CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

BLA 125277

PHARMACOLOGY REVIEW(S)

INTEROFFICE MEMO

TO: BLA125277/Resubmission
Sequence number/date/type of submission: 000/June 1, 2009/Complete Response

FROM: Jean Q Wu, M.D., Ph.D.
Acting Pharmacology/Toxicology Supervisor
Division of Pulmonary and Allergy Products

DATE: October 29, 2009

BLA 125277 was originally submitted for approval of Ecallantide on September 23, 2008. Ecallantide is a 60 amino acid protein inhibitor of plasma kallikrein developed as a potential treatment of hereditary angioedema (HAE). The original pharmacology/toxicology review was completed by Dr. Jean Wu on February 13, 2009 and a recommendation of approval was made from the nonclinical perspective. A carcinogenicity study in one species (rat) was recommended as a post-marketing requirement. The labeling review of pharmacology/toxicology relevant sections was conducted in the original review. A complete response letter was issued on March 25, 2009 due to the clinical deficiency.

In the current re-submission, no new nonclinical data was submitted. As indicated in the primary review of this re-submission by Dr. Timothy Robison dated October 13, 2009, the recommended labeling is essentially identical to that in the original Pharmacology/Toxicology review dated February 13, 2009. The post-marketing requirement for a carcinogenicity study remains the same. Therefore, I concur with the primary reviewer's recommendation that Ecallantide should be approved with suggested labeling changes from a nonclinical perspective.

JQ Wu 10/29/09

Jean Q Wu, M.D., Ph.D.
Acting Pharmacology/Toxicology Supervisor

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D.
OND IO
BLA: 125277
Submission date: September 23, 2008
Drug: ecallantide (Kalbitor)
Sponsor: Dyax Corp.
Indication: patients with acute attacks of hereditary angioedema
Reviewing Division: Division of Pulmonary and Allergy Products

Introductory Comments:
The pharm/tox reviewer and supervisor have written reviews which recommend that ecallantide be approved from a nonclinical perspective. The reviewer and supervisor also recommend a phase 4 carcinogenicity study.

Reproductive and Developmental toxicity:
The sponsor proposed a pregnancy labeling category of C and the Division agreed with this category although some minor changes in specific wording describing the relevant studies were suggested by the pharm/tox reviewer. The reviewer noted that the drug appears to produce some increase in resorptions in rats dosed intravenously at approximately 13 times the maximum recommended human dose on a mg/kg basis. Although this dose is significantly higher than the human dose and was associated with some mild maternal toxicity, it still is appropriate to describe the effects in the labeling.

Carcinogenicity:
No carcinogenicity studies have been conducted with ecallantide. The sponsor was advised in the end-of-phase 2 meeting that they should provide a rationale for not conducting a carcinogenicity study. The sponsor subsequently provided a rationale. The division, in conjunction with pharm/tox associate directors, assessed the rationale and found that there was inadequate reason not to conduct a carcinogenicity study. The intended use of the drug was considered chronic intermittent, the carcinogenicity study appeared feasible and the lifetime consequences of inhibiting kalikrein or other off-target effects were unknown. The sponsor was informed by facsimile on September 26, 2007 that evaluation of carcinogenic potential for ecallantide would be required if it was feasible in one rodent species. However, the sponsor was also informed that such a study would be acceptable as a post-marketing commitment. Allowing the study to be conducted as a phase 4 commitment was considered acceptable due to the lack of effective treatments for the indication.

The pharm/tox reviewer has recommended that a carcinogenicity study be conducted in the rat. This recommendation is based on the observation that the rat appears to be a relevant species since it responds with an appropriate pharmacologic marker (prolongation of activated partial thromboplastin time) and appears to tolerate treatment with the compound in the six month study already completed. Induction of antidrug

antibody was noted in the six month rat study; however, this did not reduce drug exposure and the pharmacologic effect was maintained.

Conclusions:
I concur with the Division pharmacology/toxicology recommendation that the non-clinical studies submitted to this BLA provide sufficient information to support the approval of ecallantide for the treatment of patients with acute attacks of hereditary angioedema but that a rat carcinogenicity study should be required as a post marketing commitment.

Paul C Brown 3-13-09
Paul C. Brown, Ph.D.
ODE Associate Director for Pharmacology and Toxicology

INTEROFFICE MEMO

TO: BLA 125277

FROM: Timothy W. Robison, Ph.D., D.A.B.T.
Acting Pharmacology/Toxicology Team Leader
Division of Pulmonary and Allergy Products

DATE: February 19, 2009

I concur with pharmacologist's (Dr. Jean Wu) recommendation that the pharmacology and toxicology of KALIBITOR (Ecallantide) have been adequately studied and the drug product should be approved from a nonclinical standpoint.

Pharmacology: Ecallantide is a 60 amino acid recombinant protein inhibitor of plasma kallikrein developed as a potential treatment for hereditary angioedema (HAE). It is intended for the subcutaneous route of administration. *In vitro* pharmacology studies demonstrated that ecallantide was a potent plasma kallikrein inhibitor with a Ki of 25 pM for the human form.

ADME: Following subcutaneous administration, the terminal half-life ranged from 143-158 minutes in rats, rabbits and minipigs. Due to the relatively short half-life, the repeat-dose PK profile was consistent with the single dose profile. Volume of distribution values in all species indicated ecallantide could distribute beyond the blood volume into the tissues. Generally, exposure increased with increasing dose in both monkeys and rats.

General Toxicology: Pivotal nonclinical toxicology studies were conducted in Sprague-Dawley rats and Cynomolgus monkeys that received subcutaneous doses up to 25 mg/kg administered once every 3 days for a total treatment duration of 6 months. Potential treatment-related deaths occurred for rats in the 25 mg/kg group. Significant increases of AST and ALT values were observed for female rats in the 25 mg/kg group that resolved during the recovery period and were not associated with any histopathological findings in the liver. For both rats and monkeys, there were test article-related findings at injections sites that included inflammation, epithelial hyperplasia, exudate, ulceration, and granulation tissue/fibrosis for rats and minimal to moderate perivascular mixed inflammatory cell infiltrates for monkeys. Findings at injection sites generally resolved during the recovery period. The NOAEL for rats was identified as 10 mg/kg. Systemic and local NOAELs for monkeys were 25 and 0.4 mg/kg, respectively. NOAELs identified in the 6-month toxicology studies with ecallantide in rats and monkeys provided adequate safety margins for the maximum recommended human dose of ecallantide (See Dr. Wu's review for further details).

In studies with rats and monkeys, ecallantide caused a dose-dependent, reversible prolongation of APTT, which was attributed to inhibition of activation of factor XII to

factor XIIa in the clotting cascade. There was no evidence of gross bleeding in the animals with increased APTT values.

Anti-ecallantide antibodies were measured in both rats and monkeys and at a higher frequency in the higher dose groups. Based upon the pharmacokinetic data, clearance of ecallantide was reduced and systemic exposure was increased following the development of ecallantide antibodies. However, there was no increase in toxicity noted with the higher exposure. Drug activity appeared to be maintained throughout the duration of the 6-month rat and monkey studies based upon measurements of ecallantide and elevated APTT values.

Reproductive Toxicology: A complete battery of reproductive toxicity studies was conducted with ecallantide. Subcutaneous doses of ecallantide administered to male and female rats up to 25 mg/kg/day had no effects on fertility or reproductive performance. Subcutaneous doses of ecallantide up to 20 mg/kg/day in rats had no effects on embryofetal development. An embryofetal development study was conducted with rats that received intravenous doses of 0, 10, 15, and 20 mg/kg/day. At 15 mg/kg/day, there were increased numbers of early resorptions and percentages of resorbed conceptuses per litter without significant reduced numbers of live fetuses. These findings occurred in the presence of relatively mild maternal toxicity. There were additional findings at 20 mg/kg/day although significant maternal toxicity was evident. The developmental NOAEL was identified at 10 mg/kg. In an embryofetal development study with rabbits, intravenous doses of ecallantide up to 5 mg/kg/day had no effects. In a pre- and postnatal development study with rats, subcutaneous doses up to 25 mg/kg had no effects.

Labeling: Ecallantide was determined to be Pregnancy C based upon the results of an intravenous teratology study with rats. At 15 mg/kg/day, there were increased numbers of early resorptions and percentages of resorbed conceptuses per litter in the presence of mild maternal toxicity.

A carcinogenicity study should be conducted in one species (rat) as a post-marketing commitment. There are no other outstanding nonclinical issues.

Timothy W. Robison 2-19-09

Timothy W. Robison, Ph.D., D.A.B.T.
Acting Pharmacology/Toxicology Team Leader

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER:	**125277**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**6/1/09**
PRODUCT:	**KALBITOR (ecallantide) Injection**
INTENDED CLINICAL POPULATION:	**Hereditary Angioedema**
SPONSOR:	**Dyax Corp**
DOCUMENTS REVIEWED:	**Electronic Submission**
REVIEW DIVISION:	**Division of Pulmonary and Allergy Products (HFD-570)**
PHARM/TOX REVIEWER:	**Timothy W. Robison, Ph.D., D.A.B.T.**
PHARM/TOX ACTING SUPERVISOR:	**Jean Wu, M.D., Ph.D.**
DIVISION DIRECTOR:	**Badrul Chowdhury, M.D., Ph.D.**
PROJECT MANAGER:	**Colette Jackson**

Date of review submission to Division File System (DFS): NA

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability
From a nonclinical standpoint, the application is recommended for approval.

B. Recommendation for nonclinical studies
A carcinogenicity study in rats is recommended as a post-marketing commitment (see Review dated February 13, 2009).

C. Recommendations on labeling
See recommended labeling attached at the end of this review. The recommended labeling is essentially identical to that in the Review dated February 13, 2009 (refer to that review for details of the labeling review). In the final version of the labeling, other review disciplines made changes in the Mechanism of Action section under Clinical Pharmacology that removed the recommended PharmTox changes, which is acceptable.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings
No new PharmTox data was provided in the resubmission. See Review dated February 13, 2009 for details of nonclinical findings.

B. Pharmacologic activity
Ecallantide is a 60 amino acid protein inhibitor of plasma kallikrein. See Review dated February 13, 2009 for details of nonclinical findings.

C. Nonclinical safety issues relevant to clinical use
None.

Recommended Labeling:

Reviewer signature: Timothy W. Robison October 13, 2009
Timothy W. Robison, Ph.D.

Supervisor signature: Concurrence - JaWu 10/13/09
Jean Wu, M.D., Ph.D.

cc: list:
BLA 125277, HFD-570
JacksonC, HFD-570
WuJ, HFD-570
RobisonT, HFD-570

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER:	125277
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	September 23, 2008
PRODUCT:	Ecallantide (DX-88)
INTENDED CLINICAL POPULATION:	Patients with acute attacks of hereditary angioedema
SPONSOR:	Dyax Corp
DOCUMENTS REVIEWED:	Electronic Submission
REVIEW DIVISION:	Division of Pulmonary and Allergy Products (HFD-570)
PHARM/TOX REVIEWER:	Jean Wu, MD, PhD
PHARM/TOX SUPERVISOR:	Timothy Robison, PhD, DABT
DIVISION DIRECTOR:	Badrul Chowdhury, MD, PhD
PROJECT MANAGER:	Colette Jackson

Date of review submission to Division File System (DFS): N/A

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

From the nonclinical perspective, approval is recommended.

B. Recommendation for nonclinical studies:

A carcinogenicity study in rats as a post-marketing commitment is recommended.

C. Recommendations on labeling (see Suggested labeling at the end of current review)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The sponsor conducted general toxicity studies mainly in rats and monkeys, reproductive/ developmental toxicity studies in rats and rabbits, and local tolerance studies in rats and minipigs.

General toxicology studies were conducted in rats, monkeys and minipigs with administration of ecallantide by the subcutaneous or intravenous route. The duration of exposure employed in these studies ranged from a single dose up to 6-month with dosing frequency from once daily, once every other day to once every three days. Pivotal repeat-dose subcutaneous GLP studies in rats and monkeys were conducted to support intended subcutaneous, intermittent/chronic dosing with a maximum frequency of once every three days in the clinic. Ecallantide was pharmacologically active both in vitro and in vivo across the species tested including mice, rats, minipigs, rabbits, dogs and monkeys.

The pharmacokinetics of ecallantide was evaluated following single and/or repeated-dose intravenous/subcutaneous administration in rats, monkeys, rabbits and minipigs. Generally, following single or repeated s.c. dosing, systemic exposure (C_{max} and AUC) increased with increasing dose with no consistent gender differences across species. T_{max} were 40-60 minutes post single s.c. dose in rats and rabbits, and 2-3 hours in minipigs. The elimination half-life ranged from 143-158 minutes following a single s.c. dose (rats, rabbits and minipigs). The exposure appeared to be greater in the cynomolgus monkeys than in the rat.

In the 6-month rat toxicity study, DX-88/vehicle was administered subcutaneously at dose levels of 0, 0.4, 10 and 25 mg/kg once every three days. Three unscheduled deaths occurred between SD 103 to 142 in the 25 mg/kg males and females and potential relationships to treatment could not be excluded; however, the incidence (4.7%) was low. For these 3 deaths, one female that died

early showed multiple necrosis in the brain including optic chiasm, the lateral internal capsule and optic tract, the fimbria of the hippocampus and the ventral midline of the brainstem, and for the other 2 animals, the cause of the death could not be determined. One control group female also died during this period. There were significant increases of AST and ALT values observed in the 25 mg/kg females that resolved during the recovery period and were not associated with any liver lesions in microscopic examination. Test article-related injection sites reactions observed in both males and females of the 25 mg/kg group and in females only of the 10 mg/kg group included inflammation, epithelial hyperplasia, exudate, ulceration, and granulation tissue/fibrosis. These injection site reactions were completely resolved after recovery period in most animals except for the 25 mg/kg females that was only partially resolved. The NOAEL was identified at 10 mg/kg (approximately 5-fold of maximum recommended human dose on an AUC basis).

In monkeys, a 3-month study with daily sc dosing up to 25 mg/kg and a 6-month study with once every three day sc dosing up to 25 mg/kg were conducted. There was no significant evidence of systemic toxicity observed in either study. Test article-related effects were limited to the injection sites, which included minimal to moderate perivascular mixed inflammatory cell infiltrates at injection sites observed in males and females at doses of 10 and 25 mg/kg in both studies. The NOAELs for systemic toxicity in both monkey studies were identified at 25 mg/kg while the NOAELs for local toxicity would be 0.2 and 0.4 mg/kg for the 3- and 6-month studies, respectively.

In these pivotal repeat-dose studies, transient APTT prolongation was observed in rats and monkeys treated with 10 and 25 mg/kg DX-88, which was considered an expected pharmacologic effect of DX-88 and clinically monitorable. No evidence of gross bleeding was observed in these animals.

With respect to immunogenicity, anti-DX-88 antibodies were measured in the pivotal rat and monkey studies by using validated ELISA or ECL methods. In both rats and monkeys, anti-DX-88 antibodies were detected in each test article-treated group with greater percentages of animals being antibody-positive in the 10 and 25 mg/kg dose groups. The 3-month monkey study (using ELISA method) showed that DX-88 exposure was increased and the clearance rate was decreased when the positive antibody titer was > 26,000 ng/mL, regardless of dose. In the 6-month rat and monkey studies, the increased DX-88 exposure and reduced clearance was also observed. However, there was no increase in toxicity noted with the higher exposure. Drug activity appeared to be maintained throughout the duration of the 6-month rat and monkey studies as evidenced by elevated APTT.

The carcinogenicity assessment was not evaluated in the current application. As indicated in the previous Agency correspondence, the potential carcinogenicity of DX-88 should be assessed, if feasible, in one species as a post-marketing commitment. Based on the 6-month rat toxicity study, it appears feasible to conduct a carcinogenicity study in rat given that exposure to DX-88 was

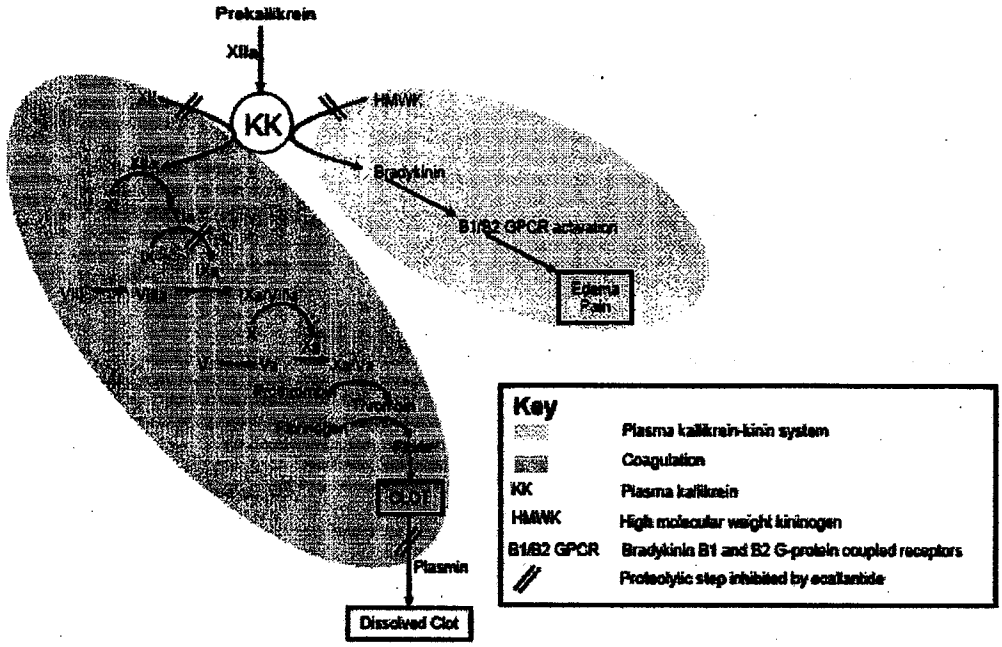
maintained based upon prolongation of APTT values. It is noted that anti-drug antibodies may increase the drug exposure and reduce the clearance rate although the biological significance is unclear.

A complete battery of reproductive toxicity studies was conducted with DX-88. In the reproductive toxicity studies, subcutaneous doses of DX-88 in rats up to 25 mg/kg/day did not show any adverse effects on male or female fertility and reproductive functions. Embryonic and fetal development studies were conducted with rats using the subcutaneous and intravenous routes. Subcutaneous doses of DX-88 in rats up to 20 mg/kg/day did not result in any developmental toxicity. Intravenous administration with DX-88 at a dose of 20 mg/kg/day in rats showed increased number of resorptions and percentage of resorbed conceptuses per litter with corresponding decreased number of live fetuses, reduced fetal body weights (-11%), and increased incidence of fetal alterations, including marked dilation of the lateral ventricle of the brain, short tail, and vertebrate/rib malformations. These findings occurred in the presence of significant maternal toxicity. Intravenous dose of DX-88 at 15 mg/kg/day in rats resulted in increased the number of early resorptions and the percentage of resorbed conceptuses per litter without significant reduced number of live fetuses and reduced fetal body weights (-4%). These findings occurred in the presence of relatively mild maternal toxicity and should be reported in the labeling. The development NOAEL in rats was identified at intravenous dose of 10 mg/kg (AUCinf=7.55 μg.hr/mL= 453 μg.min/mL after single intravenous dose of 10 mg/kg). Intravenous doses of DX-88 in rabbits at dose levels of 2, 5 and 10 mg/kg did not show any adverse development toxicity. However, the number of rabbits at 10 mg/kg dose group (n=10) was not considered sufficient for a full evaluation. Subcutaneous doses of DX-88 up to 25 mg/kg in F_0 rats did not affect the gestation, parturition, or lactation in the F_0-generation rats, growth, development, and reproductive performance of the F_1-generation rats, and the viability and growth in the offspring. DX-88 was not measured in the milk of rats.

B. Pharmacologic activity

Ecallantide is a 60 amino acid protein inhibitor of plasma kallikrein identified using an engineered phage display technology. A series of in vitro and in vivo experiments were conducted to evaluate the ability of ecallantide to inhibit plasma kallikrein. Human plasma kallikrein is known as a serine protease that is active in the intrinsic coagulation, fibrinolysis, hypotension, pain and inflammation pathways (Bhoola et al. 1992). The following figure (excerpted from page 4 of Section 2.4) outlines the contact system, or kallikrein kinin system and the role of DX-88 in the pathway.

Figure 2.4.1. Ecallantide Activity in Pathways of Intrinsic Coagulation and Inflammation



In vitro pharmacology studies demonstrated that ecallantide was a potent human plasma kallikrein inhibitor with a Ki of 25 pM. Ecallantide showed nanomolar inhibition plasma kallikrein in human monkeys, pigs, rabbits, and dogs and micromolar inhibition in rats and mice. In two *in vivo* animal models, ecallantide administered by the IV route reduced in a dose-dependent manner, increases of vascular permeability in C1-INH deficiency mice, and alleviated the carrageenan-induced paw swelling in rats at a subcutaneous dose of 30 mg/kg, which was similar to the effect of a bradykinin receptor antagonist, tested in the same model as a reference.

C. Nonclinical safety issues relevant to clinical use: None

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

BLA number: 125277
Review number: 1
Sequence number/date/type of submission: 0/September 23, 2008/BLA
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Dyax Corp.

Manufacturer for drug substance:

Avecia Biologics (Avecia)
Advanced Biologics Centre 5000 L Facility (ABC5000)
PO Box 2, Belasis Avenue
Billingham, Cleveland TS23 1YN
United Kingdom

Reviewer name: Jean Q. Wu, MD, PhD
Division name: Division of Pulmonary and Allergy Products
HFD #:570
Review completion date: February 13, 2009

Drug:

Trade name: Kalbitor™ (proposed by the sponsor)
Generic name: Ecallantide
Code name: DX-88
Chemical name: Recombinant human plasma kallikrein inhibitor produced by expression in the yeast *Pichia Pastoris*
CAS registry number: 460738-38-9
Molecular formula/molecular weight: ~7054 Daltons
Structure: Ecallantide contains 60 amino acid residues and three intramolecular disulfide bonds as depicted in the figure below.

(b) (4)

Relevant INDs/NDAs/DMFs: IND 10426, (b) (4)

Drug class: Recombinant protein which reversibly binds human plasma kallikrein.

Intended clinical population: Patients with acute attacks of hereditary angioedema.

Clinical formulation: The drug product is a sterile, preservative-free isotonic solution with an ecallantide concentration of 10 mg/mL provided in a 2 mL glass vial. Each vial is filled with 1 mL. The manufacture of drug product involves (b) (4) of the drug substance and filling into vials. There is no (b) (4) drug product manufacture and no (b) (4). The composition of the ecallantide drug product per mL is shown in the Table below (excerpted from Section 2.3.P.1.)

Table 2.3.P.1.1. Ecallantide Drug Product Composition

Component	Quantity per mL	Quality Standard	Function
Ecallantide Drug Substance	**10 mg**	**Dyax Release Specifications**	**Active Substance**
Ecallantide Drug Substance Buffer			(b) (4)
$Na_2HPO_4 \cdot 2H_2O$	0.76 mg[a]	Ph. Eur., BP, USP	
KH_2PO_4	0.2 mg[a]	Ph. Eur., BP, NF	
NaCl	8.0 mg[a]	Ph. Eur., BP, JP, USP	
KCl	0.2 mg[a]	Ph. Eur., BP, USP	
(b) (4)	(b) (4)	Ph. Eur., BP	
WFI[b]	qs to 1.0 mL	Ph. Eur., USP	

[a] Quantities are theoretical and based on targets established for drug substance manufacturing.
[b] Water for Injection.

Route of administration: Subcutaneous

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

Studies reviewed within this submission:

Primary Pharmacodynamics
Study 650-00042, Measurements of DX-88 Specificity
Study (b) (4)11268, In Vitro Pharmacology: Comprehensive Pharmacology Profile
Study (b) (4)04-0847: DX-88: Inhibition of COX-1 and COX-2 Enzymes-Interaction with Endothelin Receptors A and B
Study 640-00028, Analysis of the Effect of DX88 on Vascular Permeability in C1 Inhibitor Deficient Mice
Study 06GSTR068 (FFA00021), Pharmacokinetics and Pharmacodynamics of DX-88 in the Carrageenan Model of Rat Paw Edema

Secondary Pharmacodynamics
Study 640-00078: DX-88 Specificity and Effects on Coagulation Factor Assays
Study 640-00079: DX-88 Effects on Fibrinolysis and Protein C

Safety Pharmacology

Study (b)-446019: The Acute Central Nervous System Pharmacological Profile of Lyophilized DX-88 Following Subcutaneous Administration in Rats
Study (b)-446014: Plethysmograph-Restrained Respiratory Assessment of Lyophilized DX-88 Subcutaneously Administered To Sprague Dawley Rats
Study (b)00010: A Safety Pharmacology Study to Assess Potential Cardiovascular Effects of DX-88 Administered Subcutaneously to Cynomolgus Monkeys
Study (b) 3131, DX-88: A Hemodynamic and Cardiac Function in Conscious Cynomolgus Monkeys Following Intravenous Administration
Study 050223 (b): Effects of DX-88 on Cloned hERG Channels Expressed in Mammalian Cells (GLP)

ADME
Study (b) 1571: A Single Dose Intravenous Pharmacokinetic Study in Sprague-Dawley Albino Rats (non-GLP)
Study (b) 3127: DX-88: A Single Dose Intravenous Pharmacokinetic Study in Cynomolgus Monkeys (non-GLP)
Study (b)00019: Subcutaneous Single Dose Toxicokinetic Study of DX-88 in Male Rats
Study 05-0218PDX: Pharmacokinetics of DX-88 in Cannulated Female Sprague-Dawley Rats following a Single, Subcutaneous Injection
Study GENZ 04-0570P: Biodistribution and Pharmacokinetics of Two Different Lots of ^{125}I-DX-88 Made by Two Manufacturing Processes in Female Cannulated Sprague-Dawley Rats Following a Single Intravenous Injections

Toxicology

Single-Dose Toxicity Studies

(b) (4) 1572: DX-88: A SINGLE DOSE INTRAVENOUS TOXICITY STUDY IN SPRAGUE-DAWLEY ALBINO RATS (GLP)

Study (b) (4) 446006, A Single-Dose Intravenous Toxicity Study in Rats Comparing DX-88 Manufactured by the Original and New Processes

Repeated-Dose Toxicity Studies

1. Study (b) (4) 446020: A 6-Month Toxicity Study of DX-88 Administered Subcutaneously Once Every Three Days to Cynomolgus Monkeys With a 28-Day Recovery Period
2. Study (b) (4) 446010: A 90-Day Toxicity Study of DX-88 Administered Subcutaneously Once Every Other Day to Cynomolgus Monkeys
3. Study (b) (4) 446021: A 6-Month Toxicity Study of DX-88 Administered Subcutaneously Once Every Three Days to Sprague-Dawley Rats With a 28-Day Recovery Period
4. Study (b) (4) 3130: DX-88: 14-day Intravenous Toxicity Study in Cynomolgus Monkeys
5. Study (b) (4) 1574: DX-88: 14-day Intravenous Injection Toxicity Study in Sprague-Dawley Rats
6. Study (b) (4) 446004: A 14-day Intravenous Toxicity Study of DX-88 in Rats

Reproductive and Development Toxicity

1. Study (b) (4) 2204-003: Fertility and General Reproduction Toxicity Study of DX-88 Administered Subcutaneously in Rats
2. Study (b) (4) L 2204-001: Intravenous Development Toxicity Study of DX-88 in Pregnant Rats

Study (b) (4) 2204-001P: Intravenous Dosage-Range Development Toxicity Study of DX-88 in Rats

3. Study (b) (4) 00012: Subcutaneous Developmental Toxicity Study of DX-88 in Rats
4. Study (b) (4) 2204-002, Intravenous Development Toxicity Study of DX-88 in Rabbits.

Study (b) (4) 2204-002P, Intravenous Dosage-Range Development Toxicity Study of DX-88 in Rabbits

(b) (4) 00008: Intravenous Development Toxicity Study of DX-88 in Rabbits (supplement to Study (b) (4) 2204-002 with additional dose group of 2 mg/kg).

5. Study (b) (4) 00031, Subcutaneous Dosage-Range Developmental Toxicity Study of DX-88 in Rabbits
6. Study (b) (4) 00006: Developmental and Perinatal/Postnatal Reproduction Toxicity Study of DX-88 Administered Subcutaneously in Rats, Including A Postnatal Behavioral/Functional Evaluation

Local ToleranceStudy (b) (4)-446008: A Local Tolerance Study of Subcutaneously Administered DX-88 *(liquid)* in Rats
Study (b) (4)-446013 A Local Tolerance Assessment of Lyophilized DX-88 Subcutaneously Administered in Rats

Others
Note: The following studies were only given cursory reviews as they were not pivotal, not the relevant route of administration, non-GLP or for mechanism exploration purpose only, which were not considered to affect the safety evaluation.

Study 050224 (b) (4): Effects of DX-88 on Action Potentials in Isolated Canine Cardiac Purkinje Fibers (GLP)
Study (b) (4) Test 051220 (b) (4): Effect of DX-88 on Inward Sodium Current (INa) and Transient Outward Current (Ito) in Isolated Ventricular Myocytes From Male and Female Adult Rats (non-GLP)
Study 06GSTR077: The Effect of DX-88 in the Isolated Perfused Female Rat Heart Preparation (non-GLP)
Study (b) (4) FFA00026: A Study To Assess The Cardiovascular Effects Of DX-88 In Telemetered Female Sprague-Dawley Rats (non-GLP)
Study (b) (4) 00001: A Study To Assess The Cardiovascular Effects Of DX-88 Following Intravenous Administration in Telemetered Sprague-Dawley Rats (non-GLP)
Study GENZ 05-0103PDX: Acute Toxicity of a Single Administration of DX-88 in Normal, Ovariectomized, and Castrated Sprague-Dawley Rats (non-GLP)

Studies not reviewed within this submission:

(b) (4)

[redacted]

(b) (4)

[redacted]

PHARMACOLOGY

2.6.2.1 Brief summary

Human plasma kallikrein is known as a serine protease that is active in the intrinsic coagulation, fibrinolysis, hypotension, pain and inflammation pathways (Bhoola *et al.* 1992). Ecallantide is a 60 amino acid recombinant protein inhibitor of plasma kallikrein identified using an engineered phage display technology. A series of in vitro and in vivo experiments were conducted to evaluate the ability of ecallantide to inhibit plasma kallikrein and thus as a potential treatment for hereditary angioedema (HAE).

In vitro pharmacology studies demonstrated that ecallantide was a potent plasma kallikrein inhibitor with a Ki of 25 pM for the human form and a Ki of 17 nM for the rat form. Ecallantide inhibited plasma kallikrein in human and other species tested using activated endogenous plasma kallikrein. Nanomolar inhibition was demonstrated in human monkeys, pigs, rabbits, and dogs and micromolar inhibition in rats and mice. Ecallantide produced weak to moderate inhibition for 5 out of 18 different human proteases tested (i.e. neutrophil elastase, tissue kallikrein 2, pancreatic trypsin, plasmin and factor XIa) with Ki ranging from 1.7 nM to 0.75 μM. At a level of 5.7 μM ecallantide, there were no binding to over 100 receptors screened and significant inhibition on 2 out of 34 enzymes tested, i.e. Lyn-kinase and elastase. Ecallantide did not interact with the endothelin$_A$ and endothelin$_B$ receptors and did not inhibit COX-1 or COX-2 enzyme activity.

In two in vivo pharmacology studies, ecallantide administered by the IV route reduced increases of vascular permeability in C1-INH deficiency mice in a dose-dependent manner, and alleviated the carrageenan-induced paw swelling in rats at a subcutaneous dose of 30 mg/kg. The effect of ecallantide on the paw edema was similar to the effect of a bradykinin receptor antagonist that was assessed in the same test as a positive control.

Safety pharmacology core battery studies were conducted for respiratory, CNS and cardiovascular evaluations. Ecallantide, at single subcutaneous doses up to 25 mg/kg, did not show adverse effects in an acute CNS evaluation in rats (functional observational battery performance and local motor activity) and in a respiratory assessment in rats (respiratory frequency, tidal volume or minute volume). No test article-related adverse findings in a cardiovascular evaluation were observed in a monkey telemetry study with subcutaneous doses up to 25 mg/kg or in monkey study at an intravenous dose of 23 mg/kg. Ecallantide showed no significant inhibition on hERG channel at a level of 10 μM.

2.6.2.2 Primary pharmacodynamics

Mechanism of Action:

Study 650-00042, Measurements of DX-88 Specificity

Plasma kallikrein activity can be measured using purified enzyme or directly in plasma samples following the addition of a commercially available mixture (Chromogenix, Milan, Italy) composed of fXII, phospholipids, ellagic acid, and HMWK (high molecular weight

kininogen). The addition of this mixture to plasma specifically converts prekallikrein to active kallikrein, which is measured using the fluorogenic synthetic substrate Pro-Phe-Arg-aminomethylcoumarin (Sigma-Aldrich, St. Louis, MO). Using either purified plasma kallikrein proteins or activated plasma samples, the effect of DX-88 on plasma kallikrein activity was determined and enzyme inhibition constants (K_i or IC_{50} values) were reported. The specificity of DX-88 towards human plasma kallikrein versus 20 other selected human or nonhuman serine proteases was measured by comparing inhibition constants. As shown in Table 2 (excerpted from page 9 of the report), using purified enzymes, DX-88 inhibited both human and rat plasma kallikreins with high potency (Ki = 25 pM for human and Ki=17 nM for rats) while it inhibited human factor XIa (K_i = 1.7 nM), human plasmin (K_i = 29 nM), human trypsin (K_i = 69 nM), human tissue kallikrein 2 (K_i = 0.29 μM), and human neutrophil elastase (K_i = 0.75 μM) with potencies ranging from approximately 68-fold to 30,000-fold less than that of human plasma kallikrein. DX-88 did not inhibit other selected proteins tested (listed in Table 2 below). As shown in Table 4 (excerpted from page 11), using activated plasma cross the species, DX-88 inhibited plasma kallikreins from human and nonhuman species including Cynomolgus monkeys, pigs, dogs, rabbits, rats, and mice.

Table 4. Inhibition of Activated Kallikrein in Plasma by DX-88

Species	IC_{50}
Human	1.4 ± 0.6 nM
Monkey (cynomolgus)	1.4 ± 0.5 nM
Pig	2.7 ± 1 nM
Dog	12 ± 3 nM
Rabbit	2.5 ± 0.5 nM
Rat	0.52 ± 0.1 μM
Mouse	0.81 ± 0.02 μM

IC_{50} = amount of the inhibitor required to inhibit enzyme activity by 50%

Table 2. Summary of DX-88 Inhibition of Purified Human and Nonhuman Proteases

Protease	DX-88 K_i[a]	Fold Reduction in Potency (relative to plasma kallikrein)
Human plasma kallikrein	25 ± 4 pM	na
Rat plasma kallikrein	17 ± 3 nM	680-fold
Mouse plasma kallikrein	28 ± 3 nM	1120-fold
Human factor XIa	1.7 ± 0.2 nM	68-fold
Human plasmin	29 ± 1.2 nM	1160-fold
Human trypsin	69 ± 7 nM	2760-fold
Human tissue kallikrein 2	0.29 ± 0.05 μM	11 600-fold
Human neutrophil elastase	0.75 ± 0.01 μM	30 000-fold
Human tissue kallikrein 1	>100 μM	>4 000 000-fold
Rat tissue kallikrein 1	>10 μM	>400 000-fold
Mouse tissue kallikrein 1	>10 μM	>400 000-fold
Rabbit tissue kallikrein 1	>10 μM	>400 000-fold
Human C1 esterase	>100 μM	>4 000 000-fold
Human thrombin	>10 μM	>400 000-fold
Human factor XIIa	>10 μM	>400 000-fold
Human tissue factor/factor VIIa/factor Xa	>10 μM	>400 000-fold
Human tissue plasminogen activator (tPA)	>10 μM	>400 000-fold
Human urokinase-type plasminogen activator (uPA)	>10 μM	>400 000-fold
Human activated protein C	>10 μM	>400 000-fold
Human tryptase	>1 mM	>40 000 000-fold
Human ACE1	>8 μM	>320 000-fold
Human ACE2	>8 μM	>320 000-fold
Human chymotrypsin	>10 μM	>400 000-fold

[a] The error in all measurements, except the error associated with the K_i for human plasma kallikrein, corresponds to the standard error of the estimate associated with the fit of the data to the appropriate inhibition equation (Equation 2 or Equation 3). The error in the K_i for the human plasma kallikrein is the standard deviation from repeated determinations as shown in Appendix 2.

Study (b) (4) 11268, In Vitro Pharmacology: Comprehensive Pharmacology Profile

Effect of DX-88 (Lot 227-05-001, at 40 μg/mL or 5.7 μM) on various in vitro receptor bindings and enzyme activities was tested. Binding with each of 107 different receptors across species (human, rat, bovine, hamster, mouse or guinea pig origin) was tested with a labeled ligand specific for that receptor. The each activity of 34 different enzymes was determined with assay specific substrates. A specific reference compound was tested in each binding or enzyme assay

concurrently with DX-88. The results showed that DX-88 had no significant effect in any of 107 receptor binding assays. DX-88 significantly (>50%) inhibited elastase activity and moderately (20% -50%) inhibited Lyn Kinase activity, and had no effect on other tested enzymes.

Study (b) (4) 04-0847: DX-88: Inhibition of COX-1 and COX-2 Enzymes-Interaction with Endothelin Receptors A and B

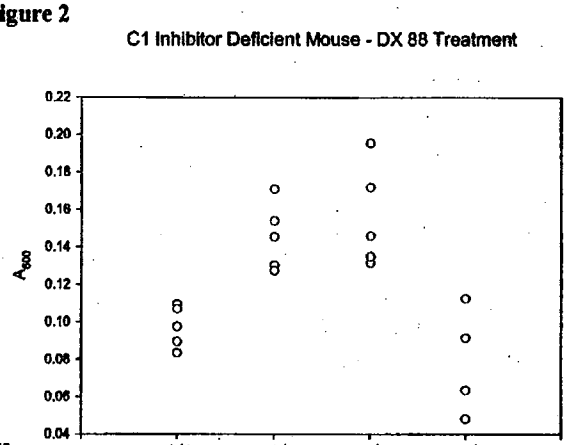
Effect of DX-88 on in vitro binding to endothelin$_A$ or endothelin$_B$ (ET-A, derived from human neuroblastoma cells) or endothelin$_B$ (ET-B, derived from human astrocytoma cells), and on enzyme activity of COX-1 (derived from rat seminal vesicle) or COX-2 (derived from bovine placenta) was tested at DX-88 (Lot 227-104-001) concentrations of 0.1, 1.0 and 10 μM. The corresponding reference compound was used in each assay. The highest concentration of DX-88 tested inhibited ET-A and ET-B receptor bindings by 5.0% and 15.7%, respectively, and inhibited enzyme activities of COX-1 and COX-2 by 3.9% and 14.0%, respectively. These levels of inhibition were considered to be within the baseline range of activity for these assays. Therefore, the results indicated that DX-88 did not interact with the ET-A or ET-B receptors and did not inhibit COX-1 or COX-2 enzyme activity.

Drug activity related to proposed indication:

Study 640-00028, Analysis of the Effect of DX88 on Vascular Permeability in C1 Inhibitor Deficient Mice

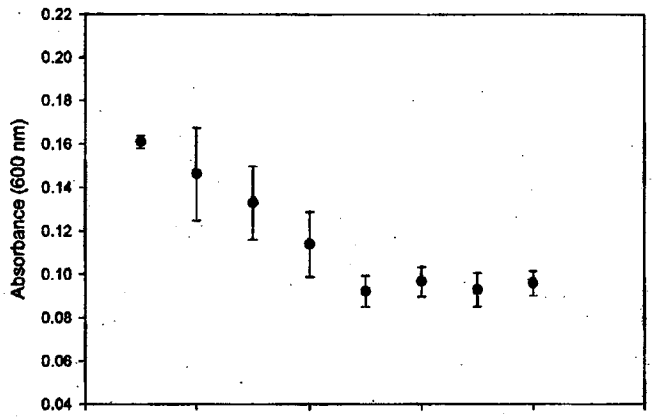
Effect of DX-88 on vascular permeability in C1-INH-/- mice was tested in three experiments. The vascular permeability was determined by vascular extravasation of Evans blue dye (30 mg/kg) which was iv. injected into the mice immediately after test article injection. In experiment 1, the vascular permeability was tested in n=5 wild type (C1-INH+/+) mice, n=5 heterozygous (C1-INH+/-) and n=5 homozygous C1-INH deficient mice, and n=4 C1-INH-/- mice receiving a single iv injection of 10 μg DX-88. As shown in Figure 2 below (excerpted from page 4), C1-INH deficient mice revealed markedly increased vascular permeability within a shorter period of time and more intense blue coloration when compared to the wild-type mice which developed blue coloration around the eyes, nose, and feet over a period of 15-30 minutes after dye injection. Intravenous injection of 10 μg DX-88 completely reversed the increased vascular permeability observed in C1-INH-/- mice. In experiment 2, a dose-dependent effect of DX-88 on vascular permeability in C1-INH-/- mice was tested when wild-type mice (n=16) received 0 DX-88, and C1-INH-/- mice received 0 (n=4), 0.001(n=6), 0.01(n=6), 0.1 (n=8), 1 (n=6) and 10 μg DX-88 (n=6). As shown in Figure 3 below (excerpted from page 5), i.v. injection of 0.001-10 μg DX-88 resulted in a dose-dependent reduction of vascular permeability in C1-INH-/- mice that was significant at doses - ≥ 0.1 μg and was equivalent to the wild-type genotype response at doses ≥ 1.0 μg. In experiment 3, increased vascular permeability in C1-INH-/- mice was determined prior to, and 2, 4, 6 and 8 hours post i.v. injection of 10 μg DX-88. As shown in Figure 4 below (excerpted from page 6), the duration of the reversal of increased permeability was maximal to 2 hours post dosing, then the increased permeability was back to pre-dosing level at 8 hours post injection of DX-88.

Figure 2



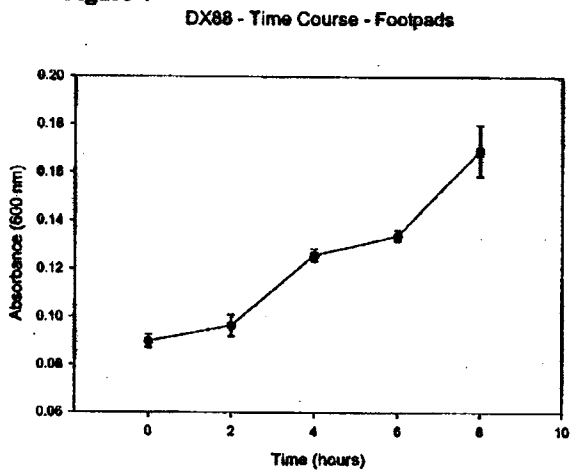
C1INH genotype	+/+	+/-	-/-	-/-
Treatment	0	0	0	DX88
Mean	0.10	0.15	0.16	0.08
S.E.	0.005	0.008	0.012	0.014

Figure 3



Genotype	-/-	-/-	-/-	-/-	-/-	-/-	-/-	+/+
DX88 (μg)	0	0.001	0.01	0.1	1	10	100	0

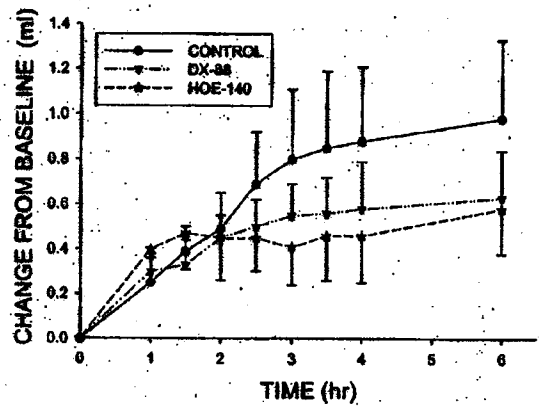
Figure 4



Study 06GSTR068 (FFA00021), Pharmacokinetics and Pharmacodynamics of DX-88 in the Carrageenan Model of Rat Paw Edema

Male rats (N=10/group) were dosed with a single s.c. injection of 0 (vehicle, 0.9% sodium chloride) or 30 mg/kg DX-88 (Lot 227-01-004), or a single iv. injection of 0.5 mg/kg of HOE-140, a bradykinin receptor antagonist. Paw edema was induced by a single subplantar injection of 0.1 mL of a 1% carrageenan suspension in the right hind footpad 1 hour prior to test article administration. The effect of treatment on rat paw edema was measured by plethysmometer at 0.5 hour prior to carrageenan injection for a baseline value, and at 1, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 6 hours post carrageenan injection. DX-88 and HOE-140 treatments resulted in reduced paw edema relative to vehicle, as shown in the figure below (Figure 1, excerpted from page 8 of the report). The observed inhibition is sustained for at least 5 hours following test article administration. The level of circulating bradykinin was measured but could not be determined reliably as the level was undetectable in 8 out of 10 animals in one group.

Figure 1. DX-88 and HOE-140 reduce carrageenan-induced paw edema in rats. Carrageenan (0.1ml of 1.0% solution) was administered via sub-plantar injection at time = 0 hours into the right hind foot pad. Vehicle (control), DX-88 (30 mg/kg, sc), or HOE-140 (0.5 mg/kg, iv) were administrations at time = 0. Data represent mean ± SD.



2.6.2.3 Secondary pharmacodynamics

Study 640-00078: DX-88 Specificity and Effects on Coagulation Factor Assays

The effect of DX-88 on coagulation factors and their assay was assessed by the in vitro mixing of buffer or DX-88 with citrated plasma from healthy normal subjects. In some experiments, aprotinin was used as a reference material. The results indicated:

- 1-stage extrinsic factor clotting assays (thromboplastin based) and FX assay (RVV, Russell's viper venom, based) were not affected. This shows that there was no inhibition of factor V, VII or X activity, or interference in the assay systems used.
- 1-stage intrinsic factor clotting assays (FVIII, FIX, FXI, FXII) were inhibited by DX-88. The effect was significant at 5 µg/mL DX-88 and higher concentrations and caused increasing non-parallelism as the DX-88 concentration increased. At low doses of DX-88 (5 µg/mL and less), these inhibitory effects should be overcome by using higher plasma dilutions in the assays. This would not cause a problem provided that the patient did not have haemophilia. 18 µg/mL Aprotinin (125 KIU/mL) gave similar inhibitory effects to 5 µg/mL DX-88.
- 2-stage FVIII amidolytic assays (based on activation of factor X with thrombin, FIXa, phospholipid and calcium ions, and measurement of FXa with a chromogenic substrate), showed no inhibition by DX-88. This shows that the inhibition of 1-stage clotting assays for FVIII was due to interference with the activation system, since kallikrein generation is required in the APTT system used in 1 -stage intrinsic system factor assays, and not due to true inhibition of FVIII activity. Since the degree of inhibition seen in 1-stage factor IX, XI and XI1 assays was similar to that in FVIII assays, it is unlikely that DX-88 has any direct inhibitory effect on these proteins.
- Amidolytic substrate assays for FXI showed an apparent reduction in FXI activity when (b) (4) was added to pooled normal plasma, but not at (b) (4) doses. The effect was abolished if two-fold higher dilutions of the plasma were used in the assay. Aprotinin had a weaker effect, with some reduction in FXI at a concentration of 40 µg/mL. This suggests that DX-88 was inhibiting kallikrein activity, which is required during FXI activation in the assay.
- Amidolytic substrate assays for FXII showed a significant reduction in activity at 5-10 µg/mL DX-88. The effect was abolished when two-fold higher dilutions of plasma were prepared in the assay. Aprotinin had a weaker effect at equivalent doses (a 12.5% reduction in FXII activity was seen at 40 µg/mL aprotinin). Kallikrein generation is required during the activation stage of the FXII assay, and it is likely that this is inhibited by DX-88.
- Amidolytic substrate assays for prekallikrein showed a dose dependant inhibition by DX-88, which was statistically significant at 1 µg/mL DX-88.This effect was not removed by preparing two-fold higher dilutions of plasma for assay. Aprotinin had no effect. These results are compatible with DX-88 being a direct inhibitor of plasma kallikrein, with greater potency than aprotinin.
- In amidolytic substrate assays for HK (high molecular weight kininogen), DX-88 caused a dose dependant inhibition at 5 µg/mL. This is likely to be due to interference with kallikrein in the assay activation stage, thereby decreasing factor Xa generation.

Study 640-00079: DX-88 Effects on Fibrinolysis and Protein C

The effect of DX-88 on plasmin and global screening tests for fibrinolysis was assessed using normal human plasma. Aprotinin was used as a reference for comparison. The results indicated the following:

- DX-88 had no effect on the commonly used screening tests for fibrinolytic defects (euglobulin clot lysis time and fibrin plate lysis), whereas aprotinin caused prolonged lysis times and abolished dextran sulphate stimulated fibrin plate lysis.
- DX-88 caused dose dependant inhibition of human plasmin, but plasmin activity was not completely abolished at the proposed therapeutic concentrations (20-50% inhibition at 1-5μg/mL). The degree of plasmin inhibition was approximately 3 fold less than that seen with aprotinin on a protein concentration basis.
- DX-88 appeared to have a weak inhibitory effect (observed using 100 μg/mL DX-88) on plasmin generation stimulated by dextran sulphate, in the presence of a fibrin clot. The pattern was disrupted when FXII deficient plasma was used. Full examination of these results would require an extensive study with the use of a variety of different plasmas, which does not seem to be justifiable, as there was no evidence of any potential clinical problem with therapeutic concentrations of DX-88.
- DX-88 showed no inhibition of t-PA stimulated plasma clot lysis in a microtitre plate method, whereas Aprotinin caused dose dependant inhibition of clot lysis at 5 μg/mL and higher concentrations, with approximately 50% inhibition of clot lysis at 20 μg/mL.
- DX-88 had no inhibitory effect on protein C in normal plasma, or on recombinant human activated protein C in solution. Therapeutic concentrations of Aprotinin showed dose dependant inhibition of both protein C (after activation by Protac) and activated protein C.

2.6.2.4 Safety pharmacology

CNS

Study (b)(4)-446019: The Acute Central Nervous System Pharmacological Profile of Lyophilized DX-88 Following Subcutaneous Administration in Rats

Ten rats/sex/group (Spraque Dawley) were subcutaneously administered with a single dose of vehicle (10 mM histidine, 10% sucrose, pH 6.5), and 5, 10 or 25 mg/kg DX-88 (lyophilized, Lot No. 05RP029, product purity: 99.6%). Functional observational battery (FOB) and locomotor activity data were recorded prior to initiation of dosing (study day -7) and approximately 60 minutes post-dosing.

All animals survived to the scheduled termination. There were no test article-related effects on FOB evaluations (the parameters involved home cage, handling, open field, sensorimotor, neuromuscular and physiological observations). There were no test article-related effects on mean ambulatory and total motor activity counts.

Respiratory

Study (b)(4)-446014: Plethysmograph-Restrained Respiratory Assessment of Lyophilized DX-88 Subcutaneously Administered To Sprague Dawley Rats

Eight rats/sex/group (Sprague Dawley) were subcutaneously administered a single dose of vehicle (10 mM histidine, 10% sucrose, pH 6.5), 5, 10 or 25 mg/kg DX-88 (lyophilized, Lot No. 05RP029, product purity: 99.6%). Clinical examinations were

performed daily and detailed physical examinations were performed on the day of dosing. Respiratory function was measured using a pneumotach to detect the air flow in and out of the head-out neck-sealed plethysmograph in response to the animal's thoracic movement. The pneumotach signals were detected by a variable reductance transducer, which was amplified and recorded by data acquisition software (PONEMAH Physiology Platform, version 4.1-SP1, LDS Test and Measurement). Respiratory data were collected continuously for at least 60 minutes prior to initiation of dosing (study day -1) and for at least 6 hours post-dosing but no more than 7 hours. Data were organized into 3 phases for analysis (analysis phase 1 consisted of hours 0-1, analysis phase 2 consisted hours 1-3 and analysis phase 3 consisted hours 3-6).
All animal survived to termination. There were no test article-related clinical observations, and there were no test article-related effects on measured respiratory parameters including respiratory frequency, tidal volume or minute volume.

Cardiovascular system

Study (b) (4) 00010: A Safety Pharmacology Study to Assess Potential Cardiovascular Effects of DX-88 Administered Subcutaneously to Cynomolgus Monkeys (GLP)

As study design listed below (excerpted from page 13 of the report), n=4 non-naïve female cynomolgus monkeys were administered with a single dose of vehicle, or 3 doses of DX-88 on the designated days. All animals instrumented with radiotelemetry devices were monitored via radiotelemetry (b) (4)). Data was collected 24 hours prior to each dosing for a baseline record and another 24 hours post each dosing for a treatment assessment. ECGs acquired by telemetry were sent to a board-certified cardiologist, for qualitative evaluation of abnormalities and for quantitative assessment of ECG intervals (PR, QRS, RR, QT, and QTc intervals). Blood samples were collected at selected time points for determination of DX-88 plasma concentration and the coagulation parameters prothrombin time (PT) and activated partial thromboplastin time (APTT).

Session Number[a]	Number of Females[b]	Test Article	Dosage Level (mg/kg)	Dosage Volume (mL/kg)	Concentration (mg/mL)	Route	Last Day on Test
1	4	Vehicle	0	2.5	0	SC	Day 16
2		DX-88	0.4	0.04	10		
3		DX-88	10	1			
4		DX-88	25	2.5			

SC = Subcutaneous.
[a] Doses were administered on Days 1, 4, 9, and 15.
[b] The same four animals were dosed at each dose session.

There were no test article–related clinical observations and there were no dose-dependent or biologically significant changes in heart rate (HR), blood pressure, core body temperature, or electrocardiograms evaluation. Mean values for the QT and QTc (Bazzett's HR correction) intervals ranged from 0.166 to 0.248 seconds and from 0.322 to 0.374 seconds over the four treatments. APTT was prolonged at 10 and 25 mg/kg as expected while no noteworthy changes were observed in PT. The C_{max} was 0.444, 7.645, or 25.739 µg/mL at the dose of 0.4, 10, or 25 mg/kg, respectively.

Study (b) 3131, DX-88: A Hemodynamic and Cardiac Function in Conscious Cynomolgus Monkeys Following Intravenous Administration (GLP)

Cynomolgus monkeys (N=1/sex/group) were administered with a single i.v. dose of 6.8 or 23 mg/kg DX-88 (in PBS, lot No. 00799DXP). The monkeys were surgically

implanted with arterial catheters for blood pressure assessment. Electrocardiograms (ECGs; bipolar limb leads I, II and III, and augmented unipolar leads aVR, aVL and aVF) were obtained for all conscious monkeys once during the pretreatment period, on the day of treatment at 0, 15 and 30 min. pre-dose (baseline period), and at 15, 30, 45 and 60 min. post-dose. Heart rate (lead II), sinus rhythm, atrioventricular rhythm, P-Q, QRS and Q-T intervals were evaluated. ECG tracings were sent to a veterinarian cardiologist for assessment of abnormalities. The following cardiac profile parameters were also collected at the same time points as for ECG tracings: cardiac output (CO), central venous pressure (CVP), diastolic arterial blood pressure (DABP), diastolic pulmonary arterial pressure (DPAP), heart rate (HR), mean arterial blood pressure (MABP), mean pulmonary arterial pressure (MPAP), pulmonary arterial wedge pressure (PAWP), pulmonary vascular resistance (PVR), respiration rate (RR), right and left cardiac work (RCW, LCW), right and left ventricular stroke work (RVSW, LVSW), stroke volume (SV), systemic vascular resistance (SVR), systolic arterial blood pressure (SABP), and systolic pulmonary arterial pressure (SPAP). All ECG tracings and cardiac profile data were evaluated by a veterinary cardiologist.
There was no test article-related mortality or adverse clinical signs observed. There were no test article-related changes in the morphology, intervals and amplitudes of the P-QRS-T complexes, cardiac rhythm or heart rate, or on the cardiac profile parameters evaluated in the study. (*Note: the results were based on the limit number of animals tested n=1/sex/group.*)

Study 050223EZD: Effects of DX-88 on Cloned hERG Channels Expressed in Mammalian Cells

The study (GLP) was conducted by (b) (4) The effect of DX-88 on hERG (human ether-a-go-go-related gene) channel current (I_{kr}, the rapidly activating, delayed rectifier cardiac potassium current), expressed in mammalian cells (HEK 293), was evaluated at 10 μM DX-88. Four cells were exposed to this concentration (n=4) at physiological temperature. The 0.6 % inhibition observed with 10 μM DX-88 was not significantly different from the inhibition (0.2%) seen with the vehicle control tested on three cells. Under identical conditions, the positive control terfenadine at 60 nM when tested on two cells produced a 74.5% inhibition of the hERG currents at 60 nM when tested on two cells (n=2). These results indicated that DX-88 at 10 μM did not significantly inhibit the hERG current. As the inhibition was less than 50% at 10 μM DX-88, the study was considered complete and the IC_{50} was not determined. Analysis of samples taken from the test formulations suggested that the solutions tested were stable for the duration of the study and homogenous. However, the test concentration used in this study was below the limit of quantitation of the validated method and the results of verification of concentration, stability and homogeneity were provided as estimates.

2.6.2.5 Pharmacodynamic drug interactions

N/A

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Table 2.4.1. Summary of Ecallantide-Mediated Protease Inhibition

Protease	Ecallantide K_i
Human Plasma Kallikrein	25 ± 4 pM
Human Factor XIa	1.7 ± 0.2 nM
Human Plasmin	29 ± 1.2 nM
Human Trypsin	69 ± 7 nM
Human Tissue Kallikrein 2	0.29 ± 0.05 μM
Human Neutrophil Elastase	0.75 ± 0.01 μM

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (mg/kg)	Gender and n per Group	Noteworthy Findings	GLP*	Study Number
Respiratory	Rat, Crl:CD(SD)	Subcutaneous	0, 5, 10, 25	8 M and 8 F	No relevant differences in respiratory frequency, tidal volume or minute volume. NOEL 25 mg/kg	Yes	(b) L-446014
CNS	Rat, Crl:CD(SD)	Subcutaneous	0, 5, 10, 25	10 M and 10 F	No effect on functional observational battery, total motor activity, or ambulatory motor activity.	Yes	(b) -446019
Cardiovascular	Monkey, cynomolgus	Subcutaneous	0, 0.4, 10, 25	4F	No ECG abnormalities and no QT/QTc prolongation. No effect on hemodynamic parameters or core body temperature. Dose dependent, prolonged APTT to 3-fold vehicle. Dose dependent C_{max} of 0.4, 7.7, 25.7 μg/ml.	Yes	(b) (4) 100010
Cardiovascular	Monkey, cynomolgus	Intravenous	6.8, 23	1 M and 1F	No ecallantide related changes in ECG measurements or cardiac profile parameters.	Yes	(b) 3131

* An entry of "Yes" indicates that the study includes a GLP compliance statement.

(table was excerpted from page 4 of section 2.6.3)

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Pharmacokinetics of ecallantide was evaluated following single and repeated-dose subcutaneous administration in rats and monkeys, and single and repeated-dose intravenous administration in rats, monkeys and rabbits. Additional single dose s.c studies were conducted in minipigs and rabbits. PK parameters in the repeated-dose toxicity studies, reproductive toxicology studies and local tolerance studies were reviewed in the individual studies in Section 2.6.6. Following single i.v. or s.c. dose, systemic exposure (C_{max} and AUC_{inf}) increased with increasing dose with no apparent gender differences across species. T_{max} were 40-60 minutes post s.c. dose in rats and rabbits, and 2-3 hours post s.c. dose in minipigs. The terminal half-life ranged from 143-158 minutes following a single sc dose (rats, rabbits and mini-pigs) and 19-99 minutes following a single i.v. dose (rats, monkeys, rabbits and mini-pigs). Due to the relatively short half-life, the repeat-dose PK profile was consistent with the single dose profile. Volume of distribution values in all species indicated ecallantide could distribute beyond the blood volume into the tissues. Generally, the exposure increased with increasing dose in both monkeys and rats with no consistent gender differences in disposition observed. The exposure appeared to be greater in the cynomolgus monkeys than in the rat. A biodistribution study showed that radiolabled iodine was detected in all tissues with highest radioactivity in kidneys at 30 minutes following a single i.v. dose of ecallantide, but majority of radioactivity was detected in the thyroid, a tissue which is known to accumulate free iodine, at 6 hour time point.

2.6.4.2 Methods of Analysis

During the drug development, Ecallantide exposure was measured using 3 different assay methods of analysis including radiochemical detection of ^{125}I-DX-88, high performance liquid chromatography with mass spectrometric detection (HPLC/MS), and enzyme-linked immunosorbent assay (ELISA). The table below (excerpted from page 7 of Application section 2.6.4) described the assays formats and analytical labs used in the studies. Except for the radiochemical method, other assay methods were validated. The validation reports were not critically reviewed separately but were checked when the correspondent PK or toxicity studies were reviewed.

Table 2.6.4.1. Assays for the Measurement of Ecallantide Concentration

Laboratory	Method of Analysis	Matrix	Species	LLOQ	Validation Report	Studies
Genzyme (Framingham, MA)	Radiochemical	Serum	Rat	(b) (4)	n/a	GNZ 04-0570P
(b) (4)	HPLC/MS	Citrated plasma	Rat		AA16809-UXZ	(b) 446004 2204-001P
(b) (4)	HPLC/MS	Serum Citrated plasma Urine	Rat Monkey		DX005	(b) (4) 1574 3130
Genzyme (Framingham, MA)	ELISA	Citrated plasma	Rat		GENZ-06GSTR062	(b) 00019 GENZ 05-0218PDX
		Citrated plasma EDTA plasma	Monkey Monkey		GENZ-05GSTR072 GENZ-05GSTR033	(b) (4) 00012 (b) (4) 46010 0010
(b) (4)	ELISA	Citrated plasma	Rabbit		(b) -0225	2204-002P
		Citrated plasma	Minipig		(b) (4) 0204	(b) 00031 (b) 446005
	ELISA	Citrated plasma Citrated plasma	Rat Monkey		7928-106 7928-107	(b) 446012 (b) 446021 (4) 446020

2.6.4.3 Absorption

Rats

Study (b) (4) 1571: A Single Dose Intravenous Pharmacokinetic Study in Sprague-Dawley Albino Rats

This is a non-GLP study conducted by (b) (4). Eight male and female rats were intravenously administered with a single dose of 0.6 mg/kg DX-88 (Lot No. 00799DXP, liquid form, DX-88 in PBS, purity, 97%). One rat per sex (one subgroup) was sampled at 0 hour (pretreatment) and at each of 0.25, 0.5, 1, 1.5, 6, 12 and 24 hour time points following the administration of DX-88. All the serum samples were analyzed by LC-MS a (b) (4) (I (b) Method DX005 Version 1). Non-compartmental analysis was conducted using WinNoLin software (version 1.1). The PK parameters were summarized in the table below. In this particular study, the half-life was prolonged in female rats as compared to male rats. The volume distribution values suggest distribution beyond the blood volume into the tissues.

Table 2. Pharmacokinetic parameters for rats receiving 0.6 mg/kg injection

Variable	Male Rats	Female Rats	All Rats Mean ± SD (%CV)
Cmax (µg/mL)	0.80	0.90	0.85 ± 0.05 (5.9)
Tmax (min)	15.0	15.0	15.0 ± 0.0 (0.0)
$t_{1/2}$ (min)	36.14	88.27	62.21 ± 26.07 (41.9)
$AUC_{(0\text{-}30\ min)}$ (µg.min/mL)	16.50	19.50	18.00 ± 1.50 (8.3)
Vss (observed, mL)	170.56	161.54	166.05 ± 4.51 (2.7)
MRT (min)	19.09	19.62	19.36 ± 0.27 (1.4)

Study (b) (4)00019: Subcutaneous Single Dose Toxicokinetic Study of DX-88 in Male Rats (non-GLP)

Method: Male Sprague-Dawley rats (n=6/group for TK assessment and n=18/group for coagulation evaluation) received a single s.c. dose of 0, 5, 10 or 20 mg/kg DX-88 (vehicle was phosphate buffered saline). The blood samples were collected from each rat at approximately 0.25, 0.5, 1, 2, 4, 8, 16, and 24 hours postdose. Serum concentrations of DX-88 were determined using an ELISA method with a low quantitation limit of 12.5 ng/ml (validation report 06GSTR062). The data was analyzed using a non-compartmental method (WinNonlin, version 5.0.1. Pharsight Corp., Mountain View, CA).

Results:
All rats survived until scheduled termination. Mean pharmacokinetic parameters are shown in the table below (excerpted from page 21 of the report). Mean $AUC_{0\text{-}inf}$ values appeared to increase in a dose-proportional manner while C_{max} values increased in a less dose proportional pattern. There were trends of prolongation of $T_{1/2}$ and reductions ofclearance with increasing dose. Volume of distribution values appeared to increase in a dose-dependent manner and suggested extensive distribution into tissues.

	Group II 5 mg/kg/day	Group III 10 mg/kg/day	Group IV 20 mg/kg/day
N	6	6	6
$T_{1/2}$ (min)	112.4 ± 23.99	146.0 ± 14.45	184.7 ± 50.16
Cl (mL/min/kg)	17.0 ± 2.31	17.6 ± 1.54	14.7 ± 2.28
Vd (mL/kg)	2814 ± 933	3713 ± 563	4008 ± 1490
C_{max} (mcg/mL)	1.69 ± 0.48	2.18 ± 0.37	3.61 ± 1.04
C_{max}/Dose (kg*mcg/mL/mg)	0.34 ± 0.10	0.22 ± 0.04	0.18 ± 0.05
T_{max} (min)	40.0 ± 15.5	50.0 ± 15.5	55.0 ± 35.1
AUC_{0-inf} (mcg*min/mL)	298 ± 38.8	573 ± 52.4	1383 ± 196
AUC_{0-inf}/Dose (mcg*min*kg/mL/mg)	59.6 ± 7.76	57.3 ± 5.24	69.2 ± 9.78

$T_{1/2}$ = elimination half-life
Cl = clearance
Vd = apparent volume of distribution
C_{max} = maximal test article concentration
T_{max} = time after dosing C_{max} observed
AUC_{0-inf} = area under concentration versus time curve extrapolated to infinity

APTT (activated partial thromboplastin times) were prolonged in the test article-treated groups at time points up to 4 hours post dose without a dose-dependent pattern and the values were comparable at 8 and 16 hours post dose among control and test article-treated groups.

Study 05-0218PDX: Pharmacokinetics of DX-88 in Cannulated Female Sprague-Dawley Rats following a Single, Subcutaneous Injection (non-GLP)

Method: Ten female Sprague-Dawley rats received a single s.c. dose of 30 mg/kg DX-88. The blood samples were collected from each rat at approximately 0.5, 1, 2, 4, 6, 8, and 24 hours postdose. Serum concentrations of DX-88 were determined using an ELISA method with a low quantitation limit of 12.5 ng/ml. The data was analyzed using a non-compartmental method (WinNonlin, version 5.0 Pharsight Corp., Mountain View, CA).

Results:
All rats survived until scheduled termination. Mean pharmacokinetic parameters are shown in the table below (excerpted from page 5 of the report). DX-88 concentrations were detected through 24 hours post dose. The volume of distribution value suggests extensive distribution into tissues.

PARAMETER	Mean ± SD
$t_{1/2}$ (hr)	2.77 ± 0.59
Cl (ml/hr/kg)	564 ± 66.5
Vd (L/kg)	2.27 ± 0.65
C_{max} (ng/ml)	6554 ± 1741
t_{max} (hr)	0.95 ± 0.72
AUC_{0-INF} (ng*hr/ml)	53789 ± 5884

Monkeys

Study (b) (4) 3127: DX-88: A Single Dose Intravenous Pharmacokinetic Study in Cynomolgus Monkeys (non-GLP)

This is a non-GLP study conducted by (b) (4). Two male and 2 female cynomolgus monkeys were intravenously administered with a single dose of 0.6 mg/kg DX-88 (Lot No. 00799DXP, liquid form, DX-88 in PBS, purity, 97%). Each monkey was sampled at 0 hour (pretreatment) and at each of 0.25, 0.5, 1, 1.5, 6, 12 and 24 hours following the administration of DX-88. All the serum samples were analyzed using a LC-MS method at (b) (4). (b) (4) Method DX005 Version 1). Non-compartmental analysis was conducted using WinNoLin software (version 1.1). The PK parameters were summarized in the table below. Volume of distribution values suggested distribution into tissues.

Variable	Male (1001A)	Male (1002A)	All Males Mean± SD (%CV)	Female (1501A)	Female (1502A)	All Females Mean± SD (%CV)	All Animals Mean± SD (%CV)
Cmax (µg/mL)	2.60	2.30	2.45 ± 0.15 (6.1)	3.40	3.90	3.65 ± 0.25 (6.9)	3.05 ± 0.63 (20.7)
Tmax (min)	15.0	15.0	15.0 ± 0.0 (0.0)	15.0	15.0	15.0 ± 0.0 (0.0)	15.0 ± 0.0 (0.0)
$t_{1/2}$ (min)	48.39	38.14	43.27 ± 5.13 (11.9)	30.99	31.54	31.27 ± 0.28 (0.9)	37.27 ± 7.01 (18.8)
$AUC_{(0\text{-}90\ min)}$ (µg.min/mL)	133.50	108.75	121.13 ± 12.38 (10.2)	143.25	118.50	130.88 ± 12.38 (9.5)	126.00 ± 13.30 (10.6)
CL (observed, mL/min)	12.22	12.70	12.46 ± 0.24 (1.9)	8.82	10.29	9.56 ± 0.74 (7.7)	11.01 ± 1.55 (14.1)
Vss (Observed, mL)	955.81	795.59	875.70 ± 80.11 (9.2)	454.58	538.88	496.73 ± 42.15 (8.5)	686.21 ± 200.00 (29.2)
Vss* (Observed, mL)	597.38	662.99	630.19 ± 32.81 (5.2)	454.58	538.88	496.73 ± 42.15 (8.5)	563.46 ± 76.67 (13.6)
MRT (min)	39.78	37.66	38.72 ± 1.06 (2.7)	35.97	33.23	34.60 ± 1.37 (4.0)	36.66 ± 2.40 (6.6)

Human

Human pharmacokinetic parameters following intravenous and subcutaneous doses of 30 mg (nominal) were shown in the table below (excerpted from page 7 of the amendment 1, study report titled "Pharmacokinetics of DX-88 in Volunteers after Intravenous and Subcutaneous Administration" from clinical protocol DX-88/13). The elimination half-life in humans was similar for both intravenous and subcutaneous routes of administration and was approximately 2 hours following a single dose. $AUC_{0\text{-}inf}$ of 3017 ng.hr/mL (equivalent to 181 µg.min/mL) has been used for safety factor calculation in this review. Since the 2nd dose within 24 hour following the initial dose was proposed in the recommended administration, the maximum recommended human dose (MRHD) would be 2 x 30 mg per day. The human exposure at MRHD would be 2 x $AUC_{0\text{-}inf}$ reported at a single s.c. dose of 30 mg (though it could be overestimated).

Table 1. Key Pharmacokinetic Parameters after Intravenous Administration of 30 mg DX-88.

Parameter	Route of Administration	
	Intravenous	Subcutaneous
$t_{1/2}$ (hr)	1.60 ± 0.62	2.00 ± 0.53
Cl (ml/min)	155 ± 30.8	168 ± 22.3
Vd (L)	20.6 ± 5.00	29.0 ± 8.55*
C_{max} (ng/ml)	3741 ± 460	586 ± 106*
t_{max} (hr)	0.17 ± 0.04	2.70 ± 0.65*
$AUC_{0\text{-}INF}$ (ng*hr/ml)	3327 ± 562	3017 ± 402

Values represent mean ± SD. $t_{1/2}$, elimination half-life; Cl, clearance; Vd, apparent volume of distribution; $AUC_{0\text{-}INF}$, area under concentration versus time curve extrapolated to infinity; C_{max}, maximal DX-88 concentration; T_{max}, time after dosing C_{max} observed.
*-indicates $p<0.05$ compared to intravenous administration.

Note: The toxicokinetic parameters in the related repeated-dose toxicity studies and reproductive toxicity studies were reviewed in toxicology section (see section 2.6.6 of this review). However, the parameters were also summarized in tables listed in the section 2.6.5 below.

2.6.4.4 Distribution

Study GENZ 04-0570P: Biodistribution and Pharmacokinetics of Two Different Lots of ^{125}I-DX-88 Made by Two Manufacturing Processes in Female Cannulated Sprague-Dawley Rats Following a Single Intravenous Injections (non-GLP)

Six female rats per group (4 groups for each lot of DX-88) received a single iv. injection of 23 mg/kg ^{125}I-DX-88. Animals were sacrificed at 0.5, 1, 3 and 6 hours post dose. Selected tissues including brain, liver, lung, heart, kidney, spleen, ovaries, stomach, small intestine, large intestine, and thyroid were collected. The radioactivity in the tissues was measured using a Cobra II Auto Gamma Counter. Both lots of DX-88 were from the same manufacturer. Lot 493034 was [redacted] (b) (4) and lot 1100184 was from the "current" process (b) [redacted] added to reduce the amount (b) (4) [redacted]. Blood samples were also collected at 10, 15, 30, 45 minutes, 1, 3, and 6 hours post dose. Non-compartmental PK analysis was performed using WinNonlin software.

The PK parameters are listed in the table below. In general, there were no significant differences in PK between two lots, except a higher Cmax for the "original" lot when compared to the "current" lot.

Pharmacokinetic Parameters of DX-88 Original Process and New Process Lots

Pharmacokinetic Parameter (± SD)	DX-88 New Lot (23 mg/kg, iv)	DX-88 Original Lot (23 mg/kg, iv)
C_{max} (μg/ml)	53.6 ± 14.3	70.9 ± 7.99*
T_{max}(hr)	0.22 ± 0.04	0.23 ± 0.04
$t_{1/2}$ (hr)	2.65 ± 1.56	2.67 ± 1.47
AUC (μg*hr/ml)	171 ± 83.6	208 ± 73.6
Vz (ml/kg)	569 ± 278	426 ±199
Cl (ml/hr/kg)	189 ± 142	122 ±39.4
Rsq	0.88 ± 0.13	0.79 ± 0.24

*- indicates $p<0.05$ compared to New Process DX-88 using Student's t test.

As shown in the table below, radioactivity was detected in all tissues evaluated through 6 hour time point. At the 30-minute time point, the highest level was observed in the kidney (approximately 200 ug/g tissue) and the lowest in the large intestine. Overall, biodistribution did not differ between the two lots. Slight differences observed in various tissues at specific time points were not consistent over the time points and tissues. At the 6-hour time point, the majority of the radioactivity was detected in the thyroid that is known to accumulate free iodine. The level of ^{125}I-DX-88 was significantly higher in thyroids of the animals treated with the "original" lot than the "current" lot. The mechanism underlying the difference in the thyroid is unknown.

Table 2.6.4.19. Mean (SD) Distribution Data Following the Single IV Administration of Ecallantide to Female Rats (GENZ 04-0570P)

	Lot 493034 (µg/g tissue) (b) (4)				Lot 1100184 (ng/g tissue) (b) (4)			
Organ	**30 min**	**60 min**	**180 min**	**360 min**	**30 min**	**60 min**	**180 min**	**360 min**
Brain	0.9 (0.24)	0.9 (0.68)	1.2 (0.38)	1.2 (0.50)	1.3 (0.46)	1.4 (0.35)	0.9 (0.43)	1.0 (0.52)
Liver	3.7 (1.15)	3.5 (0.97)	2.9 (0.30)	2.5 (0.44)	4.2 (1.64)	4.7 (1.18)	1.6 (0.74)	2.0 (1.89)
Lung	5.6 (1.89)	7.5 (3.73)	3.8 (2.12)	3.2 (1.23)	7.2 (1.63)	5.3 (1.70)	1.8 (1.07)	1.1 (0.43)
Heart	4.1 (0.74)	4.3 (0.54)	2.2 (0.94)	2.1 (1.00)	5.7 (2.19)	4.5 (1.17)	2.0 (1.45)	1.6 (1.10)
Kidney	214.2 (91.07)	121.3 (54.69)	47.4 (24.00)	22.9 (14.77)	287.4 (44.50)	140.3 (40.46)	32.2 (43.08)	10.1 (4.46)
Spleen	7.4 (1.62)	7.5 (1.13)	4.2 (2.31)	3.9 (1.80)	7.5 (1.63)	6.8 (1.64)	3.1 (2.08)	2.5 (1.20)
Ovaries	9.7 (2.14)	9.9 (3.20)	8.9 (9.25)	4.3 (1.84)	11.5 (3.61)	8.2 (2.41)	4.5 (3.09)	2.4 (1.09)
Stomach	6.5 (4.08)	4.6 (4.68)	7.7 (2.87)	11.9 (2.70)	9.1 (5.40)	7.7 (3.98)	8.8 (3.51)	11.4 (8.21)
Small intestine	12.0 (1.55)	15.9 (8.06)	8.6 (1.68)	9.7 (6.53)	12.2 (4.34)	10.1 (3.49)	10.8 (5.43)	8.8 (9.34)
Large intestine	2.9 (1.34)	2.7 (1.16)	2.5 (1.73)	2.9 (2.56)	2.3 (1.49)	3.0 (1.59)	1.9 (1.08)	2.2 (0.75)
Thyroid	33.4 (8.68)	43.5 (7.11)	205.0 (76.21)	411.3 (219.98)	20.6 (3.54)	26.3 (8.90)	55.9 (18.52)	104.8 (35.53)

2.6.4.5 Metabolism

N/A

2.6.4.6 Excretion

N/A

2.6.4.7 Pharmacokinetic drug interactions

N/A

2.6.4.8 Other Pharmacokinetic Studies

N/A

2.6.4.9 Discussion and Conclusions

The terminal half-life ranged from 143-158 minutes following a single sc dose (rats, rabbits and minipigs) and 19-99 minutes following a single i.v. dose (rats, monkeys, rabbits and minipigs). Due to the relatively short half-life, the repeat-dose PK profile was consistent with the single dose profile. The elimination half-life in humans was similar for both i.v. and s.c. routes of administration and was approximately 2 hours following a single dose.

2.6.4.10 Tables and figures to include comparative TK summary

See pharmacokinetic parameters for nonclinical species in the tables below.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

(The following tables were excerpted from pages 14-15, 18-20 of section 2.4. The PK parameter units were standardized across the studies for ease of review, therefore, units for the tabulated summary may be different from those listed in some original study reports.)

Table 2.4.2. Summary of Mean Pharmacokinetic/Toxicokinetic Parameters Following the Single SC Administration of Ecallantide

Species	Study Number	Dose (mg/kg)	C_{max} (µg/mL)	T_{max} (min)	AUC_{inf} (µg*min/mL)	CL/F (mL/kg*min)	Vz/F (mL/kg)	$t_{1/2}$ (min)
Rat[a]	(b) (4) 00019	5[m]	1.7	40	298	17.0	2814	112.4
		10[m]	2.2	50	573	17.6	3713	146.0
		20[m]	3.6	55	1383	14.7	4008	184.7
	(b) (4) 00012	5[pf]	1.2	38	228	22.5	4423	137.5
		10[pf]	1.8	56	424	25.2	5659	152.0
		20[pf]	2.5	60	856	24.2	5440	158.5
	GENZ 50-0218PDX	30[f]	6.6	57	3227	9.4	2270	166.2
Rabbit[a]	(b) (4) 00031	2[pf]	4.4	48	509	4.0	449	76.2
		5[pf]	13.7	45	1710	3.1	513	121.0
		10[pf]	23.2	38	4050	2.8	589	140.0
		20[pf]	39.7	48	9492	2.4	795	234.0
Cynomolgus Monkey[a]	(b) (4) 0010	0.4[f]	0.4	60	nc	nc	nc	nc
		10[f]	7.6	60	nc	nc	nc	nc
		25[f]	25.7	60	nc	nc	nc	nc
Minipig[a]	(b) (4) 446012	15[m]	16.1	180	5247	nc	nc	121.8
	446012	15[f]	10.7	198	4073	nc	nc	138.0
	446005	15[m]	9.6	132	5713	2.6	nc	198.0
	446005	15[f]	11.6	120	4959	3.0	nc	192.0
	446005	25[m]	13.0	198	9065	2.8	nc	156.0
	446005	25[f]	12.3	120	7739	3.3	nc	144.0

a concentration data measured by ELISA
m males
f female
pf presumed-pregnant female
nc not calculated

Table 2.4.3. Summary of Mean Pharmacokinetic/Toxicokinetic Parameters Following the Single IV Administration of Ecallantide

Species	Study Number	Dose (mg/kg)	C_{max} (µg/mL)	T_{max} (min)	AUC_{inf} (µg*min/mL)	CL (mL/kg*min)	$t_{1/2}$ (min)
Rat[b]	2204-001P	5[pf]	4.8	15	nc	nc	nc
		10[pf]	13.5	15	453	22.0	17.4
		25[pf]	33.9	15	1308	19.2	21.0
		50[pf]	145	15	5040	9.9	17.4
Rabbit[b]	2204-002P	1[pf]	7.8	30	684	1.5	39.6
		5[pf]	31.7	15	2004	2.5	31.2
		10[pf]	78.1	15	8100	1.2	34.8
		15[pf]	95.9	15	6780	2.3	40.8
Minipig[a]	(b) (4) 446005	15[m]	36.0	15	4352	3.5	132.0
		15[f]	31.4	15	3360	4.5	66.0

a concentration data measured by ELISA
b concentration data measured by HPLC/MS
m males
f females
pf presumed-pregnant females
nc not calculated due to limited concentration data

Table 2.4.4. Summary of Mean Pharmacokinetic/Toxicokinetic Parameters Following the Repeat SC Administration of Ecallantide in Rats

Study Number	Study Day	Dose (mg/kg)	C_{max} (µg/mL)	T_{max} (min)	AUC_{last} (µg*min/mL)	CL/F (mL/kg*min)	$t_{1/2}$ (min)
(b) (4) 446021	0	0.4	0.3	60	nc	nc	nc
		10	2.6	90	670	nc	nc
		25	3.7	90	1527	14.1	256.0
446021	87	0.4	0.3	60	41	nc	nc
		10	3.2	60	1459	6.8	217.2
		25	6.6	60	2620	9.3	300.3
446021	177	0.4	0.3	60	81	nc	nc
		10	4.9	60	1690	6.0	237.3
		25	7.9	60	3411	nc	nc

nc not calculated due to limited concentration data

Table 2.4.5. Summary of Mean Pharmacokinetic/Toxicokinetic Parameters Following the Repeat SC Administration of Ecallantide in Monkeys

Study Number	Study Day	Dose (mg/kg)	C_{max} (µg/mL)	T_{max} (min)	AUC_{last} (µg*min/mL)	CL/F (mL/kg*min)	$t_{1/2}$ (min)
(b) (4) 446010/	0	0.2	0.2	120	nc	nc	nc
446020		0.4	0.4	75	59	nc	nc
		10	13.8	45	2028	5.2	112.4
		25	27.2	52	6859	4.1	107.4
446020	27	0.4	0.4	83	66	nc	nc
		10	9.2	60	2301	5.0	141.0
		25	18.3	55	6009	4.4	160.2
446010	44	10	13.6	90	6318	2.3	201.0
		25	35.4	120	14607	2.2	177.0
446020	87	0.4	0.4	87	84	nc	nc
		10	8.8	57	3270	4.1	223.8
		25	23.6	73	13159	2.5	360.6
446010	88	10	21.6	120	10456	1.9	216.6
		25	48.8	75	20836	2.0	183.3
446020	177	0.4	0.5	113	143	2.3	294.6
		10	11.4	60	5034	2.6	275.4
		25	25.1	90	16702	2.2	329.7

nc not calculated due to limited concentration data

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: General toxicology studies were mainly conducted in rats and monkeys that were administered ecallantide by the subcutaneous or intravenous route. The duration of exposure employed in these studies ranged from a single dose to 6-months of once every three day dosing. Pivotal repeat-dose subcutaneous GLP studies in rats and monkeys were conducted to support subcutaneous, intermittent/chronic dosing with a maximum frequency of once every three days in the clinic.

In the 6-month rat toxicity study, DX-88/vehicle was administered subcutaneously at dose levels of 0, 0.4, 10 and 25 mg/kg once every three days. Three unscheduled deaths

(4.7%) in the 25 mg/kg males and females occurred between SD 103 to 142 and relationships to treatment could not be excluded. One female that died early showed multiple necrosis in the brain including optic chiasm, the lateral internal capsule and optic tract, the fimbria of the hippocampus and the ventral midline of the brainstem, and for the other 2 animals died early, the cause of the death could not be determined. There was one death found in control group of undetermined cause in the same period. The dose of 25 mg/kg is approximately 21-fold of a maximum clinical dose of 60 mg/day on a mg/kg basis or approximately 9-fold of maximum clinical exposure on an AUC basis. Significant increases of AST and ALT values observed in 25 mg/kg females were resolved in the recovery period and not associated with any liver lesions in the microscopic examination. Test article-related findings at injection sites included inflammation (minimal to mild), epithelial (epidermal) hyperplasia (minimal to moderate), exudate (minimal to moderate), ulceration (minimal to moderate), and granulation tissue/fibrosis (minimal to mild) in both males and females of the 25 mg/kg dose groups and in females only of the 10 mg/kg dose group. These injection site reactions were resolved completely in most animals, except for the females in the 25 mg/kg dose group that were only partially resolved. The NOAEL was identified at 10 mg/kg.

In monkeys, a 3-month study with daily sc dosing at 0, 0.2, 10 and 25 mg/kg and a 6-month study with once every three day sc dosing at 0, 0.4, 10 and 25 mg/kg were conducted. There were no overt systemic toxicities observed in both monkeys studies. The test article-related effects were limited to the injection site reactions, which included minimal to moderate perivascular mixed inflammatory cell infiltrates at injection sites observed in males and females at 10 and 25 mg/kg dose levels of both studies. The NOAELs for systemic toxicity in both monkey studies were identified at 25 mg/kg while the NOAELs for local toxicity would be 0.2 and 0.4 mg/kg for the 3- and 6-month studies, respectively.

In these pivotal repeat-dose studies, transient APTT prolongation was observed in rats and monkeys treated with 10 and 25 mg/kg DX-88, which was considered an expected pharmacologic effect of DX-88 and clinically monitorable. No evidence of gross bleeding was observed in these animals.

Anti-DX-88 antibody was measured in the pivotal rat and monkey studies by using validated ELISA or ECL methods. In both rats and monkeys, anti-DX-88 antibodies were detected in each test article-treated group with greater percentages of animals being antibody-positive in the 10 and 25 mg/kg dose groups. The 3-month monkey study (using ELISA method) showed that DX-88 exposure was increased and the clearance rate was decreased when the positive antibody titer > 26,000 ng/mL, regardless of dose. In the 6-month rat and monkey studies, anti-DX-88 antibodies increased DX-88 exposure and reduced clearance. Drug activity appeared to be maintained throughout the duration of the 6-month rat and monkey studies as evidenced by elevated APTT.

In early development, the potential toxicity of DX-88 was evaluated following a single and/or 14-day intravenous dose of DX-88 in rats, monkeys and minipigs. The test article-related acute deaths of the female rats (within 30 minutes of dosing) were observed in a

single dose i.v. study at dose levels of 25 and 50 mg/kg, and in a 14-day i.v. study at a dose level of 23 mg/kg ($AUC_{0\text{-}360\ min}$ = 4509 μg.min/mL for males or 4373 μg.min/mL for females on Day 14). Findings for dead rats in the single-dose study included mild to severe congestion within the liver, renal segmental vacuolation of the tubular epithelium (vacuolar degeneration), depletion of erythrocytes in the spleen and moderate to marked dilation of the right atrium or ventricle or both. Findings for 2 dead rats that were examined from the 14-day study included moderate congestion of the kidneys, liver, lungs and ovaries, mild congestion of the adrenal and pituitary, and vacuolation of cortical renal tubular epithelium in both females and minimal subacute inflammation and hemorrhage of the lungs and hemorrhage and minimal epidermal exudate at the injection site in one female. However, the similar test article-related mortality/moribundity was not observed in another single dose rat i.v. study at dose levels up to 23 mg/kg (vehicle was saline), another 14-day rat i.v. study at dose levels up to 23 mg/kg, a single dose minipig i.v. study at dose level up to 15 mg/kg and a 14-day monkey i.v. study at dose up to 23 mg/kg ($AUC_{0\text{-}360\ min}$ = 6361 μg.min/mL for males or 9212 μg.min/mL for females on Day 14). A series of in vitro and in vivo mechanistic studies were conducted to better understand the acute deaths of female rats following intravenous administration of DX-88. Factors investigated included the vehicle, Cmax, hormone, etc. in an effort to determine actual causes of the death.

Carcinogenicity:

In the letter dated September 26, 2007, the sponsor was informed that the current available information was not sufficient to assess the carcinogenic potential of DX-88 and a carcinogenicity study should be conducted in one species, if feasible, is to be performed as a postmarketing commitment. Based on the 6-month rat toxicity study, it seems feasible to conduct a carcinogenicity study in rats. Although the anti-drug antibodies were detected in the treated groups, the drug exposure was maintained or increased with reduced clearance rate and the drug remained active as evidenced by a prolongation of APTT observed through the study.

Reproductive toxicology: A complete battery of reproductive toxicity studies was conducted with DX-88.
In the reproductive toxicity studies, subcutaneous doses of DX-88 in rats up to 25 mg/kg/day did not show any adverse effects on male or female fertility and reproductive functions. Embryonic and fetal development studies were conducted with rats using the subcutaneous and intravenous routes. Subcutaneous doses of DX-88 in rats up to 20 mg/kg/day did not result in any developmental toxicity. Intravenous administration with DX-88 at a dose of 20 mg/kg/day in rats showed increased number of resorptions and percentage of resorbed conceptuses per litter with corresponding decreased number of live fetuses, reduced fetal body weights (-11%), and increased incidence of fetal alterations, including marked dilation of the lateral ventricle of the brain, short tail, and vertebrate/rib malformations. These findings occurred in the presence of significant maternal toxicity. Intravenous dose of DX-88 at 15 mg/kg/day in rats resulted in increased the number of early resorptions and the percentage of resorbed conceptuses per litter without significant reduced number of live fetuses and reduced fetal body weights (-4%). These findings occurred in the presence of relatively mild maternal toxicity and

should be reported in the labeling. The development NOAEL in rats was identified at intravenous dose of 10 mg/kg (AUCinf=7.55 µg.hr/mL= 453 µg.min/mL after single intravenous dose of 10 mg/kg). Intravenous doses of DX-88 in rabbits at dose levels of 2, 5 and 10 mg/kg did not show any adverse development toxicity. However, the number of rabbits at 10 mg/kg dose group (n=10) was not considered sufficient for a full evaluation. Subcutaneous doses of DX-88 up to 25 mg/kg in F_0 rats did not affect the gestation, parturition, or lactation in the F_0-generation rats, growth, development, and reproductive performance of the F_1-generation rats, and the viability and growth in the offspring. The NOAEL for the viability and growth for offspring was identified at the 25 mg/kg maternal dose. DX-88 was not measured in the milk of rats.

Local Tolerance: The local tolerance DX-88 in lyophilized form and liquid form (to be marketed) was evaluated after single s.c. doses up to 24 mg/kg in rats. Test article-related injection site injuries included chronic inflammation, hemorrhage, congestion, thrombosis, epidermal exudate, squamous hyperplasia and muscular degeneration in rats, primarily in females, treated with liquid form DX-88, and blood vessel necrosis, edema, inflammation, congestion, hemorrhage and epidermal necrosis and/or ulceration in rats treated with lyophilized form. Most local reactions were resolved by day 14 post dosing in both studies except minimal alterations remained in females treated with the lyophilized form of DX-88. The similar local reactions were observed in the 6-month s.c. dose rat studies and less severe local reactions were also observed in the repeated s.c. dose monkey studies. However, there were no significant test article-related local reactions observed in minipigs following a s.c dose of 15 mg/kg liquid or lyophilized form of DX-88.

2.6.6.2 Single-dose toxicity

(b) (4) 1572: DX-88: A SINGLE DOSE INTRAVENOUS TOXICITY STUDY IN SPRAGUE-DAWLEY ALBINO RATS (GLP)

Methods: This is a GLP study conducted by (b) (4) Sprague-Dawley rats (n=5/sex/group) were intravenously administered with a single bolus of 0 (vehicle=saline), 2.3, 6.8 or 23.0 mg/kg DX-88. All rats were observed daily for mortality and clinical signs for 14 days. Body weights were recorded prior to dosing (Day 1), and on Days 3, 7 and 14. Scheduled necropsy was performed following the 14 day observation period. No tissues were retained.

Results: All rats survived until termination. No test article related effects on clinical signs, body weight and gross pathology were observed with dose up to 23 mg/kg.

Study title: (b) (4)-446006, A Single-Dose Intravenous Toxicity Study in Rats Comparing DX-88 Manufactured by the Original and New Processes

Methods: This is a GLP study conducted by (b) (4) Eight rats (Sprague Dawley)/sex/group for toxicology groups and n=3/sex/group for APTT/PT evaluation were intravenously administered a single dose of vehicle, 10, 25 or 50 mg/kg original processed DX-88 (Lot No. 227-01-001, product purity, 98.97%) or new processed DX-88 (Lot No. 1100184, product purity, 99.6%). APTT group animals were terminated approximately 15 minutes post dosing. Toxicology group animals were terminated after a 6-day post-dose

observation period. For toxicology assessment, rats were observed twice daily for mortality and moribundity, prior to dosing, at approximately 15 minutes, 30 minutes, 1, 2, 4 and 6 hours post dosing on the dosing day for clinical examinations, and daily for clinical examinations on non-dosing days. Body weights were recorded on the day of dosing (prior to dosing), on the day before scheduled necropsy (nonfasted) and on the day of scheduled necropsy. Food consumption was recorded weekly. Samples for hematology and clinical chemistry were collected approximately 2 hours post dosing on the day of dosing for toxicology animals. Samples for APTT/PT evaluation were collected approximately 15 minutes post dosing for all APTT group animals. Samples for APTT/PT were also collected from toxicology animals at the necropsy. For toxicology animals, completed necropsies were performed, selected organs were weighed and selected tissues were microscopically examined.

Results:

Mortality: Three females in the 50 mg/kg Original process DX-88 group and 1 female each in the 25 and 50 mg/kg New process DX-88 groups were found dead within 17 minutes of dosing. Prior to death, all of these females were prostrate. Gasping and pale extremities were also noted for one female each in the Original and New process groups. As listed in the table below (excerpted from page 18 of the report), microscopic findings of these animals included mild to severe congestion (with minimal to mild midzonal hemorrhage) with diffuse effusion of erythrocytes from sinusoidal spaces into subendothelial and interstitial spaces within the liver, renal segmental vacuolation of the tubular epithelium (vacuolar degeneration) that appeared to be restricted to the distal tubules or collecting ducts, and depletion of erythrocytes in the spleen (possibly in response to the blood cell loss by intrahepatic hemorrhage). Subgross alterations of the heart, predominately characterized by moderate to marked dilation of the right atrium or ventricle or both, were observed in 4 of the 5 these unscheduled death rats without microscopic evidence of myocardial damage. The actual cause of the deaths could not be determined. Another male death in the 10 mg/kg Original process group was attributed to blood collection procedure, hence, was not considered test article-related. All other rats survived to the scheduled necropsy.

Incidence of Treatment-Related Cardiac, Hepatic, Renal and Splenic Changes (Acute Death Animals and Male No. 62710)

	Vehicle		"Original" Process						"New" Process					
Dose Group (mg/kg)	0		10		25		50		10		25		50	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Number Examined	0	0	1	0	0	0	0	3	0	0	0	1	0	1
Heart														
Right Ventricular Dilation	-	-	-	-	-	-	-	2	-	-	-	1	-	-
Right Atrial Dilation	-	-	-	-	-	-	-	1	-	-	-	1	-	1
Congestion (myocardial)	-	-	-	-	-	-	-	3	-	-	-	1	-	1
Liver														
Congestion	-	-	1	-	-	-	-	3	-	-	-	1	-	1
Kidney														
Vac. Deg., tube. epi.*	-	-	1	-	-	-	-	3	-	-	-	1	-	1
Spleen														
Hypocellular Depletion	-	-	-	-	-	-	-	2	-	-	-	1	-	-

* Vac. Deg., tube, epi = Vacuolar Degeneration, tubular epithelium

Clinical signs: Test article-related clinical findings included flushing (ear/extremities) and yellow material in the urogenital area observed in the treated males and females, and hypoactivity observed in the New process DX-88 treated females. These findings were observed within 30 minutes of dosing and subsided generally by 1 hour post dosing.

Body weight and food consumption: There were no test article-related effects on body weight and food consumption.

Hematology: Elevated neutrophils and/or monocytes were observed in all DX-88 treated females and in the 50 mg/kg males treated with both process DX-88.

Clinical chemistry: For both Original and New process DX-88, ALT, AST and BUN values were markedly elevated for males treated with a dose of 50 mg/kg and in a dose-dependent manner for females treated with doses of 25 and 50 mg/kg. APTT was prolonged in both process DX-88 treated groups at 15 minutes post dosing but was not affected by the treatment on study day 7. PT values were comparable to the control at both time points.

Pathology: Hepatic congestion and vacuolar degeneration of renal tubules were noted for one male in the 10 mg/kg Original group. Although these findings have some similarity to findings observed in the females died, they were not observed for males given higher doses (25 or 50 mg/kg) of the Original process DX-88 and for all males treated with New process DX-88, suggesting a very weak relationship to test article-treatment. There were no other gross and histopathology findings in the surviving rats that could be potentially related to the test article. The NOAEL was identified at 10 mg/kg for both processes DX-88. There was no significant difference in toxicity of DX-88 from Original and New processes.

Study Title: An Acute Toxicity and Toxicokinetic Study of DX-88 following Subcutaneous or Intravenous Administration in Mini-Pigs (Study No. (b) -446005)

The final report was previously reviewed by Jean Wu in IND 10426 Serial No. 090 (see Appendix 1). The toxicological significance of the isolated finding of lung edema in this study appeared to be minimal as it was not observed in any other repeated dose toxicity studies.

2.6.6.3 Repeat-dose toxicity

Studies conducted using the Subcutaneous Route of Administration

Study title: A 6-Month Toxicity Study of DX-88 Administered Subcutaneously Once Every Three Days to Sprague-Dawley Rats With a 28-Day Recovery Period

Key study findings:
Three unscheduled deaths in the 25 mg/kg groups (4.7% of males and females) occurred between SD 103 to 142 and their potential relationships to treatment could not be excluded. In one unscheduled death female, multiple necrosis was observed in the brain including optic chiasm, the lateral internal capsule and optic tract, the fimbria of the hippocampus and the ventral midline of the brainstem. There were no relevant microscopic findings observed for another unscheduled death female and no microscopic examinations were conducted for the unscheduled death male from the non-toxicology group. The causes of these two deaths were undetermined. It is noted that one unscheduled death of control females was also reported during this period.

There were significant increases in AST and ALT observed in 25 mg/kg females which resolved during the recovery period. There were no corresponding liver lesions observed microscopically.

APTT prolongation was observed in 25 mg/kg males and females, as an expected pharmacologic effect of DX-88.

Higher incidences and severities of the injection sites findings were observed in 25 mg/kg males and females and 10 mg/kg females at the end of the treatment period. These findings were almost completely resolved in 25 mg/kg males and 10 mg/kg females, but only partially resolved in 25 mg/kg females.

The NOAEL was identified at 10 mg/kg due to the unscheduled death for males and females at 25 mg/kg and marked increases of ALT and AST and significant non-resolved injection sites findings for 25 mg/kg females.

Study no.: (b) (4) 446021
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: December 4, 2006
GLP compliance: Yes
QA report: Yes

Drug, lot #, and % purity: DX-88 drug product in phosphate buffered saline (PBS), pH 7, Lot # 227-07-001, purity, 95.6%

Methods

Doses: 0, 0.4., 10 and 25 mg/kg, administered subcutaneously once every 3 days for up to 180 days, for a total of 60 doses.
Species/strain: Rats / Crl:CD(SD), from (b) (4)

Number/sex/group or time point (main study): See the table for Toxicology Groups below (inserted from page 27 of the report)
Route, formulation, volume, and infusion rate: The route of administration is subcutaneous. The vehicle used in preparation of the test article formulation and for administration to the control group was phosphate-buffered saline (PBS). The stock test article was diluted in vehicle to desired concentrations. Dose volumes are listed in the tables below.

Toxicology Groups ((b) -446021M, (b) -446021F)

Group Number	Test Article	Dose Level (mg/kg/dose)[a]	Dose Volume (mL/kg)	Number of Animals Males	Females
1	Vehicle	0	2.8	15	15
2	DX-88	0.4	1.12	15	15
3	DX-88	10	1.12	15	15
4	DX-88	25	2.8	15	15

[a] = The dosing formulations were not adjusted for purity.

Satellite groups used for toxicokinetics and APTT: As shown in the tables below, n=8/sex/group and n=9/sex/group were used for toxicokinetics and APTT (activated partial thromboplastin time) evaluation, respectively.

Toxicokinetic Groups ((b) -446021A, (b) -446021B)

Group Number	Test Article	Dose Level (mg/kg/dose)[a]	Dose Volume (mL/kg)	Number of Animals Males	Females
1A	Vehicle	0	2.8	8	8
2A	DX-88	0.4	1.12	8	8
3A	DX-88	10	1.12	8	8
4A	DX-88	25	2.8	8	8

APTT Groups (b) -446021Y, (b) 446021Z)

Group Number	Test Article	Dose Level (mg/kg/dose)[a]	Dose Volume (mL/kg)	Number of Animals Males	Females
1B	Vehicle	0	2.8	9	9
2B	DX-88	0.4	1.12	9	9
3B	DX-88	10	1.12	9	9
4B	DX-88	25	2.8	9	9

Age: Approximately 7 weeks at initiation of dose administration.

Weight: toxicology groups: 204-301 g for males and 163-211 g for females; toxicokinetic groups: 214-277 g for males and 158-197 g for females; APTT groups: 199-278 g for males and 154-216 g for females.
Sampling times: Toxicokinetics: blood samples were taken prior to dosing and at 1, 2, 6, 12, and 24 hours post-dose of the first, 30th and 60th dose administration.
Unique study design or methodology (if any):

Observations and times:

Mortality: All animals were observed twice daily.
Clinical signs: For toxicology groups, animals were observed twice daily, at the time of dosing and approximately 2-3 hours following dosing on dosing days. On non-dosing days, the animals were observed daily. Detailed physical examinations were conducted on all animals weekly.
Body weights: Individual body weights of all animals were recorded weekly beginning approximately 2 weeks prior to initial dosing.
Food consumption: Individual food consumptions were recorded weekly beginning approximately 1 week prior to initial dosing.
Ophthalmoscopy (toxicology groups): For toxicology groups, ocular examinations were conducted prior to initial dosing, and at the end of treatment.
EKG: Not measured
Hematology and Clinical chemistry (toxicology groups): Blood samples for hematology and serum chemistry were taken prior to dosing and approximately 2 hours post-dose for dose numbers 20, 40 and 60. In addition, blood samples for hematology, chemistry and coagulation were collected at the scheduled necropsy.
Urinalysis (toxicology groups): Urine samples were collected at the necropsy.
Gross pathology (toxicology groups): A complete necropsy was performed on all animals at the scheduled necropsy. Necropsy was also performed on the animals died early in the study.
Organ weights (toxicology groups): See "Histopathology Inventory" table.
Histopathology (toxicology groups): The tissues and organs listed in "Histopathology Inventory" table below were collected from all necropsied animals and placed in 10% neutral-buffered formalin except noted otherwise. Epididymides and testes were fixed in Bouin's solution, and the eyes with optic nerves were fixed in Davidson's solution. The collected tissues/organs were processed and stained with hematoxylin and eosin for microscopic examination. Bone marrow smears were not placed in formalin and the slides were examined only if scientifically warranted. Thyroid with parathyroids were examined if in the plane of section and in all cases where a gross lesion was present.

Adequate Battery: Yes

Peer review: No

Toxicokinetics: Blood samples (~0.75 mL) were collected via retro-orbital sinus under anesthesia. The concentration of DX-88 was determined by using a validated ELISA procedure (validation report No. 7928-106).
Immunogenicity: Blood samples (~0.75 mL) were collected from the animals of toxicology groups via retro-orbital sinus under isoflurane anesthesia prior to each dosing of the 1st dose, 10th, 30th and 60th doses. The anti-drug antibody was determined by using

a bridging electrochemiluminescent (ECL) assay formats (validation report No. TNJS07-150).
APTT: Blood samples were collected from the animals of APTT groups (3/sex/group) via the vena cava under isoflurane anesthesia at approximately 1 hour post-dose following the 1st, 30th and 60th doses.

Results

Mortality: There were a total 5 unscheduled deaths between SD 103 to 142. One (in TK group) of 32 control females was found dead. There were 4 deaths in the 25 mg/kg, 2 of 32 males (one in toxicology group and the another in APTT group) and 2 of 32 females (both in toxicology group). For 1 male (No. 7123) moribund euthanized on study day (SD) 142, the death was considered to be a complication of the retro-orbital bleeding procedure and unlikely to be test article-related. For other 3 deaths in the 25 mg/kg group, potential relationships to treatment could not be excluded. Among them, one female (No. 7256) was moribund euthanized on SD 103. The moribundity of this female was attributed to the multiple necrosis observed in the brain including optic chiasm, the lateral internal capsule and optic tract, the fimbria of the hippocampus and the ventral midline of the brainstem. Another female (No. 7310) was found dead on SD 117 with an undetermined cause of death. One male from the APTT group (No. 7067) was reported as unscheduled death during the week of 19 but no tissue collection procedure was performed on this non-toxicology group animal. It is also noted that one control female from TK group (No. 7267) were reported as unscheduled deaths during the week of 18. All other animals survived to the scheduled necropsies or termination.

Clinical signs: The test article-related clinical signs were limited at the injection sites, i.e. swelling, scabbing, reddened and purple discoloration noted at the injection sites of both male and females with sporadic occurrences in the control and 0.4 mg/kg groups, but with a higher incidence in the 10 and 25 mg/kg dose groups. In recovery period, these injection site findings were resolved completely in males, and almost completely resolved in females with a few injection site findings persisting in the 10 and 25 mg/kg females. There were no other significant test article related clinical signs observed.
Body weights: There were no test article-related effects on body weights and body weight changes.
Food consumption: There were no test article-related effects on food consumption.
Ophthalmoscopy: No test article-related ophthalmic lesions were observed.
Hematology: Increased WBC counts secondary to irritation of the test article at injections sites were observed for 25 mg/kg females. There was a slight tendency of increased white blood cell counts for females at 25 mg/kg observed in two intervals (40% at 2 hr. post Dose 20 and 47% at 2 hr. post Dose 40 comparing to the control) and for all treated females observed in one recovery interval (57% in week 29 compared to the control) without a dose-related pattern. However, there was a variation in the control females showing a decreased WBC counts (up to 62% compared to the 1st prior to dose value) in Week 29. In 25 mg/kg females, absolute neutrophil counts were increased about 69% and 211% prior to, and 2 hr post Dose 20, respectively, 74% and 90% prior to, and 2 hr post Dose 40, respectively, and 51% and 77% prior to and 2 hr post Dose 60, when comparing to the corresponding controls. Similarly, there were significant increases in

absolute monocyte counts (up to 160%) and eosinophils (up to 170%) were also observed at Doses 20, 40 or 60 in 25 mg/kg females, when comparing to the corresponding controls. The significantly higher neutrophil, monocyte and eosinophil counts along with the tendency of increased WBC counts in 25 mg/kg females indicated a dose-related inflammatory response, likely due to irritation of the test article at the injection sites (consistent with the most severe injection sites findings observed in the 25 mg/kg females).

Other differences between treated and control groups in measured parameters were of small magnitude, comparable with the prior to dose value or lacked a dose-dependent pattern, and hence, were unlikely to be related to the test article.

Clinical Chemistry: As shown in the table below (excerpted from pages 51 and 52 of the report), increased serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were observed in a dose-dependent pattern for all treated females. The increases of ALT and AST for 25 mg/kg females were considered significant (up to +485% for AST and +1379% for ALT comparing to the controls).

Text Table 2. Selected Serum Chemistry Findings - Females (continued)

		Group (mg/kg/dose)			
Analysis		**0**	**0.4**	**10**	**25**
Aspartate aminotransferase (U/L)					
Day 57 pre[a]	Mean	110	124	120	113
% Difference		--	+13	+9	+3
Day 57 post[b]	Mean	117	134	**145**	**684****
% Difference		--	+15	+24	+485
Day 117 pre	Mean	108	109	94	104
% Difference		--	+1	-13	-4
Day 117 post	Mean	142	141	**193**	**385***
% Difference		--	-1	+36	+171
Day 177 pre	Mean	108	149	106	**144**
% Difference		--	+38	-2	+33
Day 177 post	Mean	119	**214**	**178**	**361**
% Difference		--	+80	+50	+203
Week 25	Mean	128	187	122	111
% Difference		--	+46	-5	-13
Week 29	Mean	96	129	120	91
% Difference		--	+34	+25	-5

Alanine aminotransferase (U/L)					
Day 57 pre[a]	Mean	44	45	50	41
% Difference		--	+2	+14	-7
Day 57 post[b]	Mean	38	44	**59**	**562****
% Difference		--	+16	+55	+1379
Day 117 pre	Mean	62	61	55	52
% Difference		--	-2	-11	-16
Day 117 post	Mean	60	61	**102**	**281***
% Difference		--	+2	+70	+368
Day 177 pre	Mean	70	96	66	84
% Difference		--	+37	-6	+20
Day 177 post	Mean	61	**107**	**124**	**279**
% Difference		--	+75	+103	+357
Week 25	Mean	80	116	82	67
% Difference		--	+45	+3	-16
Week 29	Mean	68	83	72	56
% Difference		--	+22	+6	-18

[a] = prior to dosing
[b] = 2 hours post dosing
-- = No data available
* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

ALT and AST levels in treated females were comparable to the control females in the recovery period (Week 29). There were no test article-related liver lesions observed microscopically, indicating that direct liver toxicity was unlikely (could not be determined). The differences between treated groups and controls in total protein, albumin and globulin at 10 and 25 mg/kg females were of small magnitude (-16% to -4% over the dosing period) and the values at post-dose were comparable to the correspondent pre-dose values. There were no other significant test article related effects on the measured parameters.

Urinalysis: There were no test article-related effects on urinalysis parameters observed.

Gross pathology: Test article-related findings of scabbing were limited to the injection sites (3, 5 or 6) for both males and females treated with 10 and 25 mg/kg of DX-88. These findings correlated with microscopic findings of exudate and/or ulceration at the injection sites. There were no other test article-related gross findings observed.

Organ weights: There were no significant test article-related changes in organ weights.

Histopathology: As shown in the tables below, test article-related findings at injections sites included inflammation (minimal to mild), epithelial (epidermal) hyperplasia (minimal to moderate), exudate (minimal to moderate), ulceration (minimal to moderate), and granulation tissue/fibrosis (minimal to mild) observed in 25 mg/kg males, and 10 and 25 mg/kg females. Control animals showed minimal inflammation and epidermal hyperplasia at multiple injections sites, minimal exudate once, minimal fibrosis once and no ulceration. The incidence and severity of the findings at injection sites was higher in 25 mg/kg females than 25 mg/kg males or 10 mg/kg females. The injection sites findings were almost completely resolved in 25 mg/kg males and 10 mg/kg females (one incidence of mild fibrosis observed), and partially recovered in 25 mg/kg females.

Histopathological findings at injection sites for rats treated with DX-88 for 6 months

	Males				Females			
Dosage (mg/kg/dose):	**0**	**0.4**	**10**	**25**	**0**	**0.4**	**10**	**25**
Injection site 1 [a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Hyperplasia, epithelial	0	0	0	0	1	0	0	0
Minimal	0	0	0	0	1	0	0	0
Infiltrate, lymphocyte	1	0	0	0	0	0	0	0
Minimal	1	0	0	0	0	0	0	0
Inflammation, any type [b]	0	0	0	0	0	1	0	0
Minimal	0	0	0	0	0	1	0	0
Thrombosis	0	1	0	0	0	0	0	0
Minimal	0	1	0	0	0	0	0	0
Injection site 2 [a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Fibrosis	0	0	0	0	1	0	0	0
Minimal	0	0	0	0	1	0	0	0
Hyperplasia, epithelial	0	0	1	0	0	0	0	1
Minimal	0	0	1	0	0	0	0	0
Mild	0	0	0	0	0	0	0	1
Inflammation, any type [b]	0	1	2	0	1	1	0	0
Minimal	0	0	2	0	1	1	0	0
Mild	0	1	0	0	0	0	0	0

Histopathological findings at injection sites for rats treated with DX-88 for 6 months (continued)

	Males				Females			
Dosage (mg/kg/dose):	**0**	**0.4**	**10**	**25**	**0**	**0.4**	**10**	**25**
Injection site 3 [a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Exudate, epidermal surface	0	0	0	0	0	0	0	3
Minimal	0	0	0	0	0	0	0	1
Mild	0	0	0	0	0	0	0	1
Moderate	0	0	0	0	0	0	0	1
Hyperplasia, epithelial	0	0	0	1	1	1	2	3
Minimal	0	0	0	0	1	1	1	1
Mild	0	0	0	1	0	0	0	0
Moderate	0	0	0	0	0	0	1	2
Inflammation (any type [b])	0	0	0	3	1	0	2	3
Minimal	0	0	0	3	1	0	2	2
Mild	0	0	0	0	0	0	0	1
Ulceration	0	0	0	0	0	0	0	2
Minimal	0	0	0	0	0	0	0	1
Mild	0	0	0	0	0	0	0	1
Granulation tissue or Fibrosis	0	0	0	1	0	2	1	3
Minimal	0	0	0	0	0	2	0	1
Mild	0	0	0	1	0	0	1	2
Injection site 4 [a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Hyperplasia, epithelial	0	0	0	0	1	0	3	5
Minimal	0	0	0	0	0	0	1	2
Mild	0	0	0	0	1	0	2	2
Moderate	0	0	0	0	0	0	0	1
Inflammation (any type [b])	0	0	0	1	1	1	3	2
Minimal	0	0	0	1	1	1	3	2
Granulation tissue or Fibrosis	0	0	0	1	0	1	0	4
Minimal	0	0	0	1	0	1	0	3
Mild	0	0	0	0	0	0	0	1
Injection site 5 [a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Exudate, epidermal surface	0	0	0	2	0	0	3	5
Minimal	0	0	0	1	0	0	0	1
Mild	0	0	0	1	0	0	3	2
Moderate	0	0	0	0	0	0	0	2
Hyperplasia, epithelial	0	0	2	2	1	1	5	7
Minimal	0	0	0	0	1	1	2	1
Mild	0	0	1	2	0	0	0	4
Moderate	0	0	1	0	0	0	3	2

[a] = Number of tissues examined from each group.
[b] = Includes subacute, chronic, chronic active, and granulomatous inflammation

Histopathological findings at injection sites for rats treated with DX-88 for 6 months (continued)

	Males				Females			
Dosage (mg/kg/dose):	**0**	**0.4**	**10**	**25**	**0**	**0.4**	**10**	**25**
Injection site 5 [a] (continued)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Inflammation (any type [b])	1	0	2	1	1	3	4	6
Minimal	1	0	2	0	1	2	4	3
Mild	0	0	0	1	0	1	0	3
Ulceration	0	0	3	2	0	0	1	2
Mild	0	0	3	2	0	0	1	2
Granulation tissue or Fibrosis	0	0	1	1	0	0	1	4
Minimal	0	0	0	0	0	0	0	2
Mild	0	0	1	1	0	0	1	2
Injection site 6 [a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Exudate, epidermal surface	0	0	0	3	1	0	1	1
Minimal	0	0	0	0	1	0	0	0
Mild	0	0	0	3	0	0	1	1
Hyperplasia, epithelial	0	1	3	5	1	0	1	6
Minimal	0	1	0	1	0	0	0	3
Mild	0	0	2	4	0	0	0	1
Moderate	0	0	1	0	1	0	1	2
Inflammation (any type [b])	1	0	3	8	2	3	2	4
Minimal	1	0	2	1	2	3	1	3
Mild	0	0	1	7	0	0	1	1
Ulceration	0	0	1	2	0	0	1	4
Minimal	0	0	0	0	0	0	0	1
Mild	0	0	1	2	0	0	1	1
Moderate	0	0	0	0	0	0	0	2
Granulation tissue or Fibrosis	0	0	1	3	0	0	0	5
Minimal	0	0	0	1	0	0	0	3
Mild	0	0	1	2	0	0	0	2

[a] = Number of tissues examined from each group.
[b] = Includes subacute, chronic, chronic active, and granulomatous inflammation

Other noteworthy findings beyond findings at injection sites alterations are listed below. Liver hypertrophy centrilobular/ periportal and bile duct hyperplasia were low incidence findings for females of the 25 mg/kg dose group. The low incidences appear to be consistent with historical control background levels. Correlations to elevated ALT and AST values were unlikely since such findings were not observed in other main 25 mg/kg females with significantly elevated ALT and AST values and were observed in one recovery female when no elevated ALT and AST values were found in recovery animals.

Findings		Males				Females			
Dose (mg/kg)		Ctl.	0.4	10	25	Ctl.	0.4	10	25
Number of animals	Main	10	10	10	10	10	10	10	10
	Rec	5	5	5	4	5	5	5	4
Liver, bile duct hyperplasia	Main	0	0	0	0	0	0	0	2 (1,2)
	Rec	0	0	1(2)	0	0	0	0	0
Liver, hepatocellular hypertrophy centrilobular/periportal	Main	0	0	0	0	0	0	0	1(2)
	Rec	0	0	0	0	0	0	0	1(1)

The number in the parentheses indicates the level of severity: 1=minimal, 2= mild/slight, 3= moderate.
Ctl.= control, Rec. = recovery

Toxicokinetics: The toxicokinetic data was summarized in the table below (inserted from page 2705 of the report).

Group Number	Dosage Level (mg/kg/dose)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/dose)]	T_{max} (hr)	AUC_{0-t} (ng·hr/mL)	AUC_{0-24} (ng·hr/mL)	DN AUC_{0-24} [(ng·hr/mL)/(mg/kg/dose)]	$t_{1/2}$ (hr)	CL/F (mL/hr/kg)
					Day 0					
2A	0.4	M	335	838	1.00	NA	NA	NA	NC	NC
		F	236	591	1.00	248	294	735	NC	NC
3A	10	M	3360	336	1.00	10308	12842	1284	NC	NC
		F	1733	173	2.00	11998	12660	1266	NC	NC
4A	25	M	4773	191	1.00	26543	29849	1194	3.30	857
		F	2647	106	2.00	24330	28824	1153	5.23	834
					Day 87					
2A	0.4	M	404	1009	1.00	1026	1216	3040	NC	NC
		F	220	551	1.00	328	758	1895	NC	NC
3A	10	M	2460	246	1.00	25361	25361	2536	4.34	386
		F	3917	392	1.00	23252	23252	2325	2.90	428
4A	25	M	4920	197	1.00	38107	38107	1524	4.65	636
		F	8303	332	1.00	49215	49215	1969	5.36	482
					Day 177					
2A	0.4	M	271	677	1.00	368	755	1887	NC	NC
		F	334	836	1.00	2314	2518	6295	NC	NC
3A	10	M	2840	284	1.00	23307	23307	2331	3.17	427
		F	6973	697	1.00	33020	33020	3302	4.74	294
4A	25	M	5690	228	1.00	46701	46701	1868	4.76	518
		F	10573	423	1.00	67003	67003	2680	NC	NC

Note: Only C_{max} and T_{max} are reported for Group 2 males on Day 0 because insufficient concentration data above the limit of quantitation were available.

DN: Dose normalized
NA: Not applicable
NC: Not calculated

In general, systemic exposures (C_{max} and AUC) increased with increase in dose levels of 0.4 to 25 mg/kg. Values of C_{max} and AUC are generally greater on SDs 87 and 177 than SD 0, indicating an increase in exposure after repeating dose. The difference in gender is not clearly patterned since the values of C_{max} and AUC were higher in males than females on SD 0, not different between genders on SD 87, and lower in males than females on SD 177. The slight but not marked (>2.0 fold) decrease in CL/F values were observed after repeating dose.

Immunogenicity: Anti-DX-88 antibodies were detected in each dose group as listed below. The increased DX-88 exposure and reduced clearance was observed in the current studyalthough the trend could not be definitely attributed to anti-DX-88 antibodies since the anti-body sampling was mainly from toxicology groups not the toxicokinetic groups.

Dose (mg/kg)	N	Day 27	Day 87	Day 177
0.4	30	7	9	8
10	30	11	13	7
25	30	15	22	15

APTT evaluation: As shown in the table below (inserted from page 63 of the report), higher APTT levels were noted in 25 mg/kg males (+15 to +16%) and females (+16% to +36%) on Day 0 (1st dose), Day 87 (the 30th dose) and Day 177 (the 60th dose). The increase of APTT in 10 mg/kg males and females was small in magnitude and was not considered biologically significant. The prolonged APTT is an expected test article-related finding based upon the mechanism of DX-88.

Text Table 6. Activated Partial Thromboplastin Time Evaluations- Males

		Group (mg/kg/dose)			
Analysis:		**0**	**0.4**	**10**	**25**
APTT (seconds)					
Day 0	Mean	20.4	18.9	**22.5**	**23.4**
% Difference		--	-7	+10	+15
Day 87	Mean	21.3	21.3	**22.4**	**24.6****
% Difference		--	0	+5	+15
Day 177	Mean	17.1	16.1	**19.2**	**19.8**
% Difference		--	-6	+12	+16

Text Table 7. Activated Partial Thromboplastin Time Evaluations- Females

Analysis:		Group (mg/kg/dose) 0	0.4	10	25
APTT (seconds)					
Day 0	Mean	17.0	**18.3**	**20.3**	**23.2****
% Difference		--	+8	+19	+36
Day 87	Mean	20.1	17.4	18.8	**23.4**
% Difference		--	-13	-6	+16
Day 177	Mean	16.7	15.7	**17.9**	**19.5***
% Difference		--	-6	+7	+17

Study title: A 90-Day Toxicity Study of DX-88 Administered Subcutaneously Every Other Day to Cynomolgus Monkeys

Key study findings:
The minimal to mild perivascular inflammation at injection sites were observed in main study males and females at the 10 and 25 mg/kg dose levels.
Transient prolonged APTT was observed in 10 and 25 mg/kg animals, which is an expected pharmacologic effect based on the mechanism of DX-88.
The anti-DX-88 antibody positive rates were significantly higher in the 10 and 25 mg/kg dose groups. Regardless of dose, in the animals with a high titer (>26,000 ng/mL), DX-88 exposure increased and clearance decreased on SDs 44 and 88 compared to these parameters in the animals with a lower titer (< 26,000 ng/mL). Exposure and clearance parameters on SDs 44 and 88 for animals with a lower titer were comparable to values observed on SD 0.
The NOAEL for local toxicity is identified at 0.2 mg/kg while the NOAEL for systemic toxicity was at 25 mg/kg (high dose).

Study no.: (b) 446010
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: June 1, 2005
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: DX-88 drug product in PBS, pH 7, Lot # 227-03-001, purity, 99.51% (product) 94.93% (DX-88)
Methods

Doses: 0, 0.2, 10 and 25 mg/kg, administered subcutaneously every other day for up to 90 days (a total of 46 doses) followed with a 14-day non-dosing recovery period.
Species/strain: Cynomolgus monkeys (*Macaca fascicularis*) from (b) (4)

Number/sex/group or time point (main study): See the table below (inserted from page 28 of the report).

Group Number	Test Article	Dosage Level (mg/kg/dose)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Animals[a] Males	Number of Animals[a] Females
1	Vehicle	0	0	2.5	6	6
2	DX-88	0.2	0.08	2.5	6	6
3	DX-88	10	4	2.5	6	6
4	DX-88	25	10	2.5	6	6

[a] = Four animals/sex/group were euthanized following a minimum of 90 days of dose administration; the remaining 2 animals/sex/group were euthanized following a 14-day nondosing (recovery) period.

Route, formulation, volume, and infusion rate: The route of administration is subcutaneous. The vehicle used in preparation of the test article formulation and for administration to the control group was phosphate-buffered saline (PBS). The stock test article was diluted in vehicle at desired concentration. Dose volume is listed in the tables above.

Satellite groups used for toxicokinetics: N/A. TK samples were collected from the main study animals.

Age: 2-3 years at the initiation of dosing.

Weight: 1.5 - 1.9 kg for males and 1.5 – 2.0 kg for females.

Unique study design or methodology (if any): N/A

Observations and times:

Mortality: Animals were observed twice daily.

Clinical signs: All animals were observed prior to dosing, and approximately 15 and 30 min following dosing on SD 0. For the remaining dosing days, clinical observation was performed prior to dosing and approximately 30 min post dosing. On non-dosing days, the animals were observed daily. Detailed physical examinations were conducted on all animals weekly.

Body weights: Individual body weights of all animals were recorded weekly beginning approximately 1 week prior to initial dosing.

Food consumption: Food consumptions were assessed qualitatively daily and recorded as part of the daily observations.

Ophthalmoscopy: Ocular examinations were conducted prior to initial dosing, and prior to the primary necropsy (main animals).

EKG: Multilead ECGs were recorded for all animals prior to randomization, and for all control and 25 mg/kg animals approximately 1-2 hours post the 2nd dose SD2.

Hematology: Blood samples were collected prior to the initial dosing, prior to and approximately 2-4 hours post dosing of Dose 30 (SD 58), at the primary necropsy (SD91) and at the recovery necropsy (SD105).

Clinical chemistry: Blood samples were collected prior to the initial dosing, prior to and approximately 2-4 hours post dosing of Dose 30 (SD 58), at the primary necropsy (SD91) and at the recovery necropsy (SD105).

Urinalysis: Samples were collected prior to the initial dosing, and prior to the primary necropsy (SD91).
APTT evaluation: Blood samples were collected from designated animals prior to dosing and at approximately 0.5, 1, 4, and 14 hrs post dosing of Dose 1, Dose 23 and Dose 45.
Gross pathology: Full necropsy was performed on 4 monkeys/sex/group at the end of treatment period, and on the remaining two monkeys/sex from control and high dose groups at the end of recovery period.
Organ weights: See "Histopathology Inventory" table below.
Histopathology: The tissues and organs listed in "Histopathology Inventory" table below were collected from all necropsied animals and placed in 10% neutral-buffered formalin except noted otherwise. Epididymides and testes were fixed in Bouin's solution, and the eyes with optic nerves were fixed in Davidson's solution. The collected tissues/organs were processed and stained with hematoxylin and eosin for microscopic examination. Bone marrow smears were not placed in formalin and the slides were examined only if scientifically warranted.

Adequate Battery: Yes
Peer review: No

Toxicokinetics: Blood samples were collected via femoral vein prior to dosing, and at approximately 0.5, 1, 2, 4, 8, 14 and 24 hours post dosing of Dose 1(SD 0), Dose 23 (SD 44), and Dose 45 (SD 88). The monkey plasma samples were analyzed for levels of DX-88 by using a validated ELISA method (validation report No.GENZ-05GSTR033).
Immunogenicity: Blood samples were collected via femoral vein during the pretest period, and during the study weeks 4, 8, 13 and 14 (recovery period). The plasma samples were analyzed for levels of anti-DX-88 antibodies by using a validated ELISA method (Study(b) (4)-0184).

Results

Mortality: All animals survived to the scheduled necropsy.
Clinical signs: There were no test article-related clinical observations.
Body weights: There were no test article-related effects on body weights or body weight changes.
Food consumption: No report.
Ophthalmoscopy: There were no test article-related ophthalmic lesions observed.
EKG: There were no test article-related effects noted in ECG evaluation.
Hematology: There were no test article-related alterations of measured parameters.
Clinical Chemistry: There were no test article-related alterations of measured parameters. Some group mean differences between control and treated groups were not considered to be test article-related because the values did not show a dose-dependent response, were of a magnitude that was not toxicologically significant, present in single time point and/or within the range of the control values and/or pre-dosing values.
Urinalysis: There were no test article-related alterations of measured parameters.
APTT evaluation: The prolonged APTT was observed at 2 hours post dosing on SD 12, and SD 58 in the males and females treated with 10 and 25 mg/kg DX-88. More extensive APTT test on SDs 0, 44 and 88 showed that APTT was prolonged in 10 mg/kg and/or 25 mg/kg males and females at 0.5, 1, and 4 hours post dosing. In some cases,

there were less significant prolongations of APTT observed at 14 hours post dosing on SDs 44 and 88 in 10 and 25 mg/kg females and 25 mg/kg males. See the summary of data below.

Text Table 1: Summary of APTT Values (seconds) - Males

Dose Group (mg/kg/dose)	0	0.2	10	25
Study Day 0				
Prior to Dosing	17.8 (3)	16.7 (2)	15.4 (3)	15.6 (3)
0.5 hour post-dosing	17.8 (2)	17.5 (3)	44.6 (3)	46.7 (3)
1 hour post-dosing	16.7 (3)	17.6 (3)	37.4** (3)	43.9** (3)
4 hours post-dosing	17.5 (3)	17.6 (3)	29.7** (3)	42.0** (3)
14 hours post-dosing	15.9 (2)	16.5 (3)	15.1 (3)	15.1 (3)
Study Day 12				
Prior to Dosing	17.1 (6)	17.4(6)	16.7 (6)	17.0 (6)
2 hours post-dosing	17.5 (6)	17.3 (6)	38.1** (6)	46.8** (5)
Study Day 28				
Prior to Dosing	17.6 (6)	16.8 (6)	16.2 (6)	16.8 (5)
Study Day 44				
Prior to Dosing	17.9 (3)	17.8 (3)	16.0 (3)	16.4 (2)
0.5 hour post-dosing	17.2 (2)	17.2 (3)	41.7 (2)	48.6 (3)
1 hour post-dosing	17.6 (3)	18.3 (3)	33.4** (3)	50.6 (1)
4 hours post-dosing	18.2 (2)	17.5 (3)	37.7 (3)	48.6 (2)
14 hours post-dosing	17.0 (3)	17.1 (3)	14.7 (3)	22.0 (3)
Study Day 58				
Prior to Dosing	18.4 (6)	18.1 (6)	17.7 (6)	18.1 (6)
2 hours post-dosing	18.3 (6)	18.3 (6)	40.7** (6)	47.2** (6)
Study Day 88				
Prior to Dosing	18.1 (3)	18.0 (3)	16.1 (3)	18.4 (3)
0.5 hour post-dosing	19.9 (2)	17.6 (3)	42.0 (3)	49.9 (3)
1 hour post-dosing	19.6 (1)	18.7 (3)	37.2 (3)	44.1 (2)
4 hours post-dosing	17.9 (3)	17.5 (3)	35.8** (3)	44.6** (3)
14 hours post-dosing	17.1 (3)	17.3 (3)	15.4 (3)	29.5 (3)
Study Day 91				
Prior to necropsy	17.3 (6)	17.0 (6)	16.4 (6)	22.3 (6)
Study Day 105				
Prior to necropsy	18.0 (2)	17.3 (2)	16.2 (2)	17.2 (2)

Numbers in parentheses represent number of animals evaluated.

Text Table 2: Summary of APTT Values (seconds) - Females

Dose Group (mg/kg/dose)	0	0.2	10	25
Study Day 0				
Prior to Dosing	17.8 (3)	17.2 (3)	16.8 (3)	17.6 (2)
0.5 hour post-dosing	16.3 (3)	17.2 (3)	40.1** (3)	46.3 (2)
1 hour post-dosing	18.6 (3)	17.8 (3)	40.5** (3)	49.1** (3)
4 hours post-dosing	16.8 (3)	18.0 (3)	29.5** (3)	38.4 (2)
14 hours post-dosing	18.0 (3)	16.4 (3)	16.4 (3)	17.5 (3)
Study Day 12				
Prior to Dosing	17.5 (6)	17.2 (6)	16.9 (6)	17.4 (6)
2 hours post-dosing	16.9 (5)	17.7 (5)	36.5** (5)	43.1** (6)
Study Day 28				
Prior to Dosing	17.4 (6)	16.6 (6)	17.0 (6)	16.7 (6)
Study Day 44				
Prior to Dosing	20.1 (3)	17.2 (3)	18.0 (3)	18.6 (3)
0.5 hour post-dosing	17.0 (3)	18.9 (3)	39.5** (3)	44.9** (3)
1 hour post-dosing	19.7 (3)	17.1 (2)	42.7** (3)	50.6** (3)
4 hours post-dosing	17.2 (3)	19.9 (3)	39.6** (3)	44.5** (3)
14 hours post-dosing	18.5 (3)	16.5 (3)	22.7 (3)	26.3 (3)
Study Day 58				
Prior to Dosing	18.3 (6)	17.6 (6)	18.2 (6)	18.3 (6)
2 hours post-dosing	18.7 (6)	18.0 (6)	43.3** (6)	50.3** (6)
Study Day 88				
Prior to Dosing	18.5 (3)	17.1 (3)	18.7 (3)	18.6 (3)
0.5 hour post-dosing	17.4 (3)	18.0 (3)	39.6** (3)	46.9** (3)
1 hour post-dosing	19.0 (3)	18.0 (3)	41.7** (3)	49.3** (3)
4 hours post-dosing	17.8 (3)	17.1 (3)	38.2** (3)	40.8** (3)
14 hours post-dosing	18.1 (3)	16.5 (3)	23.1 (3)	29.0 (3)
Study Day 91				
Prior to necropsy	17.9 (6)	17.8 (6)	20.1 (6)	21.4 (6)
Study Day 105				
Prior to necropsy	17.7 (2)	16.9 (2)	17.1 (2)	16.3 (2)

Numbers in parentheses represent number of animals evaluated.

Gross pathology: There were no test article-related gross findings. The dark red areas at the injection sites were observed in control and treated groups.

Organ weights: There were no test article-related effects on organ weights.

Histopathology: Test article-related and procedure related findings were observed at the injection sites. Test article-related findings consisted of minimal to mild perivascular inflammation at the injection sites that was observed only in the treated groups (see the table listed below). High incidences were observed in the 10 and 25 mg/kg animals. In the 0.2 mg/kg animals, minimal perivascular inflammation was only observed at 1 injection site of one female and each of 3 males. The other injection sites findings including subacute inflammation, granulomatous inflammation, hemorrhage, muscle degeneration and fibroplasia were observed sporadically in control and treated animals without a clear relationship to DX-88, hence, were likely to be secondary effects of the injection procedure.

There were no other significant histopathologic findings indicating a systemic toxicity of DX-88.

Text Table 3: Incidence of Subcutaneous Perivascular Inflammation by Injection Site - Males

Injection site	1	2	3	4	5	6	7	8
No. sites examined/group	4	4	4	4	4	4	4	4
Perivascular inflammation								
Group 1 males	0	0	0	0	0	0	0	0
Group 2 males	0	0	0	1	0	2	0	0
Group 3 males	0	1	2	3	2	2	0	1
Group 4 males	2	3	3	1	3	1	2	0

Text Table 4: Incidence of Subcutaneous Perivascular Inflammation by Injection Site - Females

Injection site	1	2	3	4	5	6	7	8
No. sites examined/group	4	4	4	4	4	4	4	4
Perivascular inflammation								
Group 1 females	0	0	0	0	0	0	0	0
Group 2 females	0	0	0	0	0	1	0	0
Group 3 females	1	1	2	3	4	4	1	1
Group 4 females	1	2	3	2	3	3	2	2

Toxicokinetics: The summary of data was listed below. In general, systemic exposures (C_{max} and AUC) increased with increasing dose levels of 0.2 to 25 mg/kg. No marked (>2.0 fold) gender difference in AUC values was observed. Volume of distribution values indicated extensive distribution into tissues. The antibody positive incidence rates were higher in the 10 and 25 mg/kg groups (see below in the section of immunogenicity). High antibody levels (>26,000 ng/mL) correlated to increased DX-88 concentration in TK parameters. These levels were calculated by groups, with either low (<26,000 ng/mL) or high (>26,000 ng/mL) titers. As shown in the Table A and B below, regardless of dose, TK parameters in animals with low titers were consistent with those observed in SD 0. In animals with a high titer, the clearance decreased, exposure increased and T1/2 increased compared to those with low titers.

Table 1. DX-88 toxicokinetic parameters on Day 0 following a subcutaneous administration of either 10 or 25 mg/kg in Cynomolgus monkeys.

Day 0 Pharmacokinetic Parameters			
	Dose (mg/kg)		
	0.2*	10	25
$t_{1/2}$ (hr)	-	1.48	1.54
Cl (ml/hr/kg)	-	226	143
Vd (ml/kg)	-	481	319
C_{max} (ng/ml)	160	15373	37861
T_{max} (hr)	2.00	1.00	1.00
AUC_{0-INF} (ng*hr/ml)	-	44302	174293
AUC_{0-INF}/dose (ng*hr*kg/ml/mg)	-	4430	6971

*Toxicokinetic data could not be determined in the 0.2 mg/kg treatment group.

Day 44 Pharmacokinetic Parameters		
	Antibody Concentration	
	< 26,000 ng/ml	>26,000 ng/ml
$t_{1/2}$ (hr)	1.74	4.96
Cl (ml/hr/kg)	215	60.9
Vd (ml/kg)	539	436
C_{max} (ng/ml)	7954	19224
T_{max} (hr)	1.0	2.0
AUC_{0-INF} (ng*hr/ml)	46452	164136
AUC_{0-INF}/dose (ng*hr*kg/ml/mg)	4645	16414

Day 88 Pharmacokinetic Parameters		
	Antibody Concentration	
	< 26,000 ng/ml	>26,000 ng/ml
$t_{1/2}$ (hr)	2.99	4.23
Cl (ml/hr/kg)	188	33.9*
Vd (ml/kg)	812	207*
C_{max} (ng/ml)	11762	31309
T_{max} (hr)	2.0	2.0
AUC_{0-INF} (ng*hr/ml)	53080	295407*
AUC_{0-INF}/dose (ng*hr*kg/ml/mg)	5308	29540*

Immunogenicity: Anti-DX-88 antibodies were detected in each dose group as listed below. Higher positive incidence rates were observed in 10 and 25 mg/kg dose groups.

Text Table 7: Incidence of Antibody Positive Response

Study Day	-10	28	58	91	102
Control Group					
Total No. of Animals	10	12	12	11	4
Positive Result	0	0	0	0	0
% Positive	0%	0%	0%	0%	0%
0.2 mg/kg					
Total No. of Animals	12	12	12	11	4
Positive Result	0	0	4	4	1
% Positive	0%	8%	33%	36%	25%
10 mg/kg					
Total No. of Animals	11	12	12	11	4
Positive Result	1	10	11	10	3
% Positive	9%	83%	92%	91%	75%
25 mg/kg					
Total No. of Animals	11	12	12	11	4
Positive Result	11	12	12	11	4
% Positive	0%	100%	100%	100%	100%

Study title: A 6-Month Toxicity Study of DX-88 Administered Subcutaneously Once Every Three Days to Cynomolgus Monkeys With a 28-Day Recovery Period

Key study findings:
The minimal to moderate perivascular mixed inflammatory cell infiltrates at injection sites were observed in main study males and females at 10 and 25 mg/kg dose levels. Transient prolonged APTT was observed in 10 and 25 mg/kg animals, which is an expected pharmacologic effect based on the mechanism of DX-88.
The NOAEL for local toxicity is identified at 0.4 mg/kg while the NOAEL for systemic toxicity could be at 25 mg/kg (high dose).

Study no.: (b)-446020
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: December 13, 2006
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: DX-88 drug product in PBS, pH 7, Lot # 227-07-001, purity, 95.6%
Methods

Doses: 0, 0.4., 10 and 25 mg/kg, administered subcutaneously once every 3 days for up to 180 days, for a total of 60 doses. Doses were administered to each animal using a dosing grid consisting of 8 dose sites tattooed on the dorsoscapular region. Doses were rotated on the 8 dose sites numbered sequentially from 1-8.
Species/strain: Cynomolgus monkeys (*Macaca fascicularis*) originally from Vietnam.
Number/sex/group or time point (main study): See the table below (inserted from page 28 of the report).
Route, formulation, volume, and infusion rate: The route of administration is subcutaneous. The vehicle used in preparation of the test article formulation and for administration to the control group was phosphate-buffered saline (PBS). The stock test article was diluted in vehicle to desired concentrations. Dose volumes are listed in the tables below.

Group Number	Test Article	Dosage Level (mg/kg/dose)[a]	Dosage Volume (mL/kg)	Number of Animals[b] Males	Females
1	Vehicle	0	2.8	6	6
2	DX-88	0.4	1.12	4	4
3	DX-88	10	1.12	4	4
4	DX-88	25	2.8	6	6

[a] = The dosing formulations were not adjusted for purity.
[b] = 4 animals/sex/group were euthanized at the primary necropsy (the day following the final dose for each sex), and the remaining 2 animals/sex in the control and 25 mg/kg/dose groups were euthanized following a minimum 28-day nondosing period (recovery necropsy).

Satellite groups used for toxicokinetics: N/A. TK samples were collected from the main study animals.
Age: 2-4 years at the initiation of dosing.
Weight: 2.0 - 2.9 kg for males and 1.8 – 2.3 kg for females.

Observations and times:

Mortality: Animals were observed twice daily.
Clinical signs: All animals were observed prior to dosing, and approximately 2-3 hours following dosing on dosing days. On non-dosing days, the animals were observed daily. Detailed physical examinations were conducted on all animals weekly.
Body weights: Individual body weights of all animals were recorded weekly beginning approximately 2 weeks prior to initial dosing.
Food consumption: Food consumptions were assessed qualitatively daily and recorded as part of the daily observations.
Ophthalmoscopy: Ocular examinations were conducted prior to initial dosing, near the end of treatment period and near the end of recovery period.
EKG: Multilead ECGs were recorded prior to initial dosing, approximately 1-2 hours post dosing on SD3. Animals were anesthetized with ketamine prior to ECG recording.
Hematology: Blood samples were collected prior to the initial dosing, prior to and approximately 2 hours post dosing on SD 57, 117 and 177, and prior to the recovery necropsy on SD 206 (males)/SD 205 (females).
Clinical chemistry: Blood samples were collected prior to the initial dosing, prior to and approximately 2 hours post dosing on SD 57, 117 and 177, and prior to the recovery necropsy on SD 206 (males)/SD 205 (females).
Urinalysis: Samples were collected prior to the initial dosing, prior to and approximately 2 hours post dosing on SD 57, 117 and 177, and prior to the recovery necropsy on SD 206 (males)/SD 205 (females).
APTT evaluation: Blood samples were collected from all animals prior to dosing and at approximately 0.5, 1, 2, 4, 8, 14 and 24 hours post dosing on SD 6, 33, 93, and 171.
Gross pathology: Full necropsy was performed on 4 monkeys/sex/group at the end of treatment period, and on the remaining two monkeys/sex from control and high dose groups at the end of 28-day recovery period.
Organ weights: See "Histopathology Inventory" table below.
Histopathology: The tissues and organs listed in "Histopathology Inventory" table below were collected from all necropsied animals and placed in 10% neutral-buffered formalin except noted otherwise. Epididymides and testes were fixed in Bouin's solution, and the eyes with optic nerves were fixed in Davidson's solution. The collected tissues/organs were processed and stained with hematoxylin and eosin for microscopic examination. Bone marrow smears were not placed in formalin and the slides were not examined.

Adequate Battery: Yes
Peer review: No

Toxicokinetics: Blood samples (~1. 0 ml) were collected via femoral vein prior to dosing, and at approximately 0.5, 1, 2, 4, 8, 14 and 24 hours post dosing on SD 0, 27, 87, and 177. The monkey plasma samples were analyzed for levels of DX-88 by using a validated ELISA method (validation report No. 7928-107).

Immunogenicity: Blood samples (~1.0 mL) were collected via femoral vein during the pretest period, and prior to each dosing on SD 27, 87 and 177. The plasma samples were analyzed for levels of anti-DX-88 antibodies by using a bridging electrochemiluminescent (ECL) assay formats (validation report No. TNJS-152).

Results

Mortality: All animals survived to the scheduled necropsy.
Clinical signs: There were no test article-related clinical observations.
Body weights: There were no test article-related effects on body weights or body weight changes.
Food consumption: No report.
Ophthalmoscopy: There were no test article-related ophthalmic lesions observed.
EKG: There were no test article-related effects noted in ECG evaluation.
Hematology: There were no test article-related alterations of measured parameters. Some group mean differences between control and treated groups were not considered to be test article-related because the values did not show a dose- or time-dependent response, were of a magnitude that was not toxicologically significant, resembled trends seen in pretest values, and/or occurred at the pretest interval.
Clinical Chemistry: There were no test article-related alterations of measured parameters. Some group mean differences between control and treated groups were not considered to be test article-related because the values did not show a dose- or time-dependent response, were of a magnitude that was not toxicologically significant, involved a change in a direction of no biological significance, and/or resembled trends seen in pretest values.
Urinalysis: There were no test article-related alterations of measured parameters.
APTT evaluation: Prolongation of APTT values was observed at 2 hours post dosing on SD 57, 117 and 177 in the males and females treated with 10 and 25 mg/kg DX-88. More extensive APTT test on SD 6, 33, 93 and 171 showed that APTT was prolonged in 10 mg/kg (up to 63 seconds, +136% comparing to the control) and/or 25 mg/kg (up to 67 seconds, +149%) males and females at 0.5, 1, 2, 4 and 8 hours post dosing. The tables below (excerpted and extracted from pages 229 and 237 of the report) present the APTT values at 0.5 hr post dosing on the designated SDs. In all cases, the mean APTT levels returned to pretest levels by 24 hours post dosing while on some occasions, the APTT levels in 10 mg/kg animals returned to the pretest values earlier than 24 hours post dosing. The prolongation of APTT is an expected pharmacologic effect of DX-88 and was considered indicative of DX-88 exposure.

Males

ANALYSIS	GROUP:	0 MG/KG/DOSE	0.4 MG/KG/DOSE	10 MG/KG/DOSE	25 MG/KG/DOSE
0.5POST APTT (seconds)					
DAY 6 MEAN		27.0	26.3	56.0**	62.5**
% DIFFERENCE			-2.6	107.4	131.5
DAY 33 MEAN		26.9	27.3	62.2**	64.1**
% DIFFERENCE			1.5	131.2	138.3
DAY 93 MEAN		19.6	20.0	43.2**	50.2**
% DIFFERENCE			2.0	120.4	156.1
DAY 171 MEAN		21.2	20.8	44.0**	55.6**
% DIFFERENCE			-1.9	107.5	162.3

Females

ANALYSIS GROUP:	0 MG/KG/DOSE	0.4 MG/KG/DOSE	10 MG/KG/DOSE	25 MG/KG/DOSE
0.5POST APTT (seconds)				
DAY 6 MEAN	27.4	27.8	56.7**	58.5**
% DIFFERENCE		1.5	106.9	113.5
DAY 33 MEAN	26.8	27.2	63.2**	65.0**
% DIFFERENCE		1.5	135.8	142.5
DAY 93 MEAN	21.7	22.3	48.4**	49.6**
% DIFFERENCE		2.8	123.0	128.6
DAY 171 MEAN	21.0	21.6	48.8**	51.5**
% DIFFERENCE		2.9	132.4	145.2

Gross pathology: There were no test article-related gross findings.
Organ weights: There were no test article-related effects on organ weights.
Histopathology: The test article-related findings at the injection sites are listed in the tables below (excerpted from page 45-48 of the report). The minimal to moderate perivascular mixed inflammatory cell infiltrates at injection sites were observed in main study males and females at 10 and 25 mg/kg dose levels with higher incidence and/or severity comparing to the controls. A low incidence of minimal perivascular mixed inflammatory cell infiltrates was also observed in 0.4 mg/kg main animals which was comparable to the controls. The other observations at the injection sites including acute inflammation, foreign body, muscle degeneration or regeneration, fat necrosis, hemorrhage, fibrosis, and granulomatous inflammation (focal) were noted cross control and treated groups with low incidence and minimal severity, hence, were considered general injection procedure-related findings.

a. Table of Selected Injection Site Findings in Main Animals

	Males				Females			
Dosage (mg/kg/dose):	0	0.4	10	25	0	0.4	10	25
Injection site 1 *	4	4	4	4	4	4	4	4
Infiltrate, mixed inflammatory cell (perivascular)	0	0	0	2	0	0	3	1
Minimal	-	-	-	2	-	-	3	1
Injection site 2 *	4	4	4	4	4	4	4	4
Infiltrate, mixed inflammatory cell (perivascular)	2	2	1	3	0	0	3	1
Minimal	2	2	1	3	-	-	2	1
Mild	-	-	-	1	-	-	1	-

	Males				Females			
Dosage (mg/kg/dose):	0	0.4	10	25	0	0.4	10	25
Injection site 3 *	4	4	4	4	4	4	4	4
Infiltrate, mixed inflammatory cell (perivascular)	1	1	1	3	0	0	4	4
Minimal	1	1	1	2	-	-	2	4
Mild	-	-	-	1	-	-	1	-
Moderate	-	-	-	-	-	-	1	-
Injection site 4 *	4	4	4	4	4	4	4	4
Infiltrate, mixed inflammatory cell (perivascular)	0	0	1	2	1	0	2	1
Minimal	-	0	1	1	1	-	2	1
Mild	-	-	-	1	-	-	-	-
Injection site 5 *	4	4	4	4	4	4	4	4
Infiltrate, mixed inflammatory cell (perivascular)	0	0	2	3	0	0	3	3
Minimal	-	-	2	3	-	-	3	3
Injection site 6 *	4	4	4	4	4	4	4	4
Infiltrate, mixed inflammatory cell (perivascular)	0	0	1	2	0	1	2	1
Minimal	-	-	1	1	-	1	2	1
Mild	-	-	-	1	-	-	-	-
Injection site 7 *	4	4	4	4	4	4	4	4
Infiltrate, mixed inflammatory cell (perivascular)	1	1	1	2	0	0	4	2
Minimal	1	1	-	1	-	-	2	1
Mild	-	-	1	1	-	-	2	1
Injection site 8 *	4	4	4	4	4	4	4	4
Infiltrate, mixed inflammatory cell (perivascular)	0	1	3	3	0	0	3	2
Minimal	-	1	3	1	-	-	2	1
Mild	-	-	-	2	-	-	-	1
Moderate	-	-	-	-	-	-	1	-

* = Number of tissues examined from each group.

b. Table of Selected Injection Site Findings in Recovery Animals

	Males				Females			
Dosage (mg/kg/dose):	0	0.4	10	25	0	0.4	10	25
Injection site 1 *	2	0	0	2	2	0	0	2
Infiltrate, mixed inflammatory cell (perivascular)	0	-	-	0	0	-	-	1
Minimal	-	-	-	-	-	-	-	1
Injection site 2 *	2	0	0	2	2	0	0	2
Infiltrate, mixed inflammatory cell (perivascular)	0	-	-	0	0	-	-	1
Minimal	-	-	-	-	-	-	-	1
Injection site 3 *	2	0	0	2	2	0	0	2
Infiltrate, mixed inflammatory cell (perivascular)	0	-	-	0	0	-	-	1
Minimal	-	-	-	-	-	-	-	1
Injection site 4 *	2	0	0	2	2	0	0	2
Infiltrate, mixed inflammatory cell (perivascular)	0	-	-	1	1	-	-	1
Minimal	-	-	-	1	1	-	-	1
Injection site 5 *	2	0	0	2	2	0	0	2
Infiltrate, mixed inflammatory cell (perivascular)	0	-	-	2	0	-	-	1
Minimal	-	-	-	2	-	-	-	1
Injection site 6 *	2	0	0	2	2	0	0	2
Infiltrate, mixed inflammatory cell (perivascular)	0	-	-	0	1	-	-	2
Minimal	-	-	-	-	1	-	-	2
Injection site 7 *	2	0	0	2	2	0	0	2
Infiltrate, mixed inflammatory cell (perivascular)	0	-	-	1	0	-	-	1
Minimal	-	-	-	1	-	-	-	1
Injection site 8 *	2	0	0	2	2	0	0	2
Infiltrate, mixed inflammatory cell (perivascular)	0	-	-	0	0	-	-	1
Minimal	-	-	-	-	-	-	-	1

* = Number of tissues examined from each group.

Other observed histopathological findings (some are listed in the table below) were likely to be incidental, spontaneous or related to the experimental procedure or physiological status (sexual immature) since the findings were observed in controls or only in single animal, with low severity or lacked dose-dependent pattern. There were no significant histopathologic changes indicating a test article-related systemic toxicity.

Findings		Males				Females			
Dose (mg/kg/)		Ctl.	0.4	10	25	Ctl.	0.4	10	25
Number of animals	Main	4	4	4	4	4	4	4	4
	Rec	2	0	0	2	2	0	0	2
Kidney, infiltrates with mixed inflammatory cells	Main	3(1)	2(1)	3(1)	4(1)	2(1)	3(1)	**3(1)** **1(3)**	**2(1)** **1(2)**
	Rec	1(1)	N/A	N/A	1(1)	2(1)	N/A	N/A	**1(1)** **1(2)**
Heart, infiltrates with mixed inflammatory cells	Main	2(1)	2(1)	2(1)	1(1)	1(1)	2(1)	2(1) 1(2)	0
	Rec	1(1)	N/A	N/A	2(1)	1(1)	N/A	N/A	2(1)
Salivary gland, lymphocyte infiltration	Main	3(1)	4(1)	3 (1)	3 (1)	1(1)	3(1)	**0**	3 (1)
	Rec	2(1)	N/A	N/A	2(1)	1(1)	N/A	N/A	2(1)
Skeletal muscle, necrosis	Main	0	0	0	0	0	**0**	0	**1(2)**
	Rec	0	N/A	N/A	0	0	N/A	N/A	0

The number in the parentheses indicates the level of severity: 1=minimal, 2= mild/slight, 3= moderate. Ctl.= control, Rec. = recovery

Toxicokinetics: The toxicokinetic data was summarized in the table below (inserted from pages 1770 to 1773 of the report).

Summary of the Mean Toxicokinetic Parameters of DX-88 in Male and Female Monkeys: Day 0

Group	Dose Level (mg/kg/dose)	Sex		C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/dose)]	T_{max} (hr)	AUC_{0-t} (ng·hr/mL)	AUC_{0-24} (ng·hr/mL)	DN AUC_{0-24} [(ng·hr/mL)/(mg/kg/dose)]	$AUC_{0-\infty}$ (ng·hr/mL)	DN $AUC_{0-\infty}$ [(ng·hr/mL)/(mg/kg/dose)]	$t_{1/2}$ (hr)	CL/F (mL/hr/kg)
2	0.4	M	Mean	350	875	1.50	963	1219	3046	NA	NA	NA	NA
			SD	71	NA	0.58	102	135	NA	NA	NA	NA	NA
			N	4	NA	4	4	4	NA	0	NA	0	0
		F	Mean	371	928	1.00	1003	1264	3159	NA	NA	NA	NA
			SD	61	NA	0	132	204	NA	NA	NA	NA	NA
			N	4	NA	4	4	4	NA	0	NA	0	0
3	10	M	Mean	8235	824	0.750	30407	31259	3126	31044	3104	1.81	344
			SD	1620	NA	0.289	9221	8801	NA	8792	NA	0.27	105
			N	4	NA	4	4	4	NA	4	NA	4	4
		F	Mean	7753	775	0.500	26716	28240	2824	28286	2829	2.33	371
			SD	2379	NA	0	6023	6246	NA	6413	NA	0.37	102
			N	4	NA	4	4	4	NA	4	NA	4	4
4	25	M	Mean	20833	833	0.750	87287	88829	3553	88334	3533	1.96	293
			SD	7543	NA	0.274	15938	16532	NA	16452	NA	0.49	62
			N	6	NA	6	6	6	NA	6	NA	6	6
		F	Mean	22967	919	0.833	81362	83164	3327	82534	3301	1.87	304
			SD	11047	NA	0.258	4942	4914	NA	4832	NA	0.64	18
			N	6	NA	6	6	6	NA	6	NA	6	6

Summary of the Mean Toxicokinetic Parameters of DX-88 in Male and Female Monkeys: Day 27

Group	Dose Level (mg/kg/dose)	Sex		C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/dose)]	T_{max} (hr)	AUC_{0-t} (ng·hr/mL)	AUC_{0-24} (ng·hr/mL)	DN AUC_{0-24} [(ng·hr/mL)/(mg/kg/dose)]	$t_{1/2}$ (hr)	CL/F (mL/hr/kg)
2	0.4	M	Mean	382	955	1.50	1061	1322	3305	NA	NA
			SD	66	NA	0.58	126	112	NA	NA	NA
			N	4	NA	4	4	4	NA	0	0
		F	Mean	407	1018	1.25	1113	1435	3588	NA	NA
			SD	71	NA	0.50	187	254	NA	NA	NA
			N	4	NA	4	4	4	NA	0	0
3	10	M	Mean	10465	1047	1.25	46525	47919	4792	2.29	271
			SD	2422	NA	0.87	30306	29641	NA	1.42	151
			N	4	NA	4	4	4	NA	3	3
		F	Mean	7775	778	0.750	30163	31376	3138	2.41	322
			SD	937	NA	0.289	3989	3497	NA	0.53	38
			N	4	NA	4	4	4	NA	4	4
4	25	M	Mean	18933	757	0.833	97855	100224	4009	2.53	256
			SD	2855	NA	0.258	18094	17942	NA	0.53	49
			N	6	NA	6	6	6	NA	6	6
		F	Mean	17550	702	1.00	102435	103858	4154	2.81	265
			SD	4690	NA	0.55	36790	36126	NA	1.13	93
			N	6	NA	6	6	6	NA	6	6

Summary of the Mean Toxicokinetic Parameters of DX-88 in Male and Female Monkeys: Day 87

Group	Dose Level (mg/kg/dose)	Sex		C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/dose)]	T_{max} (hr)	AUC_{0-t} (ng·hr/mL)	AUC_{0-24} (ng·hr/mL)	DN AUC_{0-24} [(ng·hr/mL)/(mg/kg/dose)]	$t_{1/2}$ (hr)	CL/F (mL/hr/kg)
2	0.4	M	Mean	336	839	1.50	1290	1630	4075	NA	NA
			SD	77	NA	0.58	770	863	NA	NA	NA
			N	4	NA	4	4	4	NA	0	0
		F	Mean	382	956	1.38	1514	1805	4512	1.80	314
			SD	79	NA	0.75	641	626	NA	NA	NA
			N	4	NA	4	4	4	NA	1	1
3	10	M	Mean	10155	1016	1.00	69300	70248	7025	3.47	231
			SD	3100	NA	0	70673	70068	NA	2.59	130
			N	4	NA	4	4	4	NA	4	4
		F	Mean	7380	738	0.875	39684	39839	3984	3.99	249
			SD	843	NA	0.250	5165	4856	NA	1.97	39
			N	4	NA	4	4	4	NA	4	4
4	25	M	Mean	27400	1096	1.00	242474	266228	10649	6.31	140
			SD	17458	NA	0.55	167073	204281	NA	3.82	93
			N	6	NA	6	6	6	NA	6	6
		F	Mean	19667	787	1.42	196149	196579	7863	5.71	158
			SD	6949	NA	0.66	98097	97471	NA	3.01	102
			N	6	NA	6	6	6	NA	6	6

Summary of the Mean Toxicokinetic Parameters of DX-88 in Male and Female Monkeys: Day 177

Group	Dose Level (mg/kg/dose)	Sex		C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/dose)]	Tmax (hr)	AUC_{0-t} (ng·hr/mL)	AUC_{0-24} (ng·hr/mL)	DN AUC_{0-24} [(ng·hr/mL)/(mg/kg/dose)]	$t_{1/2}$ (hr)	CL/F (mL/hr/kg)
2	0.4	M	Mean	375	936	2.00	2572	2826	7065	6.90	52.3
			SD	160	NA	0	2901	2727	NA	NA	NA
			N	4	NA	4	4	4	NA	1	1
		F	Mean	491	1228	1.75	2199	2579	6446	2.92	222
			SD	175	NA	0.50	1014	1150	NA	NA	NA
			N	4	NA	4	4	4	NA	1	1
3	10	M	Mean	14075	1408	0.750	99181	99425	9942	4.00	154
			SD	3654	NA	0.289	88914	88708	NA	2.77	92
			N	4	NA	4	4	4	NA	4	4
		F	Mean	8695	870	1.25	68622	68861	6886	5.18	151
			SD	2150	NA	0.50	20729	20300	NA	3.76	64
			N	4	NA	4	4	4	NA	4	4
4	25	M	Mean	26283	1051	1.33	302441	302441	12098	5.23	129
			SD	10324	NA	0.52	196847	196847	NA	2.21	70
			N	6	NA	6	6	6	NA	5	5
		F	Mean	23883	955	1.67	254302	255034	10201	5.76	123
			SD	9901	NA	0.52	154254	153374	NA	2.23	72
			N	6	NA	6	6	6	NA	6	6

In general, systemic exposures (C_{max} and AUC) increased with increase in dose levels of 0.4 to 25 mg/kg. Values of C_{max} and AUC0-24hr were generally greater on SDs 27, 87 and 177 than SD 0, indicating an increase in exposure after repeating dose. No marked (>2.0 fold) gender difference in AUC values was observed.

Immunogenicity: Anti-DX-88 antibodies were detected in each dose group as listed below. All animals were positive in 10 and 25 mg/kg dose groups. In another monkey study (b)-446010 (see review above), presence of anti-DX-88 antibodies at a concentration of 26,000 ng/mL or greater resulted in an increased exposure to DX-88 and a reduction in DX-88 clearance. Apparently, there was a trend of increase in exposure to DX-88 and reduction in CL/F observed in the current study from 10 mg/kg to 25 mg/kg dose levels.

Dose (mg/kg)	N	Day 27	Day 87	Day 177
0.4	8	2	5	5
10	8	7	8	8
25	8	8	8	8

Studies conducted using the Intravenous Route of Administration

(Note: the following 3 toxicity studies were not conducted with the relevant route of administration. They were reviewed primarily to assess the potential systemic toxicity profile.)

Study title: DX-88: 14-day Intravenous Injection Toxicity Study in Sprague-Dawley Rats

Key study findings:

Rats were administered IV doses of 0, 2.3, 6.8 and 23 mg/kg/day.
No significant test article-related toxicity was observed.
The NOAEL is identified at 23 mg/kg (AUC0-360 min = 4509 µg.min/mL for males or 4373 µg.min/mL for females on Day 14).

Study no. (b) 1574
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: November 29, 1999
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: DX-88 in PBS, pH 7.0, Lot # 00799DXP, purity, ~97.7%, concentration 10.1 mg/mL
Methods

Doses: 0, 2.3, 6.8 and 23 mg/kg, administered intravenously once daily for 14 consecutive days.
Species/strain: Rats/Sprague Dawley, from (b) (4)

Number/sex/group or time point (main study): See the table for Toxicology Groups below (inserted from page 2 of the report)

Treatment Groups	Dose Level (mg/kg/day)	Dose Conc. (mg/mL)	Toxicology Phase		Toxicokinetic Phase	
			Males	Females	Males	Females
1. Control *	0	0	8	8	0	0
2. Low Dose	2.3	0.5	8	8	6	6
3. Mid Dose	6.8	1.48	8	8	6	6
4. High Dose	23	5.0	8	8	6	6

*The control rats received the vehicle at the same dose volume as that used for treated rats. The dose volume was 4.6 mL/kg BW for all rats.

Route, formulation, volume, and infusion rate: The route of administration is subcutaneous. The control/vehicle was 0.9% NaCl for Injection, USP. The stock

test article was diluted in vehicle to achieve the desired concentration. Dose volume is listed in the tables above.
Satellite groups used for toxicokinetics: As shown in the table above, n=6/sex/group for test article-treated groups were used for toxicokinetics evaluation.
Age: Approximately 7-12 weeks at initiation of dose administration.
Weight: 222-281 g for males and 205-247 g for females;
Unique study design or methodology (if any): N/A

Observations and times:

Mortality: All animals were observed once daily.
Clinical signs: For toxicology groups, clinical signs were observed once daily. Observations for TK animals were not reported but retained in the raw data.
Body weights: Individual body weights of all animals were recorded approximately 1 week prior to initial dosing and recorded weekly beginning on Day 1. Terminal body weights (fasted) were recorded prior to the necropsy.
Food consumption/appetite: Food consumptions were assessed once during the pretreatment period and twice throughout the treatment period.
Ophthalmoscopy: Ocular examinations were conducted once prior to initial dosing, and once at the termination.
EKG: not measured.
Hematology: Blood samples were collected at the termination.
Clinical chemistry: Blood samples were collected at the termination.
Urinalysis: Samples were collected at the termination.
Gross pathology: At the end of treatment period, full necropsy was performed on toxicology animals at the scheduled termination.
Organ weights: See "Histopathology Inventory" table below.
Histopathology: The tissues and organs listed in "Histopathology Inventory" table below were collected from all necropsied animals and placed in 10% neutral-buffered formalin except noted otherwise. Epididymides, testes, and the eyes with optic nerves were fixed in Zenker's solution. The collected tissues/organs from the control and high dose toxicology groups and gross lesions from all animals were processed and stained with hematoxylin and eosin/phloxine for microscopic examination. Parathyroids and optic nerves were only examined if present in routine sections.

Adequate Battery: Yes
Peer review: No

Toxicokinetics: Blood samples (~0.6 mL) were collected at approximately 5, 15, 45, and 360 minutes post dosing of Dose 1(Day 1) and Dose 14 (Day 14). The serum samples were analyzed for levels of DX-88 using a validated HPLC/MS method by (b) (4)

Results

Mortality: One female in the high dose group was found dead on Day 3 and was replaced by a spare female. It was considered an accidental death. All other animals survived to the scheduled necropsy.

Clinical signs: There were no test article-related clinical observations.
Body weights: There were no test article-related effects on body weights.
Food consumption: There were no test article-related effects on food consumption.
Ophthalmoscopy: There were no test article-related ophthalmic lesions observed. .
EKG: not conducted.
Hematology: There were no test article-related alterations of measured parameters. There were no significant effects on the measured APTT and PT values.
Clinical Chemistry: There were no significant test article-related adverse effects on the measured parameters.
Urinalysis: There were no test article-related alterations of measured parameters.
Gross pathology: There were no test article-related gross findings.
Organ weights: There were no test article-related effects on organ weights. The slight increase in liver/body weight (+16%) of high dose females and in adrenal/body weight (+17) of mid dose females was not considered toxicologically significant.
Histopathology: There were no significant test article-related microscopic findings indicative of toxicity of DX-88.
Toxicokinetics: The summary of data was listed below (excerpted from appendix IV, Table 4). Plasma DX-88 concentration decreases biphasically with a rapid distribution phase having a half life (α1/2) of approximately 13 - 19 minutes. The terminal elimination half lift (β1/2) could not be determined. No significant concentrations of DX-88 were detected in all animals at 360 min time points, indicating a rapid elimination without significant accumulation. Both Cmax and $AUC_{(0\text{-}360min)}$ seem to increase in a dose proportional fashion. The clearance (Cl) and volume of distribution at steady state (Vss) is found to be larger in males than in females.

Table 4. Pharmacokinetic Parameters normalized to 2.3 mg/kg/day

Day 1

Males			Females	
Dose (mg/kg/day)	Cmax (µg/mL)	$AUC_{(0\text{-}360\ min)}$ (µg.min/mL)	Cmax (µg/mL)	$AUC_{(0\text{-}360\ min)}$ (µg.min/mL)
2.3	7.30	389.25	7.20	469.50
6.8	8.50	349.14	8.90	454.42
23.0	7.50	420.93	8.60	450.68

Day 14

Males			Females	
Dose (mg/kg/day)	Cmax (µg/mL)	$AUC_{(0\text{-}360\ min)}$ (µg.min/mL)	Cmax (µg/mL)	$AUC_{(0\text{-}360\ min)}$ (µg.min/mL)
2.3	8.50	410.25	6.70	475.75
6.8	7.90	368.51	9.30	480.13
23.0	7.40	450.85	9.60	437.33

Study title: A 14-day Intravenous Toxicity Study of DX-88 in Rats

Key study findings: