7-6: Repeat-Dose Toxicity: Non-pivotal Studies

Species (Strain)	Route (Vehicle)	Duration of Dosing	Doses (mg/kg) Free Base Equivalent	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number
Rat (WIST (SPF))	SC	14 days	9.49	4F	Not Reported	9.49: Copper and zinc - Urinary excretion greatly increased at all times, concentrations in urine less than that of plerixafor, plasma concentrations not affected; Calcium and magnesium – Both show stable decrease in plasma concentrations, urine calcium concentrations progressively increased to two fold over control by study end, urine calcium concentrations higher than plerixafor, magnesium levels in urine not affected.	GT-249-TX-4
Rat (WIST (SPF))	SC (Saline)	16 days	0 3 6 9 12	4M 4F	Not Reported	≥ 3: Sedation and piloerection; ↑ blood iron, ↑ urine calcium; ↑spleen size, ↑ extramedullary haematopoiesis and megakaryocytes in the liver and spleen, Histiocyte aggregates and/or focal lymphoid hyperplasia, birefringent deposits, dermatitis, inflammation, haemorrhage, necrosis and muscle degeneration and regeneration at injection sites ≥ 6: Recumbency, twitching, and laboured respiration; ↓ blood calcium and magnesium; ↑ spleen weights ≥ 9: Mortality; ↓ rectal temperature, ↓ body weight gain, ↓ haemoglobin and/or	189DFR-tox

Species (Strain)	Route (Vehicle)	Duration of Dosing	Doses (mg/kg) Free Base Equivalent	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number
						RBC, ↑ WBC, ↑ reticulocytes, lymphoid depletion 12: ↑ blood phosphorus; hemorrhagic fluid in abdomen Toxicokinetics: Increasing plasma levels with rising doses, increase of Cmax becomes underproportional at higher doses, mean maximal plasma concentrations higher in male than female, 1 hour plasma samples higher on day 2 than day 9 or 16. Plerixafor disappears rapidly from rat plasma	
Rat (Wistar)	SC (Saline)	Up to 7 days (5 days for 6 BID and a single dose for 36 and 50)	1.5 BID 6 BID 12 BID 24 BID 36 50	2M 2F	12 BID	≥ 24 BID: Mortality; ↓ activity, cold to touch, head tilt, partly closed eyes, non-sustained convulsion, uncoordination and tremors ≥ 36: Mortality; weakness, recumbency, shallow and irregular breathing, skin pallor, and vocalization	(b) (4) 89342
Rat (SD)	SC (Saline)	14 days	0 2 BID 6 BID 12 QD	5F	12 QD	2 and 6 BID: No findings 12 QD: No findings	(b) (4) 1663

Species (Strain)	Route (Vehicle)	Duration of Dosing	Doses (mg/kg) Free Base Equivalent	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number
Dog (Beagle)	SC (Saline)	Days 1-3, 8, and 16	4.0 (Day 1-3) 5.0 (Day 16) 6.0[a] (Day 8)	1M 1F	Not Reported	≥ 4.0: Transient increase pulse rate and reduction blood pressure; slight increase in rectal temperature at 0.5 hr and decrease at 2 hours (not measured at higher dose levels) ≥ 5.0: Hypoactivity, salivation, ataxia, hunched posture, tremors prostration, retching, emesis and excessive drinking, recovery seen at 1 hour post dose; slight reduction pulse pressure and marked increase in pulse rate 6.0: Ears cold to touch, pale gums/ears, vomiting	(b) (4) 94/ SPM030 /0883
Dog (Beagle)	SC (Saline)	15 days	0 2 4 6	1M 1F	< 2	≥ 2: Diarrhoea, emesis; ↑ heart rate by 50-100 % at 1 h post dose; 2-3 fold increases in CSF calcium levels; ↑ serum aldosterone; ↑ extramedullary hematopoiesis in the liver ≥ 4: Salivation, tremor, ataxia, impaired balance, sedation, and mydriasis; ↓ body weight gains. 6: Mortality (sacrifice); abnormal posture, increased defecation, twitches, weak legs, lateral recumbency, apathy, pale oral mucous membrane, ptosis and protrusion of the nictitating membrane; ↓ food consumption; ↓ rectal	189DFD -tox

Species (Strain)	Route (Vehicle)	Duration of Dosing	Doses (mg/kg) Free Base Equivalent	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number
						temperature; ↑ serum ACTH and cortisol; moderate fibrinoid necrosis of the myocardial blood vessel wall with minimal necrosis and fibrosis in adjacent myocardium. Toxicokinetics: Increase in maximal plasma levels and AUC with rising doses, no significant effect of dose or gender on dose normalized C_{max} and AUC_{0-24}, significant increase in normalized C_{max} and AUC after prolonged administration, rapid disappearance of drug from dog plasma	
Dog (Beagle)	SC (Saline)	Up to 7 days (a single dose for 9 BID)	6 BID 9 BID	1M 1F	< 6 BID	≥ 6 BID: Uncoordination, limited use of limbs, tremors, ↓ activity; ↑ WBC 9 BID: Mortality, severe salivation, tremors/convulsions, difficult breathing; ↑ heart rate	(b) (4) 89349
Pig (Yorkshire)	SC (Saline or sodium citrate)	4 days	4.75	1M	4.75	No findings	AOM 0032

ACTH = Adrenocorticotropic hormone, CSF = cerebral-spinal fluid, NOAEL = No Observed Adverse Effect Level, SD = Sprague-Dawley
[a] The report does not indicate whether the dose levels were based on free base equivalents or salt form.

Genetic toxicology:

In Vitro Studies			
Study #	System	Concentrations	Results
Mut. Bakt. 15/94	Bacterial Ames test: *Salmonella typhimurium*: TA98, TA97a, TA100, TA102 and TA1535	Experiment #1: – S9: 8-5000 μg/plate + S9: 4-2500 μg/plate Experiment #2: 312.5-5000 μg/plate Experiment #3: 312.5-5000 μg/plate	Negative in mutagenicity. With or without S9-mix
Z48*	Chromosome aberration: V79 Chinese hamster cells	– S9: 250-5000 μg/mL (Expt. #2 & #3), or 1000-5000 μg/mL (Expt. #1) + S9: 1000-5000 μg/mL (Expt. #1 & #2)	Negative in clastogenicity
In Vivo Study			
(b) (4) 960379	Micronuleaus assay: Rat bone marrow	SC doses at 0,6.25, 12.5 and 25 mg/kg , twice (24 hr apart) ➢Micronucleus assay: n=5/sex/dose	Negative

*** SDZ SID 791, at concentrations up to 5000 mg/plate, did not demonstrate adequate cytotoxicity under the condition of the study. On the other hand, it induced a decrease in mitotic index in the absence of S9.**

Reproductive toxicology:

Study	Route	Duration	Dose (mg/kg/d)	Results
Rat (b) (4) 900519)	SC	Females dosed GD 6-17 inclusive	0.5, 3, and 15 (Or, 3, 18 and 90 mg/m^2)	➢ Maternal toxicity: ↓ corrected gestation weight gains, and food consumption. ➢ Dose-dependent embryonic toxicity (15 mg/kg): ↑ resorption & post-implantation loss, ↓ fetal weights. ➢ Dose-dependent fetal toxicity (≥ 3 mg/kg/d): external, visceral and skeletal malformations and variations.
Rabbit (6045K) (not reviewed)	SC	Females dosed GD 6-18 inclusive	1, 3 and 10 (Or, 12, 36 and 120 mg/m^2)	➢ Maternal toxicity: death, clinical signs, weight loss, ↓ food consumption. ➢ Dose-dependent embryonic toxicity (mainly 10 mg/kg): ↑ pre- and post-implantation loss, ↓ litter size. ➢ Fetal toxicity (≥ 3 mg/kg/d): external malformations.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:
Pharmacology, safety pharmacology, pharmacokinetic/ADME, and toxicology studies supporting the marketing application of plerixafor for the poposed indication were conducted in *in vitro* systems as well as in rats, rabbits, and dogs. The general toxicology studies were conducted in appropriate animal species, using the administration route, dosing schedule/duration that adequately addressed safety concerns for the indicated patient population and the intended duration of administration. The target organs of plerixafor are bone, liver, spleen, and cardiovascular and central nervous systems.

Unresolved toxicology issues (if any): None

Recommendations:
There are no pharmacology/toxicology issues which preclude the approval of plerixafor (Mozobil) for the intended indication.

Suggested labeling:
Recommendations on labeling have been provided within internal meetings and communicated to the sponsor. A separate review for labeling will be provided if deemed necessary.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

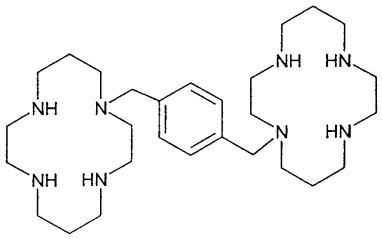
IND 55851 (N-065), Review 2, Guodong Fang, Ph.D., 2002
Reviewer's note: The letters **"Γ"** and **"E"** used in the document represent **"male"** and **"female"**, respectively.

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

IND number:	55,851
Review number:	2
Sequence number/date/type of submission:	065 05/16/2002 IND IT
Information to sponsor:	Yes ( ) No (X)
Sponsor:	AnorMed Inc., Langley, BC, Canada
Manufacturer for drug substance:	AnorMed Inc.
Reviewer name:	Guodong Fang, MD
Division name:	Division of Oncology Drug Products
HFD #:	150
Review completion date:	07/10/2002

Drug:

Trade name:	None
Generic name (list alphabetically):	AMD-3100 free base
Code name:	AMD-3100
Chemical name:	1,1'-[1,4-phenylenebis (methylene)]-bis-1,4,8,11-tetraazacyclotetradecane
CAS registry number:	110078-46-1
Mole file number:	None
Molecular formula/molecular weight:	$C_{28}H_{54}N_8$/ 502.79
Structure:	



Relevant INDs/NDAs/DMFs:	None
Drug class:	Selective antagonist of CXCR4 chemokine receptor
Indication:	Non-Hodgkins lymphoma (NHL) / Multiple myeloma (MM)
Clinical Formulation:	50 mg/ml in water
Route of administration:	Subcutaneous injection
Previous reviews:	Review 1, dated

Proposed clinical protocol:

A phase 1 study of the safety and effect on circulating CD34+ cells of a dose of 160 µg/kg or 240 µg/kg of AMD-3100 administered by subcutaneous injection to patients with non-Hodgkins lymphoma or multiple myeloma

Objectives:
To determine the safety of 160 μg/kg and 240 μg/kg of AMD-3100 administered as a single, subcutaneous injection in patients with NHL and MM.
To determine the effectiveness (hematological activity) of 160 μg/kg and 240 μg/kg of AMD-3100 administered as a single, subcutaneous injection to increase circulating $CD34^+$ cells in patients with NHL and MM.

Dose regimen:

AMD-3100:

Route:	S.C.
Dose:	160 μg/kg or 240 μg/kg
Schedule:	single dose
Cycle:	None
Escalation:	Yes

The protocol provides detailed rules for schedule delay or discontinuation.

Study design:

This is an open label, multi-center, phase 1 study designed to examine the safety and effectiveness (hematological activity) of 160 μg/kg and 240 μg/kg of AMD-3100 administered as a single, subcutaneous injection in patients with NHL and MM. The sponsor defines the patients must have been diagnosed with NHL or MM and are undergoing initial chemotherapy or first salvage therapy and have a partial response to their current therapy with evidence of platelet and PMN recovery of at lease one week. The sponsor also set up detailed inclusion and exclusion criteria.

Previous Clinical Experience

This IND was recently transferred to DODP from Division of Anti-viral Drug Products (HFD-530).

AMD-3100 has been shown to increase levels of circulating hematopoietic progenitor cells (CD34+) and is being developed for use in stem cell harvesting and transplantation in cancer patients.

AMD-3100 has been shown to exert an additive effect on the number of circulating progenitor cells when administered in combination with G-CSF.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

OVERALL SUMMARY AND EVALUATION

Introduction:

AMD-3100 is a selective antagonist of the CXCR4 chemokine receptor and inhibits binding of its cognate ligand stromal cell-derived factor-1 (SDF-1). Chemokines are known to function in the production and trafficking of blood cells. SDF-1 has been shown to act as a chemo-attractant for

T-lymphocytes, monocytes, megakaryocytes, and hematopoietic stem cells with the possibility of direct consequence for clinical use in cell therapies such as hematopoietic stem cell transplantation.

Current protocols for collection of CD34+ hematopoietic progenitor cells for clinical transplantation purposes are based on mobilization to the peripheral blood by administration of G-CSF to donors. Unlike repeated daily administration of G-CSF, which stimulates the production of progenitors resulting in overflow into the peripheral circulation, AMD-3100 is presumed to exert a direct mobilizing effect by releasing progenitor cells into the periphery. The effects of AMD-3100 are thought to result from an inhibition of the chemoattractant effect of SDF-1 that is locally produced in the bone morrow, resulting in the appearance of both mature and pluripotent cells in the systemic circulation.

Safety Evaluation

The toxicology of AMD3100 has been characterized in single and repeated-dose subcutaneous injection studies of up to 28-day duration in rats and dogs. Additional safety pharmacology studies have been performed in dogs to examine the effects of AMD3100 on cardiovascular parameters. The toxicokinetic behavior of AMD3100 has been characterized as part of twice daily repeated-dose general toxicology studies.

The acute toxicity of AMD3100 in animals is characterized by transient dose-dependent CNS effects that may include weakness, ↓activity or ataxia, recumbency or irregular posture, uncoordinated, shallow, and irregular breathing, skin pallor, ↑defecation, salivation, non-sustained convulsions and tremors. These findings were consistent with results previous neurological and behavioral studies in mice that showed that AMD3100 produced dose-dependent sedative-like effects that generally subsided with time. It was noted that clinical signs in rats and dogs generally occur within 0.5-1 hour following S.C. administration. At sub-lethal doses, evidence of recovery typically occurs 1-2 hrs after dosing. The dose-response relationship for the acute toxicity of AMD3100 appears to be relative steep. In dose range-finding study in dogs, a single 9 mg/kg dose was lethal. However a 6 mg/kg twice daily regimen (12 mg/kg/day) was tolerated for up to 5 days at which time clinical signs were noted. These signs were noted ~ 30 min after dosing and complete recovery was noted at 1 hour.

In dose range-finding studies in rats using AMD3100 single S.C. doses of 36 or 50 mg/kg were lethal. A dose of 24 mg/kg twice daily (8 mg/kg/day) produced clinical signs that commenced on day 5 for 1 animal and on day 7 for remaining animals, with 1 found dead on day 7. A dose of 12 mg/kg twice daily (24 mg/kg/day) was generally well tolerated in rats for up to 28 days. The observation that a single 50 mg/kg dose was lethal but that a total daily dose of 48 mg/kg/day given as 24 mg/kg twice daily was tolerated for 5 days indicates that the acute toxicity of AMD3100 is C_{max} dependent.

In this submission, no elevation of heart rate (HR) was found in a repeated dose study in dogs following single 4 mg/kg dose given twice daily (8 mg/kg/day) for 28 days using AMD3100 free base. This is contrast to previous findings that transient increase in HR (~ 50-100 %) in dogs 1 hr following administration of AMD3100 at S.C. dose levels of 1, 2, 4, and 6 mg/kg in repeat dose studies using the octahydrochloride salt form of the drug. The effect of AMD3100 on cardiovascular parameters was examined in both a pilot and then GLP safety pharmacology study in dogs using free base form of the compound through I.V. administration. I. V. infusion of AMD3100 free base at 5 mg/kg/hr for 1 hr followed by a rate of 3.33 mg/kg/hr for 7 hrs resulted in ↑ HR and adverse clinical signs while the plasma AMD3100 levels in the range of 10.9-14.3

μg/ml were determined. There were no test article related effects on HR at I.V. infusion rate of 2.5 mg/kg/hr for 1 hr followed by a rate of 1.67 mg/kg/hr. There was no evidence of cardiotoxicity on the ECGs. Therefore, the sponsor thought that the effects were not considered to be directly related to an effect on the heart itself.

Additional effects of AMD3100 noted in repeated dose animal studies include minor and reversible changes in clinical chemistry and hematological parameters, e.g., ↑ WBC, slight ↓ plasma Ca^{++} and Mg^{++} levels coupled to corresponding ↑ in urinary levels.

In a 1-month study in dogs in which AMD3100 free base was administered twice daily at S.C. dose levels of 0.15, 0.75, and 4 mg/kg, AMD3100 was generally well tolerated at all dose levels. The 4 mg/kg twice daily dose (8 mg/kg/day) was selected as the no adverse effect level (NOAEL), with minor, reversible changes in clinical signs, a slight change in body weight gain and food consumption, hematology (leucocytosis), clinical chemistry (↓ Mg^{++}, slight ↑ A/G ratio) and urinalysis noted. All clinical chemistry changes were reversible following a 14-day recovery period. No treatment related changes in ocular parameters, organ weights or bone morrow were noted. There were no toxicologically significant histopathological changes. The long-term implications of the observed clinical chemistry and hematological changes remain to be determined.

The proposed starting dose (160 μg/kg and 240 μg/kg) for this phase 1 clinical trial was derived from an extrapolation of the rat and dog 4-wk repeated dose toxicity studies wherein the NOAEL dose 4 mg/kg bid was converted to human equivalent doses (HED) based on surface area correction factors and further reduced by a safety factor. Based on the comparisons in rodents and dogs, acute toxicity may be predicted to occur at plasma AMD-3100 levels that exceed 25 μg/ml. Human subjects that received a single 80 μg/kg I.V. dose in phase 1 study had C_{max} values of ~ 500 ng/ml, roughly 25-fold below the proposed threshold level for the acute toxicity of cardio-vascular effects seen in non-clinical studies. C_{max} values following S.C. administration were roughly 50% of those seen at the same dose when administered as an I.V. injection over a period of 15 minutes.

Safety issues relevant to clinical use: None

Other clinically relevant issues: Higher doses (high plasma concentrations) have effect on cardiovascular parameters. At this time, it is unclear whether the sponsor is targeting a plasma concentration or MTD.

Conclusions: Based on data from nonclinical safety studies reviewed, it is reasonably safe to initiate a phase 1 study of safety and effect on circulating CD34+ cells with a starting dose of 160 μg/kg or 240 μg/kg of AMD-3100 administered by S.C. injection to patients with NHL or MM.

RECOMMENDATIONS

Internal comments:

Based on previous submitted pre-clinical, the proposed starting doses of single S.C. injection 160 or 240 μg/kg is safe. The study may proceed.

External recommendations (to the sponsor): None

Draft letter content for sponsor (if not same as above): None

Future development issue: No recommendations at this time.

Reviewer signature:

Guodong Fang, M.D. Date	John K. Leighton, Ph.D., DABT Date
Pharmacologist	Supervisory Pharmacologist

cc: list:

Studies reviewed within this submission:

PHARMACOLOGY

***In vitro* pharmacology**

#	Title	Report No.	Vol.	Page
1	Inhibition of SDF-1 ligand binding to CXCR4 by AMD-3100 free base		1	1-25
2	Inhibition of SDF-1 stimulated GTPγS binding by AMD3100 free base		1	1-28
3	Inhibition of SDF-1 stimulated chemotaxis by AMD3100 free base		1	1-31
4	Inhibition of SDF-1 stimulated calcium flux by AMD3100 free base		1	1-34
5	AMD3100 free base has no significant effect on CCR5 mediated calcium flux		1	1-37
6	The effect of AMD3100 free base on IP10 stimulated calcium flux		1	1-39
7	The effect of AMD3100 free base on MCP-1 stimulated calcium flux		1	1-42
8	Inhibition of LTB4 ligand binding to the LTB4 receptor by AMD3100 free base		1	1-45
9	Cross screening of AMD3100 free base for CCR4 and CCR7		1	1-48

***In vivo* pharmacology**

#	Title	Report No.	Vol.	Page
10	Progenitor cell mobilizing effects of AMD3100 free base in mice		1	1-14

Safety pharmacology

#	Title	Report No.	Vol.	Page
11	A pilot cardiovascular profile study following a single intravenous infusion of AMD3100 free base in the conscious unrestrained beagle dogs	93226	1	1051
12	A cardiovascular profile study following an intravenous infusion of AMD3100 free base in the conscious unrestrained Beage dogs	93227	1	1-93

TOXICOLOGY

Dosing schedule dependency studies

#	Title	Report No.	Vol.	Page
13	A 14-day dosing schedule dependency (once vs. twice daily) study in rats	1663	2	2-1 to 2-4

Dose-ranging toxicology studies

14	A range finding subcutaneous injection (twice daily) toxicity study of AMD3100 free base in the Albino rat	89342	2	2-5 to 53
15	A range finding subcutaneous injection (twice daily) toxicity study of AMD3100 free base in the Beagle dog	89349	4	4-1 to 52

28-day multi-dose toxicology studies

16	A 28-day twice daily subcutaneous injection toxicity study of AMD3100 free base in the Albino rat with a 14-day recovery period	89289	2	2-54 to 376 3-1 to 381
17	A 28-day twice daily subcutaneous injection toxicity study of AMD3100 free base in the Beagle dog with a 14-day recovery period	89290	4	4-53 to 217 5-1 to 250

Studies not reviewed within this submission: None

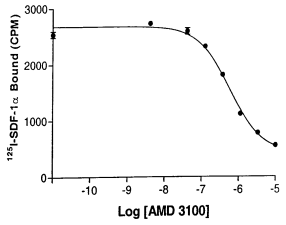
Introduction and drug history:

AMD-3100 is a selective antagonist of the CXCR4 chemokine receptor and inhibits binding of its cognate ligand stromal cell-derived factor-1 (SDF-1). Chemokines are known to function in the production and trafficking of blood cell. SDF-1 has been shown to act as a chemo-attractant for T-lymphocytes, monocytes, megakaryocytes, and hematopoietic stem cells with the possibility of direct consequence for clinical use in cell therapies such as hematopoietic stem cell transplantation

PHARMACOLOGY

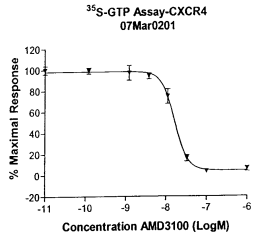
***In vitro* Pharmacology:** To demonstrate AMD-3100 is an inhibitor of SDF-1 binding to the CXCR4 receptor.

Study 1 Inhibition of SDF-1 ligand binding to CXCR4 by AMD3100 free base



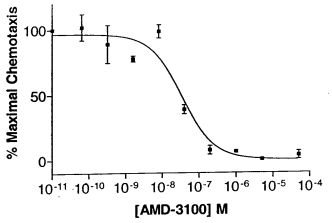
The principle of the assay is to measure the inhibition of radio-labeled SDF-1 binding to CCRF-CEM lymphoblastoid cells (expressing CXCR4 receptor) by AMD3100 using a competition-binding assay (flow cytometry). A typical result is shown in the figure and the IC_{50} = 651±37 nM. In conclusion, these data demonstrate that AMD3100 is an inhibitor of SDF-1 binding to the CXCR4 receptor.

Study 2: Inhibition of SDF-1 stimulated GTPγS binding by AMD-3100 free base



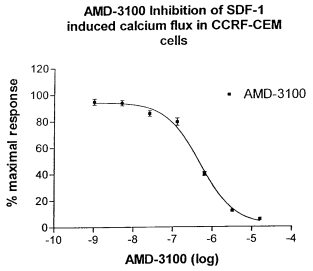
The principle of this assay is based upon the mechanism of activation of G-protein coupled receptors. As SDF-1 is the sole ligand for CXCR4 the bound GTPγS is the result of activation of the CXCR4 receptor. A typical result is shown in the figure. The IC_{50} for AMD3100 inhibition of GTPγS binding was 15.4±4.4 nM. In conclusion, these data demonstrate that AMD-3100 is an inhibitor of SDF-1 mediated activation of the CXCR4 receptor.

Study 3: Inhibition of SDF-1 stimulated chemotaxis by AMD-3100 free base



To demonstrate AMD3100 was an inhibitor of SDF-1 mediated chemotaxis *via* activation of the CXCR4 receptor. The principle is based upon the migration of fluorescently labeled CEM cells in response to SDF-1. A typical result is shown in the figure. The IC_{50} for AMD3100 induced inhibition of chemotaxis was 51±17 nM. In conclusion, these data demonstrate that AMD-3100 is an inhibitor of SDF-1 mediated chemotaxis *via* the CXCR4 receptor.

Study 4: Inhibition of SDF-1 stimulated calcium flux by AMD-3100 free base



The principle of the assay is based upon the activation of G-protein coupled receptor intracellular signaling pathways resulting in the release of calcium from intracellular stores. The calcium flux is assayed using a calcium-chelating molecule, Fluo-4. A typical result is shown in the figure. The IC_{50} for AMD3100 inhibition of SDF-1 mediated calcium flux was 572±190 nM. In conclusion, these data demonstrate that AMD-3100 is an inhibitor of SDF-1 induced calcium flux mediated *via* the CXCR4 receptor

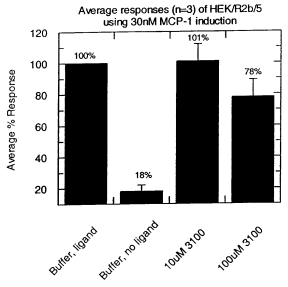
Study 5: AMD-3100 free base has no significant effect on CCR5 mediated calcium flux

HEK293F cells were transfected to express CCR5 undergo calcium flux in response to RANTES binding to CCR5. The percentage inhibition for AMD-3100 at 10 and 100 μM was 2±4.2 % and 5±2.8 %, respectively. In conclusion, these data demonstrate that AMD-3100 is not an inhibitor of RANTES induced calcium flux mediated *via* the CCR5 receptor.

Study 6: The effects of AMD-3100 free base on IP10 stimulated calcium flux

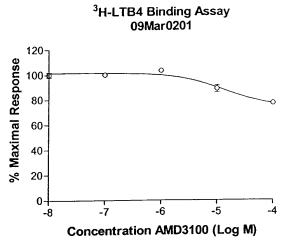
IP10 mediated calcium flux is via activation of CXCR3 receptors expressed by HEK293 cells. The principle of the assay is based upon the activation of G-protein coupled receptor intracellular signaling pathways resulting in release of calcium from intracellular stores. Both 10 and 100 μM AMD-3100 did not inhibit IP10 induced calcium flux via the CXCR3 receptor. This indicates that the IC50 for AMD-3100 against CXCR3 is greater than 100 μM. Therefore, it can be concluded that AMD-3100 is not an effective inhibitor of IP1- induced calcium flux mediated via the CXCR3 receptor.

Study 7:The effects of AMD-3100 free base on MCP-1 stimulated calcium flux



MCP-1 mediated calcium flux is via activation of CCR2b receptors expressed by HEK293F cells. The principle of the assay is based upon the activation of G-protein coupled receptor intracellular signaling pathways resulting the release of calcium from intracellular stores. 10 μM AMD3100 did not inhibit MCP-1 induced calcium flux via the CCR2b receptor while 100 μM AMD3100 caused slight (22%) inhibition of CCR2b. This indicates that the IC50 for AMD3100 against CCR2b is >100 μM. Therefore, it can be concluded that AMD3100 is not effective inhibitor of MCP-1 induced calcium flux mediated via the CCR2b receptor.

Study 8: Inhibition of LTB4 ligand binding to the LTB4 receptor by AMD-3100 free base



The principle of this assay is to measure the inhibition of radiolabelled LTB4 binding by AMD3100 using a competition binding assay through CHO-S cells which have been transfected to express LTB4 receptor on their surface. A typical result is shown in the figure . The EC50 of AMD3100 was >100 μM. The data demonstrated that AMD-3100 does not significantly inhibit LTB4 receptor binding at concentrations of at least 2 orders of magnitude higher than that for the inhibition of SDF binding.

Study 9: Cross screening of AMD-3100 free base for CCR4 and CCR7

TARC and ELC are specific ligands for CCR4 and CCR7 respectively, which are expressed by CCRF-CEM cells. TARC and ELC induce calcium flux in CCRF-CEM cells via CCR4 and CCR7 receptors. A typical result showed that the IC_{50}s of AMD-3100 for inhibition of calcium flux in CCRF-CEM cells via. CCR4 and CCR7 are >100 μM, which are more than 3 orders of magnitude higher than its IC_{50} for CxCR4, demonstrating that it is selective inhibitor of CXCR4.

***In vivo* Pharmacology:** To demonstrate AMD-3100 is an inhibitor of SDF-1 binding to the CXCR4 receptor.

Study 10: Progenitor cell mobilizing effects of AMD-3100 free base in mice

The effect of AMD3100 on circulating hematopoietic progenitor cells levels was examined in C3H/HeJ mice when administered alone and in combination with G-CSF. The resulting colonies of CFU-GM, BFU-E and CFU-GEMM are as following:

Treatment	Myeloid Progenitor Cells					
	CFU-GM		BFU-E		CFU-GEMM	
	No. / ml	ΔFold	No. / ml	ΔFold	No. / ml	ΔFold
Saline/Saline	92±10		53±16		12±3	
Saline/AMD	582±71	6.3	140±17	2.6	64±4	5.3
Saline/G-CSF	1116±275	12.1	561±61	10.6	339±118	28.3
G-CSF/AMD	4019±1039	**43.7**	1025±208	**19.3**	534±54	**44.5**

G-CSF: 2.5 µg/mouse S.C., bid for 2 days; AMD3100: single injection 16 hrs after last injection of G-CSF. Conclusion: AMD-3100 used in sequential combination with G-CSF has synergistic effect compared to either agent used alone.

Pharmacology conclusions: AMD3100 is a selective antagonist of the CXCR4 receptor, which serves as a receptor for the chemokine SDF-1. AMD3100 inhibits SDF-1 ligand binding, SDF-1 mediated G-protein activation, cellular response, and calcium flux. AMD3100 used in sequential combination with G-CSF has synergistic effect in mobilization of progenitor cells.

SAFETY PHARMACOLOGY

Study 11: A pilot cardiovascular profile study following a single I.V. infusion of AMD-3100 free base in the conscious restrained Beagle dog (# 93226, Non-GLP, conducted by (b) (4))

The objective of this non-GLP study was to investigate the cardiovascular effects of AMD3100 in the dog when administered by continuous I.V. infusion and to select infusion rates for the planned GLP study. AMD3100 was administered in a single dog at 3 dose rates using a stepwise escalation regimen and infusion was discontinued for 1-hr period following the first and second infusion periods.

Dose infusion regimen

Time Course	Dose Level (mg/kg/hr)	Infusion Rate (ml/kg/hr)
First hour	5.064	1.10128
Second hour	No infusion	No infusion
Third hour	7.530	1.506
Fourth hour	No infusion	No infusion
Fifth hour	9.9	2.0

Sample concentration of AMD3100 (µg/ml)

Sample ID	Pre-Rx	Post infusion					End infusion						
		1 hr	2 hr	3 hr	4 hr	5 hr	30’	1 hr	2 hr	4 hr	6 hr	12 hr	24 hr
AMD	<0.25	10.4	3.91	17.27	8.20	29.45	20.38	6.25	7.70	2.18	1.14	0.36	<0.25

Results: There were no direct test article related effects on the ECG during any of the 3 infusion periods, nor were there any test article-related effects on blood pressure or heart rate, or treatment-related clinical signs during the first 2 infusion periods. There were no test article-related gross necropsy findings 24 hrs after the end of the third infusion. During the third infusion period, heart rate started to slowly increase shortly following the start of infusion. Approximately 50’ into the infusion, blood pressure increased more dramatically and heart rate declined, coinciding with clinical signs of salivation, dilated pupils and non-sustained convulsions. The plasma drug level during this period was approximately 25–30 µg/ml. Within 10-30’ following the end of the infusion period, these effects had resolved.

Conclusion: The hourly I.V. infusion of AMD-3100 free base produced test article-related effects on blood pressure and heart rate and severe clinical sings at a dose infusion rate associated with plasma AMD3100 levels in the range of 25–30 µg/ml. There were no direct effects of test article on the ECG.

Study 12: A cardiovascular profile study following an I.V. infusion of AMD-3100 free base in the conscious restrained Beagle dog (# 93227, GLP, conducted by (b) (4))

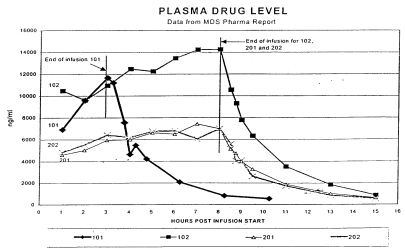
The purpose of this GLP study was to evaluate the hemodynamic effects of AMD3100 continuous I.V. infusion for a period of approximately 8 hrs in the conscious unrestrained dog.

Dose regimen

Group	Dose Level (mg/kg/hr)		Number of males
	1st hour of infusion	Remaining 7 hours of infusion	
1	5	3.33	#101, #102
2	2.5	1.67	#201, #202

Each animal was instrumented with an abdominal aortic catheter, a left ventricular catheter, a pulmonary artery catheter, a venous catheter for dosing and cutaneous leads for ECG recording.

Results



There were no test article-related deaths or ECG evidence of cardiotoxicity. The infusion of 5/3.33 mg/kg/hr resulted in adverse clinical signs that were severe enough to prematurely terminate dosing for dog #101 approximately 3.25 hrs after the initiation of infusion. These signs included decreased activity, tremors, salivation, uncoordination, dilated pupils, lateral recumbency and labored breathing. Plasma AMD3100 levels determined shortly before (3 hrs) and at the time of discontinuation of infusion (3.27 hrs) were approximately 11.7 and 11.2 µg/ml, respectively. Prolonged increases in heart rate were observed in both #101 and #102 and was considered to be abnormal (tachycardia) in #101 who also exhibited a transient elevation in pulmonary artery pressure for an approximately 1.75 hr period following termination of dosing. Analysis of the plasma for drug level revealed an approximately 2 fold higher plasma level in the 5/3.33 mg/kg/hr group (14.3 µg/ml), 8 hrs post infusion start, compared to the 2.5/1.67 mg/kg/hr dose group (7.0 µg/ml). At the time that dosing was terminated for #101 with the severe reaction to treatment, the plasma level was similar to that of #102 that did not exhibit a similar reaction. Therefore, the above reaction of the single #101 at 5/3.33 mg/kg/hr was likely to have been idiosyncratic.

Conclusion: the I.V. infusion of AMD-3100 at the higher of 2 tested dose rates resulted in elevations in heart rate and adverse clinical signs in both tested animals within the first half of the 8 hr infusion period. However, there was no evidence of cardiotoxicity on the ECGs and therefore, the effects were considered not to be directly related to an effect on the heart itself. Animals that received one half of this dose did not exhibit any clinical signs or any article-related cardiovascular effects.

Safety pharmacology conclusions: I.V. infusion of AMD3100 at higher dose rate resulted in test article related effects on heart rate and adverse clinical signs and is plasma level related but there was no evidence of cardiotoxicity on the ECGs. Proposed starting dose in the clinical trial should not be a concern.

PHARMACOKINETICS/TOXICOKINETICS: Reviewed with toxicology studies.

TOXICOLOGY

Dosing schedule dependency studies

Study 13: A 14-day dosing schedule dependency (once vs. twice daily) study in rats

Key study findings:
- S.C. injection of AMD3100 once or twice daily for 14 consecutive days had no adverse effects.

Study no: 1663
Volume #, and page #: Vol. 2, p.2-1
Conducting laboratory and location: (b) (4)
Date of study initiation: May 2000
GLP compliance: No
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: N/A
Formulation/vehicle: N/A

Methods: subcutaneous (S.C.) injection of AMD3100 into female Sprague-Dawley rats once or twice daily for 14 days and under observation for clinical signs prior to dosing, and at 0.5, 1, 2, and 4 hrs post-dosing. Mortality checks were once a day. No tests in hematology and clinical chemistry were done. Blood sampling was performed on Groups 2, 3, 4 rats on the last day of dosing (Day 14), at 1 hr post-dosing (for Groups 2 and 4, after the first injection on Day 14) for determination of test article plasma concentration.

Group	Treatment	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Frequency	Number of Erats
1	saline	0	2	once	5
2	AMD3100	4	2	twice	5
3	AMD3100	12	2	once	5
4	AMD3100	12	2	twice	5

Dosing:

Species/strain:	Rats/Sprague Dawley
#/sex/group or time point (main study):	5 E rats/group
Age:	N/A
Weight:	N/A
Doses in administered units:	once or twice dose of 0, 4, 12 mg/kg/day
Route, form, volume, and infusion rate:	subcutaneous injection

Observations and times:

Clinical signs:	prior to dosing, 0.5, 1, 2, 4 hrs post-dosing
Body weights:	prior to assignment, Days 1, 8, 14
Food consumption:	N/A
Ophthalmoscopy:	N/A
Hematology:	N/A
Clinical chemistry:	N/A
Gross pathology:	N/A (tissues were preserved)

Organs weighed: N/A
Toxicokinetics: blood samples were collected on Day 14, at 1 hr post-dosing for Groups 2, 3, 4.

Results:

No mortalities, and no adverse clinical signs. No treatment-related or dose-dependent changes were observed in body weights.

Summary of individual study findings:

S.C. injection of AMD3100 free base once or twice daily, at dose levels of 4 and 12 mg/kg/day for 14 consecutive days in female Sprague-Dawley rats had no adverse effects on clinical signs or body weight.

Dose-ranging toxicology studies

Study 14: A range finding subcutaneous injection (twice daily) toxicity study of AMD3100 free base in the Albino rat
Key findings:

- Rats received total daily dose levels of 24 (7-day), 12 (5-day) and 3 (7-day) mg/kg/day were unaffected by treatment.
- Deaths occurred within 2 hrs post treatment following a single administration of 50 mg/kg/dose (1/4) and 36 mg/kg/dose (2/4).
- At 48 mg/kg/day (24 mg/kg/dose), one animal was found dead on day 7.

Study no: 89342
Volume #, and page #: Vol. 2, p.2-5 to 2-53
Conducting laboratory and location: (b) (4)

Date of study initiation: January 2001
GLP compliance: No
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: B1047-941001, 98.0-102.0% anhydrous basis
Formulation/vehicle: dissolved in HCL with pH 6.5-7.0,diluted in 0.9% sodium chloride

Methods: S.C. injection of AMD3100 into scapular region by twice daily (approximately 7 hrs apart at equal doses) at 2 mL/kg and followed by 2 hr post-dose and then daily observation. Tests in hematology and clinical chemistry were done terminally from all surviving animals.

Group	No. of Rats		Daily Dosing Regimen	Dose Levels (mg/kg/day)	Dose Volume (mL/kg)	Concentration of AMD (mg/ml)
	Γ	E				
1	2	2	Twice	100 (50 mg/kg/dose)	2	25
2	2	2	Twice	24 (12 mg/kg/dose)	2	6
3	2	2	Twice	12/72 (6/36 mg/kg/dose)	2	3/18
4	2	2	Twice	3 (1.5 mg/kg/dose)	2	0.75
5	2	2	Twice	48 (24 mg/kg/dose)	2	12

Dosing:

Species/strain:	Rats/Albino
#/sex/group or time point (main study):	2 rats/sex/dose
Age:	5 wks old
Weight:	Γ: 150-250 g; E: 125-200 g.
Doses in administered units:	100, 24, 12/72, 3, 48 mg/kg/day
Route, form, volume, and infusion rate:	subcutaneous injection twice daily for 7 days

Observations and times:

Clinical signs:	4 times daily, 1, and 2 hr post-dose period
Body weights:	daily; prior to the initial dose and
Food consumption:	measured and recorded daily
Gross pathology:	None
Organs weighted	None
Histopathology	None
Toxicokinetics:	None

Results:

Parameter	100 mg/kg/day		24 mg/kg/day		12/72 mg/kg/day		3 mg/kg/day		48 mg/kg/day	
	Γ (N=2)	E (N=2)	Γ (N=2)	E (N=2)	Γ (N=2)	E (N=2)	Γ (N=2)	E (N=2)	Γ (N=2)	E (N=2)
Mortality	0	1/2	0	0	1/2	1/2	0	0	0	1/2
Death Time (post dose)		2 hr s			2 hrs	2 hrs				Day 7
Clinical signs	see below		None		None for 12 mg, 72 mg see below		None		See below	

Clinical signs: Weak, decreased activity, lying on side, uncoordinated, shallow, irregular breathing, skin pallor, non-sustained convulsions and tremors.
Group 1: within 2 hrs post dose, all animals; Group 3: after increasing the dose level to 72 mg/kg/day, after one dose for one male and one female; Group 5 (48 mg/kg/day) on Day 5 for one male and Day 7 for all.

Laboratory investigations: No overt treatment-related effects on hematology or clinical chemistry parameters.

Summary of individual study findings:

1. A single S.C. administration of AMD3100 at dose levels of 36 and 50 mg/kg and a 7-day twice-daily administration at 48 mg/kg/day resulted in death.
2. There were no treatment-related adverse effects on animals that received a total daily dose level of 24 mg/kg/day or lower for up to 7 consecutive days.

Conclusion of the study: The dose levels for the subsequent 28-day repeat dose study were chosen at 24, 4, 1.2 and 0.6 mg/kg/day.

Study 15: A Range finding S.C. injection (twice daily) toxicity study of AMD3100 free base in the Beagle dog

Key findings:

- Deaths occurred post single dose treatment of 9 mg/kg/dose with life threatening clinical signs.
- At 12 mg/kg/day (24 mg/kg/dose), treatment-related clinical signs were observed 30 minutes post dose on Day 5 and then recovery was followed 1 hr post dosing on each day.

Study no: 89349
Volume #, and page #: Vol. 4, p.4-1 to 4-52
Conducting laboratory and location: (b) (4)

Date of study initiation: February 7, 2000
GLP compliance: No
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: N/A
Formulation/vehicle: dissolved in HCL with pH 6.5-7.0, diluted in sterile water, further diluted with 0.9% sodium chloride

Methods: S.C. injection of AMD3100 into scapular region by twice daily (approximately 7 hrs apart at equal doses) at 1 mL/kg/dose and followed by 2 hr post-dose and then daily observation. Tests in hematology and clinical chemistry were done terminally from all surviving animals.

Group	Dose level (mg/kg/day)	Dose Level (mg/kg/dose)	Animal Numbers	
			Γ	E
1	12	6	#101	#151
2	18	9	#201	#251

Dosing:

Species/strain:	Beagle dog
#/sex/group or time point (main study):	1 dog/sex/dose
Age:	5 –6 months old
Weight:	Γ: 7.3-7.7 kg; E: 7.4-8.0 kg.
Doses in administered units:	100, 24, 12/72, 3, 48 mg/kg/day
Route, form, volume, and infusion rate:	subcutaneous injection twice daily for 7 days

Observations and times:

Clinical signs:	6 times, 0.5, 1, and 2 hr postdose period
Body weights:	Not done
Food consumption:	Not done
Gross pathology:	Yes
Organs weighted	No
Histopathology	Not done
Toxicokinetics:	Not done
Laboratory:	Hematology and clinical chemistry prior to the first dose and following the last dose

Results:

Group/Parameters	12 mg/kg/day (6 mg/kg/dose)		18 mg/kg/day (9 mg/kg/dose)	
	Γ #101	E #151	Γ #201	E #251
Mortality	alive		Euthanized post 1st dose	Died post 1st dose
Time of clinical signs	On Day 5, 30' post-Rx, disappeared 1 hr post-Rx		30' post-Rx, life threatening, and no signs of diminishing of intensity	
Clinical signs	Uncoordination, limited use of hindlimbs, tremors, activity ↓		Severe salivation. Tremor/convulsions, activity↓, uncoordination, HR↑, breathing difficulties	
Hematology WBC(10^3/mm^3) Day 1/Day 8 Pre/Post-Rx	10.0/14.2	10.1/16.1	8.2/17.8	No sample
Clin Chemistry	Non remarkable			
Gross Pathology	No treatment-related findings			

Summary

1. A single administration of AMD3100 at 9 mg/kg resulted in severe acute clinical signs and death.
2. A twice-daily administration at a total daily dose of 12 mg/kg/day for 7 days resulted in some clinical signs following 5 days treatment.
3. Leukocytosis was noted in both groups.

Conclusion: the high dose level of AMD3100 for a 28-day repeat dose study should not exceed 12 mg/kg/day (6 mg/kg/dose).

28-day multi-dose toxicology studies

Study 16: A 28-day twice daily S.C. injection toxicity study of AMD3100 free base in the Albino rat with a 14-day recovery period

Key findings:

- No toxic effect level (NTEL) was 24 mg/kg/day.
- No observed effect level (NOEL) was 0.6 mg/kg/day.

Study no:	89289
Volume #, and page #:	Vol. 2, p.2-54 to 2-376, Vol. 3, 3-1 to 3-381
Conducting laboratory and location:	**(b) (4)**
Date of study initiation:	January 31, 2000
GLP compliance:	Yes
QA report:	yes (x) no ( )
Drug, lot #, radiolabel, and % purity:	B1047-941001, 100 %
Formulation/vehicle:	dissolved in HCL with pH 6.5-7.0, diluted in sterile water, further diluted with 0.9% sodium chloride

Methods: S.C. injection of AMD3100 by twice daily (approximately 7 hrs apart at equal doses) at 2 mL/kg/dose and followed by daily observation.

Group	Treatment***	Dose Level (mg/kg/day)	Concentration (mg/mL)	Number of Animals	
				Γ	E
1	Control	0	0	15*	15*
2	AMD3100	0.6	0.15	15*+21**	15*+21**
3	AMD3100	1.2	0.3	15*	15*
4	AMD3100	4	1	15*	15*
5	AMD3100	24	6	15*+21**	15*+21**

* 5 animals/sex were retained for a 14-day recovery period

** 21 animals/sex/group in Groups 2 (low dose) and 4 (high dose) were used for provision of toxicokinetic blood samples.

*** Twice daily dosing at 2 mL/kg with equal dose 7 hrs apart.

Dosing:

Species/strain:	Albino rat
#/sex/group or time point (main study):	10 rats/sex/dose for main study, 21 rats/sex/group for toxicokinetic study, 5 rats/sex/group for 14-day recovery period
Age:	7 weeks old
Weight:	Γ: 163-222 g; E: 124-170 g.
Doses in administered units:	0, 0.6, 1.2, 4 and 24 mg/kg/day
Route, form, volume, and infusion rate:	subcutaneous injection twice daily for 28 days

Observations and times:

Clinical signs:	daily
Body weights:	weekly
Food consumption:	measured and recorded weekly
Ophthalmoscopy:	pretest, week 4, end of recovery
Hematology:	prior to and end of treatment, recovery period
Clinical chemistry:	prior to and end of treatment, recovery period
Urinalysis	prior to and end of treatment, recovery period
Gross pathology:	at terminal examination
Organs weighed:	at terminal examination
Histopathology	at terminal examination (selected animals)
Bone marrow examination	at terminal examination (selected animals)
Toxicokinetics:	30', 1, 2, 4 and 7 post first dose, 30', 1, 2, 4, 5, 6, 12 and 17 hr post second dose (day 1), pre-dose (AM on days 14 and 21), 30', 1, 2, 4, 5, 6, 12, 17 and 24 hr post second dose (day 28).

Number of. Animals (Groups 2, 4)	**DAY 1: Timepoint**												
	POST First Dose					**POST Second Dose**							
	0.5	**1.0**	**2.0**	**4.0**	**7.0**	**0.5**	**1.0**	**2.0**	**4.0**	**5.0**	**6.0**	**12.0**	**17.0**
3/sex	X			X									
3/sex		X			X								
3/sex			X			X							
3/sex							X				X		
3/sex								X				X	
3/sex									X				X
3/sex										X			

Number of Animals (Groups 2, 4)	DAY 28: Timepoint (hr) POST Second Dose								
	0.5	1.0	2.0	4.0	5.0	6.0	12.0	17.0	24.0
3/sex	X				X				X
3/sex		X				X			
3/sex			X				X		
3/sex				X				X	

Results

Groups /Parameters	Controls		0.6 mg/kg/day		1.2 mg/kg/day		4 mg/kg/day		24 mg/kg/day	
	Γ (N=15)	E (N=15)	Γ (N=15)	E (N=15)	Γ (N=15)	E (N=15)	Γ (N=15)	E (N=15)	Γ (N=15)	E (N=15)
Mortality	None							1**	None	
Body weight	Non-remarkable									
Fd. consumption	Non-remarkable									
Clinical signs	Non-remarkable									
Ophthalmology	Non-remarkable									
Hematology (wk5)										
WBC x10^3/mm^3	4.6	2.7	4.6	3.6	5.4	3.8	7.2	4.8*	9.0	7.6*
Neutrop seg.%	10.5	16.0	10.2	9.0	11.8	12.4	19.7	16.9	26.1	28.7*
Lymph %	87.5	82.3	86.5	88.6	84.8	85.2	75.4**	79.3	66.6*	66.7*
Mono %	1.1	0.8	2.8	1.1	2.4	1.1	3.7	1.8	4.6	2.0
Clin. Chemistry (wk5)										
ALT (u/L)	18.2	18.5	16.0	21.3	17.3	15.1	19.3	16.4	45.1*	17.7
Mg^{++} (mg/dL)	2.1	2.0	2.1	2.0	2.0	2.1	2.1	2.3	1.8*	2.0
Urinalysis										
Ca^{++} (mg/dL)	10.1	22.9	13.6	20.0	10.6	26.3	22.7*	27.9	41.7*	52.2*
Gross pathology	Relatively minor scapular injection site lesions, primarily dark areas were present in a proportion of animals from Groups 4 and 5, and changes were well localized.									
Organ weights (wk5)										
Thymus										
Absolute	0.570	0.376	0.493	0.430	0.490	0.444	0.433*	0.432	0.354*	0.345
Relative (%bw)	0.202	0.223	0.170*	0.248	0.181	0.258	0.160	0.245	0.137*	0.197
Spleen										
Absolute	0.610	0.417	0.631	0.451	0.594	0.441	0.664	0.480	0.699	0.522*
Relative (%bw)	0.216	0.246	0.216	0.261	0.220	0.256	0.245	0.273	0.271*	0.299*
Kidney										
Absolute	2.005	1.286	2.017	1.341	2.013	1.337	1.895	1.397	2.014	1.355
Relative (%bw)	0.710	0.764	0.694	0.774	0.742	0.779	0.702	0.794	0.786*	0.775
Liver										
Absolute	7.674	4.732	8.436	4.823	7.698	4.928	7.943	4.986	8.095	5.234
Relative (%bw)	2.719	2.798	2.897	2.783	2.840	2.870	2.937*	2.831	3.147*	3.002
Histopathology	(N=10)	(N=10)	(N=0)	(N=1)	(N=1)	(N=0)	(N=4)	(N=2)	(N=10)	(N=10)
Injection sites										
Hemorrhage	3	1		0	1		1	1	4	4
Inflammation				0	0					
Subcutis							1	1	3	2
Adipose tissue	3	1							2	4
Bone marrow	Non-remarkable									

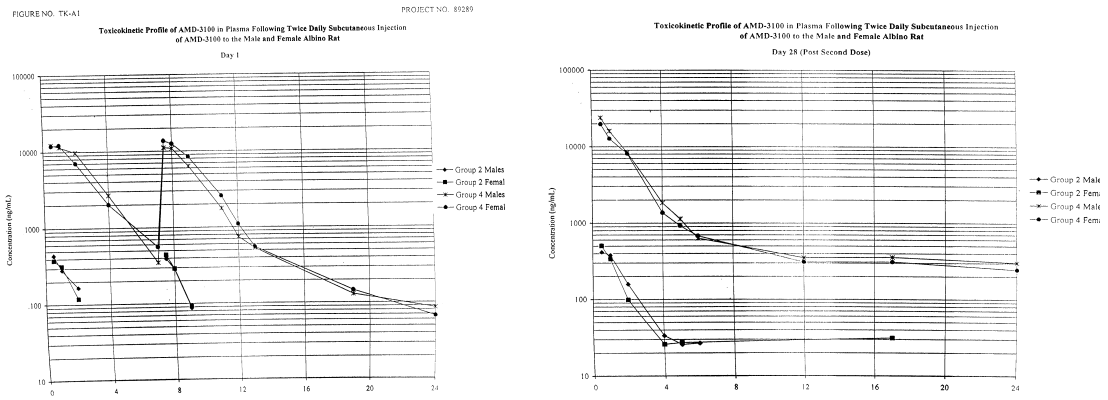
* $p<0.01$ or $p<0.05$

**Died during recovery period (day 32), due to development of urinary bladder calculi.. This death was determined not to be treatment related.

Summary of individual study findings:

1. There was no treatment-related mortality during the study. One 4 mg/kg/day recovery female was found dead 4 days into the recovery period (Day 32). The cause of death was determined to be the development of urinary bladder calculi and not treatment related.
2. Minor clinical signs were observed in two males dosed at 24 mg/kg/day on two separate study days which included respiratory rate ↑, abnormal breathing sounds and vocalization↑.
3. At the end of treatment period, WBC ↑ was noted in animals dosed at 4 mg/kg/day and 24 mg/kg/day mainly due to segmented neutrophil counts ↑ that may be partly attributable to the minor scapular injection site lesions but an effect of the test article is likely.
4. At the end of treatment period, there were ALT ↑ and slight Mg^{++} ↓ in the 24 mg/kg/day males. At the end of recovery period, only Mg^{++} levels remained slightly ↓.
5. At the study termination, weights of spleen (males and females) and kidney (males) ↑ in 24 mg/kg/day animals were noted. Also noted were thymus weights ↓ (relative and absolute) and liver weights ↑ (relative) in males dosed at 4 and 24 mg/kg/day. Without histological findings, these differences were considered of doubtful toxicological importance. There were no differences in organ weights at the end of recovery period.
6. There was a slight increase in the incidence of well localized minor subcutaneous areas of hemorrhage and inflammation seen at the scapular injection sites in animals dosed at 4 and 24 mg/kg/day. These lesions were considered to be associated with the treatment procedure, however, the possibility of a slight exacerbation of test article effect could not be ruled out. At the end of the 14 day recovery, there were no treatment related findings at the injection sites.

Toxicokinetics



Day 14 and Day 21 Plasma concentration of AMD3100 (twice daily, S.C. injection)

Gender	Group	Dose Level mg/kg/day	Pre-Rx Concentration (ng/mL)	
			Day 14	Day 21
Γ	2	0.6	N/A	N/A
	4	24	238±111	300±95
E	2	0.6	N/A	N/A
	4	24	158±26	186±16

Table TK-1 Toxicokinetic parameters of AMD3100 following twice daily S.C. injection of AMD3100 in the Γ and E Albino rat

Day 1 (post first dose)

Gender	Group	Dose Level (mg/kg/day)	t_{max} (h)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	k_{el} (h^{-1})	$t_{1/2el}$ (h)	$AUC_{0\text{-}inf}$ (ng•h/mL)	%Extrapolation $AUC_{0\text{-}inf}$
Γ	2	0.6	0.5	441	516	a	a	a	a
	4	24	0.5	12343	36577	a	a	a	a
E	2	0.6	0.5	380	488	a	a	a	a
	4	24	1.0	12232	31484	a	a	a	a

a It was not possible to estimate the k_{el} due to insufficient plasma concentrations in the elimination phase. Consequently, all parameters derived from this, $t_{1/2el}$, $AUC_{0\text{-}inf}$, and %Extrapolation $AUC_{0\text{-}inf}$ were not estimated.
k_{el} Elimination rate; $T_{1/2el}$ Terminal elimination half life; $AUC_{0\text{-}inf}$ $AUC_{0\text{-}tlast} + (C_{tlast}/k_{el})$; %Extrapolation $AUC_{0\text{-}inf}$ $(AUC_{0\text{-}inf} - AUC_{0\text{-}tlast}) / AUC_{0\text{-}tlast}$ X 100.

Day 1 (post second dose)

Gender	Group	Dose Level (mg/kg/day)	t_{max} (h)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	k_{el} (h^{-1})	$t_{1/2el}$ (h)	$AUC_{0\text{-}inf}$ (ng•h/mL)	%Extrapolation $AUC_{0\text{-}inf}$
Γ	2	0.6	0.5	394	456	a	a	a	a
	4	24	0.5	11070	29275	0.184	3.8	29739	1.6
E	2	0.6	0.5	441	485	a	a	a	a
	4	24	0.6	13582	36217	0.222	3.1	36517	0.8

Day 1 (total)

Gender	Group	Dose Level (mg/kg/day)	t_{max} (h)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	k_{el} (h^{-1})	$t_{1/2el}$ (h)	$AUC_{0\text{-}inf}$ (ng•h/mL)	%Extrapolation $AUC_{0\text{-}inf}$
Γ	2	0.6			972	a	a	a	a
	4	24			65940	0.184	3.8	66404	0.7
E	2	0.6			973	a	a	a	a
	4	24			67856	0.222	3.1	68155	0.4

Day 28 (post second dose, 0-17h)

Gender	Group	Dose Level (mg/kg/day)	t_{max} (h)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	k_{el} (h^{-1})	$t_{1/2el}$ (h)	$AUC_{0\text{-}inf}$ (ng•h/mL)	%Extrapolation $AUC_{0\text{-}inf}$
Γ	2	0.6	0.5	417	819	a	a	a	a
	4	24	0.5	23974	45541	0.086	8.08	49705	8.4
E	2	0.6	0.5	501	1055	a	a	a	a
	4	24	0.5	19809	39359	0.091	7.59	42757	8.0

Day 28 (post second dose, 0-24h)

Gender	Group	Dose Level (mg/kg/day)	t_{max} (h)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	k_{el} (h^{-1})	$t_{1/2el}$ (h)	$AUC_{0\text{-}inf}$ (ng•h/mL)	%Extrapolation $AUC_{0\text{-}inf}$
Γ	2	0.6	0.5	417	819	a	a	a	a
	4	24	0.5	23974	47851	0.059	11.8	53017	9.7
E	2	0.6	0.5	501	1055	a	a	a	a
	4	24	0.5	19809	41300	0.066	10.5	45018	8.3

Table TK-2 Plasma AMD3100 C_{max} and $AUC_{0\text{-}tlast}$ in the Γ and E Albino rat and the proportional change of each parameter relative to the target low dose following twice daily S.C. injection of AMD3100

Day 1 (post first dose)

Gender	Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	Dose Proportionality	C_{max} Ratio	$AUC_{0\text{-}tlast}$ Ratio
Γ	2	0.6	441	516	1.0	1.0	1.0
	4	24	12343	36577	40.0	28.0	70.9
E	2	0.6	380	488	1.0	1.0	1.0
	4	24	12232	31484	40.0	32.2	64.5

Day 1 (post second dose)

Gender	Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	Dose Proportionality	C_{max} Ratio	$AUC_{0\text{-}tlast}$ Ratio
Γ	2	0.6	394	456	1.0	1.0	1.0
	4	24	11070	29275	40.0	28.1	64.2
E	2	0.6	441	485	1.0	1.0	1.0
	4	24	13582	36217	40.0	30.8	74.7

Day 1 (total)

Gender	Group	Dose Level (mg/kg/day)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	Dose Proportionality	$AUC_{0\text{-}tlast}$ Ratio
Γ	2	0.6	972	1.0	1.0
	4	24	65940	40.0	67.8
E	2	0.6	973	1.0	1.0
	4	24	67856	40.0	69.7

Day 28 (post second dose, 0-17h)

Gender	Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	Dose Proportionality	C_{max} Ratio	$AUC_{0\text{-}tlast}$ Ratio
Γ	2	0.6	417	819	1.0	1.0	1.0
	4	24	23974	45541	40.0	57.5	55.6
E	2	0.6	501	1055	1.0	1.0	1.0
	4	24	19809	39359	40.0	39.5	37.3

Day 28 (post second dose, 0-24h)

Gender	Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	Dose Proportionality	C_{max} Ratio	$AUC_{0\text{-}tlast}$ Ratio
Γ	2	0.6	417	819	1.0	1.0	1.0
	4	24	23974	47851	40.0	57.5	58.4
E	2	0.6	501	1055	1.0	1.0	1.0
	4	24	19809	41300	40.0	39.5	39.1

Table TK-3 Percent difference in plasma AMD3100 C_{max} and AUC_{tlast} Day 1 to Day 28 (post second dose, 0-17h)

Gender	Group	Dose Level (mg/kg/day)	ΔC_{max} (%)	$\Delta AUC_{0\text{-}tlast}$ (%)
Γ	2	0.6	6	80
	4	24	117	56
E	2	0.6	14	118
	4	24	46	9

Summary of toxicokinetics:

No apparent differences were observed between the sexes for all reported parameters and no apparent differences were observed between the dose levels and treatment periods for the t_{max} parameter. The mean C_{max} values and the estimated $AUC_{0\text{-}tlast}$ values generally ↑ with ↑ dose level of AMD3100 in greater than proportional dose except for high dose E on Day 28. Following repeat dosing for 28 consecutive days, the C_{max} and $AUC_{0\text{-}tlast}$ parameters generally increased in comparison to single dosing, however no definitive trend was observed. The longer half-life observed on Day 28, ~ 7.8 h, in comparison to Day 1, ~ 3.5 h, at 24 mg/kg/day may indicate a decreased rate of clearance of AMD3100 from the rat plasma following repeat dosing. Steady-state AMD3100 plasma concentrations were statistically attained on Day 14 for 24 mg/kg/day males.

Conclusion of the study: Twice daily S.C. injection of AMD3100 for 28 consecutive days in rats at total daily dose levels at 0.6, 1.2, 4 and 24 mg/kg./day resulted in few clinical signs and relative minor changes in hematology, clinical chemistry, urinalysis, organ weights, mostly at 4 and 24 mg/kg/day. These changes were reversible by the end of recovery and in the absence of histopathological findings, these changes were considered not toxicologically significant. With the exception of the slight injection site changes, a total daily dose at 24 mg/kg/day was considered to be the no toxic effect level (NTEL) and the no observed effect level (NOEL) was considered to be 0.6 mg/kg/day.

Study 17: A 28-day twice daily S.C. injection toxicity study of AMD3100 free base in the Beagle dog with a 14-day recovery period

Key findings: No toxic effect level (NTEL) was 8 mg/kg/day.

Study no:	89290
Volume #, and page #:	Vol. 4, p.4-53 to 4-217, Vol. 5, 5-1 to 5-250
Conducting laboratory and location:	**(b) (4)**
Date of study initiation:	May 31, 2000
GLP compliance:	Yes
QA report:	yes (x) no ( )
Drug, lot #, radiolabel, and % purity:	B1047-941001, 100 %
Formulation/vehicle:	dissolved in HCL with pH 6.5-7.0, diluted in sterile water, further diluted with 0.9% sodium chloride

Methods: S.C. injection of AMD3100 by twice daily (approximately 7 hrs apart at equal doses) at 1 mL/kg/dose, into the scapular region, for consecutive 28 days. Injection sites were rotated daily based on predetermined schedule. and All animals were followed by daily observation

Group	Treatment***	Dose Level (mg/kg/day)	Concentration** (mg/mL)	Animal Number	
				Γ	E
1	Control	0	0	101-104*	151-154*
2	AMD3100	0.3	0.15	201-203	251-253
3	AMD3100	1.5	0.75	301-303	351-353
4	AMD3100	8	4.0	401-403*	451-453*

*1 animal/sex was retained for a 14-day recovery period

** twice daily dosing at 1 mL/kg with equal dose 7 hrs apart.

Dosing:

Species/strain:	Beagle dog
#/sex/group or time point (main study):	3-4 dogs/sex/dose for main study, 1 dogs/sex/groups 1 and 4 for 14-day recovery period
Age:	7 months old
Weight:	Γ: 7.9-10.3 kg; E: 7.0-9.3 kg.
Doses in administered units:	0, 0.3, 1.5, and 8 mg/kg/day
Route, form, volume, and infusion rate:	subcutaneous injection twice daily for 28 days

Observations and times:

Clinical signs:	twice daily
Body weights:	weekly
Food consumption:	measured and recorded daily
Ophthalmoscopy:	pretest, week 4, end of recovery
Electrocardiograph	pretreatment, weeks 1 and 4, end of recovery
Hematology:	prior to and end of treatment, recovery period
Clinical chemistry:	prior to and end of treatment, recovery period
Urinalysis	prior to and end of treatment, recovery period
Gross pathology:	at terminal examination
Organs weighed:	at terminal examination
Histopathology	at terminal examination (selected animals)
Bone marrow examination	at terminal examination (selected animals)
Toxicokinetics:	30', 1, 2, 4 and 7 post first dose, 30', 1, 2, 4, 5, 6, 12 and 17 hr post second dose (day 1), pre-dose (AM on days 14 and 21), 30', 1, 2, 4, 5, 6, 12, 17 and 24 hr post second dose (day 28).

Results

Groups /Parameters	Controls		0.3 mg/kg/day		1.5 mg/kg/day		8 mg/kg/day	
	Γ (N=4)	E (N=4)	Γ (N=3)	E (N=3)	Γ (N=3)	E (N=3)	Γ (N=4)	E (N=4)
Mortality	None							
Body weight (kg) Δ Body weight Week 1	0.17	0.30	0.07	0.03	0.20	–0.47*	0.10	–0.55*
Fd. Consumption Week 1 (g)	288.3	314.7	233.0	240.0	235.0	138.0*	180.0	170.7*
Clinical signs Salivation Appearing thin					1 (wk 2)	1 (wk 2)	1 (Day 1)	2 (Day 1)
ECG	Non-remarkable							
Ophthalmology	Non-remarkable							
Hematology (wk4) WBC x10^3/mm^3 Neut seg /mm^3	10.8 5785	10.0 5765	13.8 5960	13.5 6204	18.1* 111072	14.1 6	21.2* 14367*	14.6 9254
Clin. Chemistry (wk4) A/G Mg++ (mg/dL)	18.2 1.6	1.52 1.7	16.0 1.5	2.04* 1.7	17.3 1.5	1.90* 1.3*	19.3 1.2*	1.94* 2.1.0*
Urinalysis Ca++ (mg/dL)	4.6	7.9	4.4	2.7	10.3	12.2	14.1*	17.8*
Gross pathology	Non-remarkable							
Organ weights	Non-remarkable							
Histopathology	(N=3)	(N=13)	(N=3)	(N=3)	(N=3)	(N=3)	(N=3)	(N=3)
Injection sites Hemorrhage	1	2	–	1	2	–	1	2
Inflammation Adipose tissue	1	3	–	2	3	1	1	2
Liver Cytoplasmic rarefaction		1					3	2
Bone marrow	Non-remarkable							

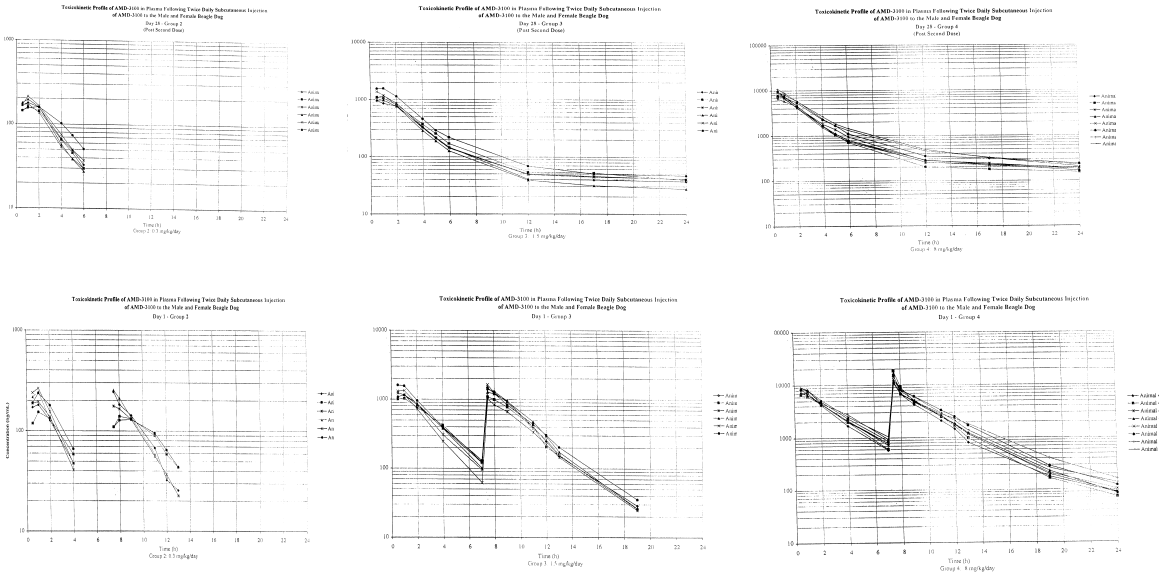
* $p<0.01$ or $p<0.05$

Summary of individual study findings:

1. There was no treatment-related mortality during the study.
2. Few clinical signs were noted including slight salivation for one Γ and two E dosed at 8 mg/kg/day, and appearing thin from Day 8 to 15 for one Γ and one E dosed at 1.5 mg/kg/day.
3. Slight transient body weights ↓ and food consumption ↓ in 8 mg/kg/day Γ and E and 1.5 mg/kg/day E were observed during Week 1. One 1.5 mg/kg/day E was provided with food supplement during Week 2 due to its thin condition and body weight loss.
4. At the end of treatment period, WBC ↑ was noted in animals dosed at 8 mg/kg/day and Γ dosed 1.5 mg/kg/day mainly due to segmented neutrophil counts ↑.
5. At the end of treatment period, there were Mg^{++} ↓ for animals dosed at 8 mg/kg/day and E dosed 1.5 mg/kg/day and slightly A/G ratio ↑ (due to a slight ↓ in globulin) was noted in E at 0.3 mg/kg/day and greater.
6. Calcium level ↑ in the urine were noted in animals dosed at 1.5 mg/kg/day and 8 mg/kg/day at the end of the treatment period but returned to control levels after 14 days of recovery.

7. There were no treatment-related ocular changes, no adverse treatment-related macroscopic or microscopic histopathological changes in the study. Also, there were no test article effects on ECG recordings during the study. There were no toxicologically significant findings at the end of the treatment or recovery periods in M:E ratios or maturation of hematopoetic cell lines in 8 mg/kg/day animals as compared to control animals.

Toxicokinetics



Day 14 and Day 21 Plasma concentration of AMD3100 following twice daily, S.C. injection to Beagle dogs

Group	Gender	Dose Level mg/kg/day	Pre-Rx Concentration (ng/mL)	
			Day 14	Day 21
2	Γ	0.3	N/A	N/A
	E		N/A	N/A
3	Γ	1.5	37±1	42±1
	E		43±6	47±12
4	Γ	8	227±25	270±63
	E	24	1308±67	288±79

Table TK-4 Toxicokinetic parameters of AMD3100 following twice daily S.C. injection of AMD3100 in the Γ and E Beagle dogs
Day 1 (post first dose)

Group	sex	Dose Level (mg/kg/day)	t_{max} (h)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	k_{el} (h^{-1})	$t_{1/2el}$ (h)	$AUC_{0\text{-}inf}$ (ng•h/mL)	%Extrapolation $AUC_{0\text{-}inf}$
2	Γ	0.3	1.0±0.0	176±22	458±29	a	a	a	a
	E	0.3	1.0±0.0	248±14	577±42	a	a	a	a
3	Γ	1.5	0.8±0.3	1132±102	3562±264	a	a	a	a
	E	1.5	0.8±0.3	1363±223	4064±447	a	a	a	a
4	Γ	8	0.5±0.0	7278±685	21171±643	a	a	a	a
	E	8	0.6±0.3	8339±828	24781±2020	a	a	a	a

a It was not possible to estimate the k_{el} due to insufficient plasma concentrations in the elimination phase. Consequently, all parameters derived from this, $t_{1/2el}$, $AUC_{0\text{-}inf}$, and %Extrapolation $AUC_{0\text{-}inf}$ were not estimated.
k_{el} Elimination rate; $T_{1/2el}$ Terminal elimination half life; $AUC_{0\text{-}inf}$ $AUC_{0\text{-}tlast}$ + (C_{tlast}/k_{el}); %Extrapolation $AUC_{0\text{-}inf}$ ($AUC_{0\text{-}inf} - AUC_{0\text{-}tlast}$) / $AUC_{0\text{-}tlast}$ X 100.

Day 1 (post second dose, mg/kg/day)

Group	sex	Dose Level	t_{max} (h)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	k_{el} (h^{-1})	$t_{1/2el}$ (h)	$AUC_{0\text{-}inf}$ (ng•h/mL)	%Extrapolation $AUC_{0\text{-}inf}$
2	Γ	0.3	0.7±0.3	186±53	576±40	0.4±0.1	1.6±0.3	649±55	22.2±5.4
	E	0.3	1.3	192	598	0.4	1.6	678	11.7
3	Γ	1.5	0.5±0.0	1158±202	3945±567	0.3±0.0	2.3±0.1	4034±575	2.2±0.1
	E	1.5	0.5±0.0	1454±180	4618±56	0.3±0.0	2.2±0.1	4709±74	1.9±0.4
4	Γ	8	0.5±0.0	14088±3638	29923±2246	0.2±0.0	3.0±0.3	30289±2199	1.2±0.3
	E	8	0.5±0.0	13235±1910	34452±2911	0.2±0.0	3.1±0.2	35042±2921	1.7±0.4

Day 1 (total)

Group	Gender	Dose Level (mg/kg/day)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	k_{el} (h^{-1})	$t_{1/2el}$ (h)	$AUC_{0\text{-}inf}$ (ng•h/mL)	%Extrapolation $AUC_{0\text{-}inf}$
2	Γ	0.3	1034±40	0.44±0.07	1.6±0.3	1108±30	6.6±3.6
	E	0.3	1166	0.44	1.6	1245	6.3
3	Γ	1.5	7533±825	0.30±0.01	2.3±0.1	7622±833	1.2±0.1
	E	1.5	8710±498	0.320.02	2.2±0.1	8801±520	1.0±0.2
4	Γ	8	51267±1940	0.23±0.02	3.0±0.3	51632±1889	0.7±0.1
	E	8	59456±4399	0.22±0.01	3.1±0.2	60046±4455	1.0±0.2

Day 28 (post second dose, mg/kg/day; 0-17h)

Group	sex	Dose Level	t_{max} (h)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	k_{el} (h^{-1})	$t_{1/2el}$ (h)	$AUC_{0\text{-}inf}$ (ng•h/mL)	%Extrapolation $AUC_{0\text{-}inf}$
2	Γ	0.3	1.0±0.0	169±10	575±36	0.34±0.05	2.1±0.3	666±39	13.7±1.9
	E	0.3	1.3±0.6	188±26	650±38	0.36±0.01	2.0±0.1	759±71	14.2±3.4
3	Γ	1.5	0.7±0.3	1070±107	4175±311	0.13±0.03	5.6±1.2	4550±431	8.1±2.0
	E	1.5	0.7±0.3	1302±299	4646±1127	0.13±0.02	5.6±0.6	4982±1177	6.8±1.4
4	Γ	8	0.5±0.0	8261±1592	24348±1071	0.13±0.01	5.7±0.3	26142±1287	6.9±0.9
	E	8	0.5±0.0	9257±1236	30048±4975	0.13±0.01	5.6±0.5	32373±5206	7.3±0.9

Day 28 (post second dose, mg/kg/day; 0-24h)

Group	sex	Dose Level	t_{max} (h)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	k_{el} (h^{-1})	$t_{1/2el}$ (h)	$AUC_{0\text{-}inf}$ (ng•h/mL)	%Extrapolation $AUC_{0\text{-}inf}$
2	Γ	0.3	1.0±0.0	169±10	575±36	0.34±0.05	2.1±0.3	666±39	13.7±1.9
	E	0.3	1.3±0.6	188±26	650±38	0.36±0.01	2.0±0.1	759±71	14.2±3.4
3	Γ	1.5	0.7±0.3	1070±106	4470±336	0.03	24.9	6101	22.9
	E	1.5	0.7±0.3	1302±299	4926±1188	0.03	22.9	6277	19.2
4	Γ	8	0.5±0.0	8261±1592	25713±1141	0.03±0.01	23.2±6.1	31433±884	18.2±3.5
	E	8	0.5±0.0	9257±1236	31812±5190	0.05±0.02	16.9±7.1	36943±3845	14.3±6.5

Table TK-5 Plasma AMD3100 C_{max} and $AUC_{0\text{-}tlast}$ in the Γ and E Beagle dogs and the proportional change of each parameter relative to the target low dose following twice daily S.C. injection of AMD3100

Day 1 (post first dose)

Gender	Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	Dose Proportionality	C_{max} Ratio	$AUC_{0\text{-}tlast}$ Ratio
2	Γ	0.3	176	458	1.0	1.0	1.0
	E	0.3	248	577	1.0	1.0	1.0
3	Γ	1.5	1132	3562	5.0	6.4	7.8
	E	1.5	1363	4064	5.0	5.5	7.0
4	Γ	8	7278	21171	26.7	41.4	46.2
	E	8	8339	24781	26.7	33.6	42.9

Day 1 (post second dose)

Gender	Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	Dose Proportionality	C_{max} Ratio	$AUC_{0\text{-}tlast}$ Ratio
2	Γ	0.3	186	576	1.0	1.0	1.0
	E	0.3	192	598	1.0	1.0	1.0
3	Γ	1.5	1158	3945	5.0	6.2	6.8
	E	1.5	1454	4618	5.0	7.6	7.7
4	Γ	8	14088	29923	26.7	75.7	51.9
	E	8	13235	34452	26.7	68.9	57.6

Day 1 (total)

Gender	Group	Dose Level (mg/kg/day)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	Dose Proportionality	C_{max} Ratio	$AUC_{0\text{-}tlast}$ Ratio
2	Γ	0.3	1034	1.0	1.0	1.0
	E	0.3	1166	1.0	1.0	1.0
3	Γ	1.5	7533	5.0	7.3	7.3
	E	1.5	8710	5.0	7.5	7.5
4	Γ	8	51267	26.7	49.6	49.6
	E	8	59456	26.7	51.0	51.0

Day 28 (post second dose, 0-17h)

Gender	Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	Dose Proportionality	C_{max} Ratio	$AUC_{0\text{-}tlast}$ Ratio
2	Γ	0.3	169	575	1.0	1.0	1.0
	E	0.3	188	650	1.0	1.0	1.0
3	Γ	1.5	1070	4175	5.0	6.3	7.3
	E	1.5	1302	4646	5.0	6.9	7.1
4	Γ	8	8261	24348	26.7	48.9	42.3
	E	8	9257	30048	26.7	49.2	46.2

Day 28 (post second dose, 0-24h)

Gender	Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	$AUC_{0\text{-}tlast}$ (ng•h/mL)	Dose Proportionality	C_{max} Ratio	$AUC_{0\text{-}tlast}$ Ratio
2	Γ	0.3	169	575	1.0	1.0	1.0
	E	0.3	188	650	1.0	1.0	1.0
3	Γ	1.5	1070	4470	5.0	6.3	7.8
	E	1.5	1302	4926	5.0	6.9	7.6
4	Γ	8	8261	25713	26.7	48.9	44.7
	E	8	9257	31812	26.7	49.2	48.9

Table TK-6 Percent difference in plasma AMD3100 C_{max} and AUC_{tlast} Day 1 to Day 28 (post second dose, 0-17h)

Group	Gender	Dose Level (mg/kg/day)	ΔC_{max} (%)	$\Delta AUC_{0\text{-}tlast}$ (%)
2	Γ	0.3	–9.1	–0.2
	E	0.3	–2.1	8.7
3	Γ	1.5	–7.6	5.8
	E	1.5	–10.5	0.6
4	Γ	8	–41.4	–18.6
	E	8	–30.1	–12.8

Summary of toxicokinetics:

No apparent differences were observed between the sexes for all reported parameters and no apparent differences were observed between the dose levels and treatment periods for the t_{max} parameter. The systemic exposure (mean C_{max} values) and the estimated $AUC_{0\text{-}tlast}$ values ↑ with ↑ dose level of AMD3100 in greater than proportional to dose for both Γ and E animals. Following repeat dosing for 28 consecutive days, the C_{max} and $AUC_{0\text{-}tlast}$ parameters generally similar in comparison to one day of dosing, however a decrease of ~ 30-40 % was observed for the C_{max} parameter at the high dose level. The longer half-life observed at a higher dose level following repeat dosing (Day 28) may indicate a decreased rate of clearance of AMD3100 from the dog plasma following repeat dosing. Steady-state AMD3100 plasma concentrations were statistically attained on Day 14 for Γ animals at dose of 8 mg/kg/day.

Conclusion of the study:

Twice daily S.C. injection of AMD3100 for 28 consecutive days in dogs at total daily dose levels at 0, 0.3, 1.5, and 8 mg/kg./day resulted in minor changes in clinical signs, hematology, clinical chemistry, urinalysis mostly at 1.5 and 8 mg/kg/day. These changes were reversible by the end of recovery and there were no toxicologically significant histopathological changes. Therefore, it was considered that no toxic effect level (NTEL) was 8 mg/kg/day.

Toxicology Summary:

Species	Daily Dose (mg/kg/day)	Schedule	Route	Findings
Rat E only	0, 4, 12	D x 14 qd or D x 14 bid	S.C.	No mortality, no adverse effects on clinical signs or body weight.
Rat	3, 12, 24, 48, 72, 100	D x 7 bid	S.C.	Deaths in single dose of 36 or 50 mg/kg or a 7-day bid 48 mg/kg/day. No treatment-related adverse effects for 24 mg/kg/day or lower.
Dog	12, 18	D x 7 bid	S.C.	Death and severe salivation, tremor/convulsions. activity ↓, uncoordination, HR ↑, breathing difficulties at single dose of 9 mg/kg. Uncoordination, limited use of hindlimbs, tremors, activity ↓, at 12 mg/kg/day. WBC ↑ for both groups.
Rat	0. 0.6, 1, 2, 4, 24	D x 28 bid	S.C.	No treatment-related mortality; respiratory rate ↑, abnormal breathing sounds, vocalization ↑ at 24 mg/kg/day Γ; segmented neutrophils ↑ at 4 & 24 mg/kg/day; ALT ↑, Mg^{++} ↓ at 24 mg/kg/day; weight of spleen (Γ and E) and kidney (E) at 24 mg/kg/day; weights of thymus and liver ↓ at 4 and 24 mg/kg/day Γ . ↑ incidence of localized minor cutaneous areas of hemorrhage and inflammation at injection sites at 4 and 24 mg/kg/day.
Dog	0, 0.3, 1.5, 8	D x 28 bid	S.C.	No treatment-related mortality; slight salivation for 1 Γ and 2 E at 8 mg/kg/day, appearing thin from Day 8 to 15 for 1 Γ and 1 E at 1.5 mg/kg/day; Slight transient body weights ↓ and food consumption ↓ at 8 mg/kg/day Γ and E and 1.5 mg/kg/day E at wk 1; segmented neutrophil ↑ at 8 mg/kg/day (Γ and E) and Γ at 1.5 mg/kg/day; Mg^{++} ↓ at 8 mg/kg/day (Γ and E) and E at 1.5 mg/kg/day and A/G ratio ↑ at E ≥ 0.3 mg/kg/day; urine Ca^{++} ↑ at 1.5 mg/kg/day and 8 mg/kg/day (Γ and E); no treatment-related ocular changes, no adverse treatment-related macroscopic or microscopic histopathological changes. no test article effects on ECGs; no toxicologically significant findings in M:E ratios or maturation of hematopoetic cell lines at 8 mg/kg/day.

Histopathology Inventory for IND # 55851

Study	#89289	#89290
Species	Rat	Dog
Adrenals	X	X
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur)	X	
Brain	X	X
Cecum	X	X
Cervix		X
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gall bladder		X
Gross lesions		
Harderian gland	X	
Heart	X	X
Ileum	X	X
Injection site	X	X
Jejunum	X	X
Kidneys	X	X
Lachrymal gland	X	
Larynx		
Liver	X	X
Lungs	X	X
Lymph nodes, cervical		
Lymph nodes mandibular	X	X
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity		
Optic nerves	X	X
Ovaries	X	X
Pancreas	X	X
Parathyroid	X	X
Peripheral nerve		
Pharynx		
Pituitary	X	X
Prostate	X	X
Rectum	X	X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles		
Skeletal muscle	X	X
Skin	X	X
Spinal cord	X	X
Spleen	X	X
Sternum		X
Stomach	X	X
Testes	X	X
Thymus	X	X
Thyroid	X	X
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X	X
Vagina	X	X
Zymbal gland		

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