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APPLICATION NUMBER:

022150Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D.
OND IO
NDA: 22-150
Date NDA received: 26 October 2007 (original) 25 February 2011 (resubmission)
Drug: icatibant acetate
Sponsor: Jerini U.S. Inc.
Indication: Treatment of hereditary angioedema in patients 18 years old and older
Reviewing Division: Division of Pulmonary, Allergy and Rheumatology Products

Comments:
The pharmacology/toxicology information submitted in this NDA was originally considered adequate to support the use of the product for up to 13 weeks duration. Pharmacology/toxicology data supporting longer term use was not submitted. Chronic toxicity study reports were provided in the resubmission. The pharm/tox reviewer and team leader reviewed these new studies and found the information adequate to support approval for the above indication including for prolonged intermittent use.

The division also recommended that carcinogenicity information be submitted to support the prolonged intermittent use of this product. The division previously indicated that they are willing to permit submission of the carcinogenicity studies post-approval based on the severity of the indication and the results of the studies reviewed to date. Therefore, the reviewer and team leader have recommended that carcinogenicity studies be submitted as post-marketing requirements.

Several impurities required qualification or lowering to levels below those requiring qualification. The sponsor was able to comply with these requirements. Several other impurities still require identification and characterization. This is primarily a CMC issue and may be handled with a post-marketing commitment.

Conclusions:
I concur that the nonclinical information submitted are adequate to support the proposed indication and that the NDA can be approved from a pharm/tox perspective. I agree that the carcinogenicity studies can be submitted as post-marketing requirements.

I have reviewed draft labeling and provided comments to the reviewer and team leader. The draft labeling of Aug. 16, 2001 appears acceptable.

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

/s/

PAUL C BROWN
08/19/2011

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

PHARMACOLOGY/TOXICOLOGY NDA CHEMISTRY CONSULT ADDENDUM

Application number:	22-150 (Amendment 0018)
Supporting document/s:	CTD Section 1 and 4
Applicant's letter date:	Original Submission: 2/25/2011
	Draft Report: 7/1/2011 (Sent by email)
	Final Report: 7/29/2011 (added to eCTD)
CDER stamp date:	Original Submission: 2/25/2011
	Draft Report: 7/1/2011 (Sent by email)
	Final Report: 8/01/2011 (added to eCTD)
Product:	icatibant/Firazyr® (Bradykinin B2 Receptor Antagonist)
Indication:	Treatment of Hereditary Angioedema (HAE)
Applicant:	Shire/Jerini U.S. Inc.
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Hans Rosenfeldt, Ph.D.
Supervisor/Team Leader:	Timothy Robison, Ph.D., D.A.B.T.
Division Director:	Badrul A. Chowdhury, M.D., Ph.D.
Project Manager:	LT Eunice Chung-Davies, PharmD.

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1 Executive Summary

1.1 Introduction

Shire has submitted this NDA amendment as a Complete Response to a Not Approvable letter sent by the FDA on April 23, 2008. The pharmacology, safety pharmacology, pharmacokinetics, single-dose and repeat-dose toxicology, genetic toxicology, carcinogenicity, reproduction toxicology, and local tolerance of icatibant are reviewed in the primary review of this NDA Amendment. Further, a chemistry consult addressed most of the issues regarding the safety evaluation of impurities in the Firazyr® drug product. The present addendum evaluates a signed final report of kidney histopathology results that define the NOAEL (b) (4) in a 13-week rat toxicology study (JE049-0171).

1.2 Brief Discussion of Nonclinical Findings

For summaries as well as detailed information regarding the pharmacology, safety pharmacology, pharmacokinetics, single-dose and repeat-dose toxicology, genetic toxicology, carcinogenicity, reproduction toxicology, and local tolerance of icatibant, please refer to the primary review of this NDA amendment. For summaries and detailed information regarding the issues surrounding impurities, please see the chemistry consult review. The data submitted with the final signed submission of the kidney histopathology results in response to an Information Request sent by the Division on 5/12/2011 are reviewed herein. The data in this final submission are identical to the data submitted earlier and reviewed in the chemistry consult review.

1.3 Recommendations

1.3.1 Approvability

Most impurity issues were resolved in communications between the review team and the sponsor. Outstanding issues do not appear to impact approvability of the application as they will be dealt with a PMC.

1.3.2 Additional Non Clinical Recommendations

None

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1.3.3 Labeling

Not applicable to this consult review.

2 Drug Information

2.1 Drug

CAS Registry Number: Not provided

Generic Name: icatibant acetate

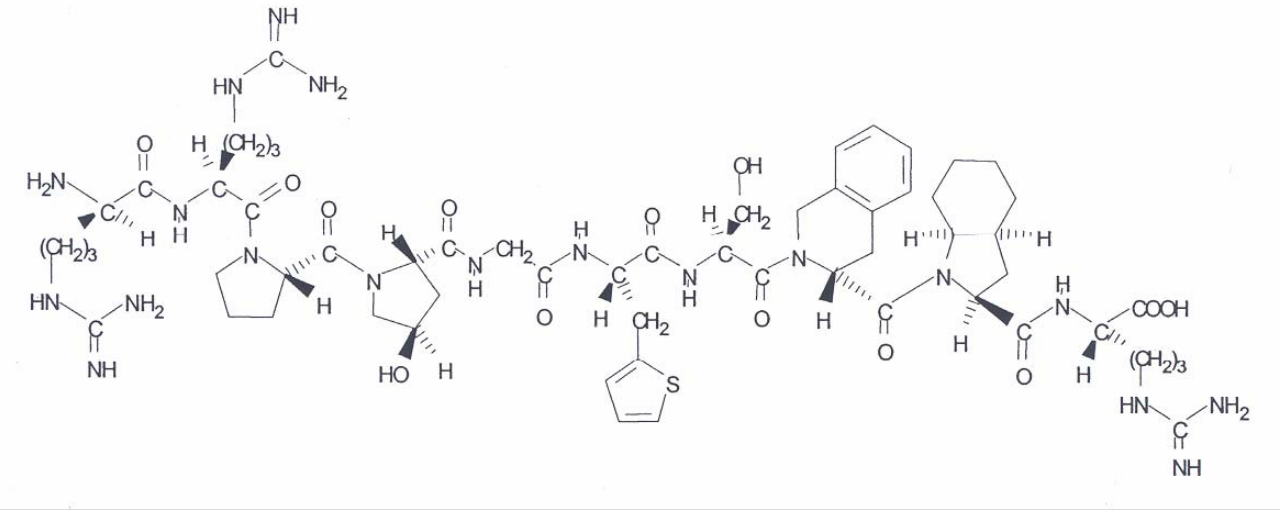
Code Name: JE049/HOE140/S890140

Chemical Name:

D-arginyl-L-arginyl-L-prolyl-L[(4R)-4-hydroxyprolyl]-glycyl-L-[3-(2-thienyl)alanyl]-L-seryl-D-(1,2,3,4-tetrahydroisoquinolin-3-yl-carbonyl)-L-3aS,-7aS)-octahydroindol-2-yl-carbonyl]-L-arginine acetate

Molecular Formula/Molecular Weight: $C_{59}H_{89}N_{19}O_{13} \cdot C_2H_4O_2$/1304.6 g/mol

Structure:



Pharmacologic Class

Bradykinin type 2 (B2) receptor antagonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND	Drug	Indication
068214	Icatibant	• Subcutaneous administration for HAE
[redacted]		(b) (4)

DMF	Material	Function
(b) (4)	Icatibant	• Active ingredient
[redacted]		(b) (4)

2.3 Drug Formulation

Icatibant 30 mg solution for injection is delivered as a 10 mg/mL sterile, isotonic, acetate-buffered solution. The following table provides the formulation of icatibant for subcutaneous injection.

Ingredient	Quantity per mL	Function
Icatibant	10.0 mg	Active ingredient
Sodium hydroxide, NF/Ph.Eur.	[redacted]	(b) (4)
Glacial acetic acid, USP/Ph. Eur.		
Sodium chloride, USP/Ph. Eur.		
Water for injection, USP/Ph. Eur.		

2.4 Comments on Novel Excipients

The sponsor did not include any novel excipients in the proposed drug formulation.

2.5 Comments on Impurities/Degradants of Concern

The sponsor submitted study #JE049-0171, a toxicology study [redacted] (b) (4) in order to qualify this degradant at the (b) (4) level in the proposed drug product. This study is reviewed in the chemistry consult review.

2.6 Proposed Clinical Population and Dosing Regimen

Clinical Population: adult patients who experience acute attacks of hereditary angioedema (HAE).

Dosing Regimen: 30 mg injected subcutaneously in the abdominal area. If response is inadequate or symptoms recur, additional injections of 30 mg may be administered at intervals of at least 6 hours. No more than 3 injections may be administered in 24 hours. Patients may self-administer upon recognition of an HAE attack.

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11 Integrated Summary and Safety Evaluation

Shire (formerly Jerini US, Inc.) has re-submitted this NDA 22-150 for Firazyr (icatibant) 30 mg, SC for the treatment of adults (≥ 18 years old) with hereditary angioedema (HAE). On 4/23/2008, FDA/ODE2 sent the sponsor a Not Approvable letter for the original submission of this NDA. This letter listed the following deficiencies involving Pharmacology/Toxicology: (1) the need for 6-month rat and 9-month dog general toxicology studies and (2) a list of impurities in the drug substance and drug product that need to be lowered to less than (b) (4) alternatively, an adequate 3 month repeat-dose toxicology study that provides a >10 fold Safety Margin should be submitted. The proposed dosing regimen is a single SC dose of 30 mg icatibant (0.6 mg/kg for a 50 kg human), with a maximum of 3 injections spaced every 6 h (90 mg; 1.8 mg/kg for a 50 kg human) allowed in one 24 h period if response is inadequate or symptoms recur.

In support of this NDA, the sponsor has submitted studies or has ongoing studies addressing the pharmacology, safety pharmacology, pharmacokinetics, single-dose and repeat-dose toxicology, genetic toxicology, carcinogenicity, reproduction toxicology, local tolerance, and impurity qualification of icatibant. For summaries and detailed information regarding the issues surrounding impurities, please see the chemistry consult review. For all other issues, please refer to the primary review of this NDA amendment. This addendum to the chemistry consult review evaluates the data included in the final signed kidney histopathology results submitted in response to an Information Request sent by the Division on 5/12/2011. These data are identical to the data evaluated in the chemistry consult review and are important because they allow for the establishment of a NOAEL for (b) (4) an impurity present in the drug product.

During a pre-NDA meeting held on January 24, 2007, discussion between the Division and the sponsor indicated that, given the peptide nature of icatibant the following qualification/identification criteria were more appropriate than the traditional ICH-Q3B/C path:

1. Any peptide-related impurity (b) (4) should be fully identified, characterized and qualified.
2. Any peptide-related impurity (b) (4) should be fully identified and characterized.
3. Any peptide-related impurity above (b) (4) should be minimally identified and characterized

Thus, it is these impurity standards that were applied to the current submission.

(b) (4)

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The Not Approvable letter of 4/23/2008 listed a set of impurities that required acceptance criteria (b) (4) or a 3-month toxicology study to qualify them:

(b) (4)

In the current submission, the sponsor chose to reduce all of the above impurities (b) (4), with the exception of the degradant (b) (4) The sponsor submitted a 13-week toxicology study (b) (4) to support an acceptance criterion of NMT (b) (4) (b) (4)

Study JE049-0171 tested doses of 0, (b) (4) 2, and 10 mg/kg/day (b) (4) in Wistar rats and is reviewed in detail in chemistry consult. This study identified the kidney as a target organ of (b) (4) toxicity. Focal cortical sclerosis in the kidney was detected in 2/10 males treated at the 2 mg/kg/day dose level and 2/10 males and 1/10 females treated with 10 mg/kg/day dose level. There was also increased incidence and severity of mineral deposits in the inner and outer medulla stripes in female rats at 10 mg/kg/day. Males at all dose levels showed decreases in white blood cell and lymphocyte counts, but there was no dose response. No other significant differences in body weight, food consumption, clinical chemistry, hematology, and opthalmoscopy were identified. Initially, a NOAEL could not be established because sponsor histopathology slides for the kidney were not read at the (b) (4) and 2 mg/kg/day dose levels. Draft histopathology results were provided July 1, 2011. A final signed response dated July 29, 2011 to an Information Request from the Division, dated May 12, 2011, indicates that the NOAEL is (b) (4) mg/kg/day based on a focal cortical sclerosis in the kidney observed at the 2 mg/kg dose level (Table 1).

Table 1. Histopathology Data from Signed Response to IR sent May 12, 2011

Microscopic Signs		Control		(b) (4)		2 mg/kg/day		10 mg/kg/day	
		M	F	M	F	M	F	M	F
	Group Size:	10	10	10	10	10	10	10	10
	Grade	Terminal							
Focal cortical sclerosis	**Min**	--	--	--	--	2	--	2	1
Mineral deposits (inner and outer medulla)	**Min**	--	3	--	1	--	3	--	2
	Mild	--	--	--	--	--	--	--	5
	All	--	3	--	1	--	3	--	7

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This NOAEL provides an appropriate Safety Margin for clinical dosing:

Table 2. Safety Margins from (b) (4) Qualification Study (JE049-0171)

Study	Species	Schedule	NOAEL (mg/kg/day)	Safety Margin Based on 30 mg*	Safety Margin Based on 90 mg**
JE049-0171	Rat	SC Daily;13 weeks	(b) (4)	33	11

*Dose at (b) (4) = 0.6 mg = 0.012 mg/kg for a 50 Kg human
**Dose at (b) (4) = 1.8 mg = 0.036 mg/kg for a 50 Kg human

Thus, the sponsor has now submitted enough information to qualify (b) (4) at NMT (b) (4).

Unresolved Impurity Qualification/Identification Issues:

The sponsor has not identified the structures of impurities that are present at levels (b) (4). The sponsor needs to propose a clear standard for "minimal characterization." The Division will issue a PMC dealing with these issues.

12 Appendix/Attachments

Submission of Draft Kidney Histopathology Data by email

From: Chung-Davies, Eunice
Sent: Friday, July 01, 2011 11:56 AM
To: Rosenfeldt, Hans
Cc: Robison, Timothy W
Subject: FW: NDA 22-150 Updated Response to 12 May 2011 Information Request

Hello Hans,

Please see the updated response from the sponsor with regard to question 2. They will provide the entire response (with the final report) in a formal amendment to the NDA. They are waiting on a signature for the final signed report but I had told them to give us whatever they have for now.

Please let me know if you have any questions.

Thanks,
Eunice

Reference ID: 2983405

From: Class, Thomas [mailto:tclass@shire.com]
Sent: Friday, July 01, 2011 11:48 AM
To: Chung-Davies, Eunice
Subject: NDA 22-150 Updated Response to 12 May 2011 Information Request

Hi Eunice,

Attached please find a copy of our updated response to question number 2 from the 12 May 2011 request for information and a histopathology report presenting results from the 0.4 and 2.0 mg/kg/day dose groups.

Please note that I am only including the update response to question 2 and the histo report in this email. In the formal amendment to the NDA I will include the entire response plus all of the attachments.

Please let me know if you have any questions.

Tom

Tom Class
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/s/

HANS M ROSENFELDT
08/03/2011

TIMOTHY W ROBISON
08/03/2011

INTEROFFICE MEMO

TO: NDA 22-150 (Shire, Firazyr® (icatibant))

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

DATE: August 1, 2011

Icatibant (Firazyr®), a Bradykinin B2 receptor antagonist, is a synthetic decapeptide analog of Bradykinin (BK) containing unnatural amino acids that that has been developed for the treatment of hereditary angioedema (HAE).

I concur with Dr. Hans Rosenfeldt's review of nonclinical pharmacology and toxicology studies and product labeling dated July 23, 2011. I also concur with Dr. Rosenfeldt's chemistry consultation review dated July 23, 2011. Dr. Molly Topper reviewed nonclinical pharmacology and toxicology studies provided in the initial NDA submission and under IND 68,214 (see review of NDA 22-150 dated March 18, 2008 and reviews of IND 68,214 dated April 21, 2005, May 12, 2008, October 24, 2008, and May 15, 2009).

The initial NDA submission was judged to be not approvable. The letter issued by the Agency on April 23, 2008 identified deficiencies relevant to pharmacology and toxicology as follows: (1) the need for 6-month rat and 9-month dog chronic toxicology studies to support chronic intermittent administration of icatibant and (2) a list of impurities in the drug substance and drug product that either needed to be lowered to NMT (b) (4) or qualified in a 3-month repeat-dose toxicology study that provides a >10-fold safety margin.

In the NDA resubmission dated February 25, 2011, the sponsor provided the 6-month rat and 9-month dog toxicology studies. In addition, the sponsor provided a 13-week toxicology study (b) (4) in rats to support a specification of (b) (4) in the drug product. Prior to the NDA resubmission, the sponsor had provided dose selection proposals and study protocols for the 2-year carcinogenicity studies with mice and rats. The carcinogenicity studies will be handled as post-marketing requirements (see below).

Pharmacology: Icatibant is a synthetic decapeptide with a structure similar to endogenous bradykinin (BK), but containing unnatural amino acids. Icatibant binds the B2 receptor with an affinity similar to BK and functions as an antagonist of the B2 receptor at lower doses. Icatibant has been shown to function as an agonist at high doses. The sponsor proposed that treatment of HAE patients with icatibant decreases BK-induced downstream events including vascular permeability, vasodilation, and inflammation.

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In consultation with SEALD and senior pharmacology/toxicology staff (Dr. Paul Brown), it was determined that icatibant constitutes a new established pharmacological class. Based upon its mechanism of action, icatibant has been designated a “bradykinin B2 receptor antagonist.”

ADME: Icatibant is rapidly absorbed after subcutaneous administration with 100% bioavailability. Icatibant is converted to two major metabolites: M1 [redacted] (b) (4) and M2 [redacted] (b) (4) These metabolites were identified and quantified in mice, rats, dogs, monkeys, and humans.

Toxicology: Subcutaneous toxicology studies up to 6 and 9 months in rats and dogs, respectively, were submitted in support of the clinical development of icatibant. Target organs of toxicity identified in rats included female reproductive organs, female mammary gland, male reproductive organs, skin, injection sites, adrenal glands, brown fat in the aorta and connective tissue, bone marrow, kidneys, liver, lung, and thymus. Target organs of toxicity identified in the dog were the male and female reproductive tracts and stomach. In addition, injection site reactions (scabbing and ulceration) occurred in rats and more generalized systemic reactions described as “histamine-like” occurred in dogs. Toxicity was generally comparable in rats and dogs as the male and female reproductive tracts were identified as targets of toxicity. In both species, these effects varied by duration of treatment and frequency of dosing.

In the 26-week subcutaneous toxicology study with rats, icatibant was administered at doses of 0, 3, 10, and 30 mg/kg/day. Injection site reactions led to the moribund sacrifices of several males and females in the high dose group between weeks 13 and 26. Target organs of toxicity included female reproductive organs (vagina, ovaries, and uterus), female mammary gland (masculinization), male reproductive organs (prostate, epididymides, testes, and seminal vesicles), skin, injection sites, adrenal glands, brown fat (macrovesicular vacuolation) in the aorta and connective tissue, bone marrow, kidneys, liver, lung, and thymus. A NOAEL could not be identified due to findings in the male and female reproductive organs, thymus, bone marrow, aorta, and adrenal glands that were observed at all doses.

In the 39-week toxicology study with dogs, icatibant was administered at doses of 0, 1, and 10 mg/kg/day and 1 mg/kg TID twice per week. Target organs of toxicity were the testes, prostate, ovaries, and uterus. In addition, significant systemic injection reactions occurred in animals treated with the high dose of 10 mg/kg/day. These reactions included face and body swelling, redness in areas surrounding the eyes, ears, and muzzle, and behaviors such as head shaking and immobility. Testicular atrophy with a concomitant 90% decrease in serum testosterone levels and profound defects of sperm counts and motility were observed in males that received 10 mg/kg/day. Males that received 1 mg/kg/day had slight deficits of sperm motility, but had >90% decreases of sperm counts relative to control. Histopathology examination indicated that testicular effects did not reverse in males that received 10 mg/kg/day. Icatibant effects on the ovaries and uterus included decreases in ovary size, a complete lack of developing follicles in the ovary, and histopathological signs of uterine atrophy in all females that

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received 10 mg/kg/day. Icatibant treatment produced decreases in heart rate, although ECG waveforms appeared normal. These clinical signs of toxicity and target organs of toxicity were generally not evident with 1 mg/kg TID twice weekly. Based on the adverse finding to the male and female reproductive tracts in animals treated with 1 and 10 mg/kg/day, the NOAEL was identified as 1 mg/kg TID twice per week.

The 39-week dog study illustrates that the observed toxicities were dependent on daily dosing; however, dosing twice per week was generally not associated with adverse effects in dogs. NOAELs could not be identified with daily dosing in repeat dose toxicology studies with rats or dogs. Less frequent dosing was not investigated with rats. The group of dogs that received icatibant twice per week provided an adequate safety margin for clinical dosing with icatibant.

Reproductive Toxicology: A complete battery of reproductive toxicology studies with icatibant was conducted that included assessments of fertility and reproductive performance in male mice and rats, embryofetal development in rats and rabbits, and peri- and post-natal development in rats. Reproduction studies in male mice and rats with daily administration of icatibant found no effects on fertility or reproductive performance with intravenous doses up 81 mg/kg or subcutaneous doses up to 10 mg/kg, respectively; however, these findings appeared to be at significant variance from those observed in toxicology studies with rats and dogs. Icatibant was not teratogenic in rats or rabbits; however, it caused delayed parturition, fetal death, and pre-implantation loss in rats and premature birth, abortion, fetal death, and pre-implantation loss in rabbits. Nonteratogenic effects consisted of impairment of pup air-righting reflex and decreased pup hair growth in rats. The sponsor has adequately completed the reproductive toxicity battery for icatibant and the data indicate that icatibant should be classified as a Pregnancy Category C based on the presence of embryotoxicity, increased spontaneous abortions, and increased pups deaths.

Carcinogenicity: Dose selection proposals and study protocols for the 2-year carcinogenicity studies with icatibant in rats and mice were reviewed by the ECAC on June 10, 2008 and November 2, 2010, respectively. Carcinogenicity studies with rats and mice will be handled as post-marketing requirements.

Impurities: There were four main issues involving impurities identified during the course of review of this NDA amendment: (1) review of dedicated toxicology studies submitted to qualify the degradants (b) (4) at the proposed limits of NMT (b) (4); (2) assessment of (b) (4) structural alerts; (3) the specification criteria for (b) (4), respectively; and (4) presence of impurities in the Firazyr drug product that have not been fully identified at levels (b) (4)

1. The sponsor submitted a 13-week toxicology study (b) (4) to support an acceptance criterion of NMT (b) (4) The NOAEL of the study provides an adequate safety margin for the proposed specification; however, final acceptance of requested histopathological examinations of tissues from lower dose groups is pending

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submission of the final report with a signature. The final signed histopathology report was received on July 29, 2011 and will be dealt with as a review addendum.

(b) (4) is a major metabolite of icatibant, designated M2, as well as a degradant. Based on the significant exposures to (b) (4) in nonclinical test species and humans, this degradant/metabolite is considered qualified at the proposed specification.

2. Results from the published literature and the CDER Computational Toxicology Consultation Service determined that (b) (4) (b) (4) were not mutagenic. Although these compounds were not detected in the drug product, they are potential leachables. Given their lack of mutagenicity in the assays described, these compounds are not of concern below limits of detection.

3. The (b) (4) limits based on the USP standard appear acceptable at this time.

One item remains outstanding in the chemistry consultation review: (1) the sponsor has not fully characterized several impurities occurring at (b) (4) or defined "minimal characterization" for impurities (b) (4)

Recommendations: From a nonclinical pharmacology and toxicology standpoint, the application is recommended for approval. The sponsor has submitted adequate nonclinical data to support the safety of Firazyr (icatibant) given on a chronic intermittent basis as a single 30 mg subcutaneous injection. Additional 30 mg injections spaced 6 hr apart, not to exceed a total of 3 injections per 24 hr period (90 mg), are supported.

The applicant has an adequate nonclinical pharmacology and toxicology program for icatibant. However, there are 2 post-marketing requirements (PMRs) and 1 post-marketing commitment (PMC).

The sponsor did not submit studies to evaluate the carcinogenic potential of icatibant, but has initiated 2-year carcinogenicity studies in mice and rats. The Division will issue two PMRs, one for the mouse study and one for the rat study, with the approval package of this NDA.

The sponsor has not identified the structures of impurities that are present at levels (b) (4). Further, the sponsor needs to propose a clear standard for "minimal characterization" for impurities that are present at levels (b) (4) The Division will develop a Post-Marketing Commitment (PMC) for their identification.

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

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/s/

TIMOTHY W ROBISON
08/01/2011
Secondary PharmTox Review

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

PHARMACOLOGY/TOXICOLOGY NDA CHEMISTRY CONSULT

Application number:	22-150 (Amendment 0018)
Supporting document/s:	CTD Section 1 and 4
Applicant's letter date:	2/25/2011
CDER stamp date:	2/25/2011
Product:	icatibant/Firazyr® (Bradykinin B2 Receptor Antagonist)
Indication:	Treatment of Hereditary Angioedema (HAE)
Applicant:	Shire/Jerini U.S. Inc.
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Hans Rosenfeldt, Ph.D.
Supervisor/Team Leader:	Timothy Robison, Ph.D., D.A.B.T.
Division Director:	Badrul A. Chowdhury, M.D., Ph.D.
Project Manager:	LT Eunice Chung-Davies, PharmD.

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1 Executive Summary

1.1 Introduction

Shire has submitted this NDA amendment as a Complete Response to a Not Approvable letter sent by the FDA on April 23, 2008. The pharmacology, safety pharmacology, pharmacokinetics, single-dose and repeat-dose toxicology, genetic toxicology, carcinogenicity, reproduction toxicology, and local tolerance of icatibant are reviewed in the primary review of this NDA Amendment. The present chemistry consult addresses the safety evaluation of impurities in the Firazyr drug product.

The identification and qualification thresholds applied to this NDA are based on a discussion that occurred during a pre-NDA meeting held on January 24, 2007. As described in the meeting minutes, discussion of additional CMC comment #2 in Question 4, indicated that the thresholds presented by Dr. Blair Fraser, Deputy Director of ONDC, OPS, CDER, at the 2006 TIDES Conference, were more appropriate given the peptide nature of icatibant. These alternative standards are as follows: (1) Any peptide-related impurity (b) (4) should be fully identified, characterized and qualified, (2) any peptide-related impurity (b) (4) should be fully identified and characterized, and (3) any peptide-related impurity above (b) (4) should be minimally identified and characterized.

1.2 Brief Discussion of Nonclinical Findings

For summaries as well as detailed information regarding the pharmacology, safety pharmacology, pharmacokinetics, single-dose and repeat-dose toxicology, genetic toxicology, carcinogenicity, reproduction toxicology, and local tolerance of icatibant, please refer to the primary review of this NDA amendment.

There were four main issues involving impurities identified during the course of review of this NDA amendment. These involve:

1. The review of dedicated toxicology studies submitted to qualify the degradants (b) (4) at the proposed limits of NMT (b) (4)
2. The assessment of (b) (4) structural alerts
3. The specification criteria for (b) (4), respectively.
4. The presence of impurities in the Firazyr drug product that have not been fully identified at levels (b) (4)

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Results of Review:

1. The sponsor submitted a 13-week toxicology study (b) (4) to support an acceptance criterion of NMT (b) (4) The NOAEL of the study provides an adequate safety margin for the proposed specification; however, final acceptance of requested histopathological examinations of tissues from the low and mid dog groups is pending submission of the final report with a signature (see Outstanding Issues below).

(b) (4) is a major metabolite of icatibant, designated M2, as well as a degradant. Based on the significant exposure of animals in the pivotal toxicology studies (b) (4) as described in the Pharmacokinetics section of the primary Pharmacology/Toxicology review of this NDA, this degradant/metabolite is qualified at the sponsor's proposed acceptance criterion.

2. Results from the public literature and from the model training sets used by the CDER Computational Toxicology Consultation Service indicate that (b) (4) are not mutagenic. Although these compounds were not detected in the drug product, they are potential leachables. The lower limits of detection were (b) (4) Given their lack of mutagenicity in the assays described, these compounds are not of concern below these limits of detection.

3. In consultation with Drs. Paul Brown and John Leighton, the (b) (4) limits based on the USP standard appear acceptable for the time being. However, the specification for (b) (4) may have to be revised once the ICH-Q3D process yields an ICH Guideline that makes specific recommendations on (b) (4) in pharmaceuticals.

Two items remain outstanding:

1. The sponsor has not provided a final, signed copy of the additional histopathology data requested by the review team for Study JE049-0171, reviewed herein. These data are required because they define the NOAEL (b) (4) and allow for the qualification of this impurity at NMT (b) (4)

2. The sponsor has not fully characterized several impurities occurring at (b) (4) Further, the sponsor has not defined "minimal characterization" for impurities (b) (4) The review team has determined that the information on impurities occurring at (b) (4) so far, such as provided only the molecular weight of an impurity, is not enough and that the sponsor needs to propose an acceptable standard. A PMC dealing with the characterization of these impurities in the drug product is being developed by the review team.

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1.3 Recommendations

1.3.1 Approvability

Most impurity issues were resolved in communications between the review team and the sponsor. Outstanding issues do not appear to impact approvability of the application as they will be dealt with as a review addendum or PMC.

The sponsor has not identified the structures of several impurities that are present at levels (b) (4), as agreed in the preNDA meeting held on January 24, 2007. The sponsor also needs to propose standards for the characterization of impurities occurring at (b) (4) This issue is detailed below. The Division will negotiate a PMC dealing with impurity characterization.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Not applicable to this consult review.

2 Drug Information

2.1 Drug

CAS Registry Number:	Not provided
Generic Name:	icatibant acetate
Code Name:	JE049/HOE140/S890140

Chemical Name:

D-arginyl-L-arginyl-L-prolyl-L[(4R)-4-hydroxyprolyl]-glycyl-L-[3-(2-thienyl)alanyl]-L-seryl-D-(1,2,3,4-tetrahydroisoquinolin-3-yl-carbonyl)-L-3aS,-7aS)-octahydroindol-2-yl-carbonyl]-L-arginine acetate

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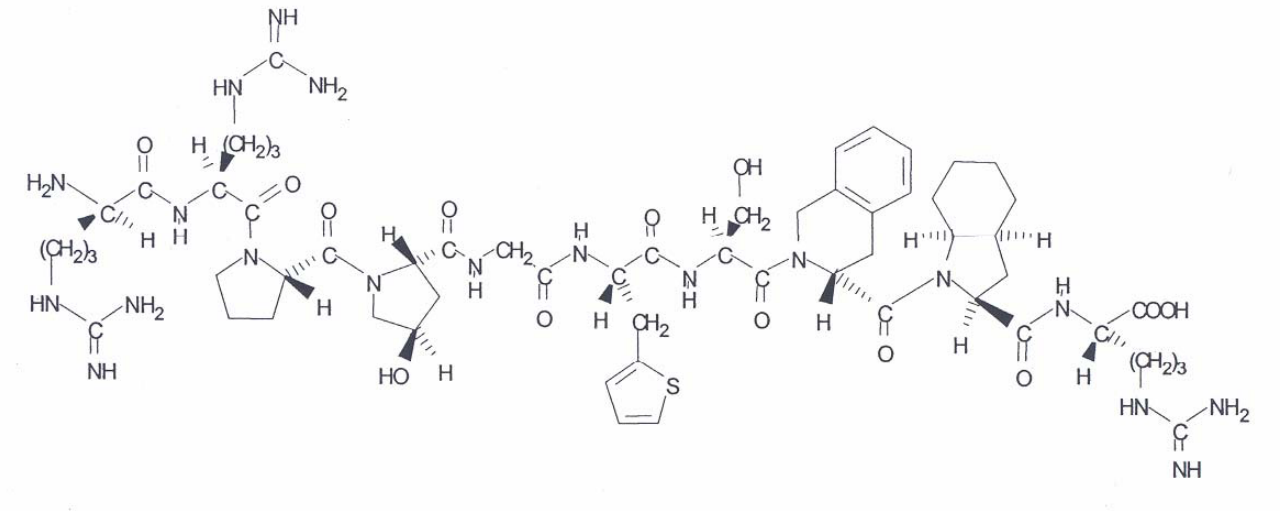
Molecular Formula/Molecular Weight: $C_{59}H_{89}N_{19}O_{13} \cdot C_2H_4O_2$/1304.6 g/mol

Structure:



Pharmacologic Class

Bradykinin type 2 (B2) receptor antagonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND	Drug	Indication
068214	Icatibant	• Subcutaneous administration for HAE

(b) (4)

DMF	Material	Function
(b) (4)	Icatibant	• Active ingredient

(b) (4)

2.3 Drug Formulation

Icatibant 30 mg solution for injection is delivered as a 10 mg/mL sterile, isotonic, acetate-buffered solution. The following table provides the formulation of icatibant for subcutaneous injection.

Ingredient	Quantity per mL	Function
Icatibant	10.0 mg	Active ingredient
Sodium hydroxide, NF/Ph.Eur.		
Glacial acetic acid, USP/Ph. Eur.		
Sodium chloride, USP/Ph. Eur.		
Water for injection, USP/Ph. Eur.		

(b) (4)

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2.4 Comments on Novel Excipients

The sponsor did not include any novel excipients in the proposed drug formulation.

2.5 Comments on Impurities/Degradants of Concern

The sponsor submitted study #JE049-0171, a toxicology study [redacted] (b) (4) in order to qualify this degradant at the [redacted] (b) (4) level in the proposed drug product. This study is reviewed in Section 10 below.

2.6 Proposed Clinical Population and Dosing Regimen

Clinical Population: adult patients who experience acute attacks of hereditary angioedema (HAE).

Dosing Regimen: 30 mg injected subcutaneously in the abdominal area. If response is inadequate or symptoms recur, additional injections of 30 mg may be administered at intervals of at least 6 hours. No more than 3 injections may be administered in 24 hours. Patients may self-administer upon recognition of an HAE attack.

2.7 Regulatory Background

On 4/23/2008, FDA/ODE2 sent the sponsor a Not Approvable letter for the original submission of this NDA. This letter listed the following deficiencies involving Pharmacology/Toxicology: (1) the need for 6-month rat and 9-month dog general toxicology studies and (2) a list of impurities in the drug substance and drug product that need to be lowered to less than [redacted] (b) (4) alternatively, an adequate 3 month repeat-dose toxicology study that provides a >10 fold Safety Margin should be submitted. The present submission, delivered on 2/25/2011, includes a 13-week toxicology study [redacted] (b) (4) [redacted] (b) (4) degradant (reviewed herein) which is specified at NMT [redacted] (b) (4) under the proposed specification in this NDA submission.

10 Special Toxicology Studies

Study title: [redacted] (b) (4) 13 Week Subcutaneous Administration Toxicity Study in the Rat

Study no.: JE049-0171

Study report location: [redacted] (b) (4)

Reference ID: 2978002

(b) (4)

Conducting laboratory and location: Same as above
Date of study initiation: Jan 13, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Lot #1, Batch #1027398; 80.2%

Key Study Findings

- Major target organ of toxicity identified by the histopathology battery was the kidney. Focal cortical sclerosis in the kidney was detected in 2/10 males and 1/10 females treated with 10 mg/kg/day (b) (4) There was also increased incidence and severity of mineral deposits in the inner and outer medulla stripes in female rats.
- Males at all dose levels showed decreases in white blood cell and lymphocyte counts. However, there was no dose response.
- No other significant differences in body weight, food consumption, clinical chemistry, hematology, and opthalmoscopy were identified.
- Initially, a NOAEL could not be established because sponsor histopathology slides for the kidney were not read at the (b) (4) and 2 mg/kg/day dose levels. Data from an unsigned response to an IR dated May 12, 2011 indicate that the NOAEL is (b) (4) mg/kg/day based on a focal cortical sclerosis in the kidney observed at the 2 mg/kg dose level.

Methods

Doses:

Doses				Number of rats	
				Main Study	
Dose Group	Dose mg/kg	Schedule	Vol ml/kg	♂	♀
Control	0	daily	2	10	10
LD	(b) (4)	daily	2	10	10
MD	2	daily	2	10	10
HD	10	daily	2	10	10

Spacer
Route of administration: Subcutaneous injection
Formulation/Vehicle: 0.9% physiological sailine
Species/Strain: HsdHan™ WIST rat
Age: 6 weeks
Weight: Males: 275.8 – 353.8 g; Females: 176.9 – 246.8 g

Unique study design: None

Deviation from study protocol:

1. Initially, a NOAEL could not be established because sponsor histopathology slides for the kidney were not read at the (b) (4) and 2 mg/kg/day dose levels and it was unknown whether the incidence of cortical sclerosis or mineral deposits in the medulla stripes decreased at these lower dose levels.
2. In response to an IR sent by the Division on May 12, 2011, the sponsor sent an unsigned report with updated results. This data is presented in the histopathology section of this report.
3. A signed version of this report has not yet been received.

Observations and Results

Mortality

- Rats were observed at the beginning and the end of the working day.
- No unscheduled mortality occurred in this study.

Clinical Signs

- Rats were given weekly detailed physical examinations.
- An individual record of the each animal's clinical condition was generated.
- Animals were observed once daily 1-2 h post dose in Week 1 and once weekly 1-2 h post-dose from Week 2 to the end of the dosing period.
- The only consistent clinical sign related to drug treatment was thinning fur and occurred in females only.

No. Affected Animals, Day					
Parameter	Sex	Control	0.4 mg/kg/day	2 mg/kg/day	10 mg/kg/day
Thinning fur	M	--	--	--	--
	F	--		1, wk 11-14	3, wk 2-6, wk 11-14

Body Weights

- Individual body-weights were recorded pre-treatment on the first day of dosing, weekly thereafter, and before necropsy.
- There were no differences between treatment groups in body weight or body weight gain.

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Feed Consumption

- The amount of food consumed by each cage of animals was determined weekly.
- Food consumption was calculated as g/animal/week.
- There were no differences between treatment groups in food consumption.

Ophthalmoscopy

- Investigations were performed on all animals pre-treatment and on all animals in the Control and 10 mg/kg/day dose groups during Week 12.
- A mydriatic agent was instilled into the eyes prior to ophthalmoscopy examination.
- No abnormalities were detected before and after 12 weeks (b) (4)

ECG

- Not conducted.

Hematology

- The sponsor conducted a standard hematology battery.
- Females treated with 10 mg/kg/day (b) (4) showed increases in mean platelet volume.
- Males at all dose levels showed decreases in white blood cell and lymphocyte counts.

Week 13					
Parameter	Sex	Control	0.4 mg/kg/day	2 mg/kg/day	10 mg/kg/day
MPV	M	7.5 fL	--	--	--
	F	8.4 fL	--	--	+10%
WBC	M	9.6 $x10^9$/L	-27%	-28%	-31%
	F	4.0 $x10^9$/L	--	--	--
Lymphocytes	M	7.4$x10^9$/L	-31%	-36%	-35%
	F	3.1$x10^9$/L	--	--	--

-- No Finding

Clinical Chemistry

- The sponsor conducted a standard clinical chemistry battery.
- There were small but dose-related and statistically significant decreases in chloride levels in both sexes at the 2 and 10 mg/kg/day dose levels.

Week 13					
Parameter	Sex	Control	0.4 mg/kg/day	2 mg/kg/day	10 mg/kg/day

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Week 13					
Parameter	Sex	Control	0.4 mg/kg/day	2 mg/kg/day	10 mg/kg/day
Chloride	M	106 mmol/L	--	-1%	-2%
	F	106 mmol/L	--	-1%	-1%

-- No Finding

Urinalysis

- Urine samples were collected during a 6 hour day time collection period from all animals in Week 12
- Food and water were removed during collection.
- There were no differences between treatment groups in standard urinalysis parameters such as volume, specific gravity, turbidity and the presence of epithelial cells.
- There were no differences between treatment groups in urinalysis parameters.

Gross Pathology

- All animals were subjected to necropsy.
- Necropsies were performed after an overnight period without food.
- Animals were euthanized with an intraperitoneal overdose of sodium pentobarbitone
- Findings in the ovary and kidney in females treated with 10 mg/kg/day (b) (4) appeared to be incidental.
- Red areas in the skin and subcutis found in males could be related to (b) (4)

Main Study					
Parameter	Sex	Control	0.4 mg/kg/day	2 mg/kg/day	10 mg/kg/day
Kidney, abnormal shape	M	--	--	--	--
	F	--	--	--	1
Liver, pale	M	--	1	1	1
	F	--	--	--	1
Skin and subcutis, red areas	M	--	--	1	1
	F	--	--	--	--
Ovary, small	F	--	--	--	1

-- No Finding

Organ Weights

- The following organs were weighed: adrenals, brain, heart, kidneys, liver, mesenteric lymph nodes, ovaries, pituitary, prostate, spleen, testes+epididymides, thymus, thyroids+parathyroids.

Reference ID: 2978002

- There were no significant differences between treatment groups.

Histopathology

Adequate Battery

- An adequate histopathological battery was performed on control and animals treated with 10 mg/kg/day (b) (4)
- Histopathological analysis in groups treated with 0.4 and 2 mg/kg/day (b) (4) was limited to liver, spleen, kidney, and gross lesions only.

Peer Review

- No peer review was conducted.

Histological Findings

- Major target organ of toxicity identified by the histopathology battery was the kidney. Focal cortical sclerosis in the kidney was detected in 2/10 males and 1/10 females treated at 10 mg/kg/day (b) (4) There was also increased incidence and severity of mineral deposits in the inner and outer medulla stripes.
- In response to an IR dated May 12, 2011, the sponsor submitted new histopathology data for the kidney that included data from the low and mid-dose, in addition to data from the high-dose. This data is listed in a separate table below the first.
- There was also an increase in cardiomyopathy. 3/10 male rats treated with 10 mg/kg/day (b) (4) developed cardiomyopathy; by comparison no control rats developed cardiomyopathy. It is unclear whether this increase is incidental or drug-related, since most male rats develop cardiomyopathy by middle age.
- The lungs, GI-tract, and prostate were also sites of (b) (4) toxicity

Histopathology Data from Original Report from Study JE049-0171

Microscopic Signs		Control		(b) (4)		2 mg/kg/day		10 mg/kg/day	
		M	F	M	F	M	F	M	F
	Group Size:	10	10	10	10	10	10	10	10
	Grade	Terminal							
GI tract, Ileum, focal mucosal congestion	**Mild**	--	--	N.D.	N.D.	N.D.	N.D.	1	--
GI tract, Ileum, focal mucosal edema	**Min**	--	--	N.D.	N.D.	N.D.	N.D.	1	--

Microscopic Signs		Control		0.4 mg/kg/day		2 mg/kg/day		10 mg/kg/day	
		M	F	M	F	M	F	M	F
	Group Size:	10	10	10	10	10	10	10	10
	Grade	Terminal							
GI tract, Caecum, focal epithelial loss	**Min**	--	--	N.D.	N.D.	N.D.	N.D.	1	1
GI tract, stomach non-glandular, keratin cyst	**Present**	--	--	--	--	N.D.	N.D.	1	--
Epididymides, Bilateral sperm granuloma	**Marked**	--	--	--	--	1	--	--	--
Epididymides Bilateral hypospermia	**Marked**	--	--	--	--	1	--	--	--
Heart, myocarditis, minimal	**Min**	--	--	N.D.	N.D.	N.D.	N.D.	3	--
Kidneys, focal cortical sclerosis	**Min**	--	--	N.D.	N.D.	N.D.	N.D.	2	1
Kidneys, mineral deposits inner and outer medulla stripes	**Min**	--	3	N.D.	N.D.	N.D.	N.D.	--	2
	Mild	--	--	N.D.	N.D.	N.D.	N.D.	--	5
	All	--	3	N.D.	N.D.	N.D.	N.D.	--	7
Kidneys, proteinaceous casts	**Min**	--	--	N.D.	N.D.	N.D.	N.D.	--	1
Lungs, perivascular inflammatory cell infiltration	**Min**	--	--	--	--	--	--	2	--
Lungs, foamy alveolar macrophages	**Min**	--	1	--	1	--	1	--	2
	Mild	--	--	--	1	1	--	1	--
	All	--	1	--	2	1	1	1	2
Lungs, agonal congestion and hemorrhage	**Present**	--	--	--	--	--	--	--	1
Lymph node, mesenteric, intrasinusal congestion, erythro-phagocytosis	**Present**	1	--	N.D.	N.D.	N.D.	N.D.	4	--

Microscopic Signs		Control		0.4 mg/kg/day		2 mg/kg/day		10 mg/kg/day	
		M	F	M	F	M	F	M	F
	Group Size:	10	10	10	10	10	10	10	10
	Grade	Terminal							
Muscle (quadriceps), focal mononuclear cell infiltration	**Min**	--	--	N.D.	N.D.	N.D.	N.D.	2	--
Pituitary gland, focal hyperplasia – pars distalis	**Min**	--	--	N.D.	N.D.	--	1	--	--
Prostate, focal mononuclear cell infiltration	**Min**	1	--	N.D.	N.D.	N.D.	N.D.	1	--
	Mild	--	--	N.D.	N.D.	N.D.	N.D.	2	--
	All	1	--	N.D.	N.D.	N.D.	N.D.	3	--
Salivary Gland, parotid, periductular mononuclear cell infiltration	**Min**	--	--	--	--	--	--	1	--
Skin and subcutaneous tissue, focal epidermal hyperplasia	**Min**	--	--	--	--	--	--	--	1
Skin and subcutaneous tissue, focal dermal inflammation	**Min**	--	--	--	--	--	--	--	1
Testes, intratubular spermatid/spermatocytes giant cells	**Min**	--	--	N.D.	N.D.	--	--	1	--
	Mild	--	--	N.D.	N.D.	1	--	--	--
	All	--	--	N.D.	N.D.	1	--	1	--

Histopathology Data from Unsigned Response to IR sent May 12, 2011

Microscopic Signs		Control		0.4 mg/kg/day		2 mg/kg/day		10 mg/kg/day	
		M	F	M	F	M	F	M	F
	Group Size:	10	10	10	10	10	10	10	10
	Grade	Terminal							

Microscopic Signs		Control		0.4 mg/kg/day		2 mg/kg/day		10 mg/kg/day	
		M	F	M	F	M	F	M	F
	Group Size:	10	10	10	10	10	10	10	10
	Grade	Terminal							
Focal cortical sclerosis	**Min**	--	--	--	--	2	--	2	1
Mineral deposits (inner and outer medulla)	**Min**	--	3	--	1	--	3	--	2
	Mild	--	--	--	--	--	--	--	5
	All	--	3	--	1	--	3	--	7

Special Evaluation

- Not conducted.

Toxicokinetics

- Not conducted.

Dosing Solution Analysis

- (b) (4) was formulated in 0.9% (w/v) saline for administration to the study animals.
- Formulations prepared for use on Weeks 1 and 13 were tested.
- Formulations were to be considered stable if the mean of the results at each time point were ±10% of the mean at 0 hour. Results were within these criteria over a 10 day room temperature (15 to 25°C) storage period.
- The target range for the preparation of the formulations was 90 to 110% of nominal. Results were within this range.

11 Integrated Summary and Safety Evaluation

Shire (formerly Jerini US, Inc.) has re-submitted this NDA 22-150 for Firazyr (icatibant) 30 mg, SC for the treatment of adults (≥ 18 years old) with hereditary angioedema (HAE). On 4/23/2008, FDA/ODE2 sent the sponsor a Not Approvable letter for the original submission of this NDA. This letter listed the following deficiencies involving Pharmacology/Toxicology: (1) the need for 6-month rat and 9-month dog general toxicology studies and (2) a list of impurities in the drug substance and drug product that need to be lowered to less than (b) (4) alternatively, an adequate 3 month repeat-dose toxicology study that provides a >10 fold Safety Margin should be submitted. The

present submission, delivered on 2/25/2011, includes the requested rat (6 month) and the dog (9 month) studies. The sponsor will submit the two carcinogenicity studies (1 mouse and 1 rat) as post-marketing requirements. The proposed dosing regimen is a single SC dose of 30 mg icatibant (0.6 mg/kg for a 50 kg human.

In support of this NDA, the sponsor has submitted studies or has ongoing studies addressing the pharmacology, safety pharmacology, pharmacokinetics, single-dose and repeat-dose toxicology, genetic toxicology, carcinogenicity, reproduction toxicology, local tolerance, and impurity qualification of icatibant. Impurity qualification issues are dealt with below.

There were four main issues involving impurities identified during the course of review of this NDA amendment. These involve the review of dedicated toxicology studies submitted to qualify the degradants (b) (4) at the proposed limit of NMT (b) (4) the assessment of (b) (4) structural alerts, the specification criteria for (b) (4) (b) (4) respectively, and the presence of impurities in the Firazyr drug product that have not been fully identified at levels (b) (4)

During a pre-NDA meeting held on January 24, 2007, discussion between the Division and the sponsor indicated that, given the peptide nature of icatibant the following qualification/identification criteria were more appropriate than the traditional ICH-Q3B/C path:

1. Any peptide-related impurity (b) (4) should be fully identified, characterized and qualified.

2. Any peptide-related impurity (b) (4) should be fully identified and characterized.

3. Any peptide-related impurity above (b) (4) should be minimally identified and characterized

Thus, it is these impurity standards that were applied to the current submission.

(b) (4)

The Not Approvable letter of 4/23/2008 listed a set of impurities that required acceptance criteria (b) (4) or a 3-month toxicology study to qualify them:

(b) (4)

(b) (4)

In the current submission, the sponsor chose to reduce all of the above impurities to (b) (4) with the exception of the degradant (b) (4). The sponsor submitted a 13-week toxicology study (b) (4) to support an acceptance criterion of NMT (b) (4)

Study JE049-0171 tested doses of 0, (b) (4) 2, and 10 mg/kg/day (b) (4) in Wistar rats. This study identified the kidney as a target organ of (b) (4) toxicity. Focal cortical sclerosis in the kidney was detected in 2/10 males treated at the 2 mg/kg/day dose level and 2/10 males and 1/10 females treated with 10 mg/kg/day dose level. There was also increased incidence and severity of mineral deposits in the inner and outer medulla stripes in female rats at 10 mg/kg/day. Males at all dose levels showed decreases in white blood cell and lymphocyte counts, but there was no dose response. No other significant differences in body weight, food consumption, clinical chemistry, hematology, and opthalmoscopy were identified. Initially, a NOAEL could not be established because sponsor histopathology slides for the kidney were not read at the (b) (4) and 2 mg/kg/day dose levels. Data from an unsigned response to an IR dated May 12, 2011 indicate that the NOAEL is (b) (4) mg/kg/day based on a focal cortical sclerosis in the kidney observed at the 2 mg/kg dose level.

Table 1. Safety Margins from (b) (4) Qualification Study (JE049-0171)

Study	Species	Schedule	NOAEL (mg/kg/day)	Safety Margin Based on 30 mg*	Safety Margin Based on 90 mg**
JE049-0171	Rat	SC Daily;13 weeks	(b) (4)	33	11

*Dose at (b) (4) = 0.6 mg = 0.012 mg/kg for a 50 Kg human
**Dose at (b) (4) = 1.8 mg = 0.036 mg/kg for a 50 Kg human

In addition to the rat toxicology study reviewed herein, the sponsor submitted a bacterial reverse mutation study (JE049-0154), an *in vitro* chromosome aberration study (JE049-0156), and an acute IV toxicity study (JE049-0155) with the original NDA submission, and a mouse micronucleus test (JE049-0167), which was submitted to IND 68214. (b) (4) was negative in the Ames test but positive in the chromosomal aberration test for inducing clastogenicity. However, intravenous doses of up to 60 mg/kg did not induce micronuclei in male ICR mice. The acute IV toxicity study submitted with the original NDA dosed rats with up to 100 mg/kg (b) (4) . No toxicity was observed in rats. However, in an IV dose-ranging study included in the report for study JE049-0167 (mouse micronucleus test) 1/1 male and 1/1 female mice injected with 300 mg/kg (b) (4) convulsed and died immediately after dosing. The sponsor conducted an additional preliminary study using 200 mg/kg IV dosing (n=2/sex). Male animals showed clinical signs of lethargy, pallor or extremities and decreased activity. One female showed decreased activity.

Reference ID: 2978002

With regard to (b) (4) the sponsor proposes an acceptance criterion of NMT (b) (4) which is higher than the qualification limit of (b) (4) contemplated at the pre-NDA meeting held January 24, 2007. However, (b) (4) is a major metabolite of icatibant, designated M2, as well as a degradant. Based on the significant exposure of animals in the pivotal toxicology studies to (b) (4) as described in the Pharmacokinetics section of the primary Pharmacology/Toxicology review of this NDA, this degradant/metabolite is qualified at the sponsor's proposed acceptance criterion.

Structural Alerts for (b) (4)

CMC review of potential leachables from the proposed Firazyr container-closure system identified three substances with structural alerts. These compounds were (b) (4) (b) (4) The structures of these compounds are detailed in **Figure 1** below:

(b) (4)

A search of the publicly available literature revealed that (b) (4) is non-mutagenic in the Ames bacterial reverse mutation assay, but positive in the *in vitro* mouse lymphoma L5178Y TK locus assay and the CHO/HGPRT chromosome aberration assay[1,2,3,4]. However, further investigation of (b) (4) *in vitro* with rat hepatocytes and *in vivo* in the rat micronucleus test, the mouse dominant lethal assay, and the *Drosophila* sex-linked recessive lethal assay indicated that (b) (4) is non-mutagenic[4]. The weight-of-evidence suggests that there is no genotoxic concern for (b) (4)

In order to assess the potential mutagenicity of (b) (4) these compounds were submitted to the CDER Computational Toxicology Consultation Service for (quantitative) structure-activity relationship (QSAR) analysis. As described in **Appendix 1**, this investigation indicated that both compounds were in the model training sets scored as negative based on actual laboratory testing.

Taken together, results from the public literature and from the model training sets used by the CDER Computational Toxicology Consultation Service indicate that (b) (4) are not mutagenic. Although these

compounds were not detected in the drug product, they are potential leachables. The lower limits of detection were (b) (4) Given their lack of mutagenicity in the assays described, these compounds are not of concern below these limits of detection.

Specification Criteria for (b) (4)

In the current submission, the sponsor proposes the following specification criteria for (b) (4)

(b) (4)

Since there is no threshold for (b) (4) toxicity, since (b) (4) is similarly highly toxic, and since the proposed product uses a parenteral route of administration, the sponsor was asked to justify the proposed specification criteria for these heavy metals in an IR dated May 12, 2011. There currently is no CDER or ICH Guidance that addresses acceptable limits of (b) (4) in pharmaceuticals. The sponsor proposed to use the recommendations set out by the United States Pharmacopeia (USP)[5]. The referenced USP chapter sets out daily exposure limits for (b) (4), respectively, for parenteral pharmaceuticals that are administered in a dose volume <100 mL. Table 2 shows that the proposed limits conform to the USP Standards:

(b) (4)

Since there is currently no CDER or ICH Guidance addressing acceptable levels of heavy metals in pharmaceuticals, the review team contacted Dr. John Leighton, who is currently involved in on-going deliberations aimed toward developing a new ICH-Q3D Guideline dealing with heavy metals[6]. Dr. Leighton was apprised of the sponsor's proposed limits on (b) (4) based on USP standards (**Appendix 2**).

In consultation with Drs. Paul Brown and John Leighton, the (b) (4) limits based on the USP standard appear acceptable for the time being. However, the specification for (b) (4) may have to be revised once the ICH-Q3D process yields an ICH Guideline that makes specific recommendations on (b) (4) in pharmaceuticals.

Impurities Found at Levels (b) (4) Not Fully Identified

During the course of evaluating the current submission, the CMC reviewer identified a series of HPLC peaks occurring at concentrations (b) (4) and not fully characterized by the sponsor, as contemplated in the pre-NDA meeting held on January 24, 2007. Since icatibant contains unnatural amino acids that may not degrade or be metabolized like natural amino acids it is important that the structures of these impurities be defined so that they can be assessed for structural alerts and/or subjected to QSAR analysis. **Table 3** is an excerpt of the sponsor's response to an IR sent by the Division on May 24, 2011 and describes four HPLC peaks occurring at (b) (4) in the drug product that have not been fully characterized: no structures were provided. Further, **Table 4** is an excerpt from the same sponsor response describing 5 HPLC peaks occurring at (b) (4), (b) (4) The discussion between the sponsor and the Division on January 24, 2007 contemplated "minimal characterization" for these species. However, the level of detail provided in this table is too little. For example, the sponsor does not provide any information on (b) (4), and only gives a molecular weight for (b) (4) In discussion with the CMC reviewer, it appears that a more reasonable definition of "minimal characterization" might be the identification of component amino acids, without necessarily knowing the sequence.

A Post-Marketing Commitment (PMC) is being developed in order to address the need for chemical structures of impurities occurring at (b) (4) and to more fully define "minimal characterization."

(b) (4)

[redacted] (b) (4)

Unresolved Impurity Qualification/Identification Issues:

The sponsor needs to provide a final, signed report for the additional histopathology data from Study JE049-0171 requested in an IR dated 5/12/2011. These data identify the NOAEL of [redacted] (b) (4) a degradant of icatibant, at [redacted] (b) (4) mg/kg/day in a 13-week rat study, and qualify this impurity at NMT [redacted] (b) (4). An addendum to the NDA review will be placed on file once the sponsor submits a final signed copy of the histopathology data submitted in response to the Division's IR.

The sponsor has not identified the structures of impurities that are present at levels [redacted] (b) (4). The sponsor needs to propose a clear standard for "minimal characterization." The Division will issue a PMC dealing with these issues.

12 Appendix/Attachments

Appendix 1. QSAR Evaluation of [redacted] (b) (4)

To: Hans Rosenfeldt
cc: Tim Robison From: FDA/CDER/OPS/OTR/DDSR: The CDER Computational Toxicology Group
Re: NDA 22150 Date: June 8, 2011

[redacted] (b) (4) were evaluated by the CDER Computational Toxicology Group for *Salmonella* mutagenicity using four (quantitative) structure-activity relationship [(Q)SAR]

computational toxicology software programs[1]. Both compounds were found to be in the model training sets scored as negative based on actual laboratory testing.

(b) (4) **-*Salmonella* Mutagenicity**

Software	*Salmonella* Mutagenicity
Derek for Windows	Experimental data are negative
Leadscope	
MC4PC	
SciQSAR	
Overall Prediction	

(b) (4) was found to be in the *Salmonella* mutagenicity model training data sets scored as negative. The following link provides access to the study results upon which the training set score was based:

http://yosemite.epa.gov/oppts/epatscat8.nsf/by+Service/AF0488232CF60A0085256F6D0059F445/$File/88950000285.pdf

(b) (4) **-*Salmonella* Mutagenicity**

Software	*Salmonella* Mutagenicity
Derek for Windows	Experimental data are negative
Leadscope	
MC4PC	
SciQSAR	
Overall Prediction	

(b) (4) was found to be in the *Salmonella* mutagenicity model training data sets scored as negative. The following two links provide access to the study results upon which the training set score was based. The study results date from 1980 and 1990, respectively: 1) http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=salmonella.salmonellaData&endpointlist=SA&study%5Fno=637375&cas%5Fno=79%2D41%2D4&activetab=detail; 2) http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=salmonella.salmonellaData&endpointlist=SA&study%5Fno=187641&cas%5Fno=79%2D41%2D4&activetab=detail.

This report has been reviewed and approved by FDA/CDER/OPS/OTR/DDSR.

[1] Derek for Windows 13, Leadscope Model Applier 1.3.3-3, MC4PC 2.3.0.36, and SciQSAR 2.2

Appendix 2. Discussion of (b) (4) Limits based on USP Standards

From: Leighton, John K
Sent: Monday, May 09, 2011 12:02 PM
To: Rosenfeldt, Hans
Cc: Robison, Timothy W; Brown, Paul C
Subject: RE: (b) (4) in a Drug Substance specification/ICH Q3D

The USP limits for parenteral administration are not based on data but by using a default of (b) (4) bioavailability. Using this default, the specification for (b) (4) are below the PDE (ug/d) described in the USP monograph.

From: Rosenfeldt, Hans
Sent: Monday, May 09, 2011 11:46 AM
To: Leighton, John K
Cc: Robison, Timothy W; Brown, Paul C
Subject: RE: (b) (4) in a Drug Substance specification/ICH Q3D

The schedule is intermittent depending on when a patient has an attack of edema. Dosing consists of 30 mg injections, with a maximum daily administration of 3 injections spaced 6 h apart.

From: Leighton, John K
Sent: Monday, May 09, 2011 11:42 AM
To: Rosenfeldt, Hans
Cc: Robison, Timothy W; Brown, Paul C
Subject: RE: (b) (4) in a Drug Substance specification/ICH Q3D

What is the schedule of administration? Daily? Weekly?

From: Rosenfeldt, Hans
Sent: Monday, May 09, 2011 11:35 AM
To: Leighton, John K
Cc: Robison, Timothy W; Brown, Paul C
Subject: RE: (b) (4) in a Drug Substance specification/ICH Q3D

John,

According to the spec and the label, the maximum daily doses of (b) (4) in subcutaneous administration of icatibant are:

(b) (4)

Thanks!

Hans

From: Leighton, John K
Sent: Monday, May 09, 2011 10:50 AM
To: Rosenfeldt, Hans
Cc: Robison, Timothy W; Brown, Paul C
Subject: RE: (b) (4) in a Drug Substance specification/ICH Q3D

Limits will likely be ug/d. What are the levels for (b) (4) in ug?

Reference ID: 2978002

From: Rosenfeldt, Hans
Sent: Friday, May 06, 2011 5:20 PM
To: Leighton, John K
Cc: Robison, Timothy W; Brown, Paul C
Subject: (b) (4) in a Drug Substance specification/ICH Q3D

Hi John,

We have an NDA submission where the sponsor is proposing specifications in the drug substance for (b) (4) (b) (4). Paul Brown recommended that we check with you to see if the latest ICH Q3D deliberations have produced any limits for (b) (4) that we might use.

NDA 22-150 is for the subcutaneous administration of icatibant, an artificial bradykinin peptide analog for the treatment of hereditary angioedema. So the limits that we would apply would be those designed for parenteral administration.

We have had a hard time finding any specific limits for (b) (4) in pharmaceuticals. We did find some proposed limits for (b) (4) mass relative to drug mass in some USP documents However, the proposed limits that we are finding are lower than the specifications proposed in the NDA. For example, we found the following USP chapter:

http://www.usp.org/pdf/EN/USPNF/2008-04-10InorganicImpuritiesStim.pdf

The data in this published chapter are tabulated in Table 1. According to this table, the limit for (b) (4) in drugs administered via a parenteral route is (b) (4)

In the end, though, we need some kind of upper exposure limit for (b) (4) in a drug that is administered parenterally, not just a limit on the mass ratio of the impurity relative to drug. We were wondering if things were far along enough with the ICH Q3D process where we could start talking about such daily exposure limits from parenteral administration.

Thank you!

Hans

[1] Lijinsky W, Andrews AW. Mutagenicity of vinyl compounds in *Salmonella typhimurium*. Teratog Carcinog Mutagen. 1980;1(3):259-67.
[2] Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K, Speck W. Salmonella mutagenicity tests: III. Results from the testing of 255 chemicals. Environ Mutagen. 1987;9 Suppl 9:1-109.
[3] Moore MM, Amtower A, Doerr C, Brock KH, Dearfield KL. (b) (4) .
McCarthy KL, Thomas WC, Aardema MJ, Seymour JL, Putman DL, Yang LL, Curren RD, Valencia R. (b) (4) .
U.S. Pharmacopeia Proposed Chapter <232> Elemental Impurities – Limits, Revised October 6, 2010. http://www.usp.org/pdf/EN/hottopics/232_ElementalImpuritiesLimits.pdf
[6]http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3D/Concept_paper/Q3D_Metal_Impurities_Concept_Paper_Final.pdf

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

/s/

HANS M ROSENFELDT
07/23/2011

TIMOTHY W ROBISON
07/23/2011
I concur.

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	22-150 (Amendment 0018)
Supporting document/s:	CTD Section 1 and 4
Applicant's letter date:	2/25/2011
CDER stamp date:	2/25/2011
Product:	icatibant/Firazyr® (Bradykinin B2 Receptor Antagonist)
Indication:	Treatment of Hereditary Angioedema (HAE)
Applicant:	Shire/Jerini U.S. Inc.
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Hans Rosenfeldt, Ph.D.
Supervisor/Team Leader:	Timothy Robison, Ph.D., D.A.B.T.
Division Director:	Badrul A. Chowdhury, M.D., Ph.D.
Project Manager:	LT Eunice Chung-Davies, PharmD.

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of 22-150 are owned by Jerini U.S. Inc. or are data for which Jerini U.S. Inc. has obtained a written right of reference. Any information or data necessary for approval of 22-150 that Jerini U.S. Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of 22-150.

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1 Executive Summary

1.1 Introduction

Icatibant, a Bradykinin B2 receptor antagonist, is a synthetic decapeptide analog of Bradykinin (BK) containing unnatural amino acids that is under development for the treatment of hereditary angioedema (HAE).

Shire has submitted this NDA amendment as a Complete Response to a Not Approvable letter sent by the FDA on April 23, 2008. Among the deficiencies listed in this letter, there were two items relevant to this Pharmacology/Toxicology review: (1) the need for 6-month rat and 9-month dog chronic toxicology studies, and (2) a list of impurities in the drug substance and drug product that either need to be lowered to NMT (b) (4) or qualified in a 3-month repeat-dose toxicology study that provides a > 10-fold Safety Margin.

The present submission, delivered on February 25, 2011, includes the requested rat (6 month) and the dog (9 month) studies. The sponsor will submit the two carcinogenicity studies (1 mouse and 1 rat) as post-marketing requirements. The proposed dosing regimen is a single SC dose of 30 mg icatibant (0.6 mg/kg for a 50 kg human), which is associated with a systemic exposure of 4107 ng*h/mL. The potential maximum human dose is 90 mg (1.8 mg/kg for a 50 kg human) which is associated with systemic exposures of 12300 ng*h/mL in humans.

1.2 Brief Discussion of Nonclinical Findings

In support of this NDA, the sponsor has submitted studies or has ongoing studies addressing the pharmacology, safety pharmacology, pharmacokinetics, single-dose and repeat-dose toxicology, genetic toxicology, carcinogenicity, reproduction toxicology, local tolerance, and impurity qualification of icatibant.

<u>Pharmacology</u>

Type 1 and Type 2 Hereditary Angioedema are thought to be caused by mutations in the gene encoding the C1-INH protein leading to elevated levels of BK. BK binds to the Bradykinin B2 Receptor in vascular tissues, leading to the main symptom of HAE: extravasation of fluid into the interstitium of the deep dermis and subcutaneous tissue. This extravasation results in severe swelling. Icatibant is an antagonist of the B2 receptor and prevents BK from eliciting the cellular responses that lead to the clinical manifestations of HAE.

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The main drug target of icatibant is the B2 receptor: the affinity of BK and icatibant for the B2 receptor is ~1000-fold higher than that of the other known bradykinin receptor, B1. Further, the B1 receptor is inducible in humans and not expressed in most tissues, while the B2 receptor is constitutively expressed. Icatibant binds the B2 receptor with an affinity similar to BK: *in vitro* receptor binding studies that measured the displacement of ^{3}H-BK in guinea pig ileum, guinea pig trachea epithelium and human synovial cells established that icatibant competed for the B2 receptor with an IC_{50} ranging from 1-4 nM. In Chinese Hamster ovary (CHO) cells expressing recombinant B1 or B2 receptors, icatibant inhibited BK with an IC_{50} of 6000 nM and 4.3 nM, respectively.

Mice engineered with two disrupted alleles in the C1-INH locus have elevated levels of BK and increased vascular permeability, among other HAE-like symptoms. Icatibant treatment reverses the effects of this loss of C1-INH protein function.

Icatibant can function as B2 receptor agonist under some circumstances. Examples of this property include agonist function at very high icatibant exposures and the responses to icatibant treatment exhibited by some non-human cell lines.

In consultation with SEALD and senior pharmacology/toxicology staff, it was determined that icatibant constitutes a new Established Pharmacological Class, a “bradykinin B2 receptor antagonist.”

Safety Pharmacology

Icatibant has effects on the CNS, including decreased motor activity, assumption of prone position, reduced respiration, ptosis, piloerection and mydriasis at IV doses of 1-10 mg/kg, on the CVS, including decreased coronary blood flow in isolated guinea pig heart, aggravated the duration of post-ischemic reperfusion arrhythmias in isolated working hearts in Wistar rats and impaired cardiodynamics, and on renal systems, including decreased urine output, sodium and potassium excretion, to a lesser extent for GFR, renal plasma flow and osmolar clearance in beagle dogs at 0.3 mg/kg, IV. Intravenous icatibant (10 mg/kg) decreased gastric acid volume and acidity in rats compared to the vehicle control. These effects were considered the result of partial agonistic properties of icatibant on the B2 receptor due to the higher exposures achievable by intravenous dosing or the use of *in vitro* systems.

Repeat Dose Toxicology

Subcutaneous toxicology studies submitted in support of the clinical development of icatibant were conducted in rats (up to 6 months) and dogs (up to 9 months). Toxicology studies indicate that the target organs of icatibant toxicity in rats include the male and female reproductive tracts, kidneys, stomach and liver. In the dogs, the target organs were the male and female reproductive tracts and stomach. In addition, injection site

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reactions (scabbing and ulceration) occurred in rats and more generalized systemic reactions described as “histamine-like” occurred in dogs. In both species, these effects varied by length (1 month vs. 6 or 9 months) and frequency (daily vs. twice weekly treatment).

As requested in the Not Approvable Letter sent to the sponsor on April 23, 2008, Shire has submitted two pivotal long-term toxicology studies in rats and dogs.

The sponsor submitted a 26-week rat toxicity study using subcutaneous doses of 0, 3, 10 and 30 mg/kg/day. Injection site reactions were a major toxicity in this study: 7 HD males and 6 HD females were euthanized *in extremis* between weeks 13 and 26 due to severe injection site toxicity. Clinical signs of toxicity included sores and lesions, staining of fur, excessive activity and thin appearance in males and females. The target organs of toxicity in male and female rats included skin, injection sites, adrenal glands, brown fat (macrovesicular vacuolation) in the aorta and connective tissue, bone marrow, kidneys, liver, lungs, and thymus. Female reproductive organs (vagina, ovaries, and uterus) and female mammary gland (masculinization) and male reproductive organs (prostate, epididymides, testes, and seminal vesicles) were also target organs of toxicity. No NOAEL could be determined due to findings in the male and female reproductive organs, thymus, bone marrow, aorta and adrenal gland that were observed in the LD group (3 mg/kg/day).

The sponsor submitted a 39-week dog toxicity study using subcutaneous doses of 0, 1, and 10 mg/kg/day and 1 mg/kg TID twice weekly in partial fulfillment of the long-term toxicology studies. Icatibant subcutaneous administration (daily or twice a week for 39 weeks) caused end-organ toxicity in the testes, prostate, ovaries, and uterus in Beagle dogs. In addition, significant systemic injection reactions occurred in animals treated at the high dose of 10 mg/kg/day. These reactions included face and body swelling, redness in areas surrounding the eyes, ears, and muzzle, and behaviors such as head shaking and immobility. Icatibant treatment resulted in testicular atrophy with a concomitant 90% decrease in serum levels of testosterone and profound defects in sperm motility and sperm count in animals treated at the 10 mg/kg/day dose level. Animals treated at the 1 mg/kg/day level had slight deficit in sperm motility, but had >90% decrease in sperm count relative to control. Although seminology was not assessed in recovery animals, histopathology data indicate that testicular effects did not reverse in animals treated at the 10 mg/kg/day dose level. Icatibant effect on the ovaries and uterus included decreases in ovary size, a complete lack of developing follicles in the ovary, and histopathological signs of uterine atrophy in all females treated at the 10 mg/kg/day dose level. Icatibant treatment produced decreases in heart rate, although ECG waveforms appeared normal. These clinical signs of toxicity and target organs of toxicity were generally not evident with 1 mg/kg TID twice weekly. Based on the adverse finding to the male and female reproductive tracts in animals treated at 1 and 10 mg/kg/day, the NOAEL is 1 mg/kg TID twice weekly.

The submitted dog studies highlight that the toxicities observed from chronic dosing with icatibant depend on daily dosing; 9 months of dosing twice a week did not cause

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adverse effects in dogs. Because the twice weekly dosing schedule was only explored in dogs, and no NOAEL could be established with daily dosing in repeat dose toxicology studies in either dogs or rats, the pivotal dog study that included a group that was dosed on a twice-weekly basis is the only long-term toxicology study that can provide a Safety Margin. Nonetheless, the data from the pivotal dog study are bolstered by the results from the 26-week rat study because the findings from both studies were similar.

Genetic Toxicology

The sponsor submitted a bacterial reverse mutation assay (a complete bacterial strain battery), an *in vitro* mammalian chromosome aberration test, and an *in vivo* bone marrow micronucleus assay in Sprague-Dawley rats using the icatibant. Icatibant did not induce genetic toxicity in these three studies under the conditions assayed.

Carcinogenicity

The sponsor did not submit studies to evaluate icatibant's carcinogenic potential but has initiated two 104-week carcinogenicity studies, one in mice and a second in rats with the concurrence of the Executive Carcinogenicity Committee. During a pre-NDA meeting held on February 22, 2007, the Division conveyed a willingness to accept carcinogenicity studies as a Phase 4 Commitment. However, under the Food and Drug Administration Amendments Act (FDAAA) of 2007, the Division now considers these studies as post-marketing requirements (PMRs) since they are safety assessments. The Division will issue two PMRs, one for the mouse study, and one for the rat study, with the approval package of this NDA.

Reproductive Toxicology

Segment 1 male fertility assessments in male mice and rats treated with IV and SC icatibant, respectively, and embryonic fetal development studies in rats and Himalayan rabbits were submitted to the NDA. The pivotal long-term general toxicology studies, also included special seminology and hormone analyses that correlated with histopathological findings of atrophy in the male and female reproductive tracts. Under the conditions tested in the rats and rabbits, icatibant was not teratogenic. However, icatibant has a potential to produce abortions/premature births, intrauterine death or resorptions. In rats and dogs, long-term daily administration of icatibant caused ovarian, uterine, and testicular atrophy/degeneration, concomitant decreases in testosterone in males, and adverse effects on the mammary and prostate glands as described in the general toxicology section, above. The sponsor has adequately completed the reproductive toxicity battery for icatibant and the data indicate that icatibant should be classified as a Pregnancy Category C based on the presence of embryotoxicity, increased spontaneous abortions and increased pups deaths.

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Local Tolerance

Studies examining intra-articular injection, primary skin irritation, and mucosal tolerance indicate that icatibant is not an irritant at the doses tested. However, injection site reaction, including scabbing, necrosis, and ulceration, was a dose-limiting toxicity in rodent toxicology studies. In dogs, more generalized reactions, involving redness and swelling of the face, ears, eyes, and body, and sometimes described as “histamine-like,” occurred shortly after dosing with higher icatibant doses.

Icatibant did not induce the formation of antibodies in guinea pigs sensitized with icatibant alone via the intranasal or SC route. However, when guinea pigs were sensitized subcutaneously with icatibant plus Freund’s adjuvant, antibody formation was observed with an icatibant-BSA conjugate challenge. In a similar study, icatibant caused erythema in guinea pigs previously sensitized with icatibant plus Freund’s adjuvant.

Icatibant did not induce hemolysis at concentrations of up to (b) (4) in whole human blood.

Impurity Qualification

Impurity qualification issues are detailed in a separate consult to ONDQA.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical pharmacology and toxicology standpoint, the application is recommended for approval. The sponsor has submitted adequate nonclinical data to support the safety of Firazyr (icatibant) given on a chronic intermittent basis as a single 30 mg subcutaneous injection. Additional 30 mg injections spaced 6 h apart, not to exceed a total of 3 injections per 24 h period (90 mg), are supported.

The sponsor did not submit studies to evaluate icatibant’s carcinogenic potential but has initiated two 104-week carcinogenicity studies, one in mice and a second in rats. The Division will issue two PMRs, one for the mouse study, and one for the rat study, with the approval package of this NDA.

As described in a separate consult to ONDQA, the sponsor has not identified the structures of several impurities that are present at levels (b) (4). The Division will issue a PMR for their identification.

1.3.2 Additional Non Clinical Recommendations

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None

1.3.3 Labeling

(b) (4)

6 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

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(b) (4)

[redacted]

2 Drug Information

2.1 Drug

CAS Registry Number:	Not provided
Trade name:	Firazyr®
Generic Name:	icatibant acetate
Code Name:	JE049/HOE140/S890140

Chemical Name:

D-arginyl-L-arginyl-L-prolyl-L[(4R)-4-hydroxyprolyl]-glycyl-L-[3-(2-thienyl)alanyl]-L-seryl-D-(1,2,3,4-tetrahydroisoquinolin-3-yl-carbonyl)-L-3aS,-7aS)-octahydroindol-2-yl-carbonyl]-L-arginine acetate

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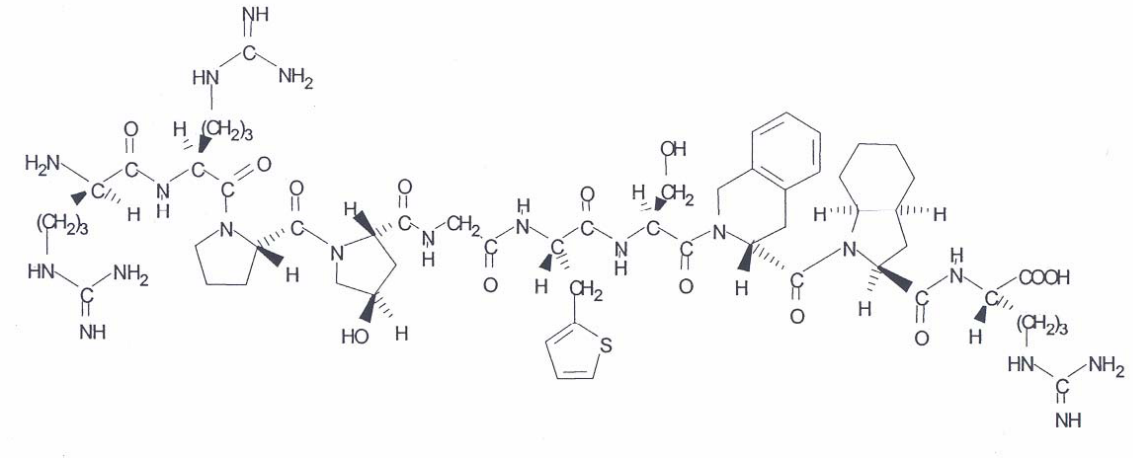
Molecular Formula/Molecular Weight: $C_{59}H_{89}N_{19}O_{13} \cdot C_2H_4O_2$/1304.6 g/mol

Structure:



Pharmacologic Class

Bradykinin type 2 (B2) receptor antagonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND	Drug	Indication
068214	icatibant	• Subcutaneous administration for HAE
[redacted]		

(b) (4)

DMF	Material	Function
[redacted]	icatibant	• Active ingredient

(b) (4)

2.3 Drug Formulation

Icatibant 30 mg solution for injection is delivered as a 10 mg/mL sterile, isotonic, acetate-buffered solution. The following table provides the formulation of icatibant for subcutaneous injection.

Ingredient	Quantity per mL	Function
Icatibant	10.0 mg	Active ingredient
Sodium hydroxide, NF/Ph.Eur.	[redacted]	
Glacial acetic acid, USP/Ph. Eur.		
Sodium chloride, USP/Ph. Eur.		
Water for injection, USP/Ph. Eur.		

(b) (4)

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2.4 Comments on Novel Excipients

The sponsor did not include any novel excipients in the proposed drug formulation.

2.5 Comments on Impurities/Degradants of Concern

The sponsor submitted study #JE049-0171, a toxicology study (b) (4) in order to qualify this degradant at the (b) (4) level in the proposed drug product.

2.6 Proposed Clinical Population and Dosing Regimen

Clinical Population: adult patients who experience acute attacks of hereditary angioedema (HAE).

Dosing Regimen: 30 mg injected subcutaneously in the abdominal area. If response is inadequate or symptoms recur, additional injections of 30 mg may be administered at intervals of at least 6 hours. No more than 3 injections may be administered in 24 hours. Patients may self-administer upon recognition of an HAE attack.

2.7 Regulatory Background

IND 68,214 for the treatment of HAE was originally submitted to the Division of Gastrointestinal and Coagulation Products April 8, 2004. On August 16, 2005, the IND 68,214 for icatibant acetate was transferred from the Division of Gastrointestinal and Coagulation Products to the Division of Pulmonary and Allergy Products (DPAP). A pre-NDA meeting was held between Jerini and DPAP on January 24, 2007 to discuss the registration process and any potential deficiencies in the drug development program. Jerini was informed that the Division considers the potential dosing of HAE patients with no more than eight 30 mg subcutaneous injections a month over the lifetime of the HAE patient as chronic intermittent dosing. To support the clinical chronic intermittent dosing schedule, Jerini was notified that nonclinical chronic repeat dose toxicity studies via the subcutaneous route of administration in the rat (6 month) and the dog (9 month) and 2 carcinogenicity studies (1 mouse and 1 rat) to address the carcinogenic potential of icatibant would be needed. Following the pre-NDA meeting, Jerini (May 1, 2007) requested comments on their proposed nonclinical study protocol designs from the Division for a 26-week subcutaneous repeat dose rat study, a 39-week subcutaneous repeat dose dog study, two 13-week subcutaneous dose range finding studies in rats and mice and 104 week subcutaneous carcinogenicity studies in rats and mice. Comments were relayed to Jerini on these proposed studies June 5, 2007. In its original comments relayed January 24, 2007, the FDA stated that carcinogenicity studies could be submitted as “phase 4 commitments.” However, in the intervening time, the Food and Drug Administration Amendments Act of 2007 was signed into law, and under this new law, the proposed carcinogenicity studies are considered post-marketing requirements (PMRs), since they involve patient safety.

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On October 22, 2007 Jerini US, Inc (Jerini) originally submitted NDA 22-150 for Firazyr (Icatibant), a new molecular entity, for the treatment of hereditary angioedema (HAE) in adults (patients ≥18 years old). Jerini provided pharmacology, pharmacokinetics and toxicology studies conducted for the development of icatibant for the intranasal/inhalation, intravenous and subcutaneous routes of administration with the original NDA submission of October 22, 2007. Pharmacology, pharmacokinetic and toxicology studies were previously reviewed under (b) (4) and 68,214 (subcutaneous administration for the treatment of HAE). (b) (4) . Jerini has a Letter of Authorization allowing cross reference (b) (4) (b) (4) , and 68,214 (subcutaneous administration of HAE) for the detailed review of the nonclinical studies. However, the original submission of this NDA did not contain the rat (6 month) and the dog (9 month) and 2 carcinogenicity studies (1 mouse and 1 rat) required by the FDA during the January 24, 2007 meeting.

On 4/23/2008, FDA/ODE2 sent the sponsor a Not Approvable letter for the original submission of this NDA. This letter listed the following deficiencies involving Pharmacology/Toxicology: (1) the need for 6-month rat and 9-month dog general toxicology studies and (2) a list of impurities in the drug substance and drug product that need to be lowered to less than (b) (4) alternatively, an adequate 3 month repeat-dose toxicology study that provides a >10 fold Safety Margin should be submitted to qualify these impurities. The present submission, delivered on 2/25/2011, includes the requested rat (6 month) and the dog (9 month) studies. The sponsor will submit the two carcinogenicity studies (1 mouse and 1 rat) as post-marketing requirements.

3 Studies Submitted

3.1 Studies Reviewed

Study #	Title
Repeat Dose Toxicity	
JE049-0164	Icatibant: 39 Week Subcutaneous Administration Toxicity Study in the Dog followed by a 4 Week Treatment-free Period
JE049-0172	Icatibant: 14 Day Subcutaneous Administration Range-Finding Toxicity Study in the Juvenile Rat
Reproductive and Developmental Toxicology	
JE049-0353	Study For Effects On Embryo-Fetal Development By Subcutaneous Route In Himalayan Rabbits: Complementary Study To Evaluate The Toxicokinetic Profile Of Icatibant And Its Metabolite M2
Local Tolerance	
JE049-0016	Icatibant And Scrambled Icatibant Induce Calcitonin

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Study #	Title
	Gene Related Peptide (Cgrp) Release From Isolated Skin In Wild-Type C57BL/6 Mice

The following study is reviewed in a consult for the ONQA review of the current submission:

Study #	Title
Impurities	
JE049-0171	(b) (4) 13 Week Subcutaneous Administration Toxicity Study in the Rat

The following studies were reviewed by Dr. Molly Topper (JE049-0159, JE049-0160, JE049-0163, JE049-0167) and Dr. Timothy Robison (JE049-0170) under IND 68,214

Study #	Title
Repeat Dose Toxicity	
JE049-0159	Icatibant: 13 Week Subcutaneous Administration Toxicity Study in the Mouse
JE049-0160	Icatibant: 13 Week Subcutaneous Administration Toxicity Study in the Rat
JE049-0163	Icatibant: 26 Week Subcutaneous Administration Toxicity Study in the Rat followed by a 4 Week Treatment-free Period
JE049-0170	Icatibant: 13 Week Subcutaneous Administration Toxicity Study in the Mouse
Genotoxicity	
JE049-0167	Induction of micronuclei in the bone marrow of treated mice

The following protocols were reviewed by Dr. Molly Topper and Dr. Timothy Robison and evaluated by the Executive Carcinogenicity Assessment Committee under IND 68214.

Protocol #	Title
Carcinogenicity	
JE049-0161	Icatibant: 104 Week Subcutaneous Administration Oncogenicity Study in the Mouse – Final Letter Report
JE049-0162	Icatibant: Subcutaneous Administration Carcinogenicity Study in the Rat – Definitive Protocol
JE049-0176	Icatibant: 104 Week Subcutaneous Administration Carcinogenicity Study in the Mouse – Draft Protocol v1

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3.3 Previous Reviews Referenced

NDA	Relevant Studies	Reviewer	Communication PK	Appendix
22-150	Original NDA Submission	Molly Topper, Ph.D.	2485833	I
IND	**Relevant Studies**	**Reviewer**	**Communication PK**	**Appendix**
[redacted] (b) (4)				
68214	Original IND Submission	Ronald Honchel, Ph.D.	1649430	II
	JE049-0159 JE049-0160	Molly Topper, Ph.D.	1750782	III
	JE049-0163		1777952	IV
	JE049-0167		1824128	V
	JE049-0170	Timothy Robison, Ph.D., D.A.B.T.	2860450	VI

4 Pharmacology

4.1 Primary Pharmacology

No new pharmacology studies were submitted with the current submission. Studies submitted with the original submission to this NDA were reviewed by Dr. Molly Topper. This NDA references INDs [redacted] (b) (4) and 68,214, and studies that were submitted to these INDs were reviewed by Dr. Topper and other colleagues. A brief updated summary is provided below:

<u>Mechanism of Action</u>

Type 1 and Type 2 hereditary angioedema (HAE) is caused by DNA mutations that result in reduced function of the C1 esterase inhibitor protein (C1-INH). C1-INH is an important inhibitor of Factor XII and plasma kallikrein, two proteases that form a proteolytic cascade leading to the release of bradykinin (BK) **(Figure 1**). In the absence of C1-INH function, the activation of these proteases can occur spontaneously, or can be precipitated by blunt trauma, infection, menstruation, the use of estrogen, or angiotensin-converting enzyme (ACE) inhibitors[1]. BK released by the Factor

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XII/kallikrein cascade causes swelling due to extravasation of fluid into the interstitium of the deep dermis and subcutaneous tissue[2]. Laryngeal edema and edema of the bowel wall can also occur by the same mechanism, causing breathing difficulties in the former case and severe abdominal pain in the latter case. BK elicits a variety of intracellular processes that are involved in the inflammation and edema observed in patients with HAE, including pain neuron excitation, vasodilatation, increased vascular permeability and smooth muscle cell contraction.

Bradykinin belongs to the kinin class of peptides that includes kallidin, desArg^9-BK, and Lys-desArg^9-BK. These peptides are generated from the proteolysis of either high MW kininogens (HK) or low MW kininogens (LK) by plasma kallikrein, and in the case of desArg^9-BK, and Lys-desArg^9-BK, further modification of bradykinin to their respective structures (desArg^9-BK = BK amino acids 1-8; Lys-desArg^9-BK = Lys + BK amino acids 1-8). Kinins bind to the bradykinin type 1 and type 2 G-protein-coupled receptors (B1 and B2, respectively) with different affinities that are species-specific. In humans and rabbits, BK binds the B2 receptor preferentially and kallidin binds both B1 and B2 receptors with similar affinity. Further, in humans, the B1 receptor is inducible, while the B2 receptor is expressed constitutively. By contrast, in mice, bradykinin binds both receptors with similar affinity[3]. BK effects relevant to HAE are thought to occur through the B2 receptor, which is coupled to the $G_{\alpha q/11}$ pathway leading to calcium mobilization (**Figure 2)**. The B2 receptor can also link to the $G_{\alpha i}$ protein cascade leading to ERK1/2 activation[4] and can stimulate cell proliferation in some cell types. This cell growth is proposed to be mediated by ERK phosphorylation[5,6].

Icatibant is a synthetic decapeptide with a structure similar to endogenous bradykinin (BK) but containing unnatural amino acids that prevent icatibant digestion by ACE or neutral endopeptidase. Icatibant binds the B2 receptor with an affinity similar to BK: *in vitro* receptor binding studies that measured the displacement of ^{3}H-BK in guinea pig ileum, guinea pig trachea epithelium and human synovial cells established that icatibant competed for the B2 receptor with an IC_{50} ranging from 1-4 nM. In Chinese Hamster ovary (CHO) cells expressing recombinant B1 or B2 receptors, icatibant inhibited BK with an IC_{50} of 6000 nM and 4.3 nM, respectively. The M1 and M2 icatibant metabolites, (b) (4) respectively, also bind to the B2 receptor. In an *in vitro* pharmacology study (JE049-0004), M1 and M2 displaced 10% and 45% of the ^{3}H-BK radiolabeled ligand from the purified human B2 receptor. By comparison, icatibant displaced 83% of the radioligand in the same study. The sponsor has not submitted studies that determine whether M1 or M2 are B2 receptor agonists.

Data published in the open literature as well as data submitted to the NDA by the sponsor indicate that while icatibant functions as an antagonist of the B2 receptor in most situations, icatibant can function as an agonist in specific scenarios, especially when administered at high doses. Many studies with isolated tissues indicate that icatibant functions as a B2 receptor inhibitor. Icatibant selectivity for the B2 receptor in a functional assay was demonstrated *in vitro* using a rabbit aorta model, which contains only BK type 1 (B1) receptors. Incubation of rabbit aorta with BK resulted in contraction of the aorta. After incubation with BK and icatibant, no inhibition of rabbit aorta

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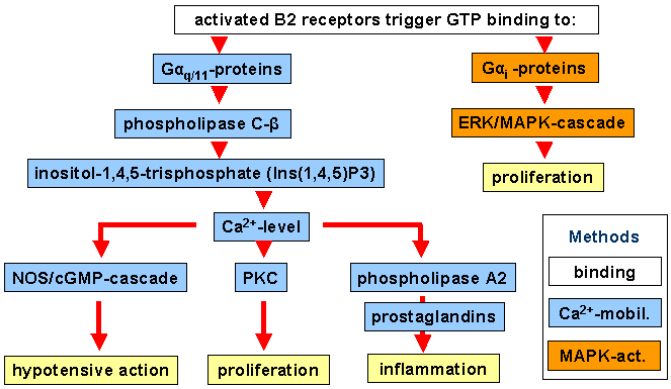
contraction was observed, demonstrating that icatibant did not competitively antagonize the B1 receptor. By contrast, there is much evidence that icatibant inhibits BK-induced effects in tissues that express the B2 receptor. In cultured endothelial cells, icatibant blocked BK-induced release of endothelium-derived relaxing factors with an IC_{50} of 10^{-8} mol/L and blocked release of intracellular calcium with an IC_{50} of 10^{-9} mol/L. Further, icatibant blocked BK-induced contraction in isolated guinea-pig hearts with an IC_{50} of 5.4×10^{-9} mol/L. However, other studies with isolated tissues and with cell cultures indicate that icatibant has agonist activity under some conditions. For example icatibant has 16% activity in comparison to BK control at a concentration of 100 µM in guinea pig ileum, and 100% that of BK in the pulmonary artery of this species (**Table 2**). Most of the submitted studies that explored the effects of icatibant on tissue-based processes used BK-dependent pathways that are also calcium-dependent, such as contraction. These processes are usually mediated intracellularly by the $G_{\alpha q/11}$ proteins and these processes appeared to be much more sensitive to icatibant inhibition, especially at low icatibant concentrations. By contrast, icatibant appears to retain agonist activity in the activation of ERK1/2 in some cell types, such as the Rat2 cell line (**Figure 3)**. Erk activation is usually a $G_{\alpha i}$–dependent process. The sponsor has submitted data suggesting that this effect is species specific, occurring only in rat derived cell lines and, to a lesser degree dog derived cell lines. However, Erk phosphorylation data for only one human cell line was presented in the submission: icatibant does not stimulate Erk1/2 phosphorylation in HF15 cells **(Figure 3)**. The fact that only one human cell line was tested, together with the lack of internal controls showing $G_{\alpha i}$ specificity (such as pre-treatment with pertussis toxin) limit the conclusions that can be drawn from these experiments. Nevertheless, the experiments testing Erk phosphorylation levels after icatibant treatment, together with experiments examining the effects of high icatibant concentrations on isolated tissues, highlight the agonist potential of icatibant.

Figure 1. Signaling Cascades triggered by the B2 receptor.



*Excerpted from the sponsor's submission (Study#JE049-0013)

<u>Drug activity related to proposed indication</u>

In addition to the above receptor binding and receptor function studies, a study using mice disrupted for the C1-INH gene indicates that the enhanced vascular permeability in

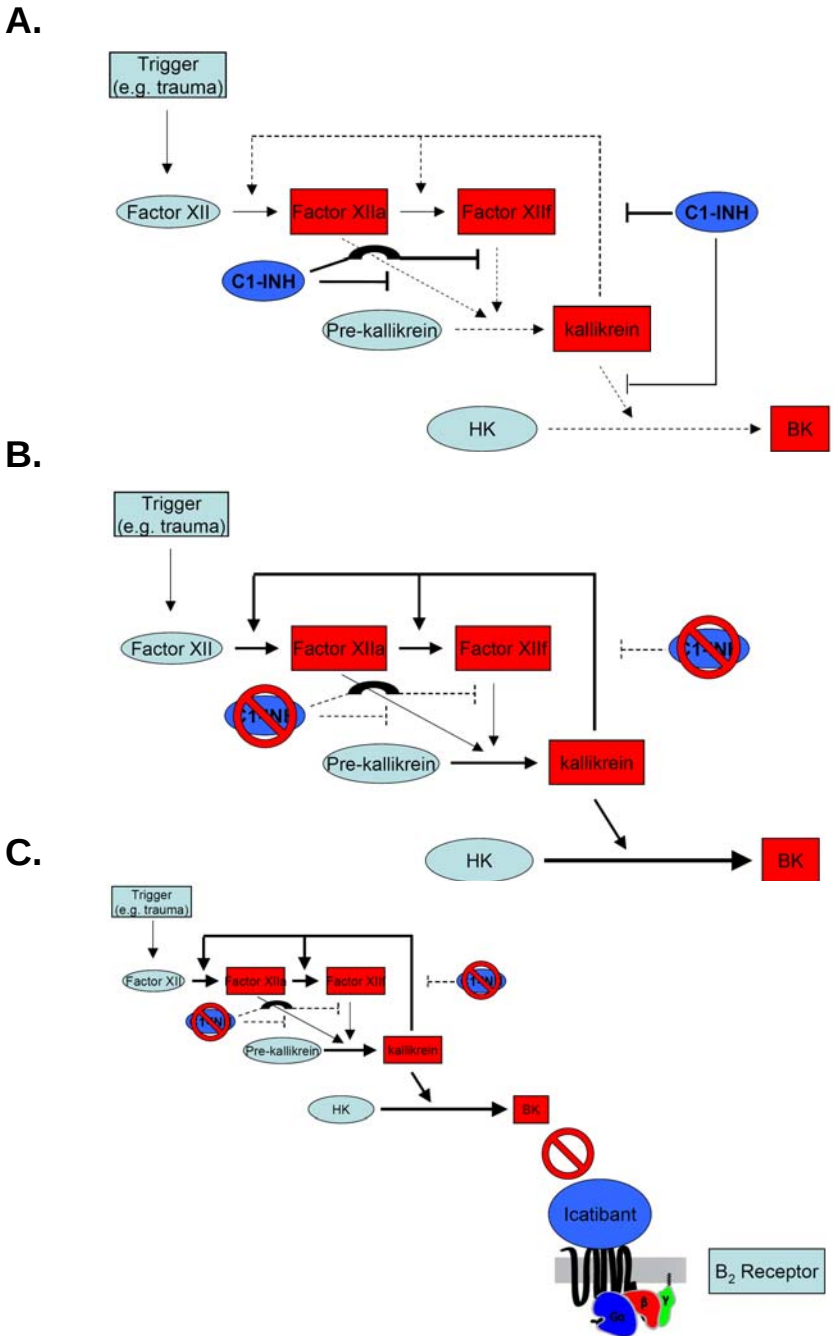
the small intestine observed in these animals relative to control is ameliorated by treatment with icatibant, but not a known antagonist of the B1 receptor desArg^9[Leu^8-icatibant^7 (**Figure 4**). Further, treatment with the ACE inhibitor captopril enhanced the vascular permeability in C1-INH -/- animals. Because these data recapitulate a very important aspect of HAE, vascular permeability, and further demonstrate the sensitivity of this vascular permeability to inhibition of ACE, a known drug effect in HAE patients, these data constitute strong evidence supporting the activity of icatibant in the context of C1-INH deficiency and HAE. In addition, the fact that double knock-out mice homozygous for disrupted versions of the genes encoding C1-INH and the B2 receptor do not show the same vascular permeability as C1-INH establishes the role of the B2 receptor as a critical player in this important component of the HAE disease process (**Figure 4**).

Table 1. Agonist and Antagonist Icatibant effects in isolated tissues

Tissue	Icatibant Inhibition (IC_{50} nM)	Icatibant agonist activity at 100 μM % Relative to BK
Guinea Pig Ileum	11.0	16%
Rat uterus	4.9	None
Guinea Pig Pulmonary artery	5.4	100%
Rabbit aorta	No effect at 10 μM	N.D.
Guinea Pig heart	31	N.D.

N.D. = No data

Figure 2. Bradykinin Production, C1 INH deficiency, and Icatibant Activity

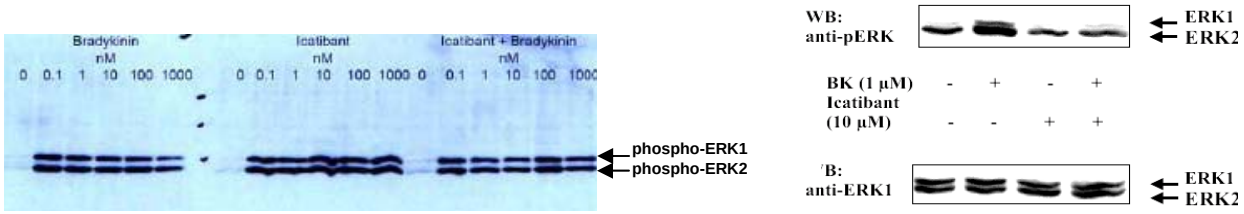


Triggers that expose negatively charged surfaces, such as trauma or infection, potentiate Factor XII auto-activation. **(A)** Active Factor XIIa begins a proteolytic cascade leading to increasing levels of bradykinin (BK). The C1-INH protein inhibits this proteolytic cascade at various steps. **(B)** In hereditary angioedema (HAE), reduced C1-INH protein function causes de-repression of the proteolytic cascade leading to BK. Increased BK levels are thought to be the main cause of HAE symptoms. **(C)** Icatibant prevents BK from activating the B2 kinin receptor.

(b) (4)

Figure 3. PhosphoERK levels: BK or icatibant-treated Rat2 and human HF15 cells

A. **B.**

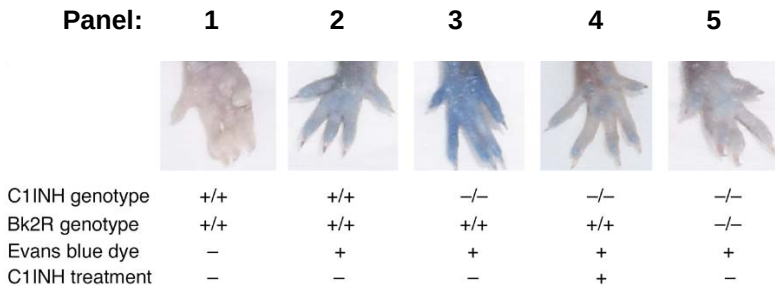


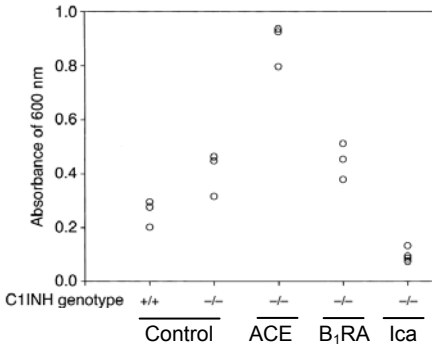
*Excerpted from sponsor submission (Study#JE049-0013)

Western blot analysis of Erk1/2 activation. **(A)** Phospho-Erk levels in Rat2 cells treated with increasing levels of BK, icatibant, or icatibant +BK. **(B)** Phospho-ERK levels in human HF15 cells treated with either BK-, icatibant, or icatibant+BK.

Figure 4. Icatibant inhibition of vascular permeability in C1-INH -/- mice

A. **B.**





(A) Extravasation of Evans blue dye 15-30 min post-administration in the rear footpads of wild-type and C1-INH-deficient mice. Some extravasation occurs in the wild-type mice, but vascular leakage is greatly enhanced in C1-INH -/- mice (compare panels 1 vs. 2, 1 vs. 3 and 2 vs. 3; panel 1 has no Evans blue injection). Injection of purified human C1-INH into C1-INH -/- mice prevented Evans blue extravasation (compare panels 3 vs. 4) and introducing homozygous mutation of the B2 receptor also prevented Evans blue extravasation (compare panels 3 vs. 5). **(B)** Evans blue extravasation in the small intestine quantified with a spectrophotometer. Control wild-type and C1-INH -/- mice replicate increased levels of extravasation in C1-INH deficient mice observed in the footpad. Treatment with the ACE inhibitor captopril (ACE) increases levels of extravasation. Treatment with the B1 receptor antagonist (B1RA; desArg^9[Leu^8]-BK) produces results equivalent to control C1-INH -/- animals. Treatment with icatibant (Ica) reduces extravasation. Data from *J Clin Invest.* 2002 Apr;109(8):1057-63. A copy of this study was submitted with the original submission of this NDA.

4.2 Secondary Pharmacology

No new studies were submitted with the current submission. Relevant secondary studies were covered by Dr. Topper in her review of the original submission of this NDA and are summarized below.

Binding of proteins other than the B2 receptor was observed with icatibant in drug-target screening studies. Of these potential targets, icatibant inhibited ACE activity (IC_{50} = 2500 nM; Study JE049-0201) and displaced the NK2 receptor ligand (IC_{50} = 420 nM; Study JE049-021). Other receptors whose normal ligands were displaced by icatibant (Study JE049-0201) included M4 (16% inhibition), opiate (19% inhibition), EP1 (13% inhibition), VIP1 (56% inhibition), and VIP2 (41% inhibition). The effects of ACE inhibition are well understood, and involve the inhibition of Angiotensin I conversion to the active Angiotensin II which leads to decreased H_20, Na^+ and Cl^- retention and decreases in vascular tone, blood volume and blood pressure. The effects of NK2 receptor inhibition are less well worked out. The NK2 receptor preferentially binds Neurokinin A, which together with Substance P, Neurokinin B, and Hemokinin forms the tachykinin family of peptides. Tachykinins are mainly localized in the central nervous system, but are also distributed in the sensory nerves and are widely distributed within the mammalian peripheral tissues[8]. Tachykinins outside the CNS are involved in vascular contraction. Neurokinin A has been shown to induce vascular contraction of human saphenous veins by stimulating smooth muscle NK2-receptor[9]. With regard to CNS effects, the NK2-receptor antagonist SR48968 has been studied as an anti-depressant and anxiolytic in rodent models of depression[10]. Further, NK2 receptor activation, along with NK1, has been linked to the initiation and control of the cardiovascular, sympathoadrenal, HPA and behavioral responses to pain stimuli and the excitation of corticotrophin-releasing hormone neurons in the PVN to activate the HPA axis[11].

Along with direct icatibant effects on B2 receptors in germ-line tissues, icatibant toxicities identified in the testes and ovary and other effects such as obesity observed in male dogs in the 39-week long-term toxicology study may be related to suppression of the HPA axis at high doses. In the 39-week dog study, C_{max} was ~12,000 ng/mL for males and females, or ~9200 nM, a value that is well above the IC_{50} of 420 nM identified in the receptor binding assay. Decreased levels of CFSH may be a direct result of NK2 inhibition by icatibant.

In addition, Jerini investigated the mechanism of injection site irritation by determining the effects of icatibant on human mast cells, nociceptive nerve fibers and measuring ERK1/2 and calcium levels using *in vitro* techniques. These data showed that icatibant does activate the release of histamine, LTC4 and PGD2 from mast cells and induces mobilization of calcium in nerve fibers, which may contribute to local injection site irritation and pain, respectively. These effects are likely related to the agonist properties of icatibant as described in the mechanism of action section above.

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4.3 Safety Pharmacology

No new safety pharmacology studies were submitted with the current submission. Studies were submitted with the original submission of this NDA and reviewed by Dr. Molly Topper included cardiovascular studies evaluating the effect of icatibant and icatibant metabolites M1 and M2 on cardiac potassium currents, respiratory function and gastrointestinal function. Icatibant did not inhibit cardiac potassium currents up at concentrations up to 300 µM under the conditions tested. No changes in respiratory function were observed compared to the vehicle control group after intravenous (IV) administration of icatibant at doses up to 10 mg/kg in Wistar rats up. In the GI studies, single IV doses of icatibant up to 10 mg/kg in Wistar rats had no effect on GI transit but did decrease gastric acid volume and acidity.

Additional safety pharmacology studies were reviewed under IND (b) (4) by Dr. Young Choi. Icatibant decreased coronary blood flow [IC_{50}= 3.1 x 10^{-8} mol/L (0.5 mL/min) resulting in inhibition ranging from 22% to 67%] in isolated guinea pig heart, aggravated the duration of post-ischemic reperfusion arrhythmias in isolated working hearts in Wistar rats and impaired cardiodynamics. At a rate of 0.5 ng/kg/min, intracoronary infusion of icatibant in an anesthetized myocardial infarction dog model increased mortality rate 2-fold (58%) over saline ischemia (28%) and produced delayed deaths. In addition, icatibant at 0.3 mg/kg given IV decreased urine output, sodium and potassium excretion (~40% for all 3 parameters), to a lesser extent for GFR, renal plasma flow and osmolar clearance in beagle dogs. Icatibant also significantly inhibited BK-induced hypotension in anesthetized rats and produced decreased motor activity, assumption of prone position, reduced respiration, ptosis, piloerection and mydriasis at 1-10 mg/kg IV in conscious rats. These effects on central nervous system (CNS), cardiovascular (CVS) and renal systems are considered the result of the partial agonistic property of icatibant on bradykinin receptors and may present toxicological concerns.

In 4-week subcutaneous (JE049-0016) and 13-week subcutaneous (JE049-0117) repeat dose toxicology studies conducted in the Beagle dog, electrocardiographic (ECG) examinations were conducted after icatibant dosing. In the 4-week study, dogs were administered up to 10 mg/kg icatibant twice weekly (administered as 3.3 mg/kg TID twice weekly). There were no statistically significant changes in ECG parameters or changes in blood pressures in icatibant treated males and females compared to the vehicle treated animals. In the 13-week study, dogs were administered daily doses of icatibant up to 100 mg/kg/day. There was a slight but statistically significant decrease in heart rate in HD males (-29%) and HD females (-21%) 6 hours post dose during Week 1 of dosing compared to the vehicle control groups. A decrease in diastolic BP was noted in HD males (-39%) 6 h post-dose on Week 1 of dosing compared to the vehicle control group. At Week 13 of dosing, there were no statistically significant differences in heart rate, PQ- QRS- or QT-intervals evaluated in the icatibant treated males and females compared to the vehicle control animals. There were no statistically significant changes in QT corrected for heart rate using either Bazett or Fridericia formulae in the icatibant

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treated animals compared to the vehicle treated animals. However, a statistically significant decrease in systolic BP (-19%) was observed in HD males 1 h post-dose in Week 12 and (-22%) was observed in HD females before dosing in Week 13, respectively.

The sponsor has evaluated icatibant's effects on the CNS, CVS, respiratory, renal and GI systems. Taking these data as whole, the safety pharmacology of icatibant has been adequately addressed.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

No new stand-alone pharmacokinetics studies were included in the current submission. A complete description of stand-alone non-clinical pharmacokinetic studies of icatibant can be found in Dr. Molly Topper's review of the original submission to this NDA (**Appendix 1**).

Previously reviewed studies submitted to this NDA and INDs 68214, (b) (4) investigated the distribution, metabolism, and excretion of icatibant in toxicological species. Distribution studies showed that icatibant and icatibant-related isotope-labeled material is distributed into the kidneys, liver, urinary bladder, lungs and spleen 1 hour after a single SC dose in rats. Icatibant signal is still detectable in the liver, kidneys, spleen, bone marrow, adrenals and the injection site 24 h post-dose. Serum protein binding of icatibant was low 44 and 49% human and rat, respectively, and 39% in monkey and dogs. Metabolic studies indicate that M1 (b) (4) and M2 (b) (4) are the primary metabolites of icatibant and are formed by hydrolysis of the peptide backbone (presumably by peptidases). Of these, M2 is disproportionally formed in humans, although still present in rats and dogs (**Table 3**). These data are similar to toxicokinetic data from the long-term general toxicology studies in which rat exposures to M2 were higher in rats than those of dogs (**Figure 5** and **Table 5**).

Table 3. M1 and M2 Exposure Relative to Icatibant

Species	% AUC M1 Exposure Relative to Icatibant	% AUC M2 Exposure Relative to Icatibant
human	>90%	~75%
dog	~60%	~3%
rat	~70%	~9%
mouse	~50%	n.d.

n.d. = No Data

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The half-life of icatibant was ~4 h in males and females in the pivotal rat toxicology study (JE049-0163) and ~2.5 h in males and females on Day 1 of the pivotal dog toxicology study (JE049-0164). The half life of icatibant was 3 h in males and 4.4 h in females on Week 39 of the pivotal dog toxicology study. The comparative pharmacokinetics of icatibant after intravenous administration in man, rat, dog and mouse were adapted from Dr. Young Choi's review of IND (b) (4) in the table below:

Table 4. Comparative Human, Rat, Dog, Mouse Pharmacokinetics

Parameter	Human	Rat	Dog	Mouse
Dose (mg/kg)	0.2	1.0	0.1	1.0
Dose (mg/m^2)	7.4	6.0	2	3
C_{max} (ng/mL)	970	1269-3600	63-70	1310
AUC (ng/mL*h)	1518	2290	120-125	1130
AUC / Dose	205	382	60	376
T_{max} (h)	1.0*	0.5**	1**	0.5**
$T_{1/2}$ (h)	4.6	-	-	-

*- After 1 h infusion; **-First sampling point; - data are not available.

5.2 Toxicokinetics

Toxicokinetic assessments were included in the general toxicology studies submitted in the current submission and toxicokinetic details can be found in the reviews of these studies. The review of the pivotal 39-week dog toxicology study (JE049-0164) can be found in the General Toxicology section of this report. The pivotal 26-week rat study (JE049-0163) was reviewed by Dr. Molly Topper in her evaluation of studies supporting the rat carcinogenicity study (**Appendix 4**). In addition, the sponsor submitted a reproductive toxicology study (JE049-0353) that generated exposure data in rabbits given icatibant on days 6-10 *post-coitum*; this study is evaluated in the Embryonic Fetal Development section of this review (Section 9.1). A tabulated listing of reproduction toxicology studies, pivotal long-term toxicology studies with associated AUC data, and comparative clinical AUC data can be found in **Table 5**.

The toxicokinetics of icatibant were generally linear in the pivotal dog and rat toxicology studies. However, the use of an alternative dosing schedule in one of the groups in the dog study caused a deviation from this pattern. Toxicokinetic analyses performed as part of the pivotal dog study show mostly similar C_{max} and AUC exposures between males and females and between Day 1 and Week 39 for icatibant, and its major metabolites M1 (b) (4) and M2 (b) (4) AUC exposures were linear relative to total daily dose, while C_{max} exposure was not. The reason for this is that the dosing schedule differed between groups in this study: the low and high doses consisted of 1 and 10 mg/kg/day while the mid dose was 1 mg/kg x 3 given every 6 h twice a week. Thus, the total daily mid-dose was 3 mg/kg, but not given all at once, generating a larger AUC while maintaining a lower C_{max} exposure similar to that of the

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low-dose of 1 mg/kg/day. M2 C_{max} and AUC exposures in females were lower in Week 1 than in Week 39. Male exposures to M2 also showed a similar decrease in Week 1 compared to Week 39, but the magnitude of the difference was much lower. Toxicokinetic analyses performed as part of the pivotal rat study show that AUC exposures to icatibant were higher in both males and females at the mid-dose and high-dose in males and at the high dose in females on Week 26, as opposed to Day 1. AUC exposures were similar on Day 1 and Week 26 for M1 (b) (4) and M2 (b) (4). A comparison of C_{max} and AUC data from both pivotal toxicology studies (**Figure 5**) indicate that icatibant and M1 exposures were generally similar between dogs and rats relative to dose calculated on a body surface area basis. However, rat AUC exposures to M2 were greater than AUC exposures in the dog.

In her review of the original submission to this NDA, Dr. Molly Topper estimated the approximate clinical AUC exposure of icatibant and the M2 metabolite from clinical data taken from volunteers treated with 0.4 mg/kg. These values are compared to icatibant and M2 exposures from Day 1 of the pivotal rat and dog long-term toxicology studies in **Table 5**.

Table 5. Relative Icatibant and M2 (b) (4) AUC Exposures

Species	Dose (mg/kg)	Dose (mg/m^2)	AUC – Icatibant	AUC – M2	M2 Relative to Icatibant (%)	M2 Relative to Human M2 at 30 mg (%)	M2 Relative to Human M2 at 90 mg (%)
Human (30 mg)	0.5	17	4107*	3023*	73%	--	--
Human (90 mg)	1.5	50	12300*	9100*	74%	--	--
Rat (JE049-0163)	30	180	43200**	5300**	12%	175%	58%
Dog (JE049-0164)	10	200	43250**	2690**	6%	89%	30%

*Estimated from clinical data taken from volunteers treated with 0.4 mg/kg icatibant
**Average of male and female values

Table 5 shows that maximum M2 exposures in the rat study exceeded the expected clinical AUC exposure of icatibant after one 30 mg dose and resulted in 58% the M2 predicted to result from the maximal clinical dose. Given the fact that toxicity assessments in long-term toxicology studies came from daily exposure over several months, compared to the intermittent exposure proposed for the clinic, these exposure levels are acceptable. Further, most patients took only a single 30 mg injection for a given attack during the pivotal clinical trials (>96% after the first attack; >85% after the fifth attack; please see Clinical Review for further information).

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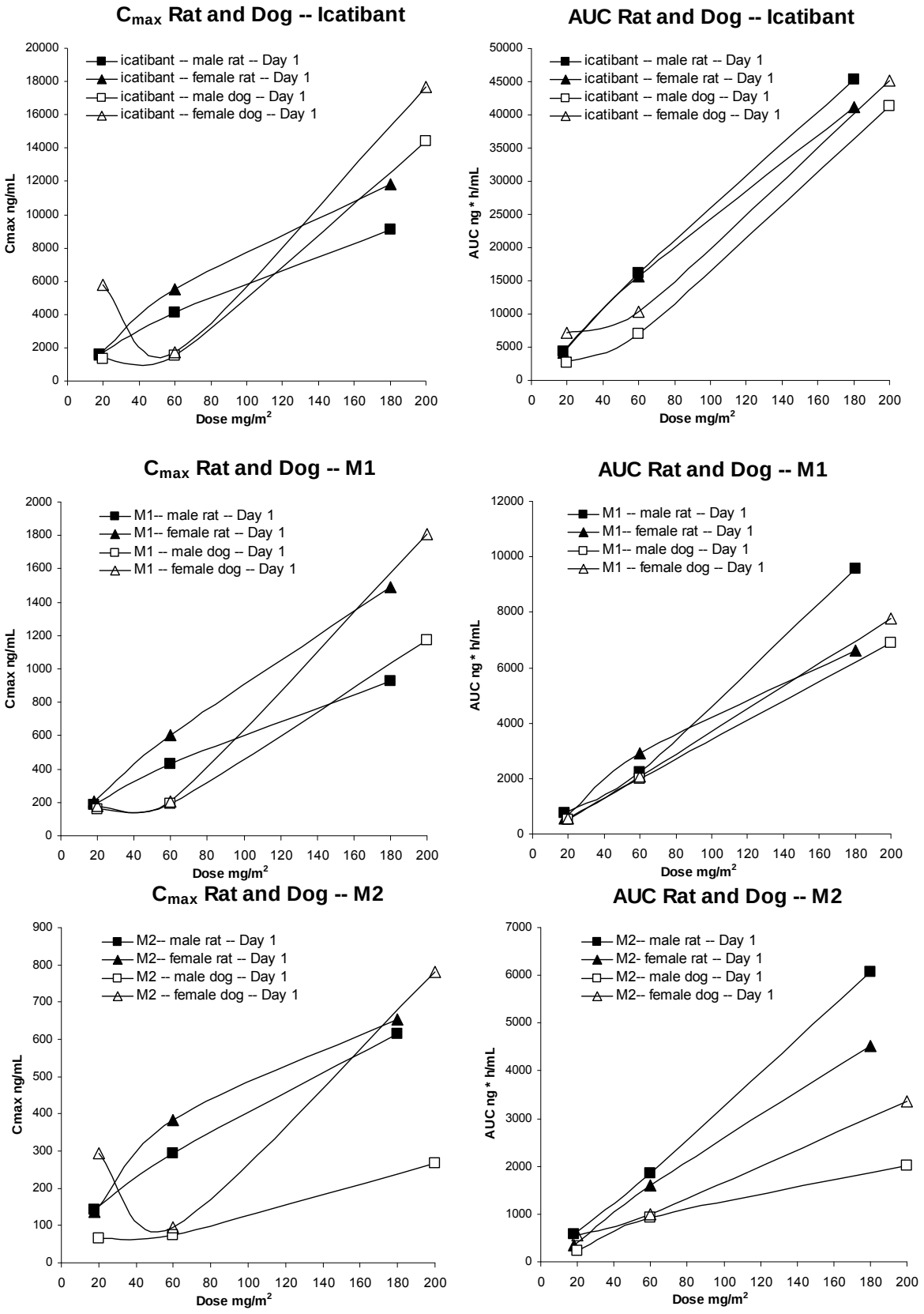
Figure 5. Comparative C_{max} and AUC Exposures in Pivotal Toxicology Studies



6 General Toxicology

6.1 Single-Dose Toxicity

No single dose toxicology studies were submitted with the current submission. These studies were reviewed under IND (b) (4) Although this review is no longer available, Dr. Ronald Honchel's review of the original submission to IND 68214 (**Appendix 3**) summarizes and quotes this review extensively. Acute toxicity studies were performed in mice and rats by intravenous and subcutaneous injections. Deaths occurred usually within minutes after IV dosing. Clinical signs included rapid respiration, twitching, and convulsions. SC dosing resulted in similar but less severe signs. Injections sites had a bluish discoloration that progressed to necrosis. These data generated the following LD_{50} values:

Table 6. LD_{50} Values in Rats and Mice treated with IV and SC Icatibant

Species	Route	LD_{50} (mg/kg)	LD_{50} (mg/m^2)
Rat	IV	12 – 19	74 - 115
Rat	SC	> 1227	>7362
Mouse	IV	48.3 – 76.6	145 – 230
Mouse	SC	614 – 760	1842 – 2280

6.2 Repeat-Dose Toxicity

In support of the proposed SC route of administration for this NDA, the sponsor conducted two 14-day SC toxicity studies in the rat, one 13-week SC toxicity study in the rat (submitted as a combined 13-week toxicity and fertility and early embryonic development study), a 26-week repeat-dose SC toxicity study in the rat, a SC dose-ranging study in the dog, a 4-week SC toxicity study in the dog (twice weekly dosing), and two 13-week SC toxicity studies in the dog (1 with daily SC dosing and 1 with daily and TID administered twice weekly SC dosing). These studies were reviewed by Dr. Molly Topper under IND 68,214. In addition, the sponsor submitted a 39-week SC toxicity study in the dog, reviewed below. The target organs of icatibant toxicity in rats include the male and female reproductive tracts, kidneys, stomach and liver. In the dogs, the target organs were the male and female reproductive tracts and stomach. In addition, injection site reactions (scabbing and ulceration) occurred in rats and more generalized systemic reactions described as "histamine-like" occurred in dogs.

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Study title: Icatibant: 39 Week Subcutaneous Administration Toxicity Study in the Dog followed by a 4 Week Treatment-free Period

Study no.:	JE049-0164
Study report location:	(b) (4)
Conducting laboratory and location:	
Date of study initiation:	June 29, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lot #: E086; 98.3%

Key Study Findings

- Icatibant subcutaneous administration (daily or twice a week for 39 weeks) caused end-organ toxicity in the testes, prostate, ovaries, and uterus in Beagle dogs. In addition, significant systemic injection reactions occurred in animals treated at the high dose of 10 mg/kg/day. These reactions included face and body swelling, redness in areas surrounding the eyes, ears, and muzzle, and behaviors such as head shaking and immobility.
- Icatibant treatment resulted in testicular atrophy with a concomitant 90% decrease in serum levels of testosterone and profound defects in sperm motility and sperm count in animals treated at the 10 mg/kg/day dose level. Animals treated at the 1 mg/kg/day level had slight deficit in sperm motility, but had >90% decrease in sperm count relative to control.
- Although seminology was not assessed in recovery animals, histopathology data indicate that testicular effects did not reverse in animals treated at the 10 mg/kg/day dose level.
- Icatibant effect on the ovaries and uterus included decreases in ovary size, a complete lack of developing follicles in the ovary, and histopathological signs of uterine atrophy in all females treated at the 10 mg/kg/day dose level. Two out of four females treated at the 1 mg/kg/day dose level showed histopathological signs of uterine atrophy.
- Mammary gland atrophy was detected in 4/4 main study and 1/2 recovery females treated at the 1 mg/kg/day dose level.
- The body weights of animals treated with 10 mg/kg icatibant were elevated relative to control. This effect was more pronounced in males where the weight increase was clinically described as obesity.
- Icatibant treatment produced decreases in heart rate, although ECG waveforms appeared normal.
- The toxicities to the reproductive organs and the heart described above appear to be related to icatibant's primary mechanism of action: inhibition of the bradykinin type 2 (B2) receptor. Effects on cardiac tissue have been described in mice that are genetically null for this receptor[1] and the role of this receptor in germ-line proliferation, prostate epithelial cell division, and ovary changes during the estrous cycle is known[2,3,4,5].

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Methods

Doses:

Doses				Number of dogs			
				Main groups		Recovery groups	
Dose Group	Dose* mg/kg	Schedule	Vol ml/kg	♂	♀	♂	♀
Control	0	daily	0.4	4	4	2	2
LD	1	daily	0.4	4	4	--	--
MD	3[§]	2x wk	0.4	4	4	--	--
HD	10	daily	0.4	4	4	2	2

*Doses expressed as free

[§]Administered in three 1 mg/kg doses 6 h apart

Route of administration: Subcutaneous administration
Formulation/Vehicle: 0.9% NaCl physiological saline
Species/Strain: Beagle dog
Age: 40 – 52 weeks
Weight: 5.0 – 10.1 kg
Unique study design: Hormone analysis, testes measurements, seminology reviewed in special evaluation section.
Deviation from study protocol: An additional opthalmoscopic examination was performed on Week 39.

Observations and Results

Mortality

- Dogs were observed at the beginning and the end of the working day.
- No unscheduled mortality occurred in this study

Clinical Signs

- Dogs were observed daily for signs of ill health or overt toxicity.
- Each animal was given a weekly detailed daily physical examination.
- An individual record was maintained of the clinical condition of each animal.
- Immediate post-dose clinical observations (0-24 h post-dose) ranged from fecal changes (soft, liquid feces) at lower doses to facial swelling and reddening, immobility, head-shaking, vomiting, panting, and salivation at the high dose of 10 mg/kg/day. Unsteadiness/immobility occurred on week 1 and did not occur later in the study. Swelling and reddening worsened over the course of the study, spreading from the face to the body in later weeks
- Weekly assessments showed thinning fur, loss of fur, warm-to-touch, and obesity in two high-dose (10 mg/kg/day) males. One high-dose male exhibited tremors on week 32.

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Post-dose Clinical observations (0 – 24 h post-dose)

No. Affected Animals, Week					
Parameter	Sex	Control	1 mg/kg/day	3 mg/kg 2x wk	10 mg/kg/day
Soft Feces	M	1, wk 8	1, wk 3 2, wk 5 1, wk 6-7 2, wk 8 1, wk 9 1, wk 11-14 1 wk 17 1, wk 19-21 1, wk 23 1, wk 25-27 1, wk 30-32 2, wk 33 1, wk 33	2, wk 1 1, wk 2 2, wk4 2, wk 6 1, wk 9-11 3, wk 13 1, wk 14 2, wk 15 1, wk 16-19 1, wk 25 1, wk 26-29 1, wk 31-32 1, wk 36	3, wk 3 1, wk 4 1, wk 5-6 1, wk 10 2, wk 25 1, wk 31 1, wk 35
	F	--	--	3, wk 1 3, wk 3 2, wk 6 1, wk 1 2, wk 8 1, wk 9 3, wk 10-11 2, wk 12 1, wk 1 3, wk 15 1, wk 16 2, wk 19 2, wk 21 2, wk 27 1, wk 28-29 2, wk 30-31 1, wk 32	1, wk 38
Mucoid feces	M	--	1, wk 3 1, wk 30 1, wk 32	1, wk 33	1, wk 1
	F	--	--	1, wk 17	--
Liquid feces	M	--	1, wk 11 1, wk 12 1, wk 13 3, wk 15	1, wk 1 1, wk 2 1, wk 4 2, wk 6 2, wk 7 2, wk 8 1, wk 9-11 2, wk 12 1, wk 13 1, wk 15-16 1, wk 18-25 2, wk 26, 1, wk 30 1, wk 37	1, wk 3

No. Affected Animals, Week					
Parameter	Sex	Control	1 mg/kg/day	3 mg/kg 2x wk	10 mg/kg/day
	F	1, wk 12	1, wk 24	1, wk 1 1, wk 13 1, wk 14 1, wk 16 1, wk 19 1, wk 22 1, wk 26	1, wk 1 1, wk 6
Unsteady on limbs	M	--	--	--	--
	F	--	--	--	1, wk 1
Immobile	M	--	--	--	--
	F	--	--	--	2, wk 1
Salivation	M	--	--	--	--
	F	--	--	--	1, wk 1
Red staining feces	M	--	1, wk 21	--	1, wk 3
	F	--	--	--	--
Excessive activity	M	--	1, wk 22	--	--
	F	--	--	--	--
rapid respiration	M	--	1, wk 22	--	--
	F	--	--	--	--
warm to touch	M	--	1, wk 22	--	--
	F	--	--	--	1, wk 13 2, wk 37
Red eyes	M	--	1, wk 22	--	1, wk 1 1, wk 4 1, wk 9 1, wk 12 1, wk 21 3, wk 25 1, wk 26 2, wk 36
	F	--	--	--	6, wk 1 1, wk 2 3, wk 3-5 4, wk 7 4, wk 9 3, wk 10 4, wk 12 4, wk 13 2, wk 14-16 2, wk 18 6, wk 19 2, wk 21-23 3, wk 24-25 2, wk 26 4, wk 27 2, wk 28-34 3, wk 35 2, wk 36 1, wk 37 2, wk 38

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No. Affected Animals, Week					
Parameter	Sex	Control	1 mg/kg/day	3 mg/kg 2x wk	10 mg/kg/day
Swelling, eyes	M	--	--	--	1, wk 9 2, wk 12 1, wk 13 1, wk 16 1, wk 19 1, wk 26
	F	--	--	--	6, wk 1 3, wk 3-5 4, wk 6-7 3, wk 8 4, wk 9 4, wk 10 2, wk 11 4, wk 12 5, wk 13 2, wk 14-16 3, wk 18 6, wk 19 3, wk 21 2 wk 22 3, wk 23-25 2, wk 27-29 1, wk 30 2, wk 31-36 2, wk 38 1, wk 39
Red ears	M	--	1, wk 22	--	4, wk 1 1, wk 4 1, wk 9-10 1, wk 16 1, wk 22 2, wk 24 3, wk 27 1, wk 30

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No. Affected Animals, Week					
Parameter	Sex	Control	1 mg/kg/day	3 mg/kg 2x wk	10 mg/kg/day
	F	--	--	--	6, wk 1 1, wk 2 3, wk 3-4 3, wk 7 4, wk 9-10 2, wk 11 4, wk 12 5, wk 13 3, wk 14 2, wk 15-16 3, wk 18 6, wk 19 2, wk 20 3, wk 21-23 4, wk 24 3, wk 25 4, wk 26-27 2, wk 28-33 1, wk 34 3, wk 35 2, wk 36 3, wk 37 2, wk 38-39
Swelling, ears	M	--	--	--	1, wk 9-12 1, wk 21 1, wk 24 2, wk 25 1, wk 27 1, wk 37
	F	--	--	--	6, wk 1 3, wk 3-5 4 wk 10 2, wk 11 4, wk 12 5, wk 13 2, wk 14 2, wk 15-16 3, wk 17-18 6, wk 19 3, wk 20 2, wk 21-23 4, wk 24 3, wk 25-26 2, wk 27-30 3, wk 31 2, wk 32 3, wk 33 1, wk 34 2, wk 35 1, wk 36 2, wk 37-39
Red gums	M	--	1, wk 22	--	--

No. Affected Animals, Week					
Parameter	Sex	Control	1 mg/kg/day	3 mg/kg 2x wk	10 mg/kg/day
	F	--	--	--	6, wk 1 1, wk 12
Red, body	M	--	--	--	--
	F	--	--	--	2, wk 1 1, wk 2 3, wk 3 3, wk 4 2, wk 5 2, wk 6 3, wk 7 2, wk 8 2, wk 9 2, wk 10 2, wk 11 3, wk 12 4, wk 13 2, wk 14-18 2, wk 19-20 3, wk 21 2, wk 22 1, wk 27 2, wk 39
Red face	M	--	--	--	--
	F	--	--	--	1, wk 2 3, wk 4-7 2, wk 8 3, wk 9 3, wk 11 2, wk 15-16 2, wk 17-18 3, wk 19 2, wk 20 3, wk 21 2, wk 22-25 3, wk 27 1, wk 28 2, wk 30-31 1, wk 32 2, wk 33-34 2, wk 38 2, wk 39
Swollen, face	M	--	1, wk 23	--	1, wk 1 1, wk 21

No. Affected Animals, Week					
Parameter	Sex	Control	1 mg/kg/day	3 mg/kg 2x wk	10 mg/kg/day
	F	--	--	--	6, wk 1 2, wk 2 3, wk 3-5 4, wk 6 3, wk 7 2, wk 8 3, wk 9-11 4, wk 12-13 1, wk 14 2, wk 15-16 3, wk 17 2, wk 18 5, wk 19 3, wk 20 2, wk 21-25 2, wk 27-28 1, wk 29 2, wk 30-31 1, wk 32 2, wk 33-34 2, wk 38-39
Vomit	M	--	--	--	2, wk 1 1, wk 23 1, wk 31
	F	--	--	--	2, wk 1
Head shaking	M	--	--	--	3, wk 1 1, wk 10 1, wk 19 1, wk 25 1, wk 27
	F	--	--	--	4, wk 1 2, wk 28
Panting	M	--	--	--	--
	F	--	--	--	2, wk 28
Lacrimation	M	--	--	--	1, wk 8 1, wk 14 1, wk 18
	F	--	--	--	--
Salivation	M	--	--	--	--
	F	--	--	--	1, wk 14 1, wk 23

-- No Finding

Weekly Clinical Observations

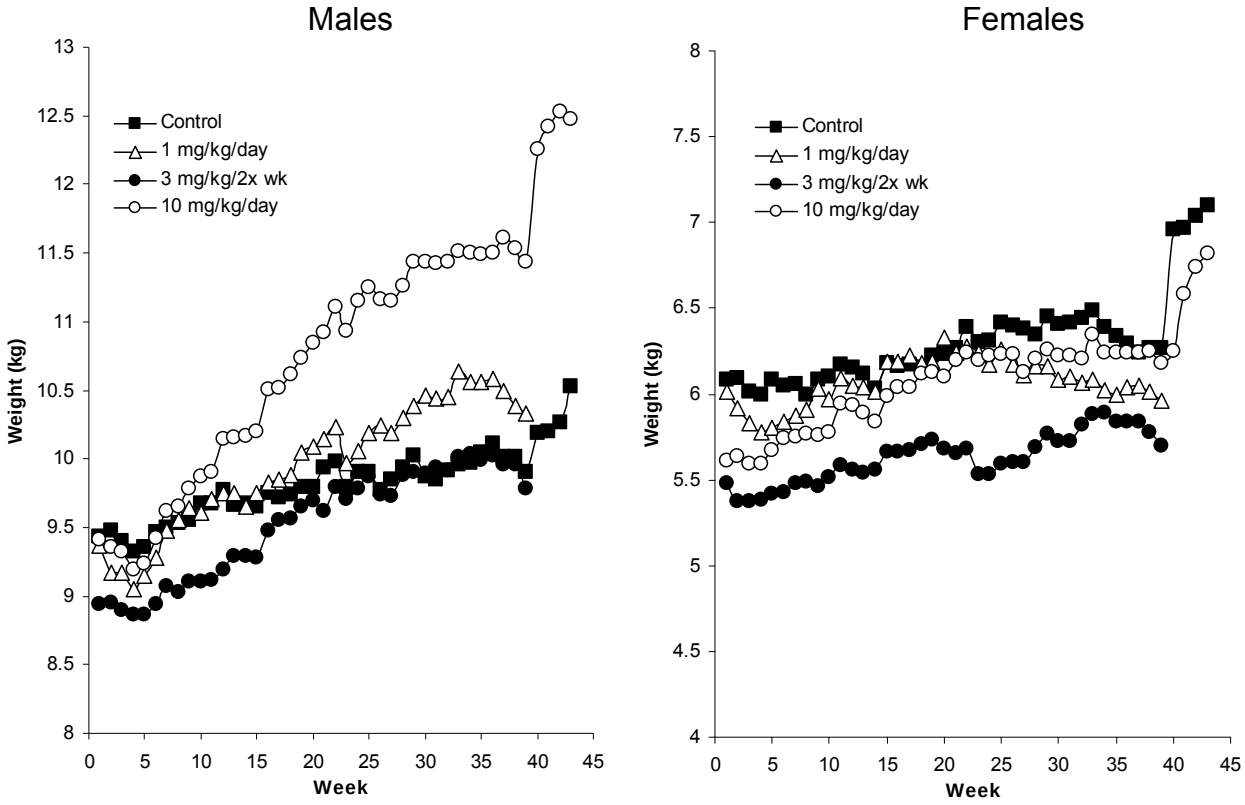
Main Study *Recovery* (No. Affected Animals, Week)					
Parameter	Sex	Control	1 mg/kg/day	3 mg/kg 2x wk	10 mg/kg/day
Small, gristly lump right side of chest	M	--	--	--	1, wk 3-39 *1, wk 40*
	F	--	--	--	--

Main Study *Recovery* (No. Affected Animals, Week)					
Parameter	Sex	Control	1 mg/kg/day	3 mg/kg 2x wk	10 mg/kg/day
Salivation	M	1, wk 9 1, wk 16-17 1, wk 26-32 1, wk 33-39 *1, wk 40*	1, wk 12 1, wk 31-32	1, wk 25-26	
	F	--	1, wk 13 1, wk 21-24 1, wk 38-39 *1, wk 40*	1, wk, 38-39 *1, wk 40*	1, wk 3-15 1, wk 19-20 2, wk 21-24 2, wk 25 1, wk 29 2, wk 30 1, wk 31-38 2, wk 39 *1, wk 40*
Warm to touch	M	--	--	--	1, wk 5-7 2, wk 25 1, wk 26-28 2, wk 29-36
	F	--	--	--	1, wk 9-24 1, wk 25
Lacrimation	M	1, wk 33-39 *1, wk 40*	--	--	1, wk 5-8 1, wk 10-16 2, wk 17-19 1, wk 20-32 1, wk 37-39 *1, wk 40-43*
	F	1, wk 24-36 1, wk 37-39 *1, wk 40*	1, wk 10-20 1, wk 24-39 *1, wk 40*	1, wk 33-39 *1, wk 40*	--
Thinning fur, body	M	--	1, wk 13-24	1, wk 17-24 1, wk 25	1, wk 13-19 2, wk 20 1, wk 24-36
	F	--	--	--	1, wk 24-25
Hair loss, body	M	--	--	--	--
	F	--	--	--	1, wk 33-39 *1, wk 40-43*
Obese	M	--	--	--	2, wk 25-39 *1, wk 40*
	F	--	--	--	--
Tremors	M	--	--	--	1, wk 32
	F	--	--	--	--

-- No Finding

Body Weights

- Individual body weights were recorded once weekly during pre-trial and at weekly intervals throughout treatment and treatment-free periods and before necropsy.
- Males treated with 10 mg/kg/day icatibant gained significantly more weight over the course of the study than controls or animals treated with lower doses of icatibant.



Weight Gain From Week 1 to Week 39

	Control	1 mg/kg/day	3 mg/kg/2x wk	10 mg/kg/day
Males	0.47	0.97	0.83	2.03
Females	0.25	-0.09	0.23	0.46

Feed Consumption

- The amount of food consumed by each animal was determined weekly. Consumption was calculated as g/animal/week.
- Males and females treated with 10 mg/kg/day icatibant consumed more food over the course of the study than controls or animals treated with lower doses of icatibant.

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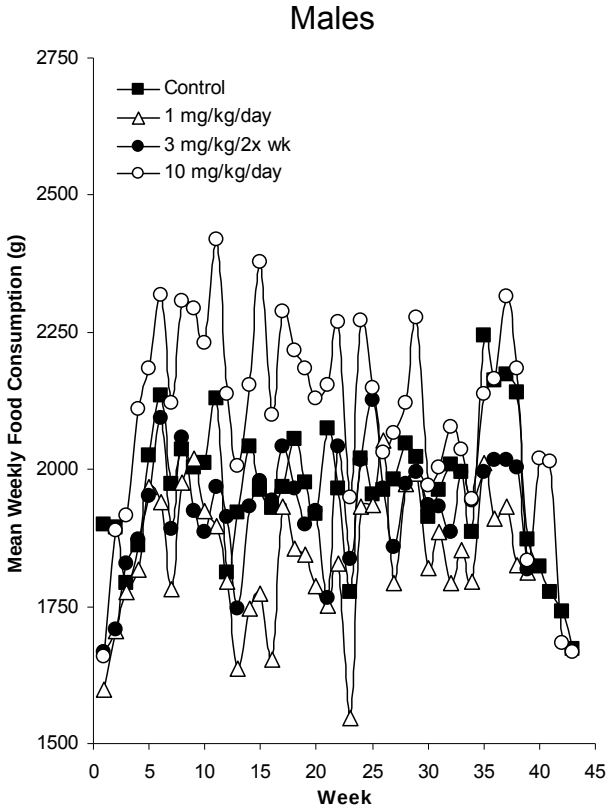
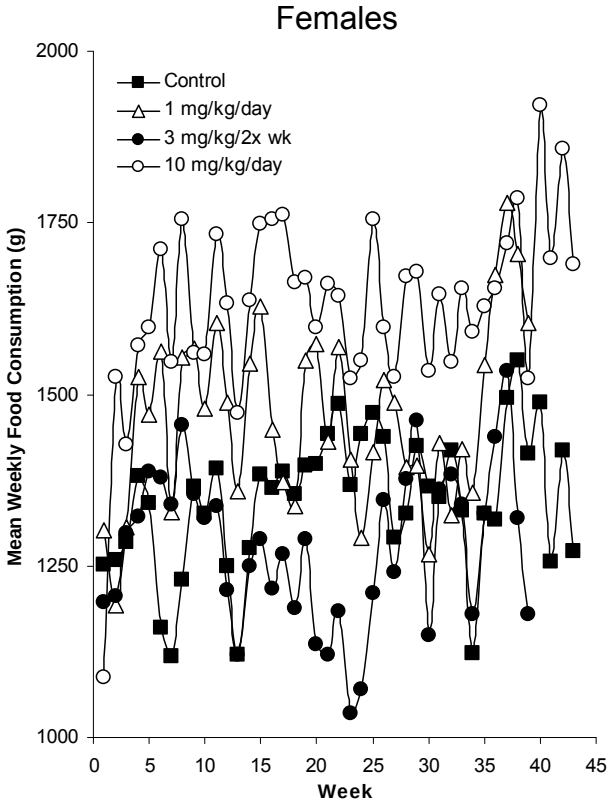




Average Weekly Food Intake From Week 1 to Week 39 (g)

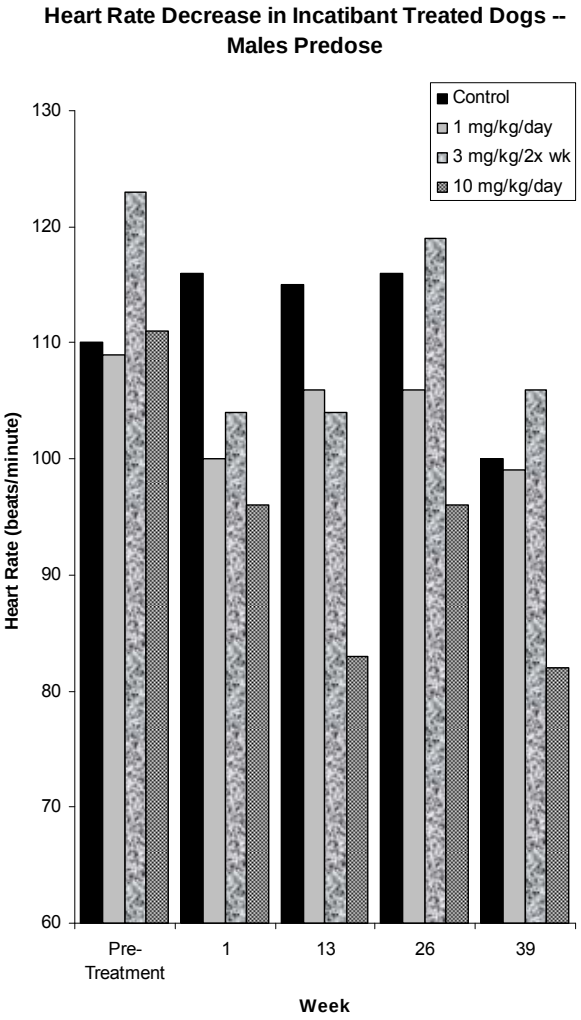
	Control	1 mg/kg/day	3 mg/kg/2x wk	10 mg/kg/day
Males	1965	1843	1931	2101
Females	1345	1467	1277	1629

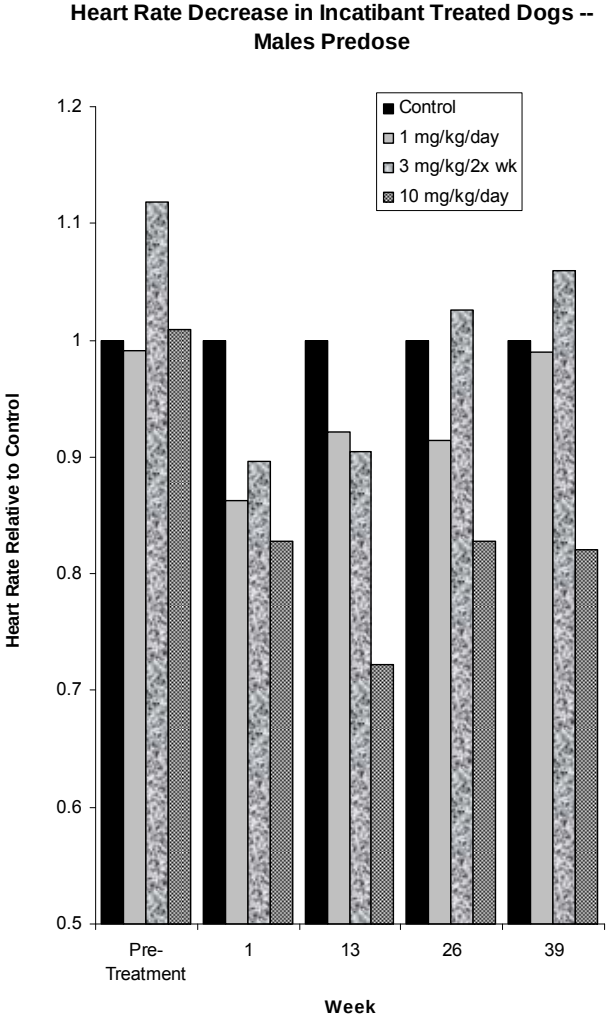
Ophthalmoscopy

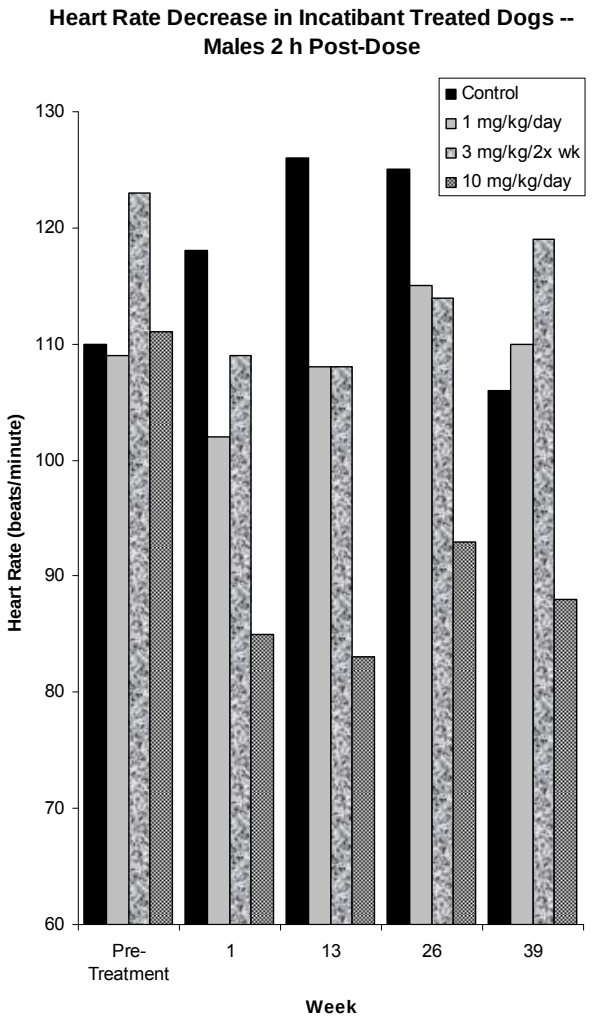
- Investigations were performed on all animals pre-treatment and in Weeks 12 and 25 and on control and high dose animals in Week 38.
- A mydriatic agent was instilled into the eyes before examinations.
- No significant findings were identified. One female treated with 3 mg/kg/2x wk icatibant had lens opacity in the right eye on days 12 and 25, but this finding did not occur in any other dose group (including the high-dose treated with 10 mg/kg/day icatibant).

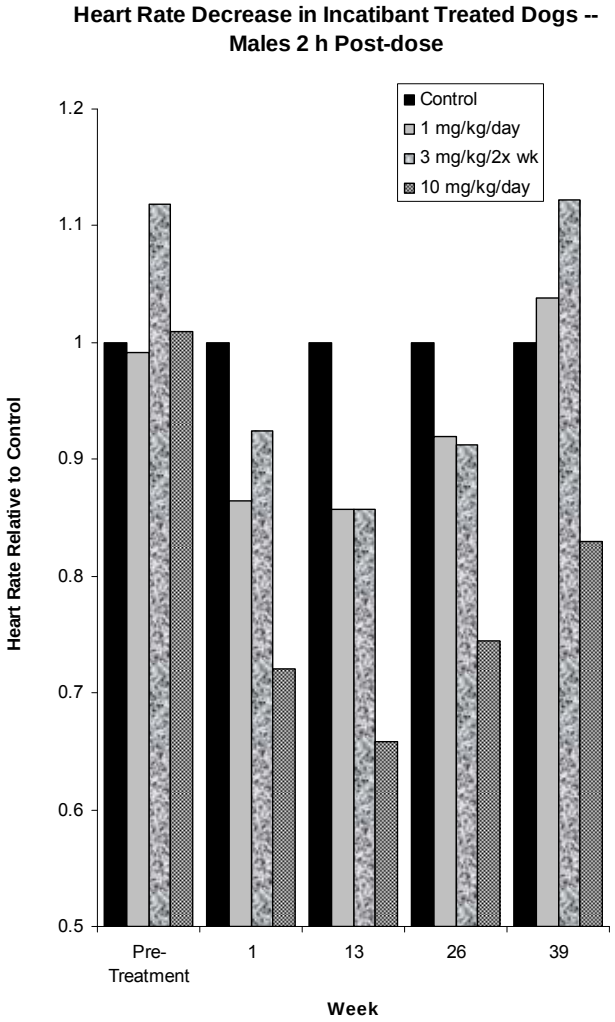
ECG

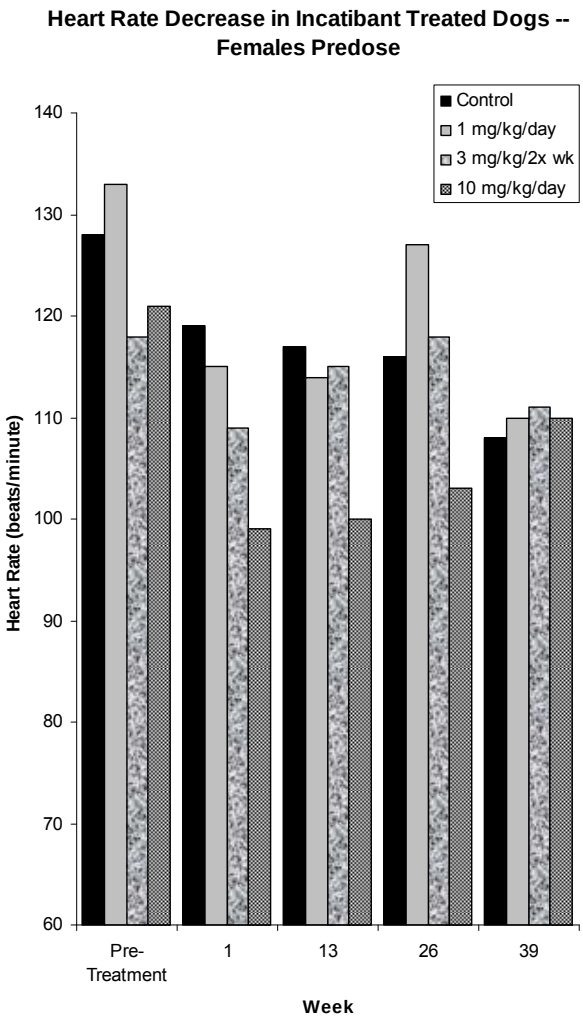
- Investigations were performed on all animals pre-treatment and on Day 2 (Control, 1 mg/kg/day and 10 mg/kg/day) or on Day 5 (3 mg/kg/2x wk and in Weeks 13, 26 and 39.
- Recovery animals were re-examined towards the end of the recovery period in Week 43.
- Readings were taken before dosing and again between 1-2 hours after dosing.
- Recordings were taken using the fixed limb leads I, II and III and the augmented leads aVR, aVL and aVF.
- A visual appraisal of the waveform was made and heart rate derived from lead II.
- There were no obvious abnormalities in ECG waveforms.
- Animals treated with 10 mg/kg/day icatibant had lower heart rates compared to control animals. This effect was most obvious in males.
- These results are consistent with cardiomyocyte dysfunction observed in mice which are null for the bradykinin type 2 (B2) receptor[12]

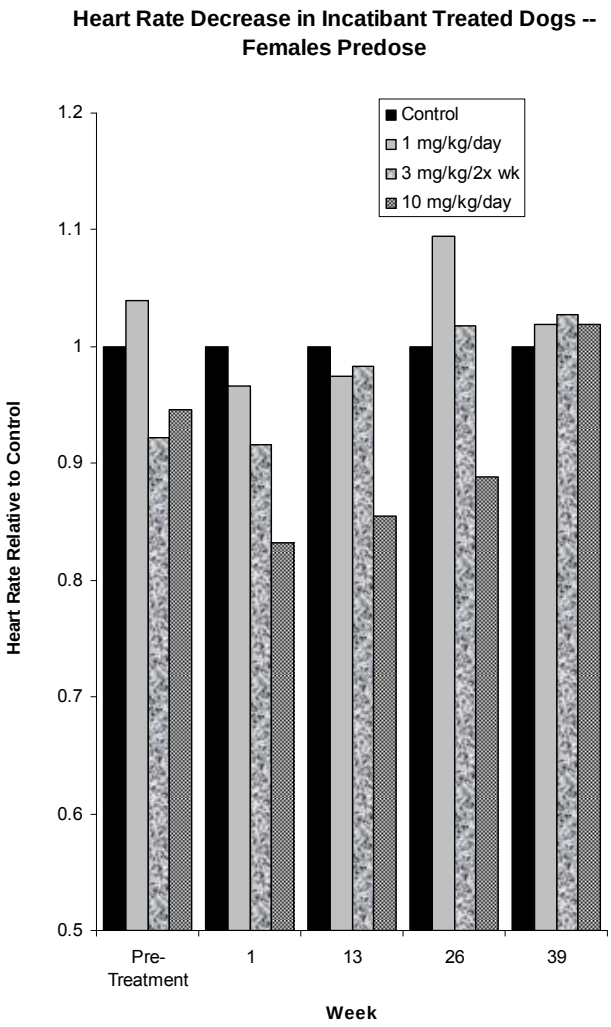


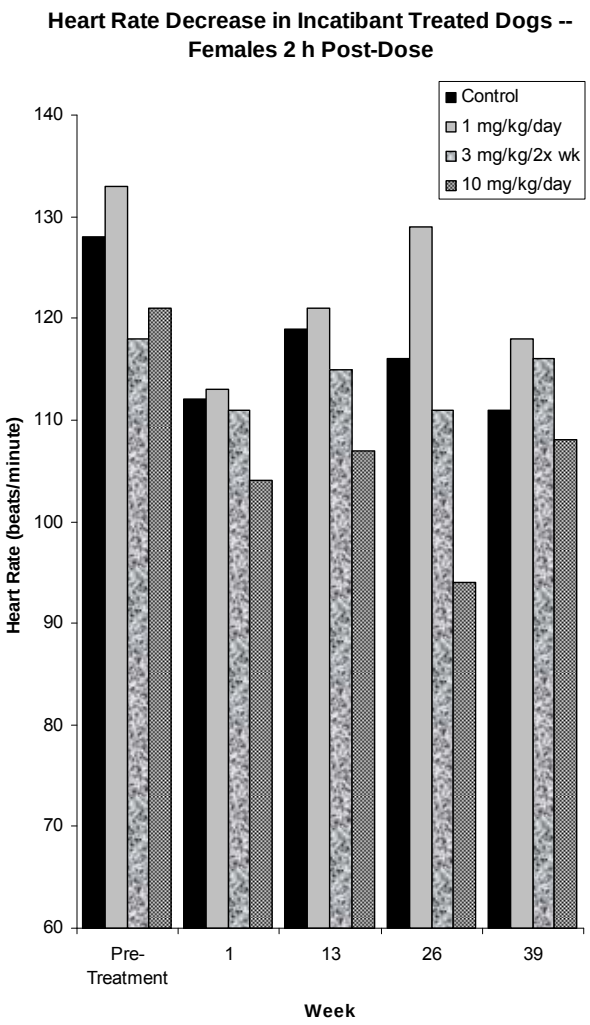


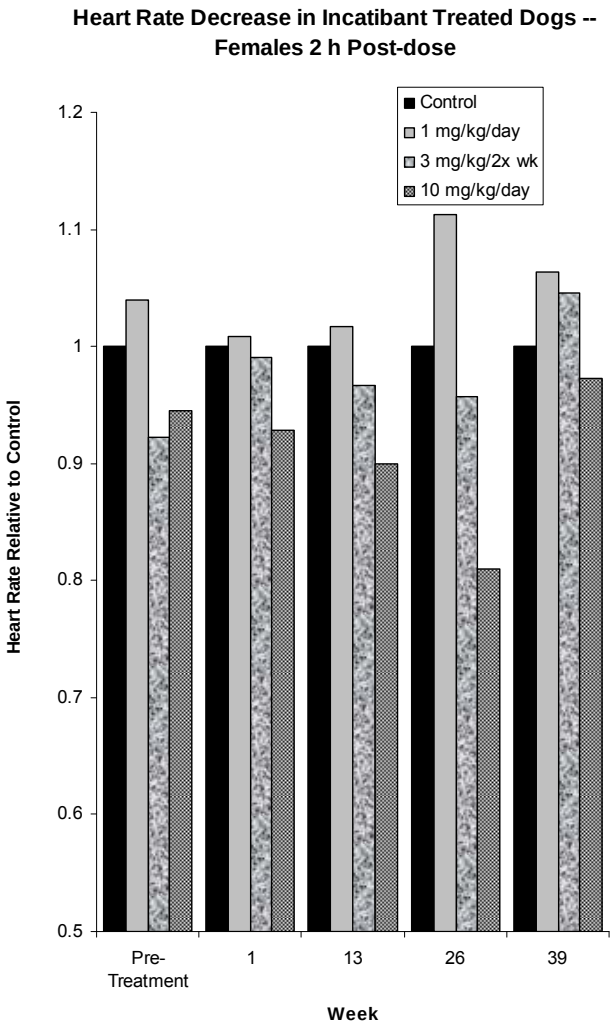

Heart Rate Decrease in Incatibant Treated Dogs -- Males 2 h Post-Dose
Control
1 mg/kg/day
3 mg/kg/2x wk
10 mg/kg/day
Heart Rate (beats/minute)
130
120
110
100
90
80
70
60
Pre-Treatment
1
13
26
39
Week


Heart Rate Decrease in Incatibant Treated Dogs -- Males 2 h Post-dose
Control
1 mg/kg/day
3 mg/kg/2x wk
10 mg/kg/day
Heart Rate Relative to Control
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
Pre-Treatment
1
13
26
39
Week


Heart Rate Decrease in Incatibant Treated Dogs -- Females Predose
Control
1 mg/kg/day
3 mg/kg/2x wk
10 mg/kg/day
Heart Rate (beats/minute)
140
130
120
110
100
90
80
70
60
Pre-Treatment
1
13
26
39
Week


Heart Rate Decrease in Incatibant Treated Dogs -- Females Predose
Control
1 mg/kg/day
3 mg/kg/2x wk
10 mg/kg/day
Heart Rate Relative to Control
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
Pre-Treatment
1
13
26
39
Week





Heart Rates

Males Predose					
Week Dose	Pre-treatment	1	13	26	39
Control	110	116	115	116	100
1 mg/kg/day	109	100	106	106	99
3 mg/kg/2x wk	123	104	104	119	106
10 mg/kg/day	111	96	83	96	82
Males 2h Post-Dose					
Week Dose	Pre-treatment	1	13	26	39
Control	110	118	126	125	106
1 mg/kg/day	109	102	108	115	110
3 mg/kg/2x wk	123	109	108	114	119
10 mg/kg/day	111	85	83	93	88

Females Predose					
Week Dose	Pre-treatment	1	13	26	39
Control	128	119	117	116	108
1 mg/kg/day	133	115	114	127	110
3 mg/kg/2x wk	118	109	115	118	111
10 mg/kg/day	121	99	100	103	110
Females 2h Post-dose					
Week Dose	Pre-treatment	1	13	26	39
Control	128	112	119	116	111
1 mg/kg/day	133	113	121	129	118
3 mg/kg/2x wk	118	111	115	111	116
10 mg/kg/day	121	104	107	94	108

Hematology

- The sponsor conducted a standard hematology battery.
- There were small decreases in white blood cells observed on Weeks 26 and 39 and a small increase in APTT on Week 39 in males treated with 10 mg/kg/day icatibant.

Week 26 **Week 39**					
Parameter	Sex	Control	1 mg/kg/day	3 mg/kg 2x wk	10 mg/kg/day
WBC	M	11.0 x10^9/L **9.3 x10^9/L**	--	--	--
	F	11.0 x10^9/L **10 x10^9/L**	--	-13% **-13%**	-16% **-17%**
APTT	M	**13.5 s**	--	--	**+13%**
	F	**15.4 s**	--	--	--

-- No Finding

Clinical Chemistry

- The sponsor conducted a standard clinical chemistry panel.
- There were small increases in cholesterol in males and decreases in potassium levels in females treated with 10 mg/kg/day icatibant.

Reference ID: 2977968

Week 13 Week 26 **Week 39**					
Parameter	Sex	Control	1 mg/kg/day	3 mg/kg 2x wk	10 mg/kg/day
Total Cholesterol	M	3.8 mmol/L 3.6 mmol/L **3.6 mmol/L**	+11% -- --	+13% -- --	+18% +28% **+36%**
	F	4.4 mmol/L 4.2 mmol/L	--	**--**	**--**
Potassium	M	4.3 mmol/L **4.2 mmol/L**	--	--	**--**
	F	4.3 mmol/L **4.2 mmol/L**	--	--	-9% **-12%**

-- No Finding

Urinalysis

- Standard urinalysis was performed.
- There were changes in specific gravity and potassium levels in males treated with 10 mg/kg/day icatibant.
- There were changes in sodium and chloride levels in females treated with 10 mg/kg/day icatibant.

Week 12 Week 25 **Week 38** *Week 43*					
Parameter	Sex	Control	1 mg/kg/day	3 mg/kg 2x wk	10 mg/kg/day
Specific Gravity	M	1.032 g/mL **1.038 g/mL** *1.043 g/mL*	-- *N.D.*	-- *N.D.*	-2% **-2%** *2%*
	F	**1.026 g/mL** *1.019 g/mL*	-- *N.D.*	-- *N.D.*	**--**
Potassium	M	105 mmol/L **93 mmol/L** *116 mmol/L*	--	--	-59% **-63%** *-60%*
	F	89 mmol/L **56 mmol/L** *83 mmol/L*	--	--	**--**
Sodium	M	113 mmol/L			
	F	95 mmol/L	--	--	+106%
Chloride	M	87 mmol/L 120 mmol/L	--	--	**--**
	F	61 mmol/L 68 mmol/L **78 mmol/L**	--	--	+149% +36% **+50%**

Gross Pathology

Reference ID: 2977968

- All animals in the study were necropsied.
- Scheduled necropsies were performed after an overnight period without food.
- Dogs were given an intravenous overdose of sodium thiopentone and exsanguinated once a suitable deep plane of anesthesia was established.
- A full macroscopic examination was performed under the general supervision of a pathologist and all lesions were recorded.
- Findings in the testes, epididymides, prostate, ovary, and uterus correlate with organ weight and histopathology results in these organs. These changes are likely associated with the known role of the bradykinin type 2 (B2) receptor in germ-line proliferation, prostate epithelial cell division, and ovary changes during the estrous cycle[2,3,4,5].

Main Study *Recovery*					
Parameter	Sex	Control	1 mg/kg/day	3 mg/kg 2x wk	10 mg/kg/day
Whole body, obese	M	--	--	--	2
	F	--	--	--	--
Connective tissue, thick	M	--	--	--	1
	F	--	--	--	--
Cecum, dark	M	--	--	--	2
	F	--	--	2	--
Colon, dark	M	--	--	--	1
	F	--	--	--	--
Ileum, dark	M	--	--	--	1
	F	--	--	--	--
Jejunum, dark	M	--	--	--	1
	F	--	--	--	--
Mass	M	--	--	--	1
	F	--	--	--	--
Pituitary, cyst	M	--	--	--	--
	F	--	--	--	2
Thymus, dark	M	--	--	1	1
	F	--	--	--	--
Thymus, thick	M	--	--	--	1
	F	--	--	--	--
Spleen, thick	M	--	--	--	--
	F	--	--	--	1
Thyroid, small	M	--	--	--	1
	F	--	--	--	--
Testes, small	M	2	4	1	4
Testes, soft	M	--	--	--	1
Epididymides, small	M	--	--	--	4
Ovary, dark area	F	--	*N.D.*	*N.D.*	*1*
Prostate, small	M	--	-- *N.D.*	-- *N.D.*	4 *1*
Urinary bladder, dark	M	--	--	--	1
	F	--	--	--	--
Urinary	M	--	*N.D.*	*N.D.*	*1*

Main Study *Recovery*					
Parameter	Sex	Control	1 mg/kg/day	3 mg/kg 2x wk	10 mg/kg/day
bladder, distension	F	--	*N.D.*	*N.D.*	*1*
Uterus, thin	F	--	--	--	1

-- No Finding; N.D. = No data

Organ Weights

- The following organs were weighed: adrenals, kidneys, liver, ovaries, pituitary, prostate, testes+epididymides, thyroids+parathyroids, uterus including cervix.
- Drug-related weight decreases occurred in the prostate, testes, epididymides, and ovaries. These changes are likely associated with the known role of the bradykinin type 2 (B2) receptor in germ-line proliferation, prostate epithelial cell division, and ovary changes during the estrous cycle[13,14,15,16].
- Icatibant caused spleen weight changes in all treated females, but not in males.
- Thymus weights were increased in recovery males and females previously treated at 10 mg/kg/day.

Main Study/ *Recovery*					
Organ	Sex	Control	1 mg/kg/day	3 mg/kg 2x wk	10 mg/kg/day
Prostate	M	12.2 g *14.3 g*	-36% *N.D.*	-30% *N.D.*	-88% *-76%*
Testes/ Epididymides	M	15.7 g *17.1 g*	-- *N.D.*	-- *N.D.*	-67% *-43%*
Ovaries	F	1.1 g	-55%	--	-67%
Spleen	M	55.6 g	--	--	--
	F	62.4 g *24.2 g*	-29%	-49%	-39% *+126%*
Thymus	M	*5.2 g*	--	--	*+185%*
	F	*4.2 g*	--	--	*+67%*

-- No Finding; N.D. = No data

Histopathology

Adequate Battery

An adequate histopathological battery was performed. All groups were evaluated.

Peer Review

Yes

Histological Findings

- Major target organs of toxicity identified by the histopathology battery included male and female reproductive organs.
- Atrophy and aspermia was detected at 10 mg/kg/day in the epididymides. Diffuse atrophy in the prostate gland was also detected. Hypospermia occurred in the epididymides in one male. Hypospermatogenesis occurred in the testes in 2/2 recovery males previously treated with 10 mg/kg/day icatibant.
- Atrophy occurred in the uterine, cervix, and uterus at 10 mg/kg/day. Atrophy also occurred in the uterus of 2/4 females treated with 1 mg/kg/day icatibant. No developing follicles were identified in the ovaries of all females treated with 10 mg/kg/day icatibant.
- Mammary gland atrophy was identified in 4/4 main study and 1/2 recovery females treated with 10 mg/kg/day icatibant.
- Lipoma was detected in 1/4 males treated with 10 mg/kg/day icatibant. Hyperplasia was detected in the urinary bladder of 1/2 recovery females previously treated with 10 mg/kg/day icatibant.

Microscopic Signs		Control		1 mg/kg/day		3 mg/kg/ 2x wk		10 mg/kg/day	
		M	F	M	F	M	F	M	F
	Group Size:	*4/2*	*4/2*	4	4	4	4	*4/2*	*4/2*
	Grade	Terminal/*Recovery*							
Adrenal Cortex, focal mononuclear cell	**Minimal**	--	--	--	--	--	--	1	--
Brainstem, perivascular inflammatory cell infiltration	**Minimal**	--	--	--	--	--	--	--	1
Epididymides, bilateral hypospermia	**Slight**	--	--	1	--	--	--	--	--
Epididymides, bilateral aspermia	**Present**	--	--	--	--	--	--	4	--
Epididymides, unilateral aspermia	**Present**	--	--	--	--	--	--	*2*	--
Epididymides, atrophic epithelium	**Present**	--	--	--	--	--	--	4	--
GI, Ileum, focal congestion	**Present**	--	--	1	1	--	--	1	1
Injection site,	**Minimal**	1	--	1	--	2	--	1	1

Microscopic Signs		Control		1 mg/kg/day		3 mg/kg/ 2x wk		10 mg/kg/day	
		M	F	M	F	M	F	M	F
	Group Size:	4/2	4/2	4	4	4	4	4/2	4/2
	Grade	Terminal/*Recovery*							
middle, inflammatory cell infiltration	**Slight**	--	--	--	--	--	--	3	--
	All	1	--	1	--	2	--	4	1
Injection site, middle, focal granuloma	**Minimal**	--	--	--	--	--	--	--	1
Injection site, middle, focal myositis	**Slight**	--	--	--	--	--	--	--	1
Kidneys, focus(i) of basophilic (regenerating) tubules	**Minimal**	--	--	--	--	--	--	--	1
Kidneys, proteinaceous cast	**Minimal**	--	--	--	--	--	--	1	--
Pituitary gland, cysts	**Present**	--	--	--	--	--	--	--	*1*
Prostate gland, diffuse atrophy	**Mod.**	--	--	--	--	--	--	*1*	--
	Marked	--	--	--	--	--	--	*1*	--
	Severe	--	--	--	--	--	--	4	--
	All	--	--	--	--	--	--	4/*2*	--
Skin, focal dermatitis	**Minimal**	--	--	--	--	--	--	--	*1*
Salivary gland, periductal mononuclear cell infiltration	**Minimal**	1	2	1	1	2	1	--	1
	Slight	--	--	--	--	--	--	1	1
	All	1	2	1	1	2	1	1	2
Lungs, agonal congestion	**Present**	--	--	--	1	--	--	--	2
Lungs, perivascular inflammatory cell infiltration	**Minimal**	--	--	--	--	--	--	--	*1*
Lungs, hair shaft embolism	**Present**	--	--	--	--	--	--	--	*1*
Lymph node, mandibular, sinusal black pigmented macrophages	**Minimal**	--	--	--	--	--	--	--	*1*

Microscopic Signs		Control		1 mg/kg/day		3 mg/kg/ 2x wk		10 mg/kg/day	
		M	F	M	F	M	F	M	F
	Group Size:	4/2	4/2	4	4	4	4	4/2	4/2
	Grade	Terminal/*Recovery*							
Lymph node, mandibular,intrasinusal congestion/ erythrophagocytosis	**Present**	--	--	--	--	--	--	--	*1*
Neoplasm, Lipoma	**Present**	--	--	--	--	--	--	1	--
Mammary gland, atrophy	**Present**	--	--	--	--	--	--	--	4/*1*
Mammary gland, inactive mammary tissue	**Present**	--	--	--	--	--	--	--	*2*
Testes, atrophy of leydig cells	**Present**	--	--	--	--	--	--	4	--
Testes, bilateral hypospermato-genesis	**Minimal**	*1*	--	--	--	--	--	--	--
	Marked	--	--	--	--	--	--	*1*	--
	Severe	--	--	--	--	--	--	*1*	--
	All	*1*	--	--	--	--	--	*2*	--
Ovaries, no corpora lutea	**Present**	--	1	--	--	--	--	--	4/*1*
Ovaries, no developing follicles	**Present**	--	--	--	--	--	--	--	4/*2*
Thymus, lymphocyte depletion	**Slight**	--	1	--	3	--	1	--	1
	Mod.	--	3	--	1	--	2	--	2
	Marked	--	--	--	--	--	1	--	1
	All	--	4	--	4	--	4	--	4
Tongue, foreign body granuloma	**Minimal**	--	--	--	--	1	--	1	--
Urinary bladder, Focal urothelial hyperplasia	**Minimal**	--	--	--	--	--	--	--	*1*
Urinary bladder, Focal hemmorrhage	**Slight**	--	--	--	--	--	--	--	*1*
Urinary bladder, congestion	**Present**	--	--	--	--	--	--	1	--

Microscopic Signs		Control		1 mg/kg/day		3 mg/kg/ 2x wk		10 mg/kg/day	
		M	F	M	F	M	F	M	F
	Group Size:	4/2	4/2	4	4	4	4	4/2	4/2
	Grade	Terminal/*Recovery*							
Uterine cervix, atrophy	**Minimal**	--	--	--	--	--	--	--	--
	Slight	--	--	--	--	--	--	--	2
	Mod.	--	--	--	--	--	--	--	2
	All	--	--	--	--	--	--	--	4
Uterus, atrophy	**Minimal**	--	--	--	2	--	--	--	--
	Slight	--	--	--	--	--	--	--	1
	Mod.	--	--	--	--	--	--	--	3
	All	--	--	--	2	--	--	--	4

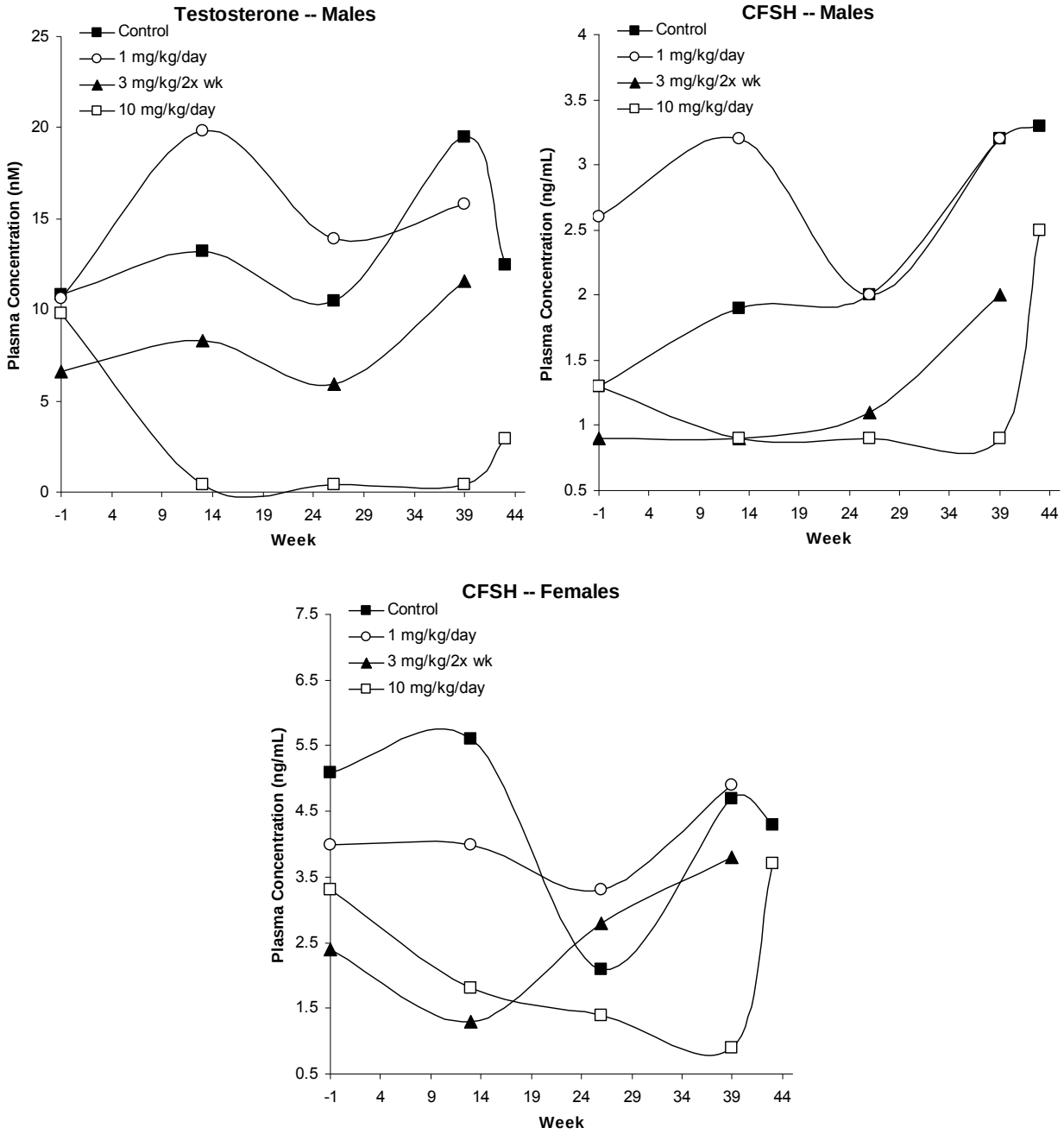
-- No Finding

Special Evaluation

Hormone Analysis

- Blood samples were taken from all animals for hormone analysis pre-treatment and in Weeks 13, 26, 39 and 43.
- Males were tested for testosterone, luteinizing hormone (LH), canine follicle stimulating hormone (CFSH).
- Females were tested for luteinizing hormone (LH), canine follicle stimulating hormone (CFSH).
- LH analysis was only performed for Weeks 26, 39 and 43.
- Samples were obtained from control, 1 mg/kg/day and 10 mg/kg/day groups at approximately 24 hours post-dose during the treatment period and at the same approximate time of day in the treatment-free period. For the 3 mg/kg/2x wk group (intermittent dosing), hormone sampling in Week 13 was 24 hours after dosing and in Week 26 immediately prior to dosing on one of the dosing days.
- No significant differences were detected in LH levels in either males or females.
- Drug-related decreases were detected in testosterone levels (males tested only). Levels recovered only partially by week 43.
- Decreases in CFSH occurred in both males and females treated with 10 mg/kg/day icatibant. The interpretation of these results is less clear because variability in CFSH levels in control animals; this variability is likely to be related to the estrous cycle in females. However, CFSH at 10 mg/kg/day was consistently below control levels in all time-points.

Figure 6. Testosterone and FSH Levels in 39 Wk Dog Study# JE049-0164



Testosterone (males) (nM)					
Week Dose	-1	13	26	39	43
Control	10.8	13.2	10.5	19.5	12.5
1 mg/kg/day	10.6	19.8	13.9	15.8	N.D.
3 mg/kg/2x wk	6.6	8.3	5.9	11.6	N.D.
10 mg/kg/day	9.8	0.4	0.4	0.4	2.9
Canine FSH (males) (ng/mL)					
Week Dose	-1	13	26	39	43
Control	1.3	1.9	2	3.2	3.3
1 mg/kg/day	2.6	3.2	2	3.2	N.D.
3 mg/kg/2x wk	0.9	0.9	1.1	2	N.D.
10 mg/kg/day	1.3	0.9	0.9	0.9	2.5
Canine FSH (females) (ng/mL)					
Week Dose	-1	13	26	39	43
Control	5.1	5.6	2.1	4.7	4.3
1 mg/kg/day	4	4	3.3	4.9	N.D.
3 mg/kg/2x wk	2.4	1.3	2.8	3.8	N.D.
10 mg/kg/day	3.3	1.8	1.4	0.9	3.7
LH (males) (ng/mL)					
Week Dose	-1	13	26	39	43
Control	N.D.	N.D.	1.9	1.9	2.6
1 mg/kg/day	N.D.	N.D.	1.6	1.6	N.D.
3 mg/kg/2x wk	N.D.	N.D.	1.6	1.6	N.D.
10 mg/kg/day	N.D.	N.D.	1.6	1.6	1.6
LH (males) (ng/mL)					
Week Dose	-1	13	26	39	43
Control	N.D.	N.D.	1.6	1.6	1.6
1 mg/kg/day	N.D.	N.D.	1.9	1.6	N.D.
3 mg/kg/2x wk	N.D.	N.D.	1.6	1.6	N.D.
10 mg/kg/day	N.D.	N.D.	1.6	1.6	1.6

N.D. = No data

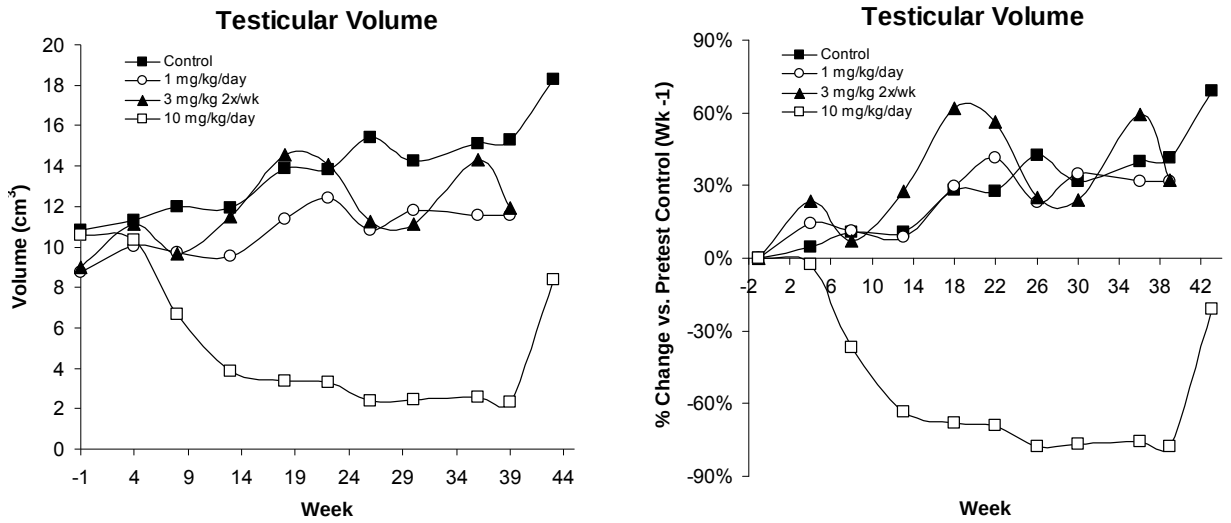
Testes Measurements

- The width (side to side) and length (head to tail) of the testes was measured and recorded once each month using digital vernier callipers (measurements were recorded in millimeters to 2 d.p.).
- No significant differences were observed during weeks -1, 4
- Testicular volume was modeled with the equation describing the volume of a cylinder:

$$V = \pi r^2 * h$$

V = volume in mm^2; r = 0.5 * (testicular width in mm); h = testicular length in mm

Figure 7. Testicular Volumes in 39 Wk Dog Study# JE049-0164



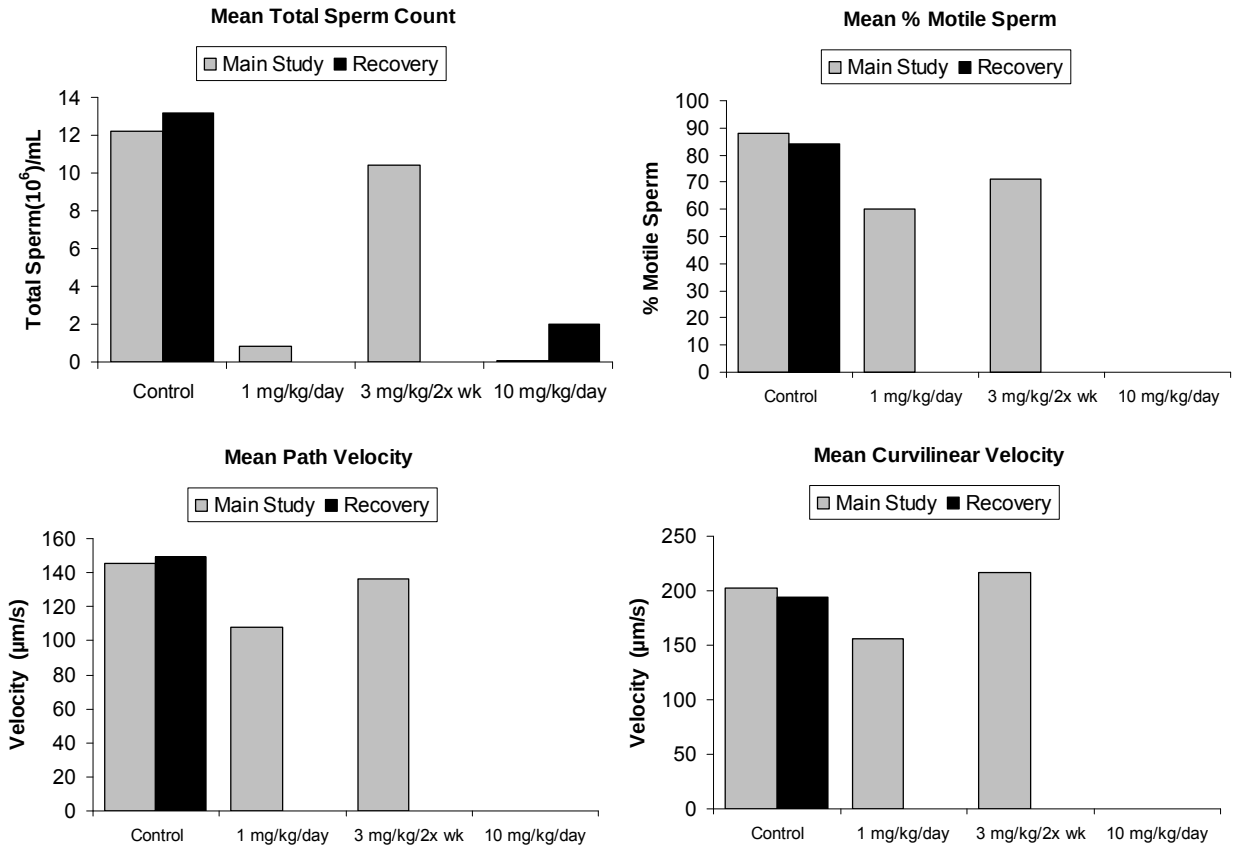
Testicle Width (mm)											
Week Dose	-1	4	8	13	18	22	26	30	36	39	43
Control	20.4	19.6	20.3	20.6	22.1	21.6	23.4	22.4	22.5	22.5	23.6
1 mg/kg/day	18.5	18.3	18.6	19.3	20.8	20.8	19.9	20.7	**20.2***	20.1	N.D.
3 mg/kg/2x wk	18.5	19.6	19.0	20.7	22.6	22.2	21.1	21.0	22.6	21.0	N.D.
10 mg/kg/day	19.7	19.3	**16.7***	**13.8***	13.4	**13.1***	21.3	**11.9***	**12.0***	11.8	18.4
Testicle Length (mm)											
Week Dose	-1	4	8	13	18	22	26	30	36	39	43
Control	32.9	36.3	36.2	35.0	35.1	37.0	34.9	35.5	37.2	37.0	38.6
1 mg/kg/day	32.4	36.8	35.6	32.9	32.8	35.8	34.3	34.9	35.4	36.0	N.D.
3 mg/kg/2x wk	33.4	36.1	33.8	33.9	35.6	36.1	**30.9***	**31.6***	35.2	33.9	N.D.
10 mg/kg/day	33.9	34.6	28.6	**23.9***	**23.5***	**23.8***	**21.3***	**20.7***	**21.7***	**20.3***	**30.8***
Testicle Volume (cm^3)											
Week Dose	-1	4	8	13	18	22	26	30	36	39	43
Control	10.8	11.3	12.0	12.0	13.9	13.8	15.4	14.3	15.1	15.3	18.3
1 mg/kg/day	8.8	10.0	9.8	9.5	11.4	12.4	10.8	11.8	11.5	11.5	N.D.
3 mg/kg/2x wk	9.0	11.1	9.6	11.5	14.6	14.1	11.3	11.2	14.3	11.9	N.D.
10 mg/kg/day	10.6	10.3	6.7	3.9	3.4	3.3	2.4	2.4	2.6	2.3	8.4

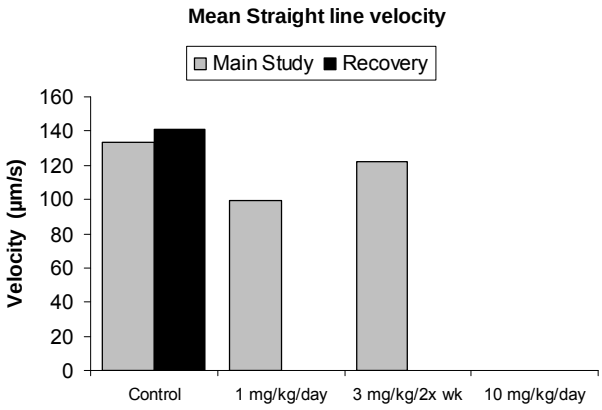
N.D. = No data; *Statistically Significant vs. Concurrent control (t-test)

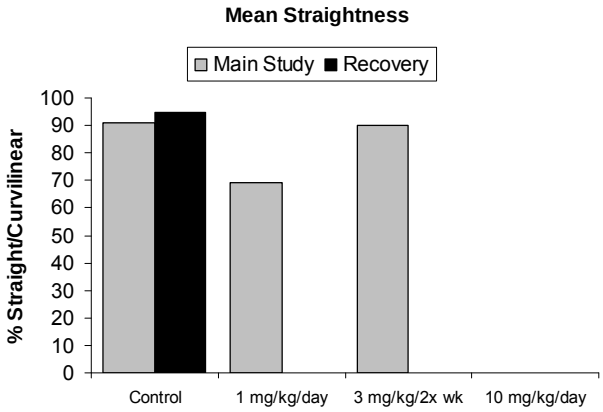
Seminology

- Sperm viability checks (including motility, velocity and sperm count) were made on samples from main study males in Week 39 and recovery males in Week 43.
- No samples could be obtained in-life from high dose males and these were therefore obtained at necropsy on both occasions.
- Decreased sperm counts detected at 1 and 10 mg/kg/day.
- Slight decreases in sperm motility detected at 1 mg/kg/day. Profound decreases in sperm motility occurred at 10 mg/kg/day.

Figure 8. Sperm Counts and Viability in 39 Wk Dog Study# JE049-0164







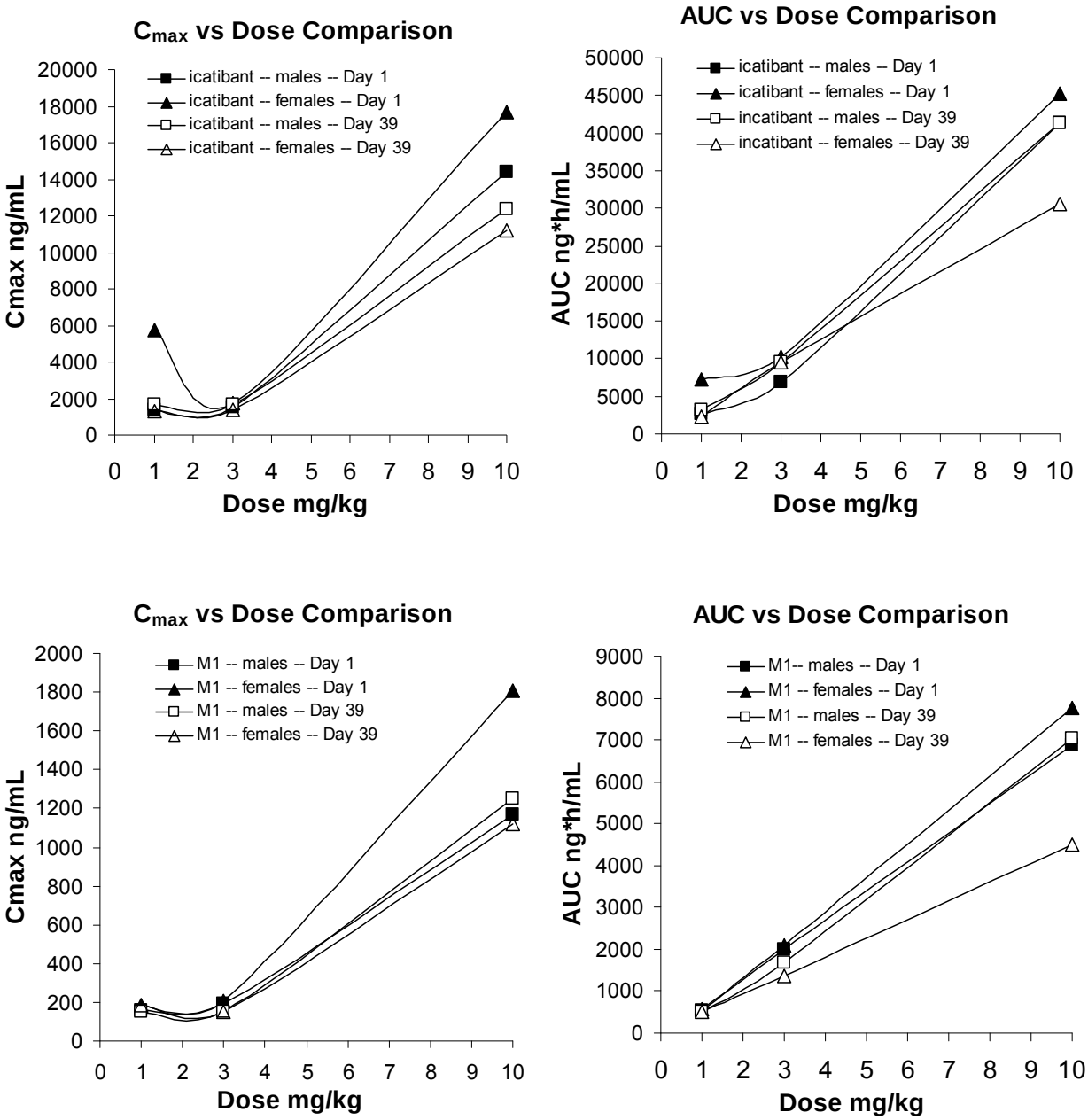
Dose	Control	1 mg/kg/day	3 mg/kg/2x wk	10 mg/kg/day
Mean total sperm count (10^6/mL)	12.2	0.8	10.4	0.1
Mean % motile	88	60	71	0
Mean Path Velocity (µm/s)	145.7	107.6	136.5	0
Mean Curvilinear Velocity (µm/s)	201.8	156.4	216.3	0
Mean Straight line velocity (µm/s)	133.4	99.2	122.3	0
Mean Straightness*	91	69	90	0

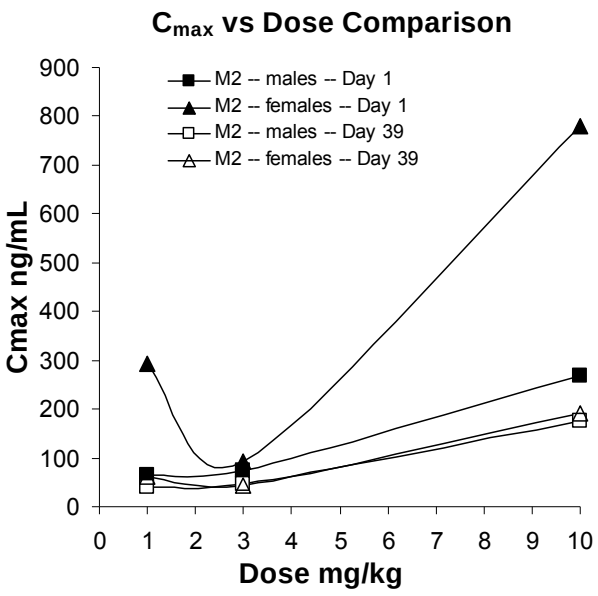
*Mean Straightness = (Mean Straight Line Velocity/Mean Path Velocity)*100

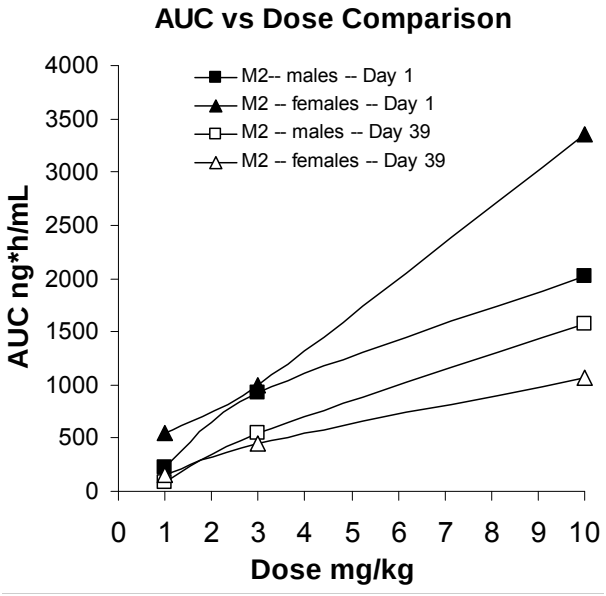
Toxicokinetics

- Analysis was performed using high performance liquid chromatography (HPLC) with tandem mass spectrometry detection (LC-MS/MS).
- The lower limit of quantification (LLOQ) was 5.00 ng/mL for icatibant, and 2.50 ng/mL for M1 and M2 using 50 µl dog serum
- Blood samples were taken 1, 2, 4, 6, 24 h post-dose on Day 1, and during Week 39.
- These data show similar C_{max} and AUC exposures between males and females and between Day 1 and Week 39 for icatibant, M1 and M2.

Figure 9. Icatabant, M1, and M2 C_{max}/AUC in 39 Wk Dog Study# JE049-0164







Day 1							
		Cmax ng/mL			AUC ng*h/mL		
Dose	Sex	icatibant	M1	M2	icatibant	M1	M2
1 mg/kg/day	M	1350	161	65.8	2650	531	230
	F	5750	176	294	7180	563	549
3 mg/kg/2x wk	M	1510	190	75.5	6930	2010	931
	F	1710	205	93.9	10300	2090	995
10 mg/kg/day	M	14400	1170	268	41300	6880	2020
	F	17700	1810	781	45200	7770	3360
Day 39							
1 mg/kg/day	M	1660	154	41.6	3220	498	89
	F	1290	185	62.3	2270	496	159
3 mg/kg/2x wk	M	1660	150	46.9	9520	1680	545
	F	1410	149	44.6	9610	1360	450
10 mg/kg/day	M	12400	1250	175	41300	7020	1570
	F	11200	1120	192	30600	4510	1070

Dosing Solution Analysis

- Analysis of the formulations for achieved concentration was performed on samples prepared for use on Day 1 and in Week 39 (Day 267), on the same day as toxicokinetic blood sampling
- The formulations were extracted and diluted to within the range of the calibration line (approximately 16.6 to 100.0 µg/ml) using diluting solvent.

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- Samples were analyzed with High Performance Liquid Chromatography (HPLC).
- Three random samples were removed from each of the formulations. Two of the samples were analyzed and the third retained in reserve.
- Two samples were taken from control formulations and one of these samples was analyzed.
- The target range for preparation of liquid formulations is 90 to 110% of nominal.
- Results were in the range 96 to 106%. Icatibant was not detected in the Group 1 control samples

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Study title: Icatibant: 14 Day Subcutaneous Administration Range-Finding Toxicity Study in the Juvenile Rat

Study no.:	JE049-0172
Study report location:	(b) (4)
Conducting laboratory and location:	
Date of study initiation:	Jan. 15, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lot#1019997, 96%

Key Study Findings

- No serious adverse effects were observed in this study.
- A small decrease in heart and thymus weight was observed. No histopathology was conducted, so the possibility that these effects reflect underlying pathology cannot be examined.
- Major clinical observations consisted of injection lesions and hair loss at 25 mg/kg/day.
- Cmax and AUC exposures were increased with dose in a linear fashion.
- The NOAEL could not be set because histopathology was not conducted.

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Methods

Doses:

Doses				Number of rats			
				Main groups		TK groups	
Dose Group	Dose mg/kg	Schedule	Vol ml/kg	♂	♀	♂	♀
Control	0	Daily from PND* 22-PND 35	2	10	10	6	6
LD	6.25			10	10	6	6
MD	12.5			10	10	6	6
HD	25			10	10	6	6

PND = Post-Natal Day

Route of administration: Subcutaneous injection
Formulation/Vehicle: 0.9% physiological saline
Species/Strain: Crl:WI(Han) rats
Age: 22 Days Old
Weight: M: 37.8 – 56.0 g; F: 38.2 – 51.8 g
Unique study design: None
Deviation from study protocol: No significant deviations

Observations and Results

Mortality

- Rats were observed at the beginning and the end of the working day.
- No unscheduled mortality occurred in this study.

Clinical Signs

- All animals were observed daily for signs of ill health or overt toxicity.
- Animal was given a detailed physical examination at weekly intervals.
- An individual record was maintained of the clinical condition of each animal.
- The toxicity animals were observed approximately 0.5, 1 and 2 hours after dosing.
- All animals were observed once daily approximately 1 to 2 hours post dose for inflammatory skin reactions.
- Major clinical observations consisted of injection lesions and hair loss at 25 mg/kg/day.

No. Affected Animals, Post-Natal Day					
Parameter	Sex	Control	6.25 mg/kg/day	12.5 mg/kg/day	25 mg/kg/day
Skin and hair, Injection lesions	M	--	--	1, Day 36	3, Day 36 2, Day 37
	F	--	--	--	2, Day 36 1, Day 37
Thinning fur	M	--	--	--	2, Day 36 1, Day 37

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No. Affected Animals, Post-Natal Day					
Parameter	Sex	Control	6.25 mg/kg/day	12.5 mg/kg/day	25 mg/kg/day
	F	--	--	--	5, Day 36 4, Day 37

Body Weights

- Individual body weights were recorded for all animals on post-natal days 14 and 21 and daily from post-natal day 22 until necropsy
- There was a slight decrease in weight gain compared to concurrent control in females treated at 12.5 and 25 mg/kg/day icatibant.

Weight Gain From Post-Natal Day 22 to 36

	Control	6.25 mg/kg/day	12.5 mg/kg/day	25 mg/kg/day
Males	61.2 g	63.8 g	61.5 g	61.1 g
Females	57.3 g	57.0 g	53.7 g	53.1 g

Feed Consumption

- The amount of food consumed by each cage of toxicity animals was determined twice weekly. Consumption was calculated as g/animal/week.
- There was a slight decrease in food consumption compared to concurrent control in females treated at 25 mg/kg/day icatibant.

Food Consumption From Post-Natal Day 22 to (35 g/animal/week)

	Control	6.25 mg/kg/day	12.5 mg/kg/day	25 mg/kg/day
Males	108 g	111 g	108 g	107 g
Females	101 g	101 g	100 g	96 g

Ophthalmoscopy

- Not conducted

ECG

- Not conducted

Hematology

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- Not conducted

Clinical Chemistry

- Not conducted

Urinalysis

- Not conducted

Gross Pathology

- Dams, TK animals, and surplus pups were euthanized by IP pentobarbitone overdose and discarded.
- Main study pups were necropsied after an overnight period without food. Pups were given an IP pentobarbitone overdose and exsanguinated once a suitable deep plane of anesthesia was established.
- A full macroscopic examination was performed under the general supervision of a pathologist and all lesions recorded.

Main Study					
Parameter	Sex	Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg
Injection site, sore	M	--	--	1	3
	F	--	--	--	2
Thymus, red	M	--	--	--	--
	F	--	--	1	1
Thyroid, small	M	--	--	--	--
	F	--	--	--	1

-- No Finding

Organ Weights

- The following organs were weighed: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, epididymides, mesenteric lymph nodes, thymus, thyroids, and parathyroids.

Main Study					
Organ	Sex	Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg
Heart	M	0.508 g	-4%	-4%	-8%
	F	0.469 g	--	--	--
Thymus	M	0.420 g	-4%	-5%	-9%
	F	0.362 g	--	-6%	-6%

-- No Finding

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Histopathology

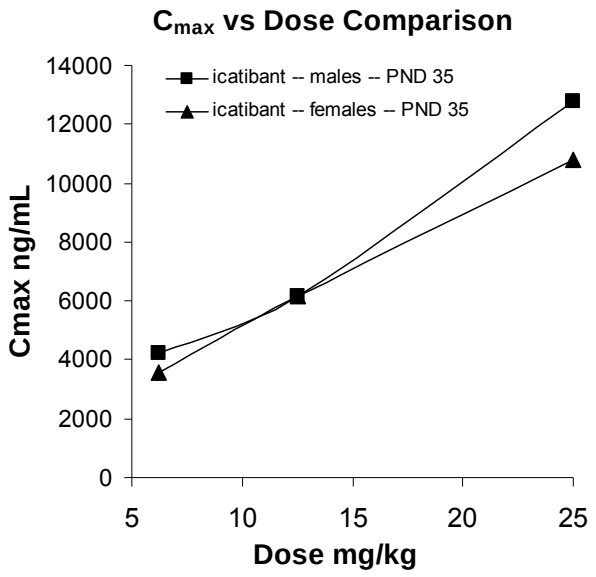
- Not conducted

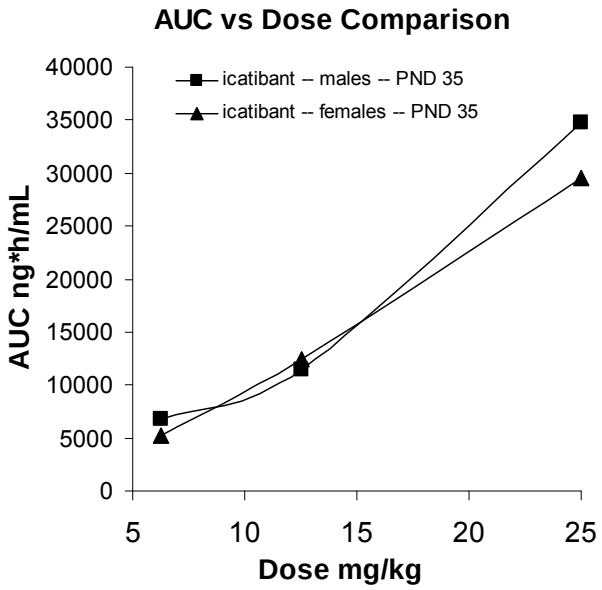
Special Evaluation

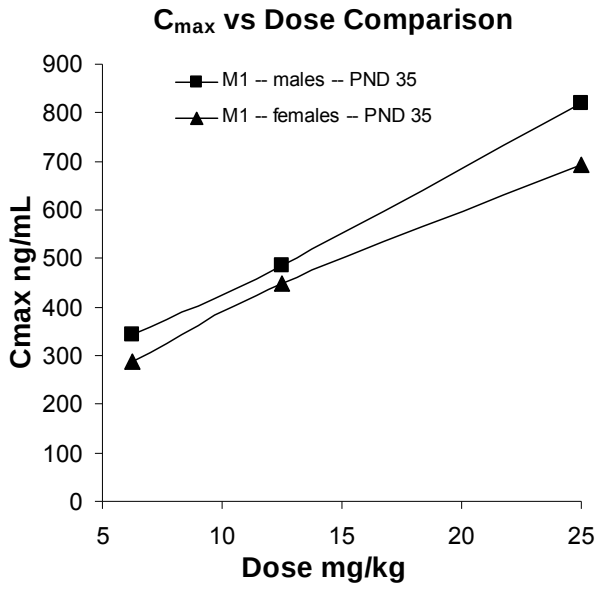
- Not conducted

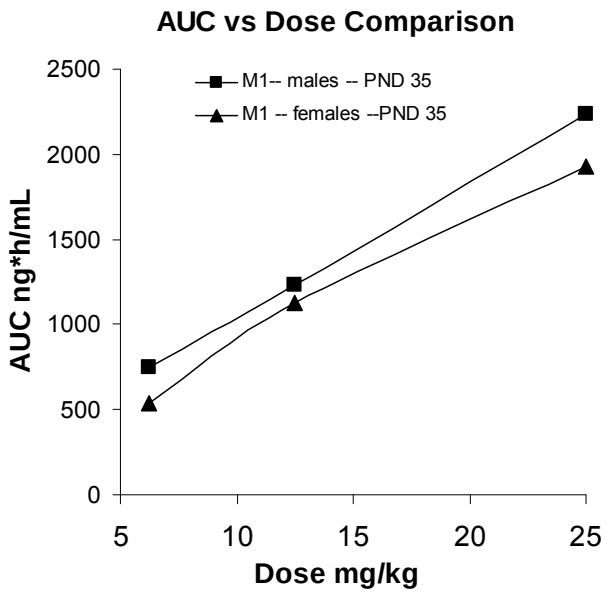
Toxicokinetics

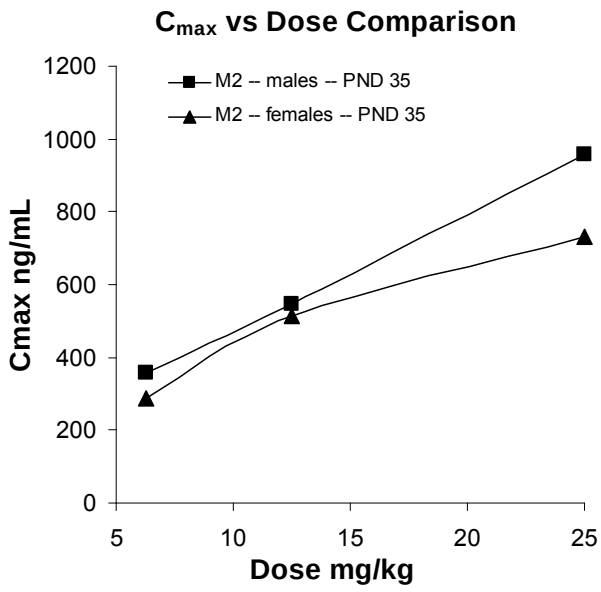
- Analysis was performed using high performance liquid chromatography (HPLC) with tandem mass spectrometry detection (LC-MS/MS).
- The low limit of quantification (LLOQ) for icatibant, icatibant M1 and icatibant M2 in rat plasma is 25, 2.5 and 2.5ng/mL respectively
- Blood samples were taken 1, 2, 4, 24 h post-dose on post-natal day 35.
- These data show similar C_{max} and AUC exposures between males and females.
- Icatibant, M1, and M2 C_{max} and AUC exposures were increased linearly with increasing icatibant dose.

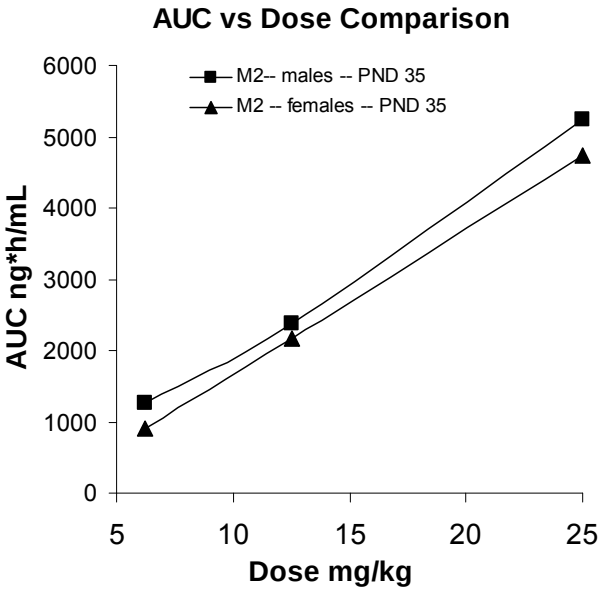












Post-Natal Day 35							
		Cmax ng/mL			AUC ng*h/mL		
Dose	Sex	icatibant	M1	M2	icatibant	M1	M2
6.25 mg/kg/day	M	4240	344	357	6840	745	1260
	F	3540	289	288	5180	538	908
12.5 mg/kg/2x wk	M	6150	487	547	11400	1230	2380
	F	6180	449	513	12500	1130	2160
25 mg/kg/day	M	12800	821	957	34700	2240	5240
	F	10800	693	733	29500	1930	4730

Dosing Solution Analysis

- Three samples were removed from the test article formulations.
- Two of the samples were analyzed with the third to be analyzed only if the samples results are outside the target range (90 to 110% of nominal concentration).
- Two samples were taken from control formulations. One of these samples was analyzed.
- Results were in the range 92 to 100%. Icatibant was not detected in the control samples

Achieved concentration

	Results as % nominal					
Day	Group 2		Group 3		Group 4	
1	100	100	99	98	99	99
14	97	97	95	96	93	92

*Excerpted from sponsor submission

7 Genetic Toxicology

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

Studies Nos. JE049-0126 (89.1177), JE049-0130 (91.0537), and JE049-0131 (92.0405), were submitted to (b) (4) and reviewed under both INDs. The study numbers in parenthesis are from the old numbering scheme used by (b) (4) when it was the sponsor of (b) (4). Although the original versions of these reviews are not available, they are quoted and summarized in Dr. Ron Honchel's review of the original submission to IND 68214. Study JE049-0132 was submitted to the original submission of this NDA and reviewed by Dr. Molly Topper. Icatibant was not mutagenic in these tests with and without metabolic activation.

7.2 *In Vitro* Assays in Mammalian Cells

Study No. JE049-0133 is an *in vitro* mammalian aberration test in cultured human lymphocytes submitted to the original submission of this NDA and reviewed by Dr. Molly

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Topper. Icatibant did not induce chromosomal aberrations under the experimental conditions of this study.

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

Study No. JE049-0134 (89.1351 using the old (b) (4) numbering scheme) is an *in vivo* bone marrow micronucleus test in male and female NMR1 mice originally submitted to IND (b) (4) The review of this study is quoted and summarized in Dr. Ron Honchel's review of the original submission to IND 68214. Mice were administered a single intravenous injection of 50 mg/kg icatibant. This study indicated that the incidence of polychromatic and normochromatic micronuclei in icatibant-treated mice was similar to the negative control.

Study No. JE049-0135 is an *in vivo* bone marrow micronucleus test in rats administered two doses of intravenous 3.75, 7.5, and 15 mg/kg icatibant with a 24 h interval between doses. This study was submitted to the original submission of this NDA and reviewed by Dr. Molly Topper. Icatibant did not induce micronucleus formation under the study conditions.

Study No. JE049-0136 (91.0123 using the old (b) (4) numbering scheme) is an *in vivo* bone marrow cell aberration test in Chinese Hamsters originally submitted to IND (b) (4) and reviewed by Dr. Young Choi. Single IP injections of 10, 50, and 100 mg/kg icatibant did not induce significant increases of bone marrow cells with chromosomal aberrations, although animals showed various toxic signals, including clonic convulsions and irregular breathing. By contrast, the positive control did produce significant increases in chromosomal aberrations.

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

The sponsor has initiated two 104-week carcinogenicity studies of icatibant, in mice and in rats. Supporting toxicological data for these on-going studies were reviewed by Drs. Molly Topper and Timothy Robison and concurrence was obtained by the sponsor from the Executive Carcinogenicity Committee.

During a pre-NDA meeting held on February 22, 2007, the Division conveyed a willingness to accept carcinogenicity studies as a Phase 4 Commitment. However, under the Food and Drug Administration Amendments Act (FDAAA) of 2007, the Division now considers these studies as post-marketing requirements (PMRs) since they are safety assessments. These PMRs are detailed in the integrated summary and safety evaluation section of this review.

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9 Reproductive and Developmental Toxicology

The sponsor submitted an adequate reproduction toxicology program with the original submission to this NDA. These studies were reviewed under IND (b) (4) and in the review for the original submission to this NDA. Although the original Pharm/Tox review for IND (b) (4) is not available, this review is summarized by Dr. Ronald Honchel in his review of IND 68,214.

Table 7. Reproductive Toxicology Studies and Clinical/Non-clinical AUC Comparison

Study No.	Species	Schedule	Dose mg/kg	Dose mg/m^2	AUC Exposure	Effect
JE049-0108	SD Rat	Daily SC 13 wks	1	6	M: 1.68 µg*h/mL F: 1.23 µg*h/mL	• Tolerated
			3	18	M: 5.57 µg*h/mL F: 4.95 µg*h/mL	• Female NOAEL
			10	60	M: 15.9 µg*h/mL F: 17.2 µg*h/mL	• Females pre-implantation loss • Females: slightly higher post-implantation loss • Male NOAEL: No effects on sperm count, motility, morphology
JE049-0137 0-91-009-086-04 Under (b) (4)	Jcl:ICR Mouse	Daily IV x35 days	16.2	48.6	No data	• Not performed according to Seg 1 Guidelines (pre-1993) • No drug-related effects on copulation or conception rate • No drug-related effects on spermatozoal number, motility, or morphology • No drug-related effects in the numbers of implantations, placental remnants, resorptions, and live/dead embryos
			80.8	242.4	No data	
JE049-0138 0-92-041-086-04 Under (b) (4)	Jcl:ICR Mouse	Daily IV x35 days	5	15	No data	• Not performed according to Seg 1 Guidelines (pre-1993) • No drug-related effects on copulation or conception rate • No drug-related effects on spermatozoal number, motility, or morphology • No drug-related effects in the numbers of implantations, placental remnants, resorptions, and live/dead embryos
			30	90	No data	

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Study No.	Species	Schedule	Dose mg/kg	Dose mg/m^2	AUC Exposure	Effect
JE049-0139 (93.0541 under (b) (4)	Wistar rat	Daily SC Days 7 – 18	All doses	All doses	No data	• Trend in dose-related increase in length of pregnancy • Intrauterine deaths in all groups, including control • Placenta unremarkable in all groups • Implantation rate, number of live pups, and pup survival similar between groups • All dams raised their pups to weaning • F1 rats: no impairment in behavior, fertility
			0.25	1.5	No data	• One fetus: malformation in head and forelimbs • One fetus: hematoma in right cerebrum • One fetus: stomach filled with soft mass • Increased incidence of poorly ossified cranial bones and 5th metatarsal bone • One pup in 2 /10 litters and all pups in 1/10 litters reported insufficient suckling • One pup in 1/10 litters: impaired coat growth
			2.5	15	No data	• One fetus: malformation in head and forelimbs • One pup in 1/10 litters: impaired coat growth
			25	150	No data	• One fetus: displacement of the heart in cranial direction, light red soft mass in thoracic cavity • One fetus bifurcated right medial lobe of the lung • One fully developed dead fetus: edema in the subcutis, poorly ossified skeleton, longitudinally displaced sternebrae, short additional rib bilaterally on the 1st lumbar vertebra • 1/20 dams no live fetuses (resorption) • One pup in 1 /10 litters reported insufficient suckling • One pup in 2/10 litters: impaired coat growth
JE-049-140 (93.0542 under (b) (4)	Himalayan Rabbit	Daily SC days 7 – 18 of pregnancy	0.1	1.2	No data	• Vaginal hemorrhage (1/15) • Premature birth (1/15) • Abortion (2/15)
			1	12	No data	• Vaginal hemorrhage (1/15) • Abortion (2/15)
			10	120	No data	• Vaginal hemorrhage (1/15) • Abortion (2/15) • Decreased implantation % • Increased intrauterine deaths
JE049-0141	SD Rat	Dams:	1	6		• Delayed parturition

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Study No.	Species	Schedule	Dose mg/kg	Dose mg/m^2	AUC Exposure	Effect
		Day 6 pc – Day 21 post-partum			F: 1.62µg*h/mL	• Icatibant found in milk • **NOAEL for F1 <u>systemic toxicity</u>** • **No NOAEL for F0 generation**
			3	18	F: 5.65 µg*h/mL	• Delayed parturition leading to fetus/pup death • Icatibant, M2 found in milk • 1/24 dams euthanized • **NOAEL for F1 <u>development</u>**
			10	60	F: 20.8 µg*h/mL	• Delayed parturition leading to fetus/pup death • Icatibant, M2 not measured in milk due to early deaths • Increased pre-implantation loss • Slight decrease in air righting and hair growth in F1 • 14/24 dams found dead or euthanized
JE-049-353	Himalayan Rabbit	Daily SC days 6 – 10 post-coitum	1	12	F: 4.38 µg*h/mL	• Thee females given 10 mg/kg/day had implant scars without live conceptuses. • All control animals were pregnant; 5/6 females treated at 1 mg/kg/day and 1/6 females treated at 10 mg/kg/day were pregnant at the end of the study.
			10	120	F: 51.4 µg*h/mL	
JE-049-0163	Wistar Rat	Daily SC 26-weeks	3	18	M: 7.2 µg*h/mL F: 5.2 µg*h/mL	• Reduced prostate secretion • Bilateral germinal epithelial degeneration in the testes • Mammary gland masculinization • Males: ~80% decrease testosterone • ~30% decrease FSH • ~70 decrease LH • Degenerate corpora lutea
			10	60	M: 25.1 µg*h/mL F: 18.1 µg*h/mL	• Reduced prostate secretion • Bilateral germinal epithelial degeneration in the testes • Mammary gland masculinization • Uterine Atrophy • Males: ~90% decrease testosterone • ~50% decrease FSH • ~70 decrease LH • Degenerate corpora lutea
			30	180	M: 53.8 µg*h/mL F: 61.3 µg*h/mL	• Reduced prostate secretion • Bilateral germinal epithelial degeneration in the testes

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Study No.	Species	Schedule	Dose mg/kg	Dose mg/m^2	AUC Exposure	Effect
						• Mammary gland masculinization • Uterine Atrophy • Degenerate corpora lutea
JE049-0164	Beagle Dog	Daily SC 38 wks	1	20	M: 2.65 µg*h/mL F: 7.18 µg*h/mL	• Decreased sperm counts • Reduced testicle size • Uterine atrophy
			10	200	M: 41.3 µg*h/mL F: 45.2 µg*h/mL	• Undetectable sperm counts • Reduced testicle size • Reduced testosterone (>90%) • Decreased ovary size developing follicles
		SC TID 2x per wk	3	60	M: 6.9 µg*h/mL F: 10.3 µg*h/mL	• NOAEL for spermatogenesis • NOAEL for uterine/ovary/mammary effects
	Human Male 18 - 45 yro	Single SC	0.5	17	1.2 µg*h/mL	Clinical Dose
		SC TID	1.5	50	1.4 µg*h/mL	
	Human Female 18 - 45 yro	Single SC	0.5	17	2.9 µg*h/mL	
		SC TID	1.5	50	3.0 µg*h/mL	
	Human Male >65 yro	Single SC	0.5	17	2.7 µg*h/mL	
		SC TID	1.5	50	3.5 µg*h/mL	
	Human Female >65 yro	Single SC	0.5	17	4.8 µg*h/mL	
		SC TID	1.5	50	5.5 µg*h/mL	

Reference ID: 2977968

9.1 Fertility and Early Embryonic Development

Studies JE049-0137 and JE049-0138 with mice were reviewed under IND (b) (4). Daily administration of icatibant revealed no impairment of fertility in mice with intravenous doses up 81 mg/kg or in rats (JE049-0108) with subcutaneous doses up to 10 mg/kg (**Table 7**). However, long term general toxicology studies conducted in rats (JE049-0163) and dogs (JE049-0164) showed that daily subcutaneous administration of icatibant to rats and dogs caused ovarian, uterine, and testicular atrophy/degeneration, decreased levels of testosterone and adverse effects on the mammary and prostate glands. In dogs, these effects led to reduced sperm counts, degenerate corpora lutea, and decreases in developing follicles in the ovary. By contrast, toxicity to the ovary, uterus, testis, mammary gland, and prostate did not occur in dogs treated twice a week for 9 months. Sperm counts and testosterone remained unaffected over the course of the study in male dogs dosed twice a week.

9.2 Embryonic Fetal Development

Studies JE049-0139 and JE049-0140 in and rats and rabbits, respectively were reviewed under IND (b) (4) These studies showed that daily administration of icatibant caused delayed parturition, fetal death and pre-implantation loss in rats and premature birth, abortion, fetal death, and pre-implantation loss in rabbits (**Table 3**). Study JE049-353, reviewed below, was designed to provide icatibant exposure data for Himalayan rabbits treated on the same schedule and time of development as rabbits treated in Study # JE-049-140. However, the low dose in Study JE-049-140, 0.1 mg/kg, which caused symptoms of premature birth or abortion, was not tested in this study JE049-353.

Study title: Study For Effects On Embryo-Fetal Development By Subcutaneous Route In Himalayan Rabbits: Complementary Study To Evaluate The Toxicokinetic Profile Of Icatibant And Its Metabolite M2

Study no.:	JE049-0353
Study report location:	(b) (4)
Conducting laboratory and location:	Same as above
Date of study initiation:	September 26, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	E085, 83.6%

Key Study Findings

- C_{max} and AUC exposures were roughly dose-proportional for both icatibant and M2.
- AUC values for icatibant were 4.38 and 51.4 µg.h/mL for the 1 and 10 mg/kg/day dose-levels, respectively; corresponding values for the M2 metabolite were 0.46 and 2.91 µg.h/mL.
- Thee females given 10 mg/kg/day had implant scars without live conceptuses.

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- All control animals were pregnant; 5/6 females treated at 1mg/kg/day and 1/6 females treated at 10 mg/kg/day were pregnant at the end of the study.

Methods

Doses:

Doses				Rabbits	
				Main groups	
Dose Group	Dose mg/kg	Schedule	Vol ml/kg	Mated ♀	Pregnant ♀
Control	0	daily days 6-10 p.c.	2	3	3
LD	1			6	5
HD	10			6	4

Route of administration: Subcutaneous injection
Formulation/Vehicle: Sterile 0.9% NaCl in water
Species/Strain: Himalayan rabbit; 17-19 weeks old; 2.1-2.8 kg
Study design: This study was designed to provide icatibant exposure data for Himalayan rabbits treated on the same schedule and time of development as rabbits treated in Study # JE-049-140 (#93.0542 under IND (b) (4); September 1993). **However, the low dose in Study 93.0542, 0.1 mg/kg, which caused symptoms of premature birth or abortion, was not tested in this study.**

Rabbits were given SC icatibant on days 6-10 *post-coitum* and necropsied. Plasma levels of icatibant and metabolite M2 were determined on day 10 *p.c.*
Deviation from study protocol: No significant deviations

Observations and Results

Mortality

- Rabbits were checked for mortality or signs of morbidity at least twice per day during the treatment period.
- One control female was found dead 0.5 h after blood sampling on Day 9 p.c.
- Gross exam of this female showed a hematoma adjacent to the blood sampling site and thick reddish contents in the thoracic cavity.

Clinical Signs

- Each animal was observed once a day, at approximately the same time of the day, for the recording of clinical signs (including evidence of abortion).
- All clinical observations involved injection site trauma.

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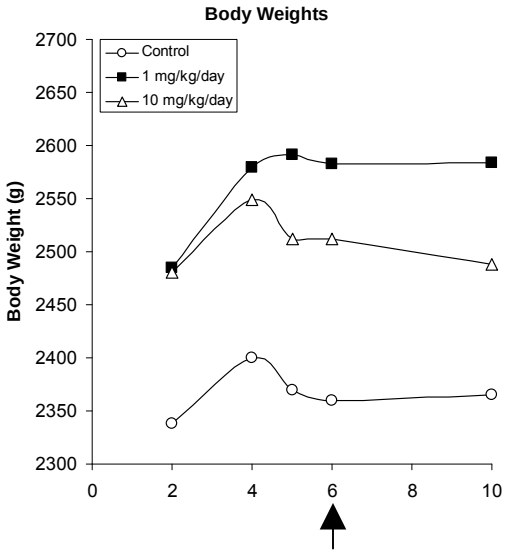
No. Affected Animals, Day				
Parameter	Sex	Control	1 mg/kg/day	10 mg/kg/day
Hematoma at injection site	F	--	2, day 8 1, day 9 1, day 10	2, day 7 1, day 10

Body Weight

- No statistically significant decreases in body weight occurred over the 5-day dosing period.
- There was a slight trend toward decrease in weight gain over the 5-day dosing period.



Days *p.c.* ; Arrow = Dosing Start

	Control	1 mg/kg/day	10 mg/kg/day
Weight Gain Days 6-10 *p.c.*	5 g	-1.7 g	-23 g

Feed Consumption

- The amount of food consumed for each animal was determined on days 2-4, 4-5, 5-6, and 5-10 *p.c.*
- There were no differences between treatment groups in food consumption.

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Toxicokinetics

- Analysis was performed using high performance liquid chromatography (HPLC) with tandem mass spectrometry detection (LC-MS/MS).
- The lower limit of quantification (LLOQ) was 10 ng/mL for icatibant, and 20 ng/mL and M2 using 200 µl rabbit serum
- TK blood draws were 0.5, 2, and 6 h post-dose for the control group and pre-dose, 0.5, 1, 2, 4, 6, 8, and 24 h post-dose for icatibant-treated groups
- These data show that C_{max} and AUC exposures were roughly dose-proportional for both icatibant and M2.

Day 10 *post-coitum*							
		C_{max} (µg/mL)		$AUC_{(0-t)}$ (µg*h/mL)		t_{max}	
Dose	Sex	icatibant	M2	icatibant	M2	icatibant	M2
1 mg/kg/day	F	1.65	0.15	4.4	0.46	0.5	2
10 mg/kg/day	F	16.8	1.02	51.4	2.91	1	2

Dosing Solution Analysis

- Icabitant concentration was determined in samples taken from each control and icatibant dosage form prepared for use on day 6 *p.c.*
- The nominal and actual concentrations of the test item in the administered dosage forms analyzed since the deviations from nominal concentration were within a range of ± 10%.

Necropsy

- After collection of their last blood sample, all the animals were euthanized with an intravenous injection of thiopental sodium.
- The pregnancy status was determined by recording the number of corpora lutea and implantation sites.
- Thee females given 10 mg/kg/day had implant scars without live conceptuses.
- All control animals were pregnant; 5/6 females treated at 1mg/kg/day and 1/6 females treated at 10 mg/kg/day were pregnant at the end of the study.
- A complete necropsy was not performed on all animals surviving to the end of the study. No histopathology or organ weight analyses were performed.
- A gross examination of a control female that was found dead on day 9 *p.c.* was performed (see Mortality section).

Cesarean Section Data

Parameter	Control	1 mg/kg/day	10 mg/kg/day
Corpora Lutea (No. per animal)	6.5	8.8	10.8

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Parameter	Control	1 mg/kg/day	10 mg/kg/day
Implantation Sites (No. per animal)	5.5	7	9.3
Preimplantation Loss (Mean%)	16.7	20.1	14.3
No. animals with implant scars	0/3	0/6	3/6
No. pregnant animals	3/3	5/6	1/6

Offspring

- Pregnancies were not allowed to progress beyond day 10 *p.c.*
- Embryos were not assessed in the study.

9.3 Prenatal and Postnatal Development

Study JE049-0141 in rats was reviewed with the original submission to this NDA. This study dosed dams from Day 6 *post coitum* to Day 21 *post-partum* and assessed icatibant effects in the F_0 and F_1 generations. Icatibant treatment delayed parturition at 1, 3 and 10 mg/kg/day, resulting in F_0 deaths (found dead or early sacrifice) in the 3 and 10 mg/kg/day groups. Clinical signs of toxicity (pallor of eyes and extremities, round back, piloerection and reddish discharge in vagina) were observed in 1 MD and in multiple HD F_0 females. After SC administration, icatibant and the M2 metabolite was found in rat milk. Based on the observed maternal toxicities including clinical signs, prolonged gestation periods for all icatibant dosage groups, F_0 spontaneous deaths and early sacrifice of icatibant treated dams, litter deaths, and necropsy findings in the MD and the HD groups, the maternal toxicity NOAEL could not be defined (< 1 mg/kg). The F_1 generation showed an increase in the number of pup deaths and cannabalism in the MD and HD compared to control groups. Therefore, the NOAEL for systemic toxicity in the F_1 generation was determined to be 1 mg/kg/day. The F_1 generation showed a slight decrease in air righting and hair growth at the HD compared to the control group. Therefore, the NOAEL for development of the F_1 generation was considered 3 mg/kg/day.

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10 Special Toxicology Studies

Study title: Icatibant And Scrambled Icatibant Induce Calcitonin Gene-Related Peptide (CGRP) Release From Isolated Skin In Wild-Type C57bl/6 Mice

Study no.:	JE-049-0016
Study report location:	(b) (4)
Conducting laboratory and location:	Same as above
Date of study initiation:	Not specified
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Not specified

Key Study Findings

- CGRP secretion is related to cutaneous nociceptor activation and the sponsor hypothesized that CGRP is related to icatibant-related local skin erythema, swelling and burning pain in a previous study (JE059-0216).
- The current study tests the specificity of icatibant-mediated CGRP release with a scrambled version of icatibant composed of identical amino acids as Icatibant but assembled in a non-sense sequence
- Icatibant administration produced statistically higher CGRP secretion from skin flaps. However, CGRP secretion from scrambled icatibant was ~60% that resulting from the icatibant.

Methods

- As an index for nociceptor activation calcitonin gene-related peptide release from isolated skin preparations was measured upon stimulation with icatibant at different concentrations (3.1 and 10 mM) or scrambled icatibant (3.1, 10 mM).
- Skin preparations derived from control C57BL/6 mice,
- Female C57BL/6 mice were killed by exposure to pure CO_2 atmosphere, and the skin from both hind paws was subcutaneously dissected below the knee level as described[17].
- The skin flaps obtained were wrapped around acrylic glass rods (∅ = 6mm) with the corium side exposed and fixed with surgical silk.
- The preparations were washed for 30 min in “synthetic interstitial fluid“ (SIF: 108 mM NaCl, 3.48 mM KCl, 3.5 mM $MgSO_4$, 26 mM $NaHCO_3$, 1.7 mM NaH_2PO_4, 1.5 mM $CaCl_2$, 9.6 mM sodium gluconate, 5.55 mM glucose, 7.6 mM sucrose at 32°C).

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- A series of six consecutive glass tubes (s1-s6) were filled with 700 µl SIF – previously bubbled with carbogen (95% O_2, 5% CO_2) - and positioned in a shaking bath (32°C).
- The skin flaps were incubated for 5 min in the first test-tubes and then forwarded to the next tube for 5 min and so forth; the third tube always contained the stimulating solution.
- Scrambled Icatibant (scrIC) was dissolved from powder in SIF to achieve the desired concentrations of 3.1 or 10 mM.
- CGRP content of the incubation fluid was measured using a commercial EIA (Cayman, USA distributed through SPIbio, France) immediately after the experiment as described[18].
- All EIA plates were determined photometrically using a microplate reader (Dynatech, UK).

Results

- Icatibant administration produced statistically higher CGRP secretion from skin flaps. However, CGRP secretion from scrambled icatibant was ~60% that resulting from the icatibant.

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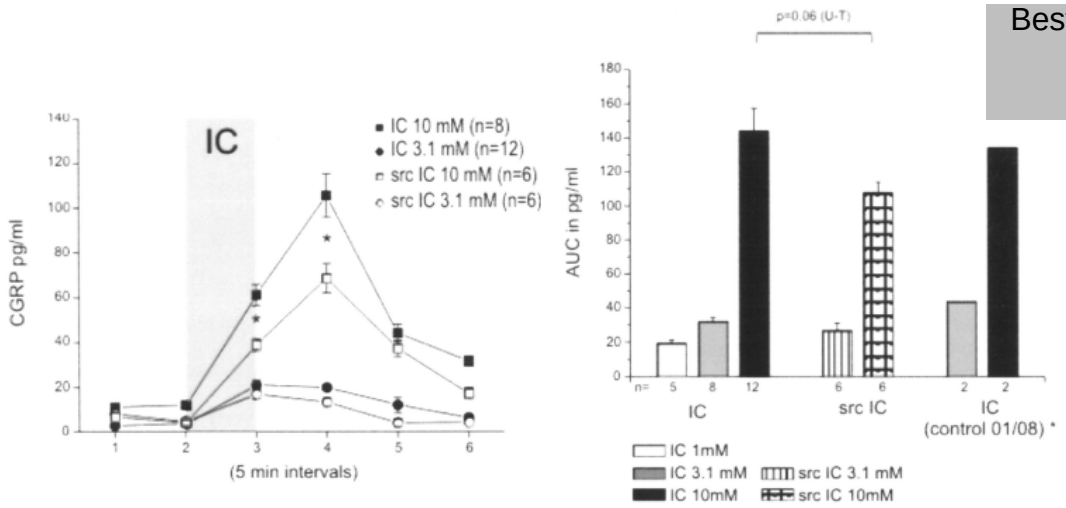

*Excerpted from sponsor's submission

11 Integrated Summary and Safety Evaluation

Icatibant (Firazyr®), a Bradykinin B2 receptor antagonist, is a synthetic decapeptide analog of Bradykinin (BK) containing unnatural amino acids that is under development for the treatment of hereditary angioedema (HAE). Shire (formerly Jerini US, Inc.) has re-submitted NDA 22-150 for Firazyr (icatibant) 30 mg, SC for the treatment of adults (≥ 18 years old) with HAE. On 4/23/2008, FDA/ODE2 sent the sponsor a Not Approvable letter for the original submission of this NDA. This letter listed the following deficiencies involving Pharmacology/Toxicology: (1) the need for 6-month rat and 9-month dog general toxicology studies and (2) a list of impurities in the drug substance and drug product that need to be lowered to less than (b) (4) alternatively, an adequate 3 month repeat-dose toxicology study that provides a >10 fold Safety Margin should be submitted. The present submission, delivered on 2/25/2011, includes the requested rat (6 month) and the dog (9 month) studies. The sponsor will submit the two carcinogenicity studies (1 mouse and 1 rat) as post-marketing requirements. The proposed dosing regimen is a single SC dose of 30 mg icatibant (0.6 mg/kg for a 50 kg human) which is associated with a systemic exposure of 4107 ng*h/mL. The potential maximum human dose is 90 mg (1.8 mg/kg for a 50 kg human) which is associated with systemic exposures of 12300 ng*h/mL in humans.

HAE patients have acute episodes of swelling of the hands, feet, trunk, face, gut and/or airway. Swelling of the upper airway can be fatal if not treated promptly. Type 1 and Type 2 hereditary angioedema (HAE) are caused by DNA mutations that result in reduced function of the C1 esterase inhibitor protein (C1-INH). A third form of HAE, Type 3, does not seem to involve genetic alterations in the C1-INH locus. In Type 1 HAE, patients have a decrease in the C1-INH protein levels; in Type 2 HAE, patients have a decrease in C1-INH protein function. C1-INH is a serine protease inhibitor that inhibits components of the complement, coagulation, fibrinolytic and kinin-releasing systems. C1-INH inhibits Factor XII and kallikrein, two proteases that contribute to the formation of bradykinin (BK) from the cleavage of high molecular weight kininogen (HK). Due to loss C1-INH protein function, HAE patients have increased levels of kallikrein activity that result in concomitantly increased levels of BK, causing swelling due to extravasation of fluid into the interstitium of the deep dermis and subcutaneous tissue[2]. BK binds to the Kinin type 1 (B1) and type 2 (B2) G-protein-coupled receptors. However, in humans, the B1 receptor is inducible, while the B2 receptor is expressed constitutively; further, BK binds the B2 receptor preferentially in humans (K_d at B1=560 nM; K_d at B2=0.2 nM). The B2 receptor is coupled to the $G_{\alpha q/11}$ and $G_{\alpha i}$ proteins, leading to calcium mobilization and MAPK activation, respectively. Through its interactions with B2 receptor, BK elicits a variety of intracellular processes that lead to the inflammation and edema observed in patients with HAE, including pain neuron excitation, vasodilatation, increased vascular permeability and smooth muscle cell contraction.

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In support of this NDA, the sponsor has submitted studies or has ongoing studies addressing the pharmacology, safety pharmacology, pharmacokinetics, single-dose and repeat-dose toxicology, genetic toxicology, carcinogenicity, reproduction toxicology, local tolerance, and impurity qualification of icatibant.

Pharmacology

Icatibant is a synthetic decapeptide with a structure similar to endogenous bradykinin (BK) but containing unnatural amino acids that prevent icatibant digestion by ACE or neutral endopeptidase. Icatibant binds the B2 receptor with an affinity similar to BK and functions as an antagonist of the B2 receptor at lower doses. Icatibant has been shown to function as an agonist at high doses and in some non-human cell-lines. The sponsor proposes that treatment of HAE patients with icatibant decreases BK-induced downstream events, including vascular permeability, vasodilation and inflammation. In support of this contention, the sponsor submitted an open-literature study of C1-INH knock-out mice indicating that, like HAE patients, C1-INH null mice have increased vascular permeability and other HAE-like symptoms, and that these effects are reversed with either icatibant treatment or by concomitant genetic disruption of the B2 receptor[7].

Safety Pharmacology

Safety pharmacology studies submitted to IND (b) (4) and reviewed by Dr. Young Choi showed that icatiabant has effects on the CNS, including decreased motor activity, assumption of prone position, reduced respiration, ptosis, piloerection and mydriasis at IV doses of 1-10 mg/kg, on the CVS, including decreased coronary blood flow in isolated guinea pig heart, aggravated the duration of post-ischemic reperfusion arrhythmias in isolated working hearts in Wistar rats and impaired cardiodynamics and on renal systems, including decreased urine output, sodium and potassium excretion, to a lesser extent for GFR, renal plasma flow and osmolar clearance in beagle dogs at 0.3 mg/kg, IV). These effects were considered the result of the partial agonistic property of icatibant on B-receptors that can occur at the higher exposures achievable by intravenous dosing or the use of *in vitro* systems and were not observed in rats or dogs in repeat dose long term toxicity studies of icatibant administered by the subcutaneous route. There was, however, a slight decrease in heart rate observed in the long-term dog toxicology study included with this submission. In other safety pharmacology studies submitted with the original submission to this NDA, icatibant did not inhibit HERG channels up to 300 μM icatibant concentration and did not have an effect on respiratory function or GI transit in rats after IV administration up to 10 mg/kg. However, studies submitted to the NDA did show that gastric acid volume and acidity were decreased in rats compared to the vehicle control treatment in rats given an intravenous dose of 10 mg/kg icatibant.

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Pharmacokinetics

Icatibant is converted to two major metabolites: M1 (b) (4) and M2 (b) (4) . These metabolites have been identified in mice, rats, dogs, monkeys and humans. The most prevalent metabolite in humans is M1, with AUC exposures >90% that of icatibant after subcutaneous administration. M1 is formed in the rat, dog, and mouse at about 70, 60, and 50% the AUC exposure of icatibant, respectively. The next most prevalent metabolite in humans is M2, with AUC exposures ~75% that of icatibant after subcutaneous administration. Unlike M1, M2 exposures were much lower in rats and dogs, with exposures of 9% and 3% relative to icatibant AUC. However, the larger doses and higher dosing frequency in the pivotal toxicology studies provides enough safety information. Maximum M2 exposures in the rat study exceeded the expected clinical AUC exposure of M2 after one 30 mg dose with 175% the clinical exposure and resulted in 58% the M2 exposure predicted to result from the maximal clinical dose. Given the fact that toxicity assessments in long-term toxicology studies came from daily exposure over several months, compared to the intermittent exposure proposed for the clinic, these exposure levels are acceptable. Further, the pivotal clinical studies show that most patients will only be taking a single 30 mg injection for a given attack.

Icatibant is rapidly absorbed after subcutaneous administration with 100% bioavailability. Protein binding is low in all species examined: 49% in rat, 44% in human, and about 38% in dog and monkey. Distribution studies showed that the isotope signal after subcutaneous administration of radiolabeled icatibant distributes to the kidneys, liver, urinary bladder, lungs and spleen at 1 h and can be detected in the liver, kidneys, spleen, bone marrow, adrenals and injection sites at 24 h in rats. Radioactivity was detected in the milk of nursing rats with peak levels observed 7 h after administration of radiolabeled drug. Icatibant is not degraded by the two main bradykinin-cleaving enzymes, angiotensin converting enzyme (ACE) and neutral endopeptidase. Neither icatibant nor the M1 or M2 metabolites are metabolized by human hepatocytes or microsomes. Additionally, icatibant is not an inducer of CYP450 isozymes. Therefore, icatibant is more metabolically stable than BK. Based on excretion studies, the primary route of elimination after SC administration of icatibant is via the kidneys in mice (~97.1%), rats ~61%) and dogs (~63%) with small amounts excreted in the feces (8.0% to 33%).

Single Dose Toxicology

Acute toxicology studies conducted in the mouse, rat and dog were reviewed under IND (b) (4) by Dr. Young Choi. The highest non-lethal IV dose was 30.7 mg/kg and the minimum lethal dose (MLD) was 48.3 mg/kg for mice. For rats, the highest non-lethal IV dose was 12.3 mg/kg and the MLD was 19.2 mg/kg. After IV dosing, rapid respiration, twitching and convulsions were observed prior to death. In an acute IV toxicity study, male dogs (n=2/group) were administered 0.4, 2 and 10 mg/kg icatibant. No mortality was observed in this study. However, numerous clinical signs, many suggestive of massive histamine release, were observed in the MD and HD groups. In a separate

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study, male dogs (n= 3/group) were administered single IV doses (infused over 5 h period) of 15, 30 and 60 mg/kg. An increase in serum CPK levels were above historical background (153 mU/mL) was noted in 1 MD animal (406 mU/mL) and two HD dogs (843 and 389 mU/mL, respectively. The NOAEL in this study was 15 mg/kg. In acute SC toxicity studies, the highest non-lethal dose was 614 mg/kg and the MLD was 767 mg/kg for mice. In rats, no mortality was observed after a single SC injection of 1227 mg/kg icatibant. After SC injection, injection sites showed bluish discoloration that progressed to necrosis. Effects occurring at very high-dose, especially when administered via the intravenous route, could reflect toxicities resulting from icatibant agonist activity.

Repeat Dose Toxicology

The icatibant development program has included repeat-dose toxicology studies that explore intranasal (IN), inhalation (IH), IV and SC routes of administration. The main repeat dose toxicology studies submitted to INDs (b) (4) investigated the intranasal (IN), inhalation (IH) and IV routes of administration with a few studies conducted using the SC route. These studies were reviewed by Dr. Young Choi. Subsequent studies were evaluated by Dr. Ronald Honchel in his review of the initial submission to IND 68214 and Dr. Molly Topper in her review of the initial submission to this NDA. Toxicology studies submitted to INDs (b) (4) 68214 and to this NDA indicate that the target organs of icatibant toxicity in rats include the male and female reproductive tracts, kidneys, stomach and liver. In the dogs, the target organs were the male and female reproductive tracts and stomach. In addition, injection site reactions (scabbing and ulceration) occurred in rats and more generalized systemic reactions described as “histamine-like” occurred in dogs. In both species, these effects varied by mode of administration (IV vs. SC vs. IN vs. IH) and length of treatment (1 month vs. 6 or 9 months). Toxicity to the male and female reproductive tracts was sensitive to length of treatment while other toxicities, such as CPK increases in observed after intravenous administration but not subcutaneous administration, are likely related to the greater C_{max} exposures achieved by intravenous dosing. No toxicity was observed in most IN and IH toxicity studies due to low bioavailability. However, reduced testicular weights and spermatogenesis in the testes were observed in a single 1-month IN toxicity study carried out in the rat at 0.125, 1.25 or 12.5 mg/kg. The NOAEL was considered to be 0.125 mg/kg based on these testicular findings.

In support of the proposed SC route of administration for this NDA, the sponsor conducted two 14-day SC toxicity studies in the rat, one 13-week SC toxicity study in the rat (submitted as a combined 13-week toxicity and fertility and early embryonic development study), a 26-week repeat-dose SC toxicity study in the rat, a SC dose-ranging study in the dog, a 4-week SC toxicity study in the dog (twice weekly dosing), and two 13-week SC toxicity studies in the dog (1 with daily SC dosing and 1 with daily and TID administered twice weekly SC dosing). These studies were reviewed by Dr. Molly Topper. In addition, the sponsor submitted a 39-week SC toxicity study in the dog, reviewed herein. The target organs of icatibant toxicity in rats include the male and

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female reproductive tracts, kidneys, stomach and liver. In the dogs, the target organs were the male and female reproductive tracts and stomach. In addition, injection site reactions (scabbing and ulceration) occurred in rats and more generalized systemic reactions described as “histamine-like” occurred in dogs. In both species, these effects varied by length (1 month vs. 6 or 9 months) and frequency (daily vs. twice weekly treatment).

During an initial 14-day repeat dose SC study (JE049-0127) using doses 0, 1, 10, and 50 mg/kg/day and a batch of icatibant synthesized by an older manufacturer, icatibant administration resulted in significant injection site irritation (at 50 mg/kg/day), decreases in prostate weights and testes weights (10 mg/kg/day and 50 mg/kg/day) and increases in thymic weights in females (50 mg/kg/day). Histopathological findings included: epididymides (oligospermia and aspermia) and testes (hypospermia). A second 14-day study with an 8-week recovery period used an incomplete histopathological battery, but was able to establish the NOAEL for male reproductive effects at 1 mg/kg/day (JE049-0128). The sponsor followed up the 14-day studies with a 13-week repeat SC study in rats of icatibant doses up to 10 mg/kg/day (JE049-0108). This study used a batch of icatibant synthesized by a new manufacturer and no systemic toxicities were observed. The fact that a NOAEL of 10 mg/kg could be identified after 13 weeks while none was identified at 14 days may be due to adaptive responses to lower drug doses over time or differences in the impurity profiles of the differently manufactured batches. However, a second 13-week rat toxicity study (JE049-0160) using does of 10, 20, and 30 mg/kg icatibant could not identify a NOAEL based on body weight effects, and findings in the male and female reproductive tracts, thymus and adrenal glands. This study is reviewed in **Appendix 3**. Male reproductive tract effects included diminished size, germinal epithelial degeneration and dystrophic tubular mineralization in the testes, reduced secretion in the seminal vesicles and prostate, and intratubular degenerate spermatozoa in the epididymides at all doses. Female reproductive tract effects included atrophy of the uterine endometrial gland, increased numbers of corpora lutea in the ovaries, and degeneration of the mucus layer and epithelial atrophy in the vagina at all doses. The main effect in the thymus was reduced size and lymphocyte depletion and the main effect in the adrenal gland was diffuse hypertrophy of the zona fasciculata; these effects also occurred at all doses.

The sponsor submitted a 26-week rat toxicity study (JE049-0163) using subcutaneous doses of 0, 3, 10 and 30 mg/kg/day icatibant in support of an ongoing rat carcinogenicity study and in partial fulfillment of the long-term toxicology studies. Dr. Molly Topper’s review of this study can be found in **Appendix 4.** The target organs of toxicity in male and female rats included skin, injection sites, adrenal glands, brown fat (macrovesicular vacuolation) in the aorta and connective tissue, bone marrow, kidneys, liver, lungs, and thymus. Female reproductive organs (vagina, ovaries, and uterus) and female mammary gland (masculinization) and male reproductive organs (prostate, epididymides, testes, and seminal vesicles) were also target organs of toxicity. Injection site reaction was a major toxicity in this study: 7 HD males and 6 HD females were euthanized *in extremis* between weeks 13 and 26 due to severe injection site toxicity. Clinical signs of toxicity included sores and lesions, staining of fur, excessive activity

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and thin appearance in males and females. Males but not females had decreased body weight gains and body weights compared to controls. This effect on male body weight is likely a result of significant decreases in testosterone secretion in icatibant treated animals, resulting in a decrease in muscle mass. This effect could be a result of icatibant interference with bradykinin regulation of pituitary hormone secretion. However, testicular atrophy also occurred in treated animals, explaining the observed testosterone deficits. Bradykinin is also known to regulate germline proliferation, and blocking its function could cause testicular atrophy. Urinalysis showed a significant increase in electrolyte levels in males and females, which coincided with an increase in urine excretion volume. Adrenal and liver weights were increased in males and females and thymic weights were decreased in males and females. In males, prostate, testes, epididymal weights were decreased. In females, ovarian weights increased. No NOAEL could be determined due to findings in the male and female reproductive organs, thymus, bone marrow, aorta and adrenal gland that were observed in the LD group (3 mg/kg/day).

In dogs, SC administration of icatibant for 13 weeks (10, 30 and 100 mg/kg/day; Study JE049-0117; **Appendix 1**), resulted in erythema of the face, injection site irritation, abnormal vocalization and diarrhea and/or vomiting at all doses. Macroscopic findings included hematomas and edema at injection sites, immature male (testes, epididymides and prostate) and female (ovaries and uterus) reproductive organs, small thymus, enlarged liver and enlarged adrenal gland. Correlating microscopic changes included histopathological confirmation of immaturity in the male (testes, epididymides, and prostate) and female (mammary gland, ovaries, uterus and vagina) reproductive organs, subcutaneous inflammation, edema, mineralization and collagen degeneration at the injection site (mainly HD groups but sporadic in LD and MD groups), vacuolated cortical cells in the adrenal gland of HD males and females, vacuolated hepatocyte in the liver (1/4 HD male) and lymphoid depletion in the thymus at all doses. No NOAEL was determined for this study due to reproductive organ effects in all icatibant-treated males and females, lymphoid depletion in the thymus and on injection site irritation.

The sponsor repeated the 13-week dog study with lower doses of 0, 3, 10 mg/kg and an additional twice weekly 3.3 mg/kg TID group in a second study (JE049-0118; **Appendix 1**) due to the excessive toxicity observed with the first dog study that used daily dosing of icatibant. No effects were observed in the male reproductive organs but females were found to have impaired maturation of the reproductive organs when dogs were administered icatibant TID twice weekly at 3.3 mg/kg dosing regimen. In addition, lymphoid depletion in the thymus was observed in both males and females after twice weekly dosing. Observed clinical signs at all dose levels included increased erythema and edema that worsened with increased dose. Erythema abated with cessation of treatment. Toxicities that occurred with daily administration of icatibant at 3 and 10 mg/kg/day, included lymphoid depletion in the thymus, immature male (testes, epididymides and prostate) and female (ovaries, oviducts, uterus and vagina) reproductive tissues. After a 14-week recovery period, 1 out of 2 males that had been treated with 10 mg/kg/day icatibant had immature testes and prostate; there were no findings in female reproductive organs after the recovery period. Hormonal analysis

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showed that icatibant induced a decrease of testosterone, LF and FSH in male dogs, which secondarily induced sexual immaturity.

The sponsor submitted a 39-week dog toxicity study (JE049-0164) using subcutaneous doses of 0, 1, 10 mg/kg/day and 1 mg/kg TID twice weekly in partial fulfillment of the long-term toxicology studies. Icatibant subcutaneous administration (daily or twice a week for 39 weeks) caused end-organ toxicity in the testes, prostate, ovaries, and uterus in Beagle dogs. In addition, significant systemic injection reactions occurred in animals treated at the high dose of 10 mg/kg/day. These reactions included face and body swelling, redness in areas surrounding the eyes, ears, and muzzle, and behaviors such as head shaking and immobility. Icatibant treatment resulted in testicular atrophy with a concomitant 90% decrease in serum levels of testosterone and profound defects in sperm motility and sperm count in animals treated at the 10 mg/kg/day dose level. Animals treated at the 1 mg/kg/day level had slight deficit in sperm motility, but had >90% decrease in sperm count relative to control. Although seminology was not assessed in recovery animals, histopathology data indicate that testicular effects did not reverse in animals treated at the 10 mg/kg/day dose level. Icatibant effect on the ovaries and uterus included decreases in ovary size, a complete lack of developing follicles in the ovary, and histopathological signs uterine atrophy in all females treated at the 10 mg/kg/day dose level. Two out of four females treated at the 1 mg/kg/day dose level showed histopathological signs of uterine atrophy. Mammary gland atrophy was detected in 4/4 main study and 1/2 recovery females treated at the 1 mg/kg/day dose level. The body weights of animals treated with 10 mg/kg icatibant were elevated relative to control. This effect was more pronounced in males where the weight increase was clinically described as obesity. Icatibant treatment produced decreases in heart rate, although ECG waveforms appeared normal. The toxicities to the reproductive organs and the heart described above appear to be related to icatibant's primary mechanism of action: inhibition of the bradykinin type 2 (B2) receptor. Based on the adverse finding to the male and female reproductive tracts in animals treated at 1 and 10 mg/kg/day, the NOAEL is 1 mg/kg TID twice weekly Effects on cardiac tissue have been described in mice that are genetically null for this receptor1 and the role of this receptor in germ-line proliferation, prostate epithelial cell division, and ovary changes during the estrous cycle is known[2,3,4,5].

Genetic Toxicology

The sponsor submitted three new genetic toxicology studies with the original NDA submission. These studies were: a bacterial reverse mutation assay (a complete bacterial strain battery), an *in vitro* mammalian chromosome aberration test, and an *in vivo* bone marrow micronucleus assay in Sprague-Dawley rats using the icatibant. The bacterial reverse mutation assay and the *in vivo* micronucleus study in rats were studies used to bridge the change in manufacturer for the drug substance. Icatibant did not induced genetic toxicity in these three studies under the conditions assayed. The results of these studies are consistent with numerous others that were previously reviewed under INDs (b) (4).

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Carcinogenicity

The sponsor did not submit studies to evaluate icatibant's carcinogenic potential but has initiated two 104-week carcinogenicity studies, one in mice and a second in rats. Supporting toxicological data for these on-going studies were reviewed by Drs. Molly Topper and Timothy Robison (**Appendix 4 and 6**) and concurrence for these protocols was obtained by the sponsor from the Executive Carcinogenicity Committee. During a pre-NDA meeting held on February 22, 2007, the Division conveyed a willingness to accept carcinogenicity studies as a Phase 4 Commitment. However, under the Food and Drug Administration Amendments Act (FDAAA) of 2007, the Division now considers these studies as post-marketing requirements (PMRs) since they are safety assessments. These PMRs are detailed in the integrated summary and safety evaluation section of this review. The Division will issue two PMRs, one for the mouse study, and one for the rat study, with the approval package of this NDA.

Reproductive Toxicology

The sponsor submitted reproduction toxicology studies under IND (b) (4) (JE049-0137, JE049-138, and JE049-139) and with the original submission of this NDA (JE049-0108, JE049-0141). These studies included male fertility assessments in mice and rats treated with IV and SC icatibant (JE049-0108, JE049-0137, JE049-138), respectively, and embryonic fetal development studies in rats and Himalayan rabbits (JE049-139 and JE049-0141, respectively). In addition, the sponsor submitted one study in Himalayan rabbits to provide maternal toxicokinetic exposures for SC administration of icatibant on days 6 – 10 *post coitum* with the current submission (JE049-353). The pivotal long-term general toxicology studies, JE049-0163 in rats (reviewed by Dr. Molly Topper under IND 68214; **Appendix 4**) and JE049-0164 in dogs (reviewed herein), also included special seminology and hormone analyses that correlated with histopathological findings of atrophy in the male and female reproductive tracts. Under the conditions tested in the rats and rabbits, icatibant was not teratogenic. However, icatibant has a potential to produce abortions/premature births, intrauterine death or resorptions. In rats and dogs, long-term daily administration of icatibant caused ovarian, uterine, and testicular atrophy/degeneration, concomitant decreases in testosterone in males, and adverse effects on the mammary and prostate glands as described in the general toxicology section, above. The sponsor has adequately completed the reproductive toxicity battery for icatibant and the data indicate that icatibant should be classified as a Pregnancy Category C based on the presence of embryotoxicity, increased spontaneous abortions and increased pups deaths.

Daily administration (daily x35) of icatibant revealed no impairment of male fertility in mice with intravenous doses up 81 mg/kg or in rats with subcutaneous doses up to 10 mg/kg. These studies were completed in 1993 and did not follow current Segment 1 study guidelines, although this difference does not appear to have affected their validity.

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These results appear to be inconsistent with the seminology, hormone analyses, and histopathological data observed in the pivotal long-term toxicology studies in rats and dogs (JE049-0163 and JE049-0164). However, sperm counts were not assessed in these studies and the 35-day dosing duration may not have been long enough to reduce sperm counts to a point where fertility is affected.

In the definitive embryofetal development study with rats (JE049-139), the effects of icatibant SC administration on morphological and post-natal development including fertility in Wistar rats was examined. Female rats (30 pregnant rats/group) were treated with 0 (vehicle control- 0.9% NaCl), 0.25, 2.5 and 25 mg/kg/group of icatibant SC from Days 7 through 18 of pregnancy. There were no significant major external and internal anomalies reported in pups for F_1 or F_2 generations, although 1 or 2 fetuses in the 0.25 and 2.5 mg/kg group had multiple minor skeletal malformations or variations. Post-natal development of treated fetuses and fertility of F_1 generation were comparable to the control group. No major terata were produced in the study although 1 dam in the 25 mg/kg/day group had empty implantation sites in the uterus, and fetuses in the 25 mg/kg/day group had slightly greater numbers with less ossification of skeletons and variations of liver and kidneys than the control group (4 fetuses had lobus dexter of liver and 3 fetuses had distended pelvises in the kidney, while 1 fetus in the control group had the same findings). Therefore, icatibant was not teratogenic in rats under the conditions studied.

A second reproductive toxicity study in rats submitted in this NDA (JE049-0141) assessed the effects of daily SC administration of icatibant on pre- and post-natal development of the progeny of pregnant rats treated with 1, 3 and 10 mg/kg/day icatibant from gestation day 6 to postpartum day 21. Icatibant treatment delayed parturition at all doses, resulting in F_0 deaths (found dead or early sacrifice) in the 3 and 10 mg/kg/day groups. Maternal clinical signs of toxicity (pallor of eyes and extremities, round back, piloerection and reddish discharge in vagina) were observed in 1 MD and in multiple HD F_0 females. After SC administration, icatibant and the M2 metabolite was found in rat milk. Based on the observed maternal toxicities including clinical signs, prolonged gestation periods for all icatibant dosage groups, F_0 spontaneous deaths and early sacrifice of icatibant treated dams, litter deaths, and necropsy findings in the MD and the HD groups, the maternal toxicity NOAEL could not be defined. Within the F_1 generation, there was an increase in the number of pup deaths and in cannabalism in the MD and HD compared to the control groups. Therefore, the NOAEL for systemic toxicity in the F_1 generation was determined to be 1 mg/kg/day. The F_1 generation showed a slight decrease in the air righting reflex and in hair growth compared to the control group. Therefore, the NOAEL for development of the F_1 generation was considered 3 mg/kg/day.

In a preliminary dose-ranging study in rabbits (submitted with study JE049-0140), 2 pregnant rabbits/group were treated daily with SC injections up to 50 mg/kg/day of icatibant from Days 6 through 18 (no vehicle control was used). Rabbits were sacrificed on Day 29 of pregnancy and their uteri were examined. One dam in the 10 mg/kg group had only empty implantation sites in the uterus, indicating a clinically unnoticed abortion,

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but the second dam had 8 normally developed live fetuses. All dams in the 25 and 50 mg/kg/ groups had empty implantation sites in the uterus. Based on this study, doses of 0.1, 1 and 10 mg/kg/day were selected.

In the definitive rabbit embryofetal development study (JE049-0140), Himalayan rabbits were treated with 0 (vehicle control- 0.9% NaCl), 0.1, 1.0 and 10 mg/kg/day of icatibant, SC once daily from Days 7 through 18 of pregnancy. Four unexpected abortions were produced in 2 dams each in all 3 treated groups and a premature birth in 0.1 mg/kg group, while none of these occurred in the control group. No other toxic effects were produced in the icatibant treated dams. At autopsy, light-grey depression was found on the surface of kidneys in 5-7 dams each in all 3 icatibant treated groups but only 1 dam in the control group. To determine if these effects were drug induced, the study was repeated but with 2 lower doses only and the vehicle group. The abortions or premature births were not reproduced in this study, and incidence of light grey colored kidneys in the treated groups was not increased, but it was also reported in 1 dam each in treated groups only. The depression on the kidney was due to chronic interstitial nephritis, and it was attributed to the strain of rabbits by the sponsor. Morphological examination of fetuses showed no increase in incidence of major malformations in the treated groups. Minor anomalies or variations of liver or kidneys were reported in some fetuses, but they were reported as within the normal ranges. Among the dead fetuses examined (6 in control, 4 in 1 mg/kg and 15 in the 10 mg/kg groups), 10 mg/kg had significantly more stunted (8/15) or retarded fetuses (7/15) over the control group (2/6 and 1/6, respectively). Icatibant was not teratogenic under the test conditions. All 3 doses of icatibant did not have significant toxic effects on the dams. However, 10 mg/kg was toxic to the embryo reducing the litter sizes as a result of increased intrauterine embryofetal death rate. Icatibant treatment resulted in dose-related decreases for total implantations and total number of live fetuses as well as dose-related increases for percent of pre-implantation loss when compared to the control values.

Local Tolerance

A local tolerance study in rabbits (JE049-0144; submitted under IND (b) (4) showed that intra-articular injection of icatibant was well tolerated. Two local irritation studies (JE049-0145, primary skin irritation study of rabbit skin and JE049-0145, mucosal tolerance study in rabbit eye) icatibant was considered a non-irritant at 0.5 mg/mL icatibant solution. Icatibant administered via the SC route up to 40 mg in acetate buffer, pH 5.5 was comparable to the vehicle control for local irritation. However, injection site reaction, including scabbing, necrosis, and ulceration, was a dose-limiting toxicity in rodent toxicology studies. In dogs, more generalized reactions, involving redness and swelling of face, ears, eyes, and body, and sometimes described as “histamine-like,” occurred shortly after dosing with higher icatibant doses. In an antigenicity study in guinea pigs (JE049-0147), no anti-icatibant antibodies were produced in animals sensitized with icatibant alone via the intranasal or SC route. However, when guinea pigs were sensitized subcutaneously with icatibant plus Freund’s adjuvant, antibody formation was observed with an icatibant-BSA conjugate challenge. In another study

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(JE049-0148), a guinea pig maximization test, 8/10 icatibant treated guinea pigs plus Freund's adjuvant had slight erythema, while the control group showed no reaction. Therefore, icatibant sensitized animals to further icatibant challenges when administered with Freund's adjuvant in 2 studies. An *in vitro* hemolysis test of icatibant (JE049-0150) showed that 0.1% of icatibant did not induce hemolysis of human whole blood after incubation for 30 min under conditions in which incubation with the positive control (distilled water for injection) did induce hemolysis.

Impurity Qualification

Impurity qualification issues are detailed in a separate consult to ONDQA.

Conclusion

Repeat dose toxicity studies conducted in dogs and rats up to 26 and 39 weeks duration identified the target organs of icatibant toxicity to be the male and female reproductive organs, particularly in the germline-containing tissues of the ovary and testes, injection reactions, lymphoid depletion in the thymus, cellular vacoulation in the adrenal cortex and vacuolated hepatocytes in the liver. No NOAEL was identified in the 26-week pivotal rat study due toxicity in the male and female reproductive organs, thymus, bone marrow, aorta and adrenal gland that were observed at the lowest dose of 3 mg/kg/day. In the pivotal dog study, the NOAEL was not defined by a reduction in dose; rather it was defined by a reduction in dosing frequency from daily administration to dosing twice weekly. No adverse effects were observed when icatibant was dosed TID twice weekly at 1 mg/kg. By contrast, atrophy in the uterus and testes was observed in dogs treated with daily with 1 mg/kg icatibant. The twice weekly schedule was not explored in toxicology studies performed in rats.

Reproductive toxicology studies show that icatibant is not teratogenic, but induces pre-implantation loss, spontaneous abortions, increased pup deaths and induces delayed parturition that results in difficulties in delivery. Although male fertility studies in mice show no negative effects on fertility after icatibant treatment, general toxicology studies in rats and dogs did show significant toxicity to the male and female reproductive tracts. Based on these reproductive toxicity data icatibant should be classified as Pregnancy Category C.

The recommended clinical dose of icatibant is one 30 mg subcutaneous injection; if response is inadequate or symptoms recur, additional 30 mg injections not to exceed a total of 3 injections per 24 hour period (90 mg) are recommended. The submitted dog studies highlight that the toxicities observed from chronic dosing with icatibant depend on daily dosing; 9 months of dosing twice a week did not cause adverse effects in dogs. Because the twice weekly dosing schedule was only explored in dogs, and no NOAEL could be established with daily dosing in repeat dose toxicology studies in either dogs or rats, the pivotal dog study that included a group that was dosed on a twice-weekly basis

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is the only long-term toxicology study that can provide a Safety Margin (**Table 8**). Given the dependence on dosing frequency for toxicity as described in dogs, the much lower dosing frequency in the clinic, and the clearly favorable benefit-risk ratio of icatibant as described in the clinical review of this NDA, the safety margins identified in the pivotal dog study, along with the elucidation of similar toxicities in the pivotal rat and dog toxicology studies, provide enough nonclinical information to support the safety of icatibant dosing in the clinic as proposed.

Table 8. NOAELs and Safety Margins From Pivotal Toxicology Studies

Study	Species	Schedule	NOAEL (mg/kg)	Safety Margin Based on AUC* (30 mg)	Safety Margin Based on AUC* (90 mg)
JE049-0163	Rat	Daily; 26 weeks	None	Not Available	Not Available
JE049-0164	Dog	Daily; 39 weeks Twice Weekly TID (3 doses every 6 h); 39 weeks	1 mg/kg Twice Weekly TID	10.5	3.5

*AUC in human: 4107 ng*hr/ml at 30 mg/day; 12300 ng*hr/ml at 90 mg/day.

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Unresolved toxicology issues (if any): The applicant has a complete nonclinical pharmacology and toxicology program for icabitant with the exception of ongoing carcinogenicity studies in mice and rats and identification of impurities as described below.

The sponsor did not submit studies to evaluate icatibant's carcinogenic potential but has initiated two 104-week carcinogenicity studies, one in mice and a second in rats. The Division will issue two PMRs, one for the mouse study, and one for the rat study, with the approval package of this NDA.

As described in a separate consult to ONDQA, the sponsor has not identified the structures of impurities that are present at levels (b) (4) Further, the sponsor needs to propose a clear standard for "minimal characterization" for impurities that are present at levels (b) (4) The Division will develop a Post-Marketing Commitment (PMC) for their identification.

In addition, as detailed in the separate consult review to ONDQA, the sponsor needs to provide a final, signed report for the additional histopathology data from Study JE049-0171 requested in an IR dated 5/12/2011. These data identify the NOAEL of (b) (4), a degradant of icatibant, at (b) (4) mg/kg/day in a 13-week rat study, and qualify this impurity at NMT (b) (4) An addendum to this NDA review will be placed on file

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once the sponsor submits a final signed copy of the histopathology data submitted in response to the Division's IR.

Recommendation: From a nonclinical pharmacology and toxicology standpoint, the application is recommended for approval.

12 Appendix/Attachments

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12.1 Appendix 1: Pharmacology and Toxicology Review NDA 22-150 (Serial No. 000), March 18, 2008

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-150**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**10/22/07**
PRODUCT:	**Firazyr (Icatibant)**
INTENDED CLINICAL POPULATION:	**Patients with hereditary angioedema 18 years of age or older**
SPONSOR:	**Jerini US, Inc.**
DOCUMENTS REVIEWED:	**Electronic document**
REVIEW DIVISION:	**Division of Pulmonary and Allergy Products**
PHARM/TOX REVIEWER:	**Molly E. Shea, Ph.D.**
PHARM/TOX SUPERVISOR:	**Timothy J. McGovern, Ph.D.**
DIVISION DIRECTOR:	**Badrul Chowdhury, M.D, Ph.D.**
PROJECT MANAGER:	**Carol Hill**

Date of review submission to Division File System (DFS): March 18, 2008

APPEARS THIS WAY ON ORIGINAL

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The nonclinical discipline recommends an approval of the application (NDA 22-150). The sponsor has submitted adequate nonclinical data to support the safety of Firazyr (Icatibant) for up to 13-weeks of clinical administration. Clinical administration beyond 13-weeks is not supported by the currently available nonclinical data. In the Division's Filing Letter dated January 8, 2008, the sponsor was informed that the icatibant labeling will reflect a clinical dosing regimen that is supported by the nonclinical toxicology studies in both dose and duration.

In the future, Jerini plans to complete two repeat dose subcutaneous chronic toxicity studies (one in rats and one in dogs) and two carcinogenicity studies (one in mice and one in rats) to support their proposed chronic intermittent indication. Additionally, Jerini will conduct two carcinogenicity studies (1 mouse and 1 rat) with icatibant. The toxicity profile of icatibant has been characterized adequately up to 13-weeks with the completion of repeat-dose subcutaneous studies up to 13-weeks. Each of the excipients included in the subcutaneous injection formulation are at or below levels found in other currently approved products that are administered via the same route of administration. However, impurities have been identified in the substance and the drug product that require qualification studies. Comments to the sponsor will be forthcoming from the ONDQA reviewer.

B. Recommendation for nonclinical studies

Chronic toxicity studies in two species (at least one non-rodent will be required to support chronic clinical use of the drug product. In addition, carcinogenicity studies will be needed to address the carcinogenic potential of icatibant under the anticipated chronic intermittent usage to treat hereditary angioedema. The following comment should be forwarded to the sponsor based on the Division's filing letter of January 2008:

The completion of nonclinical chronic repeat dose toxicity studies (6-month in rat and 9- month in dog) via the subcutaneous route of administration is required to support chronic intermittent clinical dosing. The icatibant labeling will reflect a clinical dosing regimen that is supported by the nonclinical toxicology studies in both dose and duration. At this time you have nonclinical support for subcutaneous clinical dosing for up to 13-weeks duration. We acknowledge your submission stating that chronic toxicity studies have been initiated as of June 2007 (dog study) and July 2007 (rat study) and the carcinogenicity studies will be initiated April 8, 2008 and July 7, 2008 for mice and rats, respectively.

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C. Recommendations on labeling

The review team has concluded that the recommended action for this NDA is Not Approvable. Therefore, a detailed review of the proposed labeling will not be performed at this time.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The toxicity profile of icatibant acetate has been well characterized up to 13-weeks administration via the subcutaneous route of administration. Exposures of the parent compound in animals have achieved 1.4- to 3.6-fold multiples of the anticipated human exposure at the recommended maximum clinical dose of 90 mg. The primary toxicities observed in animal studies are the male (immature testes, epididymides, and prostate) and female reproductive organs (immature ovaries and uterus) and the injection site irritation (with possible histamine release). In males, testosterone levels were reversibly reduced between 90 to 95% when icatibant was administered daily. However, when icatibant was administered twice weekly, the effects on the male reproductive organs were significantly blunted and testosterone levels recovered between icatibant doses. For females, twice weekly dosing did not reduce the icatibant-induced effects on reproductive organs. Additional toxicities in animals were observed in the thymus (lymphoid depletion), adrenal (cortical cellular vacuolation) and liver (vacuolated hepatocyte). In rats treated with a newly manufactured batch of icatibant (different manufacturer) for 13-weeks via SC administration resulted in no toxicities (NOAEL of 10 mg/kg, associated with icatibant exposure that is 1.4-fold greater than maximum clinical exposure at 90 mg). In studies of a shorter duration (14-weeks) and at higher icatibant doses using an icatibant batch manufactured at a different site than that used in the 13-week study, the primary target organs of toxicity were also the male and female reproductive organs, the injection site and thymus. In dogs treated with icatibant daily via SC administration for 13-weeks, the primary target organs of toxicity were the male and female reproductive organs, injection sites, adrenal gland, liver and thymus. Although a NOAEL was not identified in 13-week studies with daily dosing, the dosing regimen provided adequate exposure coverage compared to that anticipated in the clinic.

During icatibant's drug development, Jerini has evaluated icatibant's effects on the CNS, CVS, respiratory, renal and GI systems. Icatibant had no effect on cardiac potassium currents using *in vitro* test systems, on respiratory function or on GI transit but did decrease gastric acid volume and acidity. Partial agonistic properties of icatibant were observed on CNS (decreased motor activity, reduced respiration, ptosis, piloerection and mydriasis), CVS (decreased coronary blood flow and aggravated duration of post-ischemic

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reperfusion arrhythmias in isolated heart models) and renal systems (decreased urine output, sodium and potassium excretion renal plasma flow and osmolar clearance). In a 13-week dog study dosed up to 100 mg/kg/day, dogs showed a decrease in heart rate (-25%) and a decrease in diastolic BP (-39%) 6 h post-dose on Week 1 of dosing at 100 mg/kg/day. No effects on QT interval or other safety pharmacology findings were observed.

A complete genetic toxicology battery for icatibant was evaluated. Under the conditions tested, icatibant was not mutagenic. Carcinogenicity studies have not been conducted. Jerini plans to initiate 1 mouse and 1 rat carcinogenicity study using icatibant April 2008.

The reproductive and development toxicity battery has been completed for icatibant. The sponsor conducted a 5-week fertility study in male mice, a fertility and early embryonic development to implantation study in rats, 2 definitive teratogenic studies (1 in rats and 1 in rabbits), and a pre- and post-natal development in pregnant rats. Icatibant had no effect on male fertility in mice up to 80.8 mg/kg SC. In rats treated up to 10 mg/kg/day SC, Icatibant had no effects on male sperm count, motility, or morphology. An increase in pre-implantation loss in females was observed at 10 mg/kg/day. Icatibant was not teratogenic in rats (up to 25 mg/kg/day, SC) or rabbits (up to 10 mg/kg/day, SC). However, at 10 mg/kg/day, icatibant was embryo toxic in rabbits reducing the litter sizes as a result of increased intrauterine embryofetal death rate. Icatibant treatment resulted in dose-related decreases for total implantations and total number of live fetuses as well as dose-related increases for percent of pre-implantation loss when compared to the control values. In the pre- and post-natal development study conducted in rats (up to 10 mg/kg/day, SC), icatibant did not effect fertility or have any significant effect on pup development. However, icatibant did prolong gestation, resulting in F_0 spontaneous deaths and litter deaths. The F_1 generation showed an increase in the number of pup deaths and cannabalism in the MD and HD compared to the VC groups. After SC administration, icatibant and the M2 metabolite is found in rat milk. Based on these results, icatibant should be classified as Pregnancy Category C.

B. Pharmacologic activity

In Type I (quantitative deficiency) and Type II (qualitative deficiency) hereditary angioedema (HAE) a deficiency of C1-esterase inhibitor (C1-INH) results in an increased release of bradykinin (BK). BK binds to the bradykinin type-2 receptor (B2) to trigger activation of many cascades leading to cellular proliferation, excitation of neurons, vasodilatation, increased vascular permeability and smooth muscle cell contraction. These events are believed to contribute to the increased vascular permeability and acute inflammation observed in HAE patients.

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Icatibant is a synthetic decapeptide with similar structure as endogenous bradykinin. It is a potent, selective bradykinin-2 (B_2) receptor antagonist that binds to the B_2-receptor with similar affinity as bradykinin. Icatibant binding at the B_2 receptor inhibits bradykinin induced processes and is hypothesized to contribute to the decrease of vascular permeability and acute inflammation observed in HAE patients.

C. Nonclinical safety issues relevant to clinical use

The primary toxicities in animal studies treated with icatibant were injection site irritation and immature male (testes, epididymides and prostates) and female (ovaries and uterus) reproductive organs. As observed in reproductive studies, icatibant treatment may induce pre-implantation loss (embryo-toxic), increase spontaneous abortions and prolong gestation length. Icatibant should not be administered to pregnant women. Icatibant has been shown to be excreted in milk of lactating rats. The sponsor has identified injection site irritation in clinical studies.

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

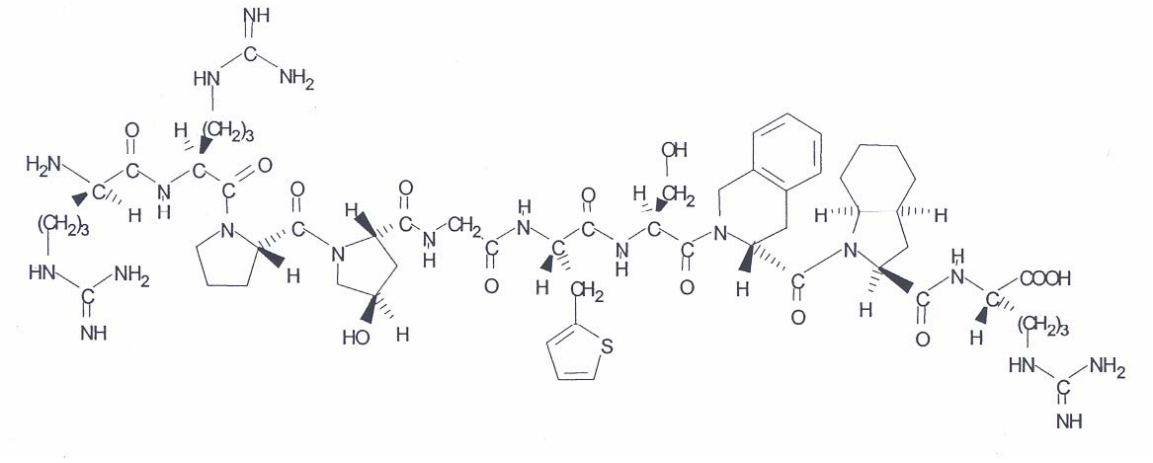
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-150
Review number: 1 Original review
Sequence number/date/type of submission: 000
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Jerini US Inc.
Manufacturer for drug substance: (b) (4)

Reviewer name: Molly E. Shea, Ph.D.
Division name: Division of Pulmonary and Allergy Products (DPAP)
Review completion date: March 18, 2008

Drug:

Trade name: Firazyr
Generic name: Icatibant acetate
Code name: JE 049/HOE 140/S 89 0140
Chemical name: D-arginyl-L-arginyl-L-prolyl-L[(4R)-4-hydroxyprolyl]-glycyl-L-[3-(2-thienyl)alanyl]-L-seryl-D-(1,2,3,4-tetrahydroisoquinolin-3-yl-carbonyl)-L-3aS,-7aS)-octahydroindol-2-yl-carbonyl]-L-arginine acetate
CAS registry number: NA
Amino acid sequence: H-D-Arg-L-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-OH· nCH_3COOH
Molecular formula/molecular weight:
Structure:



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Relevant INDs/NDAs/DMFs: IND 68,214 (Subcutaneous for HAE); (b) (4) (b) (4) DMF (b) (4) DMF (b) (4)

Drug class: Bradykinin type 2 (B_2) receptor antagonist

Intended clinical population: Patients 18 years of age or older with acute hereditary angioedema (HAE).

The proposed labeling recommends repeated injections, if required, at intervals not less than 6 hours and not to exceed 3 injections in 24 hours for a maximum daily dose of 90 mg/day.

Clinical formulation: Icatibant 30 mg solution for injection is delivered as a 10 mg/mL sterile, isotonic, acetate-buffered solution. The following table provides the formulation of icatibant for subcutaneous injection.

Ingredient	Quantity per mL	Function
Icatibant	10.0 mg	Active ingredient
Sodium hydroxide, NF/Ph.Eur.	(b) (4)	
Glacial acetic acid, USP/Ph. Eur.		
Sodium chloride, USP/Ph. Eur.		
Water for injection, USP/Ph. Eur.		

Route of administration: Subcutaneous injection (SC)

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

Studies reviewed within this submission:

Pharmacology

JE049-0003	In vitro pharmacology: Human B_1 and B_2 Receptors. Study of icatibant
JE049-0004	In vitro pharmacology: Human B_1 and B_2 Receptors. Study of M1, M2 and Icatibant
JE049-0201	Study of icatibant (HOE 140) in various receptor binding and enzyme assays
JE049-0218	In vitro pharmacology: high throughput profile. Study of icatibant, M2 and M1
JE049-0217	In vitro pharmacology: LH-RH receptor binding assay. Study of icatibant, M2 and M1
JE049-0215	Characterization of the effects of icatibant on human mast cells
JE049-0216	Icatibant dose- and calcium-dependently induces calcitonin gene-related peptide release from isolated skin in wild-type, B2 -/- and KITW/KITW-v but less in TRPV1 -/- mice

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JE049-0013	Project summary for: Analysis of species specificity and potential agonistic activity of icatibant using MAPK activation and calcium mobilization assays
JE049-0202	Effect of HOE 140 on cardiac potassium channels expressed in Xenopus oocytes
JE049-0203	Electrophysiological examination of the effects of icatibant and its metabolites M1 and M2 on the HERG-mediated potassium current
JE049-0208	Icatibant acetate safety pharmacology study on respiratory function after intravenous administration in the conscious rat (whole body plethysmography)
JE049-0209	Icatibant acetate evaluation of effects on gastrointestinal transit after intravenous administration in the rat
JE049-0210	Icatibant acetate evaluation of effects on gastric acid secretion after intravenous administration in the rat

Pharmacokinetics

JE049-0318	Icatibant metabolites M1, M2: Metabolic stability in human liver microsomes
JE049-0312	HOE 140- (oic-6,7-^{3}H): Whole-body autoradiographic studies in male rats after a single subcutaneous dose of 1 mg/kg body weight
JE049-0309	HOE 140- (oic-6,7-^{3}H): Radiokinetics study in male dogs after single subcutaneous administration of approx. 0.2 mg/kg body weight
JE049-0306	HOE 140- (oic-6,7-^{3}H): Radiokinetics study in male rats after single subcutaneous administration of approx. 1 mg/kg body weight
JE049-0311	HOE 140- (prolyl-3,4-^{3}H): Metabolism studies in male rats after subcutaneous administration of approx. 1 mg/kg body weight
JE049-0310	HOE 140-^{3}H: Determination of protein binding *in vitro* in human, monkey, dog and rat serum
JE049-0321	HOE 140- ^{3}H: Metabolism studies in male mice after a single subcutaneous dose of 1 mg/kg body weight
JE049-0323	Metabolism studies in rat following subcutaneous administration of 12 µg/kg ^{3}H-HOE 140
JE049-0326	HOE 140- ^{3}H: Excretion route, kinetics and organ distribution in male mice after a single subcutaneous dose of about 1 mg/kg body weight
JE049-0327	HOE 140- ^{3}H: Excretion route, kinetics and organ distribution in male rats after a single subcutaneous dose of about 1 mg/kg body weight
JE049-0330	Icatibant: Cytochrome P450 inhibition in human liver microsomes
JE049-0331	Icatibant: Measurement of cytochrome P450 induction on enzyme activity level in freshly isolated, pre-plated human hepatocytes
JE049-0317	Icatibant acetate: Metabolic stability in liver microsomes and S9 of Dog and Human

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JE049-0329	HOE 140- ^{3}H: Radiokinetic study of excretion with the milk in lactating rats after a single subcutaneous dose of about 1 mg/kg body weight
	Repeat Dose Toxicology
JE049-0127	HOE 140: 14-day subcutaneous toxicity study in rats
JE049-0128	HOE 140: 14-day subcutaneous toxicity study in male rats with an 8-week recovery period
JE049-0108	Combined 13-week toxicity and effects on fertility and early embryonic development to implantation in rats by subcutaneous administration
JE049-0115	Two-phase dose range-finding study by subcutaneous administration of icatibant in Beagle dogs
JE049-0016	4-week toxicity study by subcutaneous route in Beagle dogs with twice weekly T.I.D. dosing and an 8-week treatment free period
JE049-0117	13-week toxicity study by subcutaneous route in Beagle dogs
JE049-0118	13-week toxicity study by subcutaneous route in sexually immature Beagle dogs with daily or twice weekly T.I.D. dosing followed by a 14-week treatment from period
	Genetic Toxicology
JE049-0132	Bacterial reverse mutation test
JE049-0133	*In vitro* mammalian chromosome aberration test in cultured human lymphocytes
JE049-0135	Bone marrow micronucleus test by intravenous route in rats
	Reproductive Toxicology
JE049-0108	Combined 13-week toxicity and effects on fertility and early embryonic development to implantation in rats by subcutaneous administration
JE049-0141	Study for effects on pre- and post-natal development in rats by subcutaneous administration
	Local Tolerance
JE049-0142	Local tolerance after a single administration by subcutaneous route in rabbits
JE049-0143	Single-dose local tolerance study in rabbits (intra-arterial/intravenous/paravenous)
	Special Toxicology
JE049-0151	H-Oic-OH: Single-dose subcutaneous study in mice
JE049-0152	H-D-Tic-OH: Single-dose subcutaneous toxicity study in mice
JE049-0159	*In vitro* hemolysis test of HOE 140
JE049-0166	Assessment of antibody responses towards icatibant in hyperimmunization studies
JE049-0155	Acute intravenous toxicity in rats

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JE049-0154 Bacterial reverse mutation test
JE049-0156 *In vitro* mammalian chromosome aberration test in cultured human lymphocytes

Studies not reviewed within this submission: Pharmacology, pharmacokinetic and toxicology studies (IV, IN, IH and SC routes of administration) have been previously reviewed under INDs (b) (4) 68,214. Additionally, the sponsor submitted to their NDA new toxicology studies that have not been reviewed but used different routes of administration than the subcutaneous route proposed for icatibant. The following table outlines the studies previously reviewed under INDs (b) (4) 68,214. Following this table is a list of studies that have not been reviewed previously and are not reviewed here as they do not contribute to the support of icatibant's safety for the subcutaneous injection route of administration.

Previously Reviewed Nonclinical Studies

Study No.	Study Title	IND No.
Pharmacology studies		
JE049-0001	• Antagonistic effect of HOE 140 in BK-stimulated cultured endothelial cells • HOE 140 and D-Arg-[Hyp^{2}, Thi^{5,8}, D-Phe]-BK in isolated organs • Effects of HOE 140 and D-Arg-[Hyp^{2}, Thi^{5,8}, D-Phe]-BK on changes of coronary flow in isolated guinea pig hearts • Effect of HOE 140 on bradykinin induced endothelium dependent relaxations in isolated bovine coronary arteries • Inhibition of BK-induced hypotension in anesthetized rats by HOE 140 • Effect of HOE 140 on BK-induced fall in blood pressure in rats after nasal application • Antiinflammatory effect in the carrageenan-induced paw edema in rats • Inhibitory effect on S 89 0140 on burn edema in rat paws • Effect of HOE 140 on carrageenan paw edema in the rat and delayed-type hypersensitivity (DTH) in the rat and mouse • Analgesic activity in the modified Randall-Selitto test (yeast-induced motor impairment in rats) • Analgesic effect in mice: acetic acid writhing test • Bronchodilatory activity in anesthetized guinea pigs • IgE-mediated allergic reaction: passive cutaneous anaphylaxis (PCA) in rats • The effects of HOE 140 (S 89 0140) on the release of histamine from rat peritoneal mast cells • Inhibition of endotoxin-induced shock • Lack of effect of the bradykinin-antagonist HOE 140 on the endotoxin-induced drop of blood pressure in anesthetized rats • Effects of IV administration of HOE 140 on blood pressure and heart rate in conscious dogs • Effects of HOE 140 on postischemic reperfusion arrhythmias in isolated working rat hearts • Influence of intravenous infusion of the bradykinin-antagonist S 89 0140 on the development of acute renal vascular hypertension in anesthetized rats	(b) (4)

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Study No.	Study Title	IND No.
	• Effects of S 89 0140 on plasma rennin activity in comparison with D-Arg-[Hyp^{2}, Thi^{5,8}, D-Phe]-BK in rats • Behavioural observation in mice • Spontaneous motor activity in mice (6 hours) • Inhibition of electroschock-induced tonic convulsions • Protection from Pentetrazol-induced convulsions in mice by S 89 0140 • Effects of S 89 0140 on prevention of tetrabenazine-induced ptosis • Effects of S 89 0140 on D,L-5-hydroxytryptophan-potentiation in mice • Effects of S 89 0140 on potentiation of hexobarbital sleep in mice • Effects of S 89 0140 on inhibition on apomorphine-induced climbing in mice • Effects of sodium nitrite-induced hypoxia in mice • Inhibitory avoidance (step-through avoidance) in mice scopolamine-induced amnesia (subcutaneous treatment) • Blood glucose in normal rats after intravenous treatment with S 89 0140	
JE049-0002	Effects of HOE 140 on bradykinin receptors and on bradykinin-induced responses, in guinea pig tracheal epithelial cells and in human synovial cells	(b) (4)
JE049-0005	Novel bradykinin analogues act as dual antagonists on the guinea pig ileum	
JE049-0007	Effect of HOE 140 after intraduodenal application	
JE049-0008	Effect of HOE 140 after nasal application	
JE049-0010	Inhibition of nociceptive responses to intradermal injections of bradykinin by HOE 140	
JE049-0211	Inhibitory effect of HOE 140 on histamine-stimulated gastric acid secretion in stomach lumen perfused rats	
JE049-0212	Salidiuretic effect of HOE 140 after subcutaneous administration in rats	
JE049-0204	Intravenous bolus dose of the bradykinin antagonist HOE 140 lead to a short depressor response on rats in contrast to high subcutaneous and intranasal dose	
JE049-0213	Effect of HOE 140 on urinary excretion of sodium and potassium, and on renal clearances in Beagle dogs	
JE049-0214	Effect of HOE 140 on histamine release from human skin mast cells	
JE049-0206	Effects of the bradykinin antagonist HOE 140 in a model of myocardial infarction in the anesthetized dog	
JE049-0207	The effects of HOE 140 on ischemia-induced arrythmias in anesthetized rats with and without preconditioning	
JE049-0205	The hemodynamic and electrocardiographic effects on HOE 140 in conscious dogs under conditions of exercise	
JE049-0009	Effect of HOE 140 on bradykinin-induced bronchoconstriction in anesthetized guinea pigs: comparison of aerosol with intravenous administration	
JE049-0012	Effects of S 89 0140, a bradykinin antagonist, and endothelin on carrageenin-induced footpad edema and vascular permeability in urate crystal-induced inflammation rats	
JE049-0006	HOE 140 is a competitive bradykinin antagonist in anesthetized rats	
Pharmacokinetics		
JE049-0305	HOE 140-^{3}H: Studies on excretion and distribution after a single	

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Study No.	Study Title	IND No.
	subcutaneous administration of 12 µg/kg body weight to rats	(b) (4)
JE049-0319	*In vitro* metabolism of HOE 140	
JE049-0303	Pilot study on toxicokinetics in mice after subcutaneous and intravenous administration	
JE049-0304	Exploratory toxicokinetics in rats after subcutaneous and intravenous administration	
JE049-0307	Pilot study of toxicokinetics in dogs after subcutaneous administration	
Single Dose Toxicity		
JE049-0101	HOE 140: Single dose subcutaneous toxicity study in mice	
JE049-0102	HOE 140: Single dose intravenous toxicity study in mice	
JE049-0103	HOE 140: Single dose subcutaneous toxicity study in rats	
JE049-0104	HOE 140: Single dose intravenous toxicity study in mice	
JE049-0105	Single-dose intravenous infusion toxicity study of HOE 140 in rats	IND 68,214
JE049-0106	Single-dose intravenous toxicity study of HOE 140 in dogs	IND 68,214
JE049-0107	Single-dose intravenous infusion toxicity study of HOE 140 in dogs	IND 68,214
Repeat-Dose Toxicity		
JE049-0109	Repeated-dose (1 month) intravenous toxicity study of HOE 140 in rats	(b) (4)
JE049-0110	HOE 140 (S 890140): Comparative repeated-dose (1-month) toxicity study in rats (nasal./intravenous/subcutaneous)	
JE049-0111	Subacute (28-day) repeated dose inhalation toxicity study with HOE 140 in rats	
JE049-0112	Repeated-dose (1-month) nasal toxicity study of HOE 140 (S890140) in rats	
JE049-0121	Repeated-dose (1-month) intravenous toxicity study of HOE 140 (S890140) in dogs	
JE049-0123	28-Day inhalation toxicity study with HOE 140 (S890140) in the dog	
JE049-0124	Repeated-dose (1-month) nasal toxicity study of HOE 140 (S 890140) nasal spray in Beagle dogs	
Genetic Toxicology		
JE049-0126	S 89 0140 (HOE 140): Study of the mutagenic potential in strains of *Salmonella typhimurium* (Ames test)	
JE049-0130	S 89 0140: Study of the mutagenic potential in strains of *Salmonella typhimurium* (Ames test) and *Eschericia coli*	
JE049-0131	S 89 0140: Study of the mutagenic potential in strains of *Salmonella typhimurium* (Ames test) and *Eschericia coli*	
JE049-0134	S 89 0140 (HOE 140): Micronucleus test in male and female NMRI mice after intravenous administration	
JE049-0136	Evaluation of S 89 0140 (HOE 140) in the *In vivo* cytogenetic test in bone marrow cells of the Chinese hamster-chromosome analysis	
Reproductive and Developmental Toxicity		
JE049-0137	Effects of 5-week subcutaneous administration of HOE 140 on fertility of male mice	
JE049-0138	Effects of 5-week intravenous administration of HOE 140 on fertility of male mice	
JE049-0139	HOE 140: Subcutaneous embryotoxicity study in Wistar rats (effect no morphological and postnatal development including fertility) Segment II	
JE049-0140	HOE 140 (S 89 0140): Subcutaneous embryotoxicity study in Himalayan rabbits (Segment II)	
Local Tolerance		
JE049-0144	Single-dose intra-articular local tolerance study of HOE 140 (S 89 0140) injection solution in rabbits	
JE049-0145	HOE (S 89 0140): Primary skin irritation study on shorn intact and abraded rabbit skin (Patch test)	

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Study No.	Study Title	IND No.
Special Toxicology Studies		
JE049-0146	HOE 140 (S 89 0140): Mucosal tolerance in the rabbit eye	(b) (4)
JE049-0147	Antigenicity study of HOE 140 in Guinea pigs. Homologous 4-hour passive cutaneous anaphylaxis test	
JE049-0148	HOE 140, drug substance (S 89 0140): testing for sensitizing properties in the Pirbright-White guinea pig in the maximization test	
JE049-0149	Bradykinin antagonist HOE 140: S.C. injection vs SC infusion in male rats: effect on pituitary-testicular axis	

The following studies have not been reviewed. These studies do not contribute to the safety of icatibant or do not support the subcutaneous route of administration proposed for this NDA.

Methods of Analysis

JE049-0301	Radioimmunoassay method of icatibant
JE049-0302	HOE 140: Determination in human plasma by high performance liquid chromatography and method comparison vs radioimmunoassay
JE049-0141	Method validation in rat plasma and rat milk
JE049-0332	Icatibant and metabolite M2: Determination of freeze thaw stability in rat plasma
JE049-0333	Icatibant and metabolite M2: Determination of short term stability in rat plasma
JE049-0334	Icatibant and metabolite M2: Determination of long term stability in rat plasma
JE049-0335	Icatibant and metabolite M2: Determination of post preparative stability in rat plasma
JE049-0341	Icatibant and metabolite M2: Determination of freeze and thaw stability in rat milk
JE049-0342	Icatibant and metabolite M2: Determination of short term stability in rat milk
JE049-0343	Icatibant and metabolite M2: Determination of long term stability in rat milk
JE049-0344	Icatibant and metabolite M2: Determination of post preparative stability in rat milk
JE049-0122	Icatibant and metabolite M2: Bioanalytical method validation for and analysis of dog plasma samples
JE049-0337	Icatibant and metabolite M2: Determination of freeze and thaw stability in Beagle plasma
JE049-0338	Icatibant and metabolite M2: Determination of short term stability in Beagle plasma
JE049-0339	Icatibant and metabolite M2: Determination of long term stability in Beagle plasma
JE049-0340	Icatibant and metabolite M2: Determination of post preparative stability in Beagle plasma

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JE049-0336	Icatibant, metabolite M2 and internal standard (S 91 1486): Determination of stock solution stability
	Pharmacology
JE049-0011	Effect of HOE 140 on antigen-induced airway hyperresponsiveness in guinea pigs
	Pharmacokinetics
JE049-0320	*In vitro* metabolism of HOE 140 in the 9000g lung fractions of man and rat
	Repeat Dose Toxicity
JE049-0113	HOE 140 (S 890140): Repeated-dose (6 months) nasal toxicity study in rats
JE049-0114	HOE 140 (S 890140): Repeated-dose (6 months) nasal toxicity study in rats
JE049-0119	HOE 140 (S 890140): Pilot toxicity study (non-GLP). Testing for toxicity by repeated intravenous administration to dogs
JE049-0120	2-week toxicity study of icatibant by intravenous route in Beagle dogs
JE049-0122	4-week toxicity study by intravenous route (1-hour infusion) in Beagle dogs
JE049-0125	Repeated-dose (6-month) nasal toxicity study of HOE 140 (S 890140) in dogs

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INTRODUCTION AND DRUG HISTORY

On October 22, 2007 Jerini US, Inc (Jerini) submitted this New Drug Application (NDA) for Firazyr (Icatibant), a new molecular entity, for the treatment of hereditary angioedema (HAE) in adults (patients ≥18 years old). Icatibant is formulated as a pre-filled syringe for a single 30 mg subcutaneous injection. No more than 3 injections of icatibant within a 24-hour period are recommended. Currently, there are no approved treatments for HAE.

The original Investigational New Drug application (IND 68,214) for the treatment of HAE was submitted to the Division of Gastrointestinal and Coagulation Products April 8, 2004. On August 16, 2005, the IND 68,214 for icatibant acetate was transferred from the Division of Gastrointestinal and Coagulation Products to the Division of Pulmonary and Allergy Products (DPAP). A pre-NDA meeting was held between Jerini and DPAP on January 24, 2007 to discuss the registration process and any potential deficiencies in the drug development program. Jerini was informed that the Division considers the potential dosing of HAE patients with no more than eight 30 mg subcutaneous injections a month over the lifetime of the HAE patient as chronic intermittent dosing. To support the clinical chronic intermittent dosing schedule, Jerini was notified that nonclinical chronic repeat dose toxicity studies via the subcutaneous route of administration in the rat (6 month) and the dog (9 month) and 2 carcinogenicity studies (1 mouse and 1 rat) to address the carcinogenic potential of icatibant would be needed. Following the pre-NDA meeting, Jerini (May 1, 2007) requested comments on their proposed nonclinical study protocol designs from the Division for a 26-week subcutaneous repeat dose rat study, a 39-week subcutaneous repeat dose dog study, two 13-week subcutaneous pre-carcinogenicity screening study in rats and mice and two 104 week subcutaneous carcinogenicity study in rats and mice. Comments were relayed to Jerini on these proposed studies June 5, 2007.

For this NDA, Jerini submitted pharmacology, pharmacokinetics and toxicology studies conducted for the development of icatibant for the intranasal/inhalation, intravenous and subcutaneous routes of administration. Pharmacology, pharmacokinetic and toxicology studies have been previously reviewed under INDs (b) (4)), and 68,214 (subcutaneous administration of HAE). IND (b) (4) includes reviews of studies that were reviewed in IND (b) (4) Jerini has a Letter of Authorization allowing cross reference of IND (b) (4). Reference is made to the nonclinical reviews of INDs (b) (4) 68,214 (subcutaneous administration of HAE) for the detailed review of the nonclinical studies. The nonclinical studies that support the development of icatibant for the subcutaneous route of administration are reviewed herein.

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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Icatibant is a synthetic decapeptide with similar structure as endogenous bradykinin (BK). It is a potent, selective BK type 2 (B_2) receptor antagonist that binds to B_2 with similar affinity as BK. Icatibant binding at the B_2 receptor inhibits BK induced processes and is hypothesized to contribute to the decrease of vascular permeability and acute inflammation observed in HAE patients. The pharmacology of icatibant has been well characterized using various *in vitro* and *in vivo* models, which were previously reviewed under INDs (b) (4) 68,214. Under these reviews, the selectivity for the B_2 receptor demonstrated using a rabbit aorta model, which contains only BK type 1 (B_1) receptors. Incubation of rabbit aorta with BK resulted in contraction of the aorta. After incubation with BK and icatibant, no inhibition of rabbit aorta contraction was observed, demonstrating that icatibant did not competitively antagonized the B_1 receptor. However, icatibant was shown to inhibit BK-induced effects in cultured endothelial cells (IC_{50}= 10^{-8} and 10^{-9} mol/L for BK-induced release of endothelium derived relaxing factors and release of intracellular calcium, respectively) and isolated guinea-pig hearts (IC_{50}= 5.4 x 10^{-9} mol/L for BK-induced contraction).

In addition to these previously reviewed studies, the sponsor submitted additional pharmacology studies that further characterized the binding affinity and activity of icatibant and two of its major metabolites, M1 (b) (4) and M2 (b) (4) on BK receptors. These studies demonstrated that using *in vitro* receptor binding studies and measuring the displacement of ^{3}H-BK in guinea pig ileum, guinea pig trachea epithelium and human synovial cells, icatibant was a competitive inhibitor of the B_2 receptor with an IC_{50} within the 1-4 nM range. In Chinese Hamster ovary (CHO) cells expressing recombinant B_1 or B_2 receptors, icatibant had an IC_{50} of 6.0 µM and 4.3 nM, respectively. Slight binding of additional receptors was observed with icatibant in a receptor screening study. However, this binding did not interfere with the receptors enzymatic activity with the exception of ACE and NK2 enzymatic activity.

Additionally, Jerini investigated the mechanism of observed injection site irritation by exposing icatibant to human mast cells, nociceptive nerve fibers and measuring MAPK and calcium levels using *in vitro* techniques. These data showed that icatibant does activate the release of histamine, LTC4 and PGD2 from mast cells and induces mobilization of calcium nerve fibers, which may contribute to local injection site irritation.

Several new safety pharmacology studies were submitted to the NDA. These studies included 2 cardiovascular studies evaluating icatibant and metabolites M1 and M2 effects on cardiac potassium currents, one respiratory function safety study conducted in rats and 2 gastrointestinal (GI) studies conducted in rats. Icatibant did not inhibit cardiac potassium currents up to 300 µM concentrations under the conditions tested. After intravenous (IV) administration of icatibant to Wistar rats up to 10 mg/kg, no changes in

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respiratory function were observed compared to the vehicle control group. In the GI studies, single IV doses of icatibant up to 10 mg/kg in Wistar rats had no effect on GI transit but did decrease gastric acid volume and acidity.

Additional safety pharmacology studies were reviewed under IND (b) (4). Reference is made to Dr. Young Choi's review of IND (b) (4) for the detailed review of these studies. Based on Dr. Choi's review, icatibant decreased coronary blood flow [IC_{50}= 3.1 x 10^{-8} mol/L (0.5 mL/min) resulting in a % inhibition ranging from 22% to 67%] in isolated guinea pig heart, aggravated the duration of post-ischemic reperfusion arrhythmias in isolated working hearts in Wistar rats and impaired cardiodynamics. At a rate of 0.5 ng/kg/min, intracoronary infusion of icatibant in an anesthetized myocardial infarction dog model increased mortality rate 2-fold (58%) over saline ischemia (28%) and produced delayed deaths. Also, icatibant at 0.3 mg/kg, IV decreased urine output, sodium and potassium excretion (~40% for all 3 parameters), to a lesser extent for GFR, renal plasma flow and osmolar clearance in beagle dogs. Icatibant also significantly inhibited BK-induced hypotension in anesthetized rats and produced decreased motor activity, assumption of prone position, reduced respiration, ptosis, piloerection and mydriasis at 1-10 mg/kg IV. These effects on central nervous system (CNS), cardiovascular (CVS) and renal systems are considered the result of the partial agonistic property of icatibant on B-receptors and may present toxicological concerns.

In the 4-week subcutaneous (JE049-0016) and 13-week subcutaneous (JE049-0117) repeat dose toxicology studies conducted in the Beagle dog, electrocardiographic (EKG) examinations were conducted in the presence of icatibant. In the 4-week study, dogs were dose up to 10 mg/kg twice weekly (administered as 3.3 mg/kg TID twice weekly). There were no statistically significant changes in EKG parameters or changes in blood pressures in icatibant treated males and females compared to the vehicle treated animals. In the 13-week study, dogs were administered daily doses of icatibant up to 100 mg/kg/day. There was a slight but statistically significant decrease in heart rate in HD males (-29%) and HD females (-21%) 6 hours post dose during Week 1 of dosing compared to the vehicle control groups. A decrease in diastolic BP was noted in HD males (-39%) 6 h post-dose on Week 1 of dosing compared to the vehicle control group. At Week 13 of dosing, there were no statistically significant differences in heart rate, PQ- QRS- or QT-intervals evaluated in the icatibant treated males and females compared to the vehicle control animals. There were no statistically significant changes in QT corrected for heart rate using either Bazett or Fridericia formulae in the icatibant treated animals compared to the vehicle treated animals. However, a statistically significant decrease in systolic BP (-19%) was observed in HD males 1 h post-dose in Week 12 and (-22%) was observed in HD females before dosing in Week 13, respectively.

During icatibant's drug development, Jerini has evaluated icatibant's effects on the CNS, CVS, respiratory, renal and GI systems. Taking these data as whole, the safety pharmacology of icatibant has been adequately addressed.

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2.6.2.2 Primary pharmacodynamics

Mechanism of action: In Type I (quantitative deficiency) and Type II (qualitative deficiency) hereditary angioedema (HAE) a deficiency of C1 esterase inhibitor (C1-INH) results in an increased release of bradykinin (BK). BK binds to the BK type-2 receptor (B_2) to trigger activation of many intracellular processes. Included are cascades, two of which are coupled by the $G_{\alpha q/11}$ and $G_{\alpha i}$ proteins, leading to calcium mobilization and MAPK activation, respectively. Calcium mobilization and MAPK activation induces many cellular events including cellular proliferation, excitation of neurons, vasodilatation, increased vascular permeability and smooth muscle cell contraction. These events are believed to contribute to the increased vascular permeability and acute inflammation observed in HAE patients.

Icatibant is a synthetic decapeptide with similar structure as endogenous BK. It is a potent, selective B_2 receptor antagonist that binds to B_2 with similar affinity as BK. Icatibant binding at the B_2 receptor inhibits BK induced processes and is hypothesized to contribute to the decrease of vascular permeability and acute inflammation observed in HAE patients. *In vitro* studies have shown that icatibant binds to both B_1 and B_2 receptors (Ki values of 2 nM and 1.2 µM, respectively) and inhibits BK with IC_{50} of 6 µM and 4.3 nM, respectively. These data show that icatibant is selective for the B_2 receptor subtype over the B_1 receptor (Study no. JE049-0003).

The sponsor submitted 5 pharmacology studies that used isolated receptors (*in vitro* and using high-throughput screening), cell cultures and tissue models to evaluate the binding affinity and activity of icatibant and two of its major metabolites, M1 [redacted] (b) (4) and M2 [redacted] (b) (4) on BK receptors. Briefly, these studies showed that icatibant bound preferentially to the B_2 receptor subtype over the B_1 receptor which resulted in significant inhibition of native BK binding. Icatibant metabolites M1 and M2 also bound to B2 receptors but with less inhibition than the parent drug and M2 having greater inhibition of BK than the M1 metabolite. Neither icatibant nor M1 or M2 bound to the LH-RH receptor. Slight binding of additional receptors was observed with icatibant in a receptor screening study. However, this binding did not interfere with the receptors enzymatic activity with the exception of ACE and NK2 enzymatic activity. These data are summarized in the following table.

Study no/Title	Study Design	Study Outcomes
JE049-0003 *In vitro* pharmacology: Human B_1 and B_2 receptors-study of icatibant (non-GLP, Dec 20003)	*In vitro* competitive binding assay using human recombinant B_1 and B_2 receptors isolated from Chinese hamster ovary cells. B_1 and B_2 receptors were incubated with radiolabeled ligand and unlabeled icatibant (batch no. E084) to determine the IC_{50} and K_i of icatibant at each receptor.	B_1 : IC_{50}= 6.0E-06 K_i= 1.2E-06 B_2 : IC_{50}= 4.3E-09 K_i= 2.0E-09
JE049-0004 *In vitro* pharmacology: Bradykinin receptors-	*In vitro* competitive binding assay using human recombinant B_1 and B_2 isolated from Chinese hamster ovary cells and	Human B_1 M1= -8 % inhibition M2= 20% inhibition

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Study no/Title	Study Design	Study Outcomes
Study of M1, M2 and Icatibant (non-GLP, Nov 2003)	guinea pig ileum isolated B_2 receptor B_1 and B_2 receptors (human and guinea pig) were incubated with radiolabeled bradykinin ligand and unlabeled icatibant (batch no. E084), M1 and M2 metabolites to determine the % inhibition of control specific binding	Icatibant= 83% inhibition Human B_2 M1= 10 % inhibition M2= 45 % inhibition Icatibant= 100% inhibition Guinea pig B_2 M1= 48 % inhibition M2= 58% inhibition Icatibant= 100% inhibition
JE049-0217 *In vitro* pharmacology: LH-RH receptor binding assay-Study of icatibant, M2 and M1 (non-GLP, Nov 2006)	LH-RH receptor was isolated from rat pituitary gland and incubated with radiolabeled LH-RH receptor ligand and unlabeled icatibant (batch no. E084), M2 and M1 metabolites to determine the % inhibition of control specific binding	There was no binding of icatibant, M1 or M2 to the LH-RH receptor.
JE049-0201 Study of icatibant (HOE 140) in various receptor binding and enzyme assays (non-GLP, Dec 2001)	30 different receptor types were incubated with their radiolabeled ligand and with unlabeled icatibant to determine if icatibant could bind with any affinity to these receptors. 7 different enzymes were assayed in the presence of radiolabeled substrate and icatibant to determine whether icatibant had any effect on their activities Icatibant was assayed in these experiments up to 10 µM	Receptor binding: < 10% inhibition of 25/30 receptors Slight inhibition in 5 receptors: M4 (16%), opiate (19%), EP1 (13%), VIP1 (56%) and VIP2 (41%)] Enzymatic assays: Icatibant resulted in an IC_{50} = 2.3 µM in the ACE assay. No other enzymes were affected by icatibant.
JE049-0218 *In vitro* pharmacology: high throughput profile-study of icatibant, M2 and M1 (non-GLP, Dec 2006)	Using a high throughput screening method consisting of competitive binding assays ~76 different receptor types were assayed to test for binding of icatibant or either the M2 or M1 metabolites. The percent inhibition of the radiolabeled ligand by icatibant, M2 or M1 was determined.	B_2 bound icatibant (98% inhibition), M2 (62% inhibition) and M1 (2% inhibition) M3-receptor bound icatibant (52% inhibition) but neither M1 nor M2 affected this receptor NK2 bound icatibant (96% inhibition) but neither M1 nor M2 bound this receptor IC_{50} for NK2 receptor= 4.2E-07 M V1a bound icatibant (66% inhibition), M2 (10% inhibition) but not M1.

2.6.2.3 Secondary pharmacodynamics

In the nonclinical toxicology studies (see Toxicology Section of this review) injection site irritation is observed after subcutaneous injection of icatibant. This has also been observed after subcutaneous injection in humans. Jerini investigated the mechanism of this injection site irritation by exposing human mast cells and nociceptive nerve fibers to icatibant, and measuring MAPK and calcium levels using *in vitro* experimental methods. These data showed that icatibant does activate the release of histamine, LTC4 and PGD2

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from mast cells. Additionally, icatibant mobilizes calcium release from nerve fibers. Each of these actions may contribute to icatibant irritation after subcutaneous injection. These studies are summarized below.

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Study no/Title	Study Methods	Study Outcomes
JE049-0215 Characterization of the effects of icatibant on human mast cells (non-GLP, Nov 2006)	Mast cells harvested from human skin were exposed to various concentrations of icatibant and to determine the concentration at which histamine, tryptase, leukotriene-C4 (LTC4) and prostaglandin (PGD2) may be released. Concentrations of icatibant (E084) tested in each assay ranged from 1 x 10^{-3} to 1 x 10^{-10} M.	• Human mast cells are activated by icatibant • More than 40% of preformed histamine is released at 10^{-4}M icatibant concentration • Icatibant at 10^{-3} and 10^{-4} M increased the release of tryptase by ~5% compared to no treatment • LTC4 and PGD2 was released from mast cells at icatibant concentrations from 10^{-3} to 10^{-6} M.
JE049-0013 Analysis of species specificity and potential agonistic activity of icatibant using MAPK activation and calcium mobilization (non-GLP, Dec 2006)	Agonistic and antagonistic properties of B_2 receptor from human (HF15 and HEK-293 cells), rat (Rat2, PC-12, RFL-6, RBL-2 and A7r5 cells) and dog (MDCK and CF52.Tr cells) cells were determined by measuring MAPK activation and calcium mobilization	• Nanomolar concentrations of icatibant exerted partial agonistic activity on MAPK activation using the rat and dog B_2R but not the human B_2R. • At icatibant concentrations < 50 µM, no partial agonistic activity on calcium mobilization was detected in rat, dog and human cells.
JE049-0216 Icatibant dose- and calcium-dependently induces calcitonin gene-related peptide release from isolated skin in wild-type, B2 -/- and KITW/KITW-v but less in TRPV1 -/- mice (non-GLP, Feb 2006)	Neuropeptide release was measured after icatibant-stimulation (1, 3.1 and 10 mM) in isolated mouse skin (5 mice/sex/strain) from several different mouse strains. C57BL/6 =Controlcontrol B2 -/- = bradykinin 2 receptor knockout model TRPV -/- = capsaicin receptor knockout mouse KITW/KITW-v = Mast cell deficient mice	• Icatibant induces a dose-dependent and partially extracellular calcium dependent release of iCGRP from nociceptive nerve fibers in skin • 10 mM icatibant regularly induced a sustained release of iCGRP • Acute action of icatibant (3.1 and 10 mM) mobilizes internal calcium stores

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2.6.2.4 Safety pharmacology

Cardiovascular effects: Two *in vitro* experiments were conducted to evaluate the effects of icatibant and metabolites M1 and M2 on the inward rectifying potassium currents expressed in either Xenopus oocytes or Chinese hamster ovary cells. In the first study (study no. JE049-0202, non-GLP), Xenopus oocytes expressing cardiac potassium channels were incubated in the presence of icatibant at 10, 30 and 100 µM or vehicle control (physiological buffer solution) alone and potassium currents were measured using patch clamp. There was no statistically significant change in potassium currents in the icatibant treated cells compared to the vehicle control currents.

In a second study (study no. JE049-0203, non-GLP), the effects of icatibant, M1 (b) (4) and M2 (b) (4) on whole cell potassium currents were tested in Chinese hamster ovary cells that expressed the HERG potassium channel. Icatibant and the two metabolites were tested at 10, 100 and 300 µM concentrations (n= 3 assays/concentration). Compared to the vehicle (physiological saline and DMSO) treated cells, icatibant and the two metabolites did not inhibit potassium currents. At 10, 100 and 300 µM, icatibant induced a current change of 1, 3 and 6%; M1 at 3, 5 and 5%; and M2 at 0, 0 and 2%, respective to concentration. The negative (vehicle; n=3) and positive controls (100 nM Haloperidol; n=3) resulted in no inhibition of potassium currents and significant inhibition of potassium currents (90% inhibition), respectively.

Taken these two studies together, icatibant and metabolites M1 and M2 did not inhibit potassium channel currents under these assay conditions.

Pulmonary effects: The effects of single intravenous doses of icatibant on respiratory parameters using whole body plethysmography were investigated in conscious rats (study no. JE049-0208, GLP conditions). Single IV doses of icatibant (batch no. E084) were administered at 0 (vehicle control-physiological saline), 1, 3 and 10 mg/kg to male Wistar rats (mean BW range 309-359 g; n=8/dose). To serve as the positive control, theophylline (16 mg/kg) was administered intravenously under the same experimental conditions. The respiratory parameters assessed were inspiratory time, expiratory time, peak inspiratory flow, peak expiratory flow, tidal volume, respiratory rate, relaxation time, pause and enhanced flow. Icatibant had no statistically significant effects on inspiratory time, expiratory time, peak inspiratory flow, peak expiratory flow, tidal volume, respiratory rate, relaxation time, pause or enhanced flow compared to the vehicle control group. The theophylline treated rats produced the expect changes in respiratory parameters demonstrating the validity of the assay.

Gastrointestinal effects: Two studies investigated icatibant's effects on the gastrointestinal (GI) system. The first study, (JE049-209, GLP) intravenously administered icatibant to male Wistar rats (mean BW range 140-174 g; n=8/group) and the GI transit time was evaluated. Rats were administered single doses of 0 (vehicle-physiological saline), 1, 3 or 10 mg/kg icatibant intravenously 10 minutes prior to charcoal suspension gavage. Atropine (1 mg/kg, IV) was administered as a positive control. The distance covered by charcoal in the small intestine in the icatibant treated rats was compared to the vehicle treated rats. At all doses tested, icatibant had no statistically significant effect on the distance covered in the small intestine by charcoal compared to the vehicle control group (+6%, +8% and -5% for 1, 3 and 10 mg/kg IV,

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respectively, compared to VC). Atropine reduced charcoal transit by 27% compared to the vehicle control group.

In the second study (JE049-0210, GLP) the effects of icatibant on gastric acid secretion were investigated in rats. Male Wistar rats (mean BW range 216-234 g; n=8/group) were intravenously administered 0 (vehicle-physiological saline), 1, 3 and 10 mg/kg of icatibant and the gastric fluid volume, pH free chlorhydric acidity and total acidity were measured. To serve as a positive control, atropine sulfate (2 mg/kg, IV) was administered. At 1 and 3 mg/kg, IV, of icatibant, no effect on the gastric fluid volume, pH, free chlorhydric acidity and total acidity was observed compared to the vehicle control animals. The HD group (10 mg/kg) reduced gastric fluid volume (-48%), free chlorhydric acidity (-42%) and total acidity (-26%) compared to the vehicle control group. The positive control, atropine, reduced gastric fluid volume (-83%), free chlorhydric acidity (-32%) and total acidity (-26%) compared to the vehicle control group.

2.6.2.5 Pharmacodynamic drug interactions

No new data were submitted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

No new data were submitted.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Jerini conducted two 14-day toxicity studies (each in rats), a 10-day dose-ranging toxicity study in dogs, a 4-week toxicity study in dogs and three 13-week toxicity studies (1 in rats and 2 in dogs) using the subcutaneous route of administration. These studies showed that after subcutaneous administration, icatibant is rapidly distributed and metabolized with the M2 metabolite [redacted] (b) (4) appearing from 0.2 to 3 hours post-dose in rats and dogs. In rats and dogs, increases in icatibant doses from LD to MD doses (0.1 to 3 mg/kg in rats and 10 to 30 mg/kg/day in dogs) produced approximately dose proportionate increases in systemic exposures. Increases in icatibant doses from MDs to HDs resulted in super-proportionate increases in systemic exposures in rats (increased ~128-fold instead of the expected ~50-fold) and approximately dose proportionate increases in dogs. Using an alternate dosing schedule in dogs (1 or 3.3 mg/kg TID of icatibant twice weekly), AUC levels of icatibant and M2 increased in an approximate dose proportionate increase. The t_{max} was achieved from 0.5 to 3.0 h post-dose in rats and dogs. Neither icatibant nor M2 accumulated after repeat dosing in males and females up to 13-weeks dosing. There were no gender differences observed in rats or dogs in the systemic exposures of males and females.

Distribution studies showed that icatibant (or its metabolites) is distributed into the kidneys, liver, urinary bladder, lungs and spleen 1 hour and it still detectable in the liver, kidneys, spleen, bone marrow, adrenals and injection sites at 24-hours post SC dose in rats. Serum protein binding of icatibant was low with 44 and 49% in man and rat, respectively, and 39% in monkey

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and dogs. Icatibant is excreted in the milk of rats after IV administration. M1 (b) (4) and M2 (b) (4) are the primary metabolites of icatibant and are formed by hydrolysis of the peptide backbone, thought to be induced by peptidases. Neither icatibant nor the M1 or M2 metabolites are metabolized by human hepatocytes or microsomes. Additionally, icatibant is not an inducer of CYP450 isozymes. Based on excretion studies, the primary route of elimination after SC administration of icatibant is via the kidneys in mice (~97.1%), rats (~61%) and dogs (~63%) with small amounts excreted in the feces (8.0% to 33%).

As reported in the nonclinical review of IND (b) (4) (reviewed by Dr. Choi), the major metabolite in man is M1 (>90%). M1 is formed in rat (~70%), dog (~60%) and mouse (~50%). M2 is formed in rat (~9% of observed parent levels), dog (~3%) and human (~75%). The biological half-life was more than 5 hours after 26 µg/kg SC in rats and terminal half-life was over 6 days in rats after 10 mcg/kg, SC. The comparative pharmacokinetics of icatibant after intravenous administration in man, rat, dog and mouse were summarized by Dr. Choi in the table below:

Parameter	Man	Rat	Dog	Mouse
Dose (mg/kg)	0.2	1.0	0.1	1.0
AUC (ng/mL*h)	1518	2290	120-125	1130
C_{max} (ng/mL)	970	1269-3600	63-70	1310
T_{max} (h)	1.0*	0.5**	1**	0.5**
$T_{1/2}$ (h)	4.6	-	-	-

*- After 1 h infusion
**-First sampling point
- data are not available.

After single dose SC and IV administration of icatibant to healthy subjects, clinical pharmacokinetic analysis was conducted. As observed in the following table, after 0.4 mg/kg SC and IV administration, icatibant was rapidly absorbed with a T_{max} achieved for icatibant at ~0.5 to 0.6 h post-dose. The M2 metabolite T_{max} was observed at ~ 2 h post-dose after SC administration and ~ 1.5 h post-dose after IV administration, each T_{max} being significantly longer in humans than in mouse, rat or dog. After a 0.4 mg/kg SC dose in man, AUC_{0-t} (ng*h/mL) values for icatibant were significantly lower than those observed in rats and dogs from 13-week toxicology studies. The M2 metabolite exposure was ~ 25% lower than exposure to the parent compound in humans. This is in contrast to data in rats and dogs where M2 accounted for only ~9% and ~3%, respectively, of the parent compound exposure.

Parameter	Icatibant Dose (mg/kg)					
	PK of Icatibant			PK of M_2 Metabolite		
	0.4 SC (20 mg/mL)	0.4 SC (10 mg/mL)	0.4 IV (1 mg/mL)	0.4 SC (20 mg/mL)	0.4 SC (10 mg/mL)	0.4 IV (1 mg/mL)
C_{max} (ng/mL)	1133	1387	2876	354	373	463
T_{max} (h)	0.561	0.595	0.500	2.24	2.182	1.52
AUC_{0-t}	2492	2984	3034	2057	1974	2079

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(ng*h/mL)						

Extrapolating from these data, the proposed clinical dose of 30 mg icatibant SC (0.6 mg/kg for 50 kg human) is speculated to achieve an approximate systemic exposure of 4107 ng*h/mL and 3023 ng*h/mL for icatibant and the M2 metabolite, respectively. At the maximum recommended dose of 90 mg/day, the respective exposures project to 12.3 µg*h/ml and 9.1 µg*h/mL.

2.6.4.2 Methods of Analysis

For evaluation of plasma levels of icatibant and the M2 metabolite (the formation of the M1 metabolite was not evaluated in these studies) from rat and dog plasma samples were prepared from whole blood samples. These plasma samples were evaluated using liquid chromatography-MS/MS analytic methods. In the rat, the lower limit of quantitation (LLOQ) ranged from 2 to 30 ng/mL plasma for icatibant and was 10 ng/mL plasma for the M2 metabolite. In the dog, the LLOQ for icatibant ranged from 1.3 to 26.54 ng/mL plasma and for M2 ranged from 0.431 to 65.14 ng/mL plasma. Using the data obtained from LC-MS/MS the TK parameters C_{max}, t_{max} and AUC could be determined.

2.6.4.3 Absorption

Jerini conducted two 14-day toxicology studies and one 13-week toxicology study in Sprague-Dawley rats using the subcutaneous route of administration. In the Beagle dogs, the sponsor conducted a dose-ranging study up to 10 days dosing, a 4 week toxicology study and two 13-week toxicology studies. In each of these studies, blood samples were collected and concentrations of icatibant or icatibant and the M2 metabolite were determined.

In a 14-day subcutaneous rat study (JE049-0127) rats were dosed with 0 (vehicle-water for injection), 1, 10 or 50 mg/kg/day of icatibant, SC. Blood samples were taken on Day 1 and Day 14 from the TK animals (3/sex/group) to determine icatibant levels. Blood samples were taken at 0.5, 1, 2, 3, 7 and 24 h post-dose on each day from the retrobulbal venous plexus. Plasma samples were prepared from whole blood and analyzed using LC-MS/MS analytical methods. TK parameters determined included: C_{max}, t_{max} and AUC. The lower limit of quantitation (LLOQ) for icatibant was 10 ng/mL plasma.

Sampling period	TK Parameter	Icatibant Dose (mg/kg/day)					
		1		10		50	
		M	F	M	F	M	F
Day 1	C_{max} (µg/mL)	1.35	1.11	7.49	7.51	20.6	16.9
	T_{max} (h)	0.5	0.5	1.0	1.0	0.5	0.5
	AUC_{0-t} (µg*h/mL)	1.36	1.0	17.4	15.4	85	94.1
Day 14	C_{max} (µg/mL)	1.36	1.15	9.48	10.1	31.6	37.7
	T_{max} (h)	0.5	0.5	1.0	0.5	1.0	0.5
	AUC_{0-t} (µg*h/mL)	1.47	0.98	22.1	16.9	93.7	80

There were no significant gender differences in the TK parameters evaluated in rats on Days 1 and 14 of study. The systemic exposure levels increased in an approximately dose proportionate manner from the LD to the MD. However, systemic exposure increased less than dose

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proportional from the MD to the HD with an approximate increase of 2.5-fold instead of the expected 5-fold on Day 1. However, at Day 14, the systemic exposure levels were approximately dose proportional. The t_{max} was achieved from 0.5 to 1.0 h post-dose for both males and females. After repeated SC administration for 14-days, there was no accumulation of icatibant in either male or female rats.

In a second 14-day subcutaneous rat study (JE049-0128), male rats (females were not included in this study) were dosed with 0 (vehicle-water for injection), 0.1, 1 or 50 mg/kg/day of icatibant, SC. Blood samples were taken on Day 1 and Day 14 from the TK animals (3/sex/time point) to determine icatibant levels. Blood samples were taken at 0.5, 1, 2, 3, 7 and 24 h post-dose on each day from the retrobulbal venous plexus. Plasma samples were prepared from whole blood and analyzed using LC-MS/MS analytical methods. TK parameters determined included: C_{max}, t_{max} and AUC. The lower limit of quantitation (LLOQ) for icatibant was 2 ng/mL plasma.

Sampling period	**TK Parameter**	**Icatibant Dose (mg/kg/day)**		
		0.1	**1**	**50**
Day 1	C_{max} (µg/mL)	0.073	0.738	11.258
	T_{max} (h)	0.5	0.5	3.0
	AUC_{0-24}(µg*h/mL)	0.04	0.956	122.423
Day 14	C_{max} (µg/mL)	0.078	1.355	26.786
	T_{max} (h)	0.5	0.5	3.0
	AUC_{0-24}(µg*h/mL)	0.044	1.0	177.5

On Day 1 of dosing, the C_{max} and AUC increased in an approximately dose-proportionate manner from LD to MD. From MD to HD, the C_{max} increased but in a less than dose-proportional manner (increased by 15-fold instead of ~50-fold). The AUC increased from the MD to the HD by ~128-fold instead of ~50-fold, making it a super-proportional increase to dose. After repeated dosing for 14 days, the C_{max} increased in an approximately dose proportional manner from the LD to the MD. From the MD to the HD, the C_{max} increased in a sub-proportional manner by ~20-fold instead of the ~ 50-fold. The AUC increased in an approximately dose-proportional manner from the LD to the MD. However, from the MD to the HD the AUC increased in supra-proportional manner by ~177-fold. Based on these data, after 14-day repeat dosing, icatibant does not accumulate in males. The T_{max} ranges from 0.5 h to 3.0 h on Day 1 of dosing and from 0.5 h to 1.0 h on Day 14 of dosing.

In study no. JE049-0108, male and female rats were subcutaneously administered 0 (vehicle-0.9% NaCl), 1, 3 and 10 mg/kg/day for 13 weeks. Satellite males and females (6 rats/sex/group) had blood samples collected on Day 1 and during Week 13 at 0.5, 1, 2 and 4 h post-dosing. Plasma was prepared from samples and assessed for icatibant and the metabolite M_2 (b) (4) levels using LC-MS/MS analytical methods. TK parameters determined included: t_{max} and AUC. The lower limit of quantitation (LLOQ) for icatibant was 30 ng/mL plasma and for M_2 metabolite was 10 ng/mL plasma, respectively.

	Parameter	**Icatibant Dose (mg/kg/day)**											
		TK of Icatibant						**TK of M_2 Metabolite**					
		1		**3**		**10**		**1**		**3**		**10**	
		M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**

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Day 1	C_{max} (µg/mL)	0.96	0.81	2.1	2.4	5.4	6.1	0.14	0.06	0.20	0.17	0.47	0.25
	T_{max} (h)	0.5	0.5	0.5	1	1	1	1	1	1	1	2	2
	$AUC_{0\ 5\text{-}4\ h}$ (µg*h/mL)	0.82	0.66	2.5	3.7	12.4	12.6	0.13	0.07	0.42	0.29	1.12	0.64
Wk 13	C_{max} (µg/mL)	1.0	1.1	2.3	2.7	6.1	6.8	0.09	0.06	0.18	0.13	0.39	0.18
	T_{max} (h)	0.5	0.5	0.5	1	2	1	1	1	2	1	2	2
	$AUC_{0\ 5\text{-}4\ h}$ (µg*h/mL)	1.7	1.2	5.6	5.0	15.9	17.2	0.15	0.08	0.48	0.31	0.88	0.59

NC- not calculated because individual values were invalid (QC rejected).

On Day 1 and Week 13 of dosing, icatibant C_{max} and AUC levels increased in an approximate dose proportional manner in both males and females. There were no gender differences and icatibant did not accumulate after 13 weeks of daily SC dosing. The M2 metabolite C_{max} increased with increased icatibant dose on Day 1 and Week 13 in a sub-proportional at all doses. AUC levels were proportional from LD to MD and sub-proportional from MD to HD. The metabolite formed between 1 and 2 hours post-icatibant dose. The metabolite formed between 1 and 2 hours post-icatibant dose, demonstrating rapid metabolism of the parent molecule.

In a dose-ranging study (JE049-0115), a single Beagle dog/sex was dosed 100 mg/kg/day for 10 days. Blood samples were taken on Day 1 and Day 10 from each animal from Phase 2 to determine icatibant levels and the metabolite M_2 (b) (4) levels. On Day 1 and Day 10 samples were taken at: predose, 0.5, 1, 2, 4, 8 and 24 h post-dose. Plasma samples were prepared from whole blood and analyzed using LC-MS/MS analytical methods. TK parameters determined included: C_{max}, t_{max} and AUC. The lower limit of quantitation (LLOQ) for icatibant was 11.4-29.07 ng/mL plasma and for M2 metabolite was 18.59-65.14 ng/mL plasma, respectively. These data showed that dogs were exposed to icatibant and that the M2 metabolite was present in dogs.

Day	**Parameter**	**Icatibant 100 mg/kg/day**			
		TK for Icatibant		**TK for M2 metabolite**	
		M	**F**	**M**	**F**
Day 1	C_{max} (µg/mL)	52.2	54.2	2.01	0.73
	T_{max} (h)	1	2	24	24
	$AUC_{0\text{-}24}$ (µg*h/mL)	458	507	19	10
Day 10	C_{max} (µg/mL)	77.4	57.8	3.38	1.13
	T_{max} (h)	1	1	0	0
	$AUC_{0\text{-}24}$ (µg*h/mL)	415	522	6	13

Blood samples were taken from 1 dog/sex at the indicated time points at one dose (100 mg/kg/day). Based on the available data, icatibant C_{max} and AUC levels were similar between the male and the female at Day 1 and at day 10 of the study. There was no accumulation of icatibant over the 10 day dosing period in either the male or the female. The M2 metabolite was ~3-fold higher in males than in females for C_{max} on day 1 and day 10 of the study. The AUC of the M2 metabolite was approximately the same in males and females on Day 1 and Day 10.

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In a 4-week toxicity study (JE049-0116), Beagle dogs were subcutaneously administered 0 (vehicle-0.9% NaCl), 1 or 3.3 mg/kg TID of icatibant twice weekly. These doses equaled to 3 and 10 mg/kg icatibant twice weekly. Blood samples were taken on Day 22 from all animals to determine icatibant levels and the metabolite M2 (b) (4) levels. Samples were taken at: predose, 1, 3, 6, 7, 12, 13 and 24 h post-dose. Plasma samples were prepared from whole blood and analyzed using LC-MS/MS analytical methods. TK parameters determined included: t_{max} and AUC. The lower limit of quantitation (LLOQ) for icatibant was 2.27 and 8.49 ng/mL plasma and 0.431 and 1.40 ng/mL plasma for M2 metabolite, respectively.

	TK Parameter	Icatibant Dose (mg/kg/day)			
		1 TID		3.3 TID	
		M	F	M	F
TK for Icatibant	AUC_{Tot}(µg*h/mL)	18.2	10.9	43.4	44.5
TK for M2	AUC_{Tot} (µg*h/mL)	0.575	NC	0.735	0.851

NC- not calculated because individual values were invalid (QC rejected).

On Day 22, AUC levels of icatibant increased with increased dosage in both males and females in an approximately dose proportionate manner. The M_2 metabolite also increased with increased dose but in a sub-proportionate manner for males (no data were available for females at the low dose). The t_{max} was reached between 1 and 2 hours post-dose in both males and females. No gender differences were observed in systemic exposure levels on Day 22 of the study.

Study no. JE049-0117, dosed Beagle dogs with 0 (vehicle- 0.9% NaCl), 10, 30 and 100 mg/kg/day of icatibant for 13 weeks. Blood samples were taken on Day 1 and in week 13 from all animals to determine icatibant levels and the metabolite M_2 (b) (4) levels. On Day 1 and during Week 13 samples were taken at: predose, 0.5, 1, 2, 4, 8 and 24 h post-dose. Plasma samples were prepared from whole blood and analyzed using LC-MS/MS analytical methods. TK parameters determined included: C_{max}, t_{max} and AUC. The lower limit of quantitation (LLOQ) for icatibant was 6.94-26.54 ng/mL plasma and for M2 metabolite was 4.14-9.36 ng/mL plasma, respectively.

	Parameter	Icatibant Dose (mg/kg/day)											
		TK of Icatibant						TK of M2 Metabolite					
		10		30		100		10		30		100	
		M	F	M	F	M	F	M	F	M	F	M	F
Day 1	C_{max} (µg/mL)	10.9	11.2	47.7	41.4	NC	74.4	0.40	0.32	1.16	1.01	NC	1.38
	T_{max} (h)	1.0	1.0	1.5	1.75	NC	2.0	1.75	2.00	3.00	2.50	NC	4.0
	AUC_{0-t} (µg*h/mL)	24.6	22.9	190	164	NC	383	1.14	0.90	6.14	5.26	NC	7.65
Wk 13	C_{max} (µg/mL)	17.6	17.7	34.4	41.6	82.3	71.0	0.39	0.31	NC	NC	0.63	0.77
	T_{max} (h)	0.75	0.75	1.75	1.50	0.88	0.75	2.0	2.0	NC	NC	2.0	2.0
	AUC_{0-t} (µg*h/mL)	38.2	31.6	80.9	83	246	213	1.15	0.77	NC	NC	3.10	3.46

NC- not calculated because individual values were invalid (QC rejected).

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On Day 1 and Week 13 of dosing, the C_{max} and AUCs of icatibant were similar between males and females, suggesting that there were no significant gender differences. This was also observed for the M2 metabolite. On Day 1 of dosing, the C_{max} increased in an approximately dose-proportional manner from the LD to the MD. However, from the MD to the HD, the C_{max} of icatibant was sub-proportional with an increase by ~1.8-fold instead of the expected ~ 3-fold increase. The AUCs for icatibant increased in a supra-proportional manner from LD to MD (8-10 fold increase) on Day 1. At week 13, the C_{max} and AUC increased in a dose proportional manner form the LD to the MD but C_{max} was sub-proportional from the MD to the HD (~1.7-fold increase instead of ~ 3-fold increase). AUC increased approximately dose-proportionately. However, the dosing schedule was modified throughout the study period in the HD group.

The M2 metabolite formed within ~ 2-4 hours post-icatibant administration. The M2 metabolite C_{max} and AUCs increased from the LD to the MD in an approximately dose related manner. From the MD to the HD, M2 only slightly increased in C_{max} and AUC levels for those levels that were quantifiable. This increase was sub-proportionate to dose. However, the dosing schedule was modified throughout the study period in the HD group.

In a second 13-week subcutaneous repeat dose dog study (JE049-0118), sexually immature Beagle dogs were treated with icatibant daily at 3 or 10 mg/kg or with 3.3 mg/kg TID administered twice weekly. Venous blood samples were taken on Day 1 and in week 13 from all animals to determine icatibant levels and the metabolite M_2 (b) (4) levels. On Day 1 and during Week 13 samples were taken at: predose, 0.5, 1, 2, 4, 8 and 24 h post-dose for the VC, 3 and 10 mg/kg/day Groups 1, 2 and 3, respectively. For Group 4, samples were taken at pre-dose, 1, 3, 6, 7, 12, 13 and 24 h post-dosing. Plasma samples were prepared from whole blood and analyzed using LC-MS/MS analytical methods. TK parameters determined included: C_{max}, t_{max} and AUC. The lower limit of quantitation (LLOQ) for icatibant was 1.3-3.2 ng/mL plasma and for M2 metabolite was 0.37-1.05 ng/mL plasma, respectively.

	Parameter	**Icatibant Dose (mg/kg/day)**											
		TK of Icatibant						**TK of M2 Metabolite**					
		3		**10**		**3.3 TID**		**3**		**10**		**3.3 TID**	
		M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Day 1	C_{max} (µg/mL)	5.1	4.4	14.6	16.1	-	-	0.16	0.14	0.28	NC	-	-
	T_{max} (h)	0.7	1.1	0.9	1.0	-	-	3.6	2.0	3.3	NC	-	-
	AUC_{0-t} (µg*h/mL)	13.1	11.3	44.2	41.4	-	-	0.99	0.78	1.98	NC	-	-
	AUC_{tot} (µg*h/mL)	-	-	-	-	47.1	50.8	-	-	-	-	NC	1.52
Wk 13	C_{max} (µg/mL)	5.1	4.8	21.8	20.6	-	-	0.19	0.24	0.23	0.32	-	-
	T_{max} (h)	0.6	0.8	0.8	0.9	-	-	3.3	3.2	4.0	3.2	-	-
	AUC_{0-t} (µg*h/mL)	13.9	13.6	55.2	58.2	-	-	1.4	1.5	2.3	2.7	-	-
	AUC_{tot} (µg*h/mL)	-	-	-	-	NC	NC	-	-	-	-	1.9	NC

NC- not calculated because individual values were invalid (QC rejected).
- Values not determined.

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Exposure levels of icatibant increased with increased daily dose (3 to 10 mg/kg/day) in both males and females in an approximately dose-proportional manner. There was no accumulation after repeated daily SC administration of icatibant in males or females when comparing exposure levels at Day 1 to Week 13. The t_{max} for icatibant was achieved rapidly (0.6- 1 h post-dose) after SC administration in males and females. There were no PK gender differences observed after daily administration of icatibant to males and females. After twice weekly administration of icatibant, plasma concentrations and systemic exposure levels were below the level of detection in both males and females.

The M_2 metabolite of icatibant increased in systemic exposure with increased icatibant dose in supra-proportional manner. There was no accumulation of M_2 after repeat daily SC administration of icatibant and there were no significant PK gender differences in M_2. Systemic exposure to M_2 after twice daily administration of icatibant was not detectable.

2.6.4.4 Distribution

Two whole body autoradiographic studies were completed in male Wistar rats. Each study administered a single subcutaneous dose of radiolabeled icatibant. The first study (study no. JE049-0311) dosed rats with 1 mg/kg of icatibant radiolabeled at prolyl-3,4-^{3}H and the second study (study no. JE049-0312) dosed rats with 1 mg/kg of icatibant radiolabeled at oic-6,7-^{3}H at 1 mg/kg. The distribution of the test compound was examined at 5 min and 1, 4 and 24 h post-dose. The quantitative level of radioactivity distributed in tissues was not provided. However, an overall distribution summary was reported.

Study no JE049-0311 showed that from 1 h post-dose, radioactivity occurred in the kidneys, liver, lungs and spleen. Radioactivity was present in the pancreas, testes, epididymides, wall of both the stomach and intestines, walls of large vessels, wall of the eye, adrenals and in tissues chiefly or exclusively connective structures and bone marrow. Radioactivity of relatively high concentration was found in the epiphysial cartilage and radioactivity of lower concentration was also found in the articular cartilage of the tubular bones. The highest concentrations were contained in the kidneys, urinary tract and liver. In the brain, radioactivity slightly accumulated in the anterior hypophysis and in the wall of the great cerebral vein. In the vascular system, the radioactivity concentrations in the vessel wall were higher than in the blood itself, where they remained low and at all times below those in the large organs. At 24 h post-dose, detectable radioactivity was still to be found in several organs, the liver, kidneys, bone marrow, adrenals and lymph nodes being among these.

Study no. JE049-0312 the radioactivity absorbed into the body from the site of administration was negligible after 5 mins and could only be observed in the kidneys. From 1 h post-dose, radioactivity occurred in the kidneys, liver, and in the urinary tracts and urine. The lymph nodes and epiphyseal cartilage exhibited similar or slightly lower concentrations. The concentrations in these organs and tissues were higher than those in the blood. The lungs, spleen and skin showed similar concentrations as the blood, and the testes and epididymides slightly lower concentrations. The values in the skeletal and cardiac muscle were lower than the above mentioned organs. No radioactivity was detectible in the CNS and adipose tissue. In the intestines, the radioactivity was chiefly found in the anterior segments and more in the contents than in the wall. Four hours after dosing, the concentrations were lower but the highest

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concentrations remained in the liver, lymph nodes and the kidneys. At the end of the 24 h period, radioactivity had further decreased but was still detectable in the live, lymph nodes, spleen, kidneys, contents of intestines and bone marrow and at the site of administration. The liver had the highest concentration.

In study no. JE049-0310 (non-GLP), the binding of icatibant to the serum proteins of man, monkey, dog and rat was determined. Icatibant (concentrations ranging from 0.01 to 10 µg/mL) was incubated at room temperature for 30 minutes with serum proteins and then ultrafiltration was used to determine the percentage of protein binding, The protein binding in man and rat was 44 and 49%, respectively, which was ~6 and 11% higher than the protein binding in monkey and dog (~38%).

Under GLP conditions (study no. JE049-0329), 1 mg/kg of radiolabeled icatibant (propyl-3,4-^{3}H) was intravenously administered into 4 lactating female Wistar rats. The concentrations of radioactivity in blood and milk were determined up to 48 h post-dose. As early as 0.5 h post-dose, radioactivity was detected in the milk of rats (0.32 µg equivalents/g). Blood levels at 0.5 h were 1.11 µg equivalents/g. In the milk, radioactivity concentrations increased with time during the first 7 hours post-dose with maximal levels at 1.06 µg equivalents/g. The elimination radioactivity from blood was rapid at the beginning with a $t_{1/2}$= 0.5 h. In the milk, the $t_{1/2}$= 5.3 h. As from 24 h post-dose, there was no further decrease of levels in milk. At the last time point (48 h), the mean concentration was 0.10 µg equivalents/g in blood and 0.34 µg equivalents/g in milk. The portion of radioactivity excreted with the milk was estimated to be approximately 2% of the original dose. In conclusion, icatibant is excreted in the milk of rats after IV administration.

PK parameter	1.0 mg/kg IV Icatibant	
	Blood	Milk
C_{max} (µg equivalent/g)	1.109	1.061
T_{max} (h)	0.75	7.0
$T_{1/2}$ (h)	0.5	5.3

2.6.4.5 Metabolism

Jerini conducted 4 *in vitro* human and dog hepatocyte and microsome studies examining the potential effects of icatibant on cytochrome P450 enzymes (CYP450). In the first study, study no. JE049-0330 (GLP), the inhibitory effect of icatibant (batch no. E084) on catalytic activity of cytochrome P450 isoforms was tested. Nine major human hepatic CYP isoenzymes (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4) from pooled human liver microsomes were incubated with icatibant at 2, 10 and 50 µg/mL for 30 mins. The positive controls used for each isoenzyme showed clear inhibition (>30%). Icatibant did not exert any inhibitory effect on the activity of the 9 CYP isoenzymes tested up to 50 µg/mL under the experimental conditions tested. The following table summarizes the inhibition of CYP marker reactions by icatibant (mean of triplicates).

CYP	Marker Reaction	% Inhibition by Icatibant		
		2 µg/mL	10 µg/mL	50 µg/mL
1A2	7-ethoxyresorufin O-deethylation	-17.3 ± 8.6	-46 ± 5.2	-43.1 ± 4.3
2A6	Coumarin 7-hydroxylation	-9.6 ± 10.1	-2.6 ± 8.5	-10.3 ± 11.1

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2B6	S-mephenytoin N-demethylation	1.2 ± 9.7	8.4 ± 16.9	-11.6 ± 16.0
2C8	Paclitaxel 6a-hydroxylation	-3.1 ± 15.0	4.2 ± 12.7	-0.3 ± 9.8
2C9	Diclofenac 4'-hydroxylation	11.6 ± 11.5	5.9 ± 10.4	14.1 ± 14.2
2C19	S-mephenytoin 4'-hydroxylation	-1.7 ± 10.8	12.5 ± 8.3	10.3 ± 12.6
2D6	Bufuralol –hydroxylation	3.4 ± 14.0	-13.1 ± 13.0	0.7 ± 10.8
2E1	Chlorzoxazone 6-hydroxylation	-7.6 ± 4.3	-5.5 ± 6.3	-9.0 ± 9.2
3A4	Testosterone 6 b-hydroxylation	-23.2 ± 12.5	-9.7 ± 12.6	-13.9 ± 7.4

The sponsor stated that inhibition rates ±20% were considered mainly due to statistical variation.

In the second study (study no. JE049-0317), the metabolic stability of icatibant acetate in liver microsomes and S9 of dog and humans was investigated. Icatibant (75 µg/mL) was incubated for 60 or 120 minutes at 37°C with dog liver microsomes or human liver microsomes. Additionally, icatibant (75 µg/mL) was incubated with dog or human S9 fraction for 2 hrs at 37°C with samples taken at 15, 30, 45, 60 and 120 minutes to test for metabolism. Icatibant incubated up to 2 hours with liver microsomes of dog or humans or with S9 fractions from dog or humans, did not metabolize icatibant significantly. The positive controls were rapidly metabolized in the presence of dog or human microsomes and dog or human S9 fractions. These data taken together, suggest that icatibant is metabolized independently from the CYP450 enzymes.

The metabolic stability of icatibant metabolites, M1 and M2, were evaluated in human hepatocytes in study JE049-0318 (non-GLP). M1 (6.09 µg/mL) and M2 (6.83 µg/mL) were incubated with 0.5 microsomal protein for 60 min at 37°C. Samples of M1 and M2 were taken at 0, 15, 30, 45 and 60 minutes and analyzed for metabolism. Under these study conditions, neither M1 nor M2 were metabolized by human microsomal proteins.

In study no. JE049-0331 (GLP), icatibant was incubated with freshly prepared human hepatocytes to test for icatibant's potential to induce CYP450 1A2 and 3A4 enzyme activity. Icatibant at 2, 10 and 50 µg/mL concentrations was incubated with hepatocytes from 3 different donors for 1A2 and 7 different donors for 3A4. A known inducer for each 1A2 (omeprazole) and 3A4 (rifampicine) was incubated with respective donors to serve as a positive control. Icatibant did not induce enzymatic activity for either 1A2 or 3A4 in freshly prepared human hepatocytes.

2.6.4.6 Excretion

The sponsor conducted 6 excretion studies administering radiolabeled icatibant subcutaneously and collecting urine and feces in mice, rats and dogs. Male mice were administered 1 mg/kg of radiolabeled icatibant (prolyl-3,4-^{3}H) formulated in 0.9% NaCl in 3 GLP studies (study no. JE049-0321, JE049-0326, and JE049-0327). A mean total of ~ 91.7% of radioactivity administered was excreted via urine and only 8.0% was excreted into feces. Renal excretion was complete by 24 h post-dose. The renal elimination was bi-phasic with a $t_{1/2}$ at 4.5h and a second $t_{1/2}$ at 15.2 h. Excretion in the feces was ~8.8% of the injected dose. Radioactivity in mice was distributed to the liver, kidneys, and urinary bladder with the highest concentrations. The recovery rate was ~97.1% of injected dose.
The chromatographic analysis of urine showed ~64.3% of unchanged icatibant and 36% of M1 metabolite in the urine from 0-8 h urine collection. At 8-24 h urine collection, the proportion of M1 increased slight to 48.6%.

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Two excretion studies were conducted in rats. In study no. JE049-0323 (non-GLP), 3 male Wistar rats were subcutaneously administered a single 12 µg/kg radiolabeled icatibant. Approximately 61% of radioactivity was collected in urine and less than 3% was detected in feces. After chromatographic analysis, it was determined that less than 0.5% of parent product was recovered from urine. One metabolite (M1) was recovered at 57% in urine.

In the second rat study (study no. JE049-0306, GLP), 6 male Wistar rats were administered a single 1 mg/kg subcutaneous dose of radiolabeled icatibant (Oic-6,7-^{3}H). Five min post-administration, measurable concentrations of radioactivity (mean 0.048 µg equivalents/gram) were already present in animal blood. The maximum concentrations were reached after 1 h in all animals and were between 0.425 and 0.499 µg equivalents/g. The elimination of the radioactivity from blood was a 2-phase process. A rapid first phase occurred with individually different half-lives of between 0.38 and 0.89 h and a terminal phase with half-lives between 5.5 and 20.6 h. The greater part of the excretion occurred via the kidneys (mean 68.5%). In a dried sample, the corresponding portion was 67.5%. The elimination via feces was appreciably less with a mean values being 30.1%. The majority of radioactivity excreted via this route was eliminated 8-24 h post-administration. The mean balance of radioactivity recovered was 100.6 % (samples measured directly) and 99.6% (dried samples).

PK parameter	**1 mg/kg SC Icatibant**
C_{max} (µg equivalent/g)	0.4601
T_{max} (h)	1
$T_{1/2}$ Phase 1 (h)	0.63
Time range Phase 1 (h)	1-4
$T_{1/2}$ Phase 2 (h)	13.93
Time range phase 2 (h)	8-48

In study no JE049-0309 (GLP), 2 male Beagles were administered a single 0.2 mg/kg subcutaneous dose of radiolabeled icatibant (labels on Oic-6,7-^{3}H). Five min post-administration, measurable concentrations of radioactivity were already present in blood and plasma. These amounted to mean 0.03 or 0.06 µg equivalents/g in blood and plasma, respectively. The maximum concentration was reached after 0.75 h and was 0.20 and 0.33 µg equivalents/g in blood and plasma, respectively. The elimination of the radioactivity from blood was a monophasic with a half-life of 1.6 h. The last measurable concentrations in the blood were detected 8 h post-dose. The mean radioactivity excreted via the kidneys was 63% of the total radioactivity administered. This renal excretion mainly occurred within 24 h post-dose. The elimination via feces was appreciably less with a mean values being 33.4%. The majority of radioactivity excreted this route was eliminated 24-48 h post-administration. The mean balance of radioactivity recovered was 97 % (samples measured directly) and 96.4% (dried samples).

PK parameter	**0.2 mg/kg SC Icatibant**	
	Blood	**Plasma**
C_{max} (µg equivalent/g)	0.20	0.33
T_{max} (h)	0.75	0.75
$T_{1/2}$ Phase 1 (h)	1.6	1.4
Time range Phase 1 (h)	1-8	0.5-8
$T_{1/2}$ Phase 2 (h)	-	91.5
Time range phase 2 (h)	-	24-72

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Based on excretion studies conducted in mice, rats and dogs, the primary route of elimination of total radioactivity of radiolabeled icatibant administered subcutaneously is via the kidneys with a small amount of radioactivity excreted into the feces. After subcutaneous administration, radiolabeled icatibant is excreted rapidly with the majority recovered in the urine within 24 hours.

2.6.4.7 Pharmacokinetic drug interactions

No new data were submitted.

2.6.4.8 Other Pharmacokinetic Studies

No new data were submitted.

2.6.4.9 Discussion and Conclusions

- Icatibant is rapidly metabolized to M1 and M2: the major metabolite in man is M1 (>90%). Icatibant metabolism is through hydrolysis of the peptide backbone believed to result from peptidase activity. M1 is formed in rat (~70%), dog (~60%) and mouse (~50%). The M2 metabolite [redacted] (b) (4) appears from 0.2 to 3 hours post-dose in rats and dogs. Neither icatibant nor the M1 or M2 metabolites are metabolized by human hepatocytes or microsomes. Additionally, icatibant is not an inducer of CYP450 isozymes.
- Neither icatibant, nor M2 accumulated after repeat dosing in males and females up to 13-weeks dosing. There were no gender differences observed in rats or dogs in the systemic exposures of males and females.
- The t_{max} was achieved from 0.5 to 3.0 h post-dose in rats and dogs.
- In rats and dogs, increases in icatibant doses from LD to MD doses (0.1 to 3 mg/kg in rats and 10 to 30 mg/kg/day in dogs) produced approximately doses proportionate increases in systemic exposures. Increases in icatibant doses from MDs to HDs resulted in super-proportionate increases in systemic exposures in both rats (increased ~128-fold instead of the expected ~50-fold) and approximately dose proportionate in dogs. Using an alternate dosing schedule in dogs (1 or 3.3 mg/kg TID of icatibant twice weekly), AUC levels of icatibant and M2 increased in an approximate dose proportionate increase.
- Icatibant (and/or its metabolites) is distributed into the kidneys, liver, urinary bladder, lungs and spleen 1 hour and it still detectable in the liver, kidneys, spleen, bone marrow, adrenals and injection sites at 24-hours post SC dose in rats.
- Serum protein binding of icatibant was low with 44 and 49% in man and rat, respectively, and 39% in monkey and dogs. Icatibant is excreted in the milk of rats after IV administration.
- Based on excretion studies, the primary route of elimination after SC administration of icatibant is via the kidneys in mice (~97.1%), rats (~61%) and dogs (~63%) with lesser amounts excreted in the feces (8.0% to 33%).

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2.6.4.10 Tables and figures to include comparative TK summary

Not applicable because no new data were submitted.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Not applicable because no new data were submitted.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: Under IND (b) (4) acute toxicology studies conducted in the mouse, rat and dog were reviewed (Dr. Choi's review). The highest non-lethal IV dose was 30.7 mg/kg and the minimum lethal dose (MLD) was 48.3 mg/kg for mice. For rats, the highest non-lethal IV dose was 12.3 mg/kg and the MLD was 19.2 mg/kg. After IV dosing, rapid respiration, twitching and convulsions were observed prior to death. In an acute IV toxicity study, male dogs (n=2/group) were administered 0.4, 2 and 10 mg/kg icatibant. No mortality was observed in this study. However, numerous clinical signs, many suggestive of massive histamine release, were observed in the MD and HD groups. In a separate study, male dogs (n= 3/group) were administered single IV doses (infused over 5 h period) of 15, 30 and 60 mg/kg. An increase in serum CPK levels were above historical background (153 mU/mL) was noted in 1 MD animal (406 mU/mL) and two HD dogs (843 and389 mU/mL, respectively. The NOAEL in this study was 15 mg/kg. In acute SC toxicity studies, the highest non-lethal dose was 614 mg/kg and the MLD was 767 mg/kg for mice. In rats, no mortality was observed after a single SC injection of 1227 mg/kg icatibant. After SC injection, injection sites showed bluish discoloration that progressed to necrosis.

During icatibant's development, intranasal (IN), inhalation (IH), IV and SC routes of administration have been explored. Under INDs (b) (4), the main repeat dose toxicology studies investigated the intranasal (IN), inhalation (IH) and IV routes of administration with a few studies conducted using the SC route. Due to low bioavailability, no toxicity was observed in most IN toxicity studies. However, reduced testicular weights and spermiogenesis in the testes were observed in a single 1-month IN toxicity study carried out in the rat at 125, 1250 or 12500 µg/kg. The NOAEL was considered 125 µg/kg based on these testicular findings.

In a comparative IV/SC/IN 1-month toxicity study, male rats were either intravenously (10 mg/kg/day), subcutaneously (10 mg/kg/day) or intranasally (2.5 mg/animal/day) administered icatibant. Renal tubular atrophy was observed in 7/10 rats and renal tubular necrosis in 4/10 rats treated via the IV route. Impairment in sperm maturation was observed in 8/10 rats and oligospermia in the epididymides in 7/10 rats after IV administration. Tubular atrophy (4/10) and tubular necrosis (1/10) were also observed in SC treated rats. Despite similar exposure levels, oligospermia in the epididymides in 1/10 animals was the only male reproduction effect observed in SC treated animals. No control groups were included in this study. TK analysis showed the exposure (AUC_{0-4h}) to be similar between IV (20.33 µg/mL) and SC (17.3 µg*h/mL) treated animals. No adverse effects were reported in the intranasal group.

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In a 1-month toxicity study, rats were intravenously administered 0.1, 1 and 10 mg/kg/day of icatibant. Renal toxicity was observed in 10/10 males and 5/10 females with areas of epithelial necrosis, areas of repair and foci of tubular atrophy at the 10 mg/kg/day dose. Irreversible tubular atrophy was present in 5/5 males and 1/5 females after a 28-day recovery period. The NOAEL was 1 mg/kg/day. In a 1-month study, dogs were intravenously administered 0.1, 0.25 and 0.8 mg/kg/day. All HD males 3/3 had swelling of flews that was attributed to histamine release. The major part of the glandular portions in the basal mucosa was missing from the stomach of 1 HD male that was reported as a relatively rare change. The NOAEL was 0.25 mg/kg/day.

Based on the previous reviews (b) (4) of the 1-month repeat dose toxicity studies in rats and dogs, the target organs toxicity in the rats were the male reproductive organs (decreased spermatogenesis and decreased weights of testes, epididymis, prostate, and/or seminal vesicles), kidneys (uneven color, increased weight, epithelial necrosis of proximal tubules, foci of tubular atrophy, increased blood urea, uric acid, creatinine, hemoglobin and epithelial cells in urine), stomach (taut and enlarged) and liver (increased wt). In the dogs, the target organs were swelling of flews (histamine release reaction) and the stomach (chronic gastritis).

In support of the proposed SC route of administration for this NDA, the sponsor conducted two 14-day SC toxicity studies in the rat, one 13-week SC toxicity study in the rat (submitted as a combined 13-week toxicity and fertility and early embryonic development study), a SC dose-ranging study in the dog, a 4-week SC toxicity study in the dog (twice weekly dosing), and two 13-week SC toxicity studies in the dog (1 with daily SC dosing and 1 with daily and TID administered twice weekly SC dosing). In the first 14-week SC toxicity study, male and female rats were administered 0 (vehicle), 1, 10 or 50 mg/kg/day of icatibant. Only 5 rats/sex/group were dosed, which is considered an inadequately powered rat study to identify potential drug induced toxicities. Clinical signs of toxicity were limited to injection site irritation at the high-dose (50 mg/kg/day). Decreases in prostate weights in the MD (-33%) and HD (-39), and testes weights relative to brain weights in MD (-29%) and HD (-30%) were observed in males and increases in thymic weights in the HD group (30-38%) were observed in females. Histopathological findings included: epididymides (oligospermia and aspermia) and testes (hypospermia) were observed in all icatibant treated groups and injection site irritation was observed in the HD group. No NOAEL could be defined by the reviewer due to male reproductive effects of icatibant at all doses.

A second 14-day SC rat study (this study was followed by an 8-week recovery period), was conducted only in males (n=10 rats/group) to explore the reproductive organ effects in older animals. Rats were administered 0 (vehicle), 0.1, 1 or 50 mg/kg/day of icatibant SC and 1 group of untreated juvenile male rats were maintained as a second control group. A slight decrease in creatinine levels (-18%) in the HD group compared to the VC group was observed. Males in the HD had decreased testicular and prostate weights compared to the VC group. The sponsor did not evaluate a full histopathological battery of tissues in this study, with the rationale that no systemic toxicity other than reproductive organ effects was observed in the first 14-day SC study in males or females. Therefore, the sponsor selected only the male reproductive organs for microscopic examination. Histopathological findings included injection site irritation in the HD group (observed macroscopically and microscopically), oligospermia and cellular debris in the

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lumen of the epididymis and hypospermia in the testes. No NOAEL could be determined by the reviewer based on the incomplete histopathological battery in this study. For the male reproductive organ findings alone, the NOAEL is considered 1 mg/kg by the reviewer; this NOAEL was associated with an AUC of 22 µg*h/Ml.

In the 13-week SC toxicity study, male and female rats were administered 0 (vehicle), 1, 3 and 10 mg/kg/day of icatibant. In addition to extending the exposure period of rats to icatibant, this study was used to bridge the change in manufacturer of the drug substance. There were slight decreases in thyroid absolute weights (-6 to -18%) and weights relative to BWs (-3 to -16%) in all icatibant treated males but this was not associated with any macroscopic or microscopic findings. There were no microscopic findings in this study with the exception of injection site irritation. The NOAEL was 10 mg/kg/day based on the absence of systemic toxicity; this NOAEL was associated with an $AUC_{0.5\text{-}4\,h}$ of 16.55 µg*h/mL. This study was used to bridge the change in manufacturer for the drug substance. Using this new batch of the drug substance, a NOAEL was established in rats compared to no NOAEL identified in the 14-day toxicity studies conducted with the old drug substance manufacturer. Possible explanations include dosing in the 13-week study was only up to 10 mg/kg while the 14-day studies dosed up to 50 mg/kg, adaptive responses at lower doses from 14-days to 13 weeks, and the possible presence of impurities in the earlier batches.

A 13-week SC toxicity study was conducted in dogs that dosed animals once daily with 0 (vehicle), 10, 30 and 100 mg/kg/day. The top dose was selected based on a dose-range finding study conducted in dogs that observed minimal toxicity at 100 mg/kg/day. In the 13-week study, clinical signs of toxicity included erythema of the face, injection site irritation and several incidences of gastrointestinal disturbances (diarrhea and/or vomiting) at all doses. Due to abnormal vocalization in the HD group at day 12, the dose was decreased to 2 x 30 mg/kg/day. Decreases in leukocyte counts in HD males and increases in platelet counts in HD females were observed. Triglycerides and ALP were increased in HD males and decreased CK, creatinine, LDH and ASAT levels in HD males were observed compared to the vehicle control animals. Male and female reproductive organ weights (absolute and relative to BWs) were decreased, adrenal weights relative to BWs were increased in males and females, liver weights relative to BW were increased in MD and HD males, and thymic weights (absolute and relative to BWs) were decreased in both males and females compared to respective vehicle control treated animals. Macroscopic findings were observed at injection sites (hematomas and edema), the male (testes, epididymides and prostate) and female (ovaries and uterus) reproductive organs (organs all immature at all doses), thymus, liver and adrenal gland. Microscopic changes in the male (testes, epididymides, and prostate) and female (mammary gland, ovaries, uterus and vagina) reproductive organs, at the injection site (mainly HD groups but sporadic in LD and MD groups), in the adrenal gland (vacuolated cortical cell in HD males and females), liver (vacuolated hepatocyte in 1/4 males) and thymus (all doses) were observed. Based on the arrest of sexual maturity in all icatibant treated males and females, lymphoid depletion in the thymus and on injection site irritation, no NOAEL can be determined for this study. Exposure levels (AUC) in dogs ranged from 35 µg*h/mL at the low dose to 230 µg*h/mL at the high dose.

Based on the findings observed in dogs after daily administration of icatibant, the sponsor proposed an alternative dosing schedule to minimize the histamine release reactions (erythema of

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face) and to reduce systemic toxicity. In the 4-week SC toxicity study, Beagle dog were administered 0 (vehicle), 1 and 3.3 mg/kg TID as twice weekly dosing. This resulted in 3 and 10 mg/kg of icatibant administered twice weekly. Erythema was observed in low incidence in males and females at each dose. Gross and microscopic findings were limited to injection site irritation (hematomas) which did not follow a dose response. Testosterone levels were significantly decreased in males treated with icatibant at 1 or 3.3 mg/kg TID twice weekly but these levels rebounded between dosing intervals. A NOAEL of 3.3 T.I.D. (10 mg/kg) administered twice weekly (associated with an AUC of 44 µg*h/mL) was determined by the reviewer based on no microscopic drug-related findings.

The sponsor conducted a second 13-week SC toxicity study in dogs, dosing dogs either daily with 0 (vehicle), 3 or 10 mg/kg/day of icatibant or administered icatibant at 3.3 mg/kg TID twice weekly (10 mg/kg twice weekly). Observed clinical signs included increased erythema and edema at multiple sites that increased with increased dose. With cessation of treatment erythema abated. After daily administration of icatibant at 3 and 10 mg/kg/day, the thymus (lymphoid depletion) and the male (immature testes, epididymides and prostate) and female (immature ovaries, oviducts, uterus and vagina) reproductive tissues showed toxicity as reflected by reduction in these organ weights relative to body weights, macroscopic findings of reduced size and microscopic findings of reduced size. After the 13-week recovery period, 1/2 males showed the testes and prostate as immature where 1/2 males had recovered and there were no findings in female reproductive organs suggesting these adverse effects were reversible. When males were treated twice weekly with icatibant, no affects were observed in the male reproductive organs but females treated with SC administration of icatibant twice weekly showed delayed or impaired maturation of the reproductive organs. Lymphoid depletion in the thymus was observed in both males and females after twice weekly dosing. Hormonal analysis showed that icatibant induced a decrease of testosterone, LF and FSH in male dogs, which secondarily induced sexual immaturity. No NOAEL was determined in this study based on the adverse effects on male and female reproductive organs and lymphoid depletion observed in the thymus. However, twice weekly administration of icatibant resulted in less severe toxicity in males than with daily dosing.

At 13-weeks daily SC dosing of rats showed no toxicity after administration of a newly manufactured batch of icatibant from a different site manufacturing site. However, after IV and SC administration of icatibant for 1-month or 14-days, respectively, the target organs of toxicity were the male reproductive organs, kidneys, stomach and the injection sites (using old icatibant manufacturer). Dogs administered icatibant SC daily for 13 weeks showed erythema of the face (histamine reaction), male and female reproductive organ immaturity, thymus (lymphoid depletion), adrenal (cortical cellular vacuolation), liver (vacuolated hepatocyte) and injection site irritation. After 1-month IV administration, erythema and chronic gastritis were observed in dogs. When altering the dosing schedule in dogs from daily administration to twice weekly dosing, the effects on the male reproductive organs were reduced. However, females showed immature reproductive organs.

Genetic toxicology: Throughout the development of icatibant, the sponsor has conducted multiple genetic toxicology studies for icatibant and unnatural amino acids. The preliminary studies were evaluated under IND (b) (4). An Ames test (tested only 2 microbial strains) and an

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in vivo micronucleus test conducted in mice were conducted. The results of the Ames test with TA98 and TA100 up to 4000 µg/plate were negative with and without metabolic activation and the results of the micronucleus test were also negative. Under IND (b) (4), Dr. Choi reviewed five genetic toxicology studies: 4 Ames assays (2 with icatibant and 2 with the unnatural amino acids (b) (4)) and an *in vitro* cytogenetic test of bone marrow cells from Chinese hamsters. The results of all Ames tests with *Salmonella typhimurium* strains and an *Escherichia coli* strain, WP2uvrA, were negative with and without metabolic activation up to 5,000 µg/mL for all compounds. Icatibant did not produce significant increases in chromosomal aberrations of bone marrow cells in the *in vitro* cytogenetic test.

The sponsor submitted three new genetic toxicology studies in this NDA. These studies were: a bacterial reverse mutation assay (a complete bacterial strain battery), an *in vitro* mammalian chromosome aberration test, and an *in vivo* bone marrow micronucleus assay in Sprague-Dawley rats using the icatibant. The bacterial reverse mutation assay and the *in vivo* micronucleus study in rats were studies used to bridge the change in manufacturer for the drug substance. Icatibant did not induced genetic toxicity in these three studies under the conditions assayed.

Carcinogenicity: The sponsor has not submitted studies to evaluate icatibant's carcinogenic potential. At the pre-NDA meeting held on January 24, 2007 between the Division of Pulmonary and Allergy Products and Jerini, Jerini was informed of the need for 2 carcinogenicity studies to address the carcinogenic potential of icatibant. In an information request sent to Jerini following the Mid-Cycle Review meeting on January 23, 2008. Jerini was asked to provide a status update of their carcinogenicity studies. In Jerini's January 28, 2008 response to the request for information, Jerini indicated that the carcinogenicity studies would be initiated April 8, 2008 and July 7, 2008 for mice and rats, respectively.

Reproductive toxicology: Under IND (b) (4), a 5-week fertility study in male mice was conducted. Mice were dosed with 0 (vehicle-physiological saline) 16.2 and 80.8 mg/kg/day SC for 5 weeks then mated with untreated F mice for 1 week. There were no effects on copulation rate in icatibant treated animals compared to the vehicle control group. However, the conception rate was reduced in the 80.8 mg/kg/day group compared to the vehicle control group by 14%. The results showed no significant differences between control and treated groups for general health, body weight gain, mating ability, organ weight, number of spermatozoa or motility in left or right epididymis or female reproductive parameters. Therefore, 5 weeks SC treatment of male mice did not affect male fertility. The male fertility study in mice was carried out, but this study is known to be one of the most insensitive tests for spermatogenesis or testicular activity. Also, it has been known that fertility of rodents is not affected unless testicular activity or sperm counts are reduced by 90-95%. Additionally, this study did not follow current Segment 1 study guidelines.

Also under IND (b) (4), two teratogenic studies were reviewed (1 in rats and 1 in rabbits), which were preceded by dose-ranging studies. In the dose-ranging study conducted in rats, 3 pregnant rats/group were treated with 25, 50 and 100 mg/kg/day (no vehicle control was used) of icatibant SC from Days 7 through 18 of pregnancy. On Day 21 of pregnancy, pregnant rats were sacrificed and their uteri examined. At 25 mg/kg/day slight swelling at the injection site in 2/3 dams (Day 3) was observed. No additional toxicities were observed in dams or in their concepti. The third

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25 mg/kg/day female died after the 10th injection on Day 16 of pregnancy. This female had blue discoloration of skin, panting, reduced activity, abdominal position and then death. The cause of death was unclear. Dosing for the MD group (50 mg/kg/day) stopped after the 7th injection due to local intolerance but the dams were kept until Day 21 of pregnancy. From the 4th injection, swelling, hardening and scabbing occurred at the injection sties. Food consumption and body weight of dams or concepti were not affected. Pregnant rats in the HD group (100 mg/kg/day) had to be killed after the 4th injection on Day 10 of pregnancy due to local intolerance: marked swelling and reddening. One dam also had scabbing and another was observed with a "weeping skin wound". Concepti from this group were considered unremarkable with no teratogenicity observed.

In the definitive study (reviewed under IND (b) (4) by Dr. Choi), the effects of icatibant SC administration on morphological and post-natal development including fertility in Wistar rats was examined. Female rats (30 pregnant rats/group) were treated with 0 (vehicle control- 0.9% NaCl), 0.25, 2.5 and 25 mg/kg/group of icatibant SC from Days 7 through 18 of pregnancy. Twenty females/group were sacrificed and delivered by Cesarean-section on Day 21 of pregnancy and the fetuses were examined for morphological changes (skeletal examination on 50% and body cross section on 50%). The remaining 10 pregnant females/group were allowed to litter and raise their young and the fetuses were examined for viability, physical development and functional behavioral changes. One M and 1 F (10 pairs) from each litter of F_1 generation were raised to maturity for mating and their fertility was examined. After the birth of F_2 generation, the parent and pups were sacrificed. SC injection of icatibant did not produce any significant effects on dams for the following: general physical condition, food consumption, body weight gain, organ weight change (liver, spleen and kidney), pregnancy rate, duration of pregnancy in F_0 or F_1 generation, live or dead pups or sex distribution. There were no significant major external of internal anomalies reported in pups for F_1 or F_2 generations. However, 1 or 2 fetuses in the 0.25 and 2.5 mg/kg group had multiple minor skeletal malformations or variations. Post-natal development of treated fetuses and fertility of F_1 generation were comparable to the control group. Besides a kinky tail, especially 1 fetus in the 25 mg/kg/group, no major terata were produced in the study although 1 dam in the 25 mg/kg/day group had empty implantation sites in the uterus, and fetuses in the 25 mg/kg/day group had slightly greater numbers with less ossification of skeletons and variations of liver and kidneys than the control group (4 fetuses had lobus dexter of liver and 3 fetuses had distended pelvises in the kidney, while 1 fetus in the control group had the same findings). Therefore, icatibant was not teratogenic in rats under the conditions studied.

In a preliminary dose-ranging in rabbits (b) (4) 2 pregnant rabbits/group were treated daily with SC injections of 10, 25 or 50 mg/kg/day of icatibant from Days 6 through 18 (no vehicle control was used). Rabbits were sacrificed on Day 29 of pregnancy and their uteri were examined. The reviewer stated that impairment of pregnancy was observed in all groups, although no impairment of body weights was noted. One dam in the 10 mg/kg group had only empty implantation sites in the uterus, indicating a clinically unnoticed abortion, but the second dam had 8 normally developed live fetuses. All dams in the 25 and 50 mg/kg/ groups had empty implantation sites in the uterus. Based on this study, doses of 0.1, 1 and 10 mg/kg/day were selected.

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In the definitive rabbits study, Himalayan rabbits (15 pregnant females/group) were treated with 0 (vehicle control- 0.9% NaCl), 0.1, 1.0 and 10 mg/kg/day of icatibant, SC once daily from Days 7 through 18 of pregnancy. On Day 29 of pregnancy, surviving dams were sacrificed and fetuses delivered by C section. Fetuses were kept in an incubator for 24 h to determine survival rate and then examined morphologically for developmental disorders. Due to the occurrence of abortions, premature births, increased light-grey depression on the surface of kidneys in the icatibant treated groups, this study was repeated using 0.1 and 1.0 mg/kg icatibant SC and a 0.9% Na Cl group. Rabbits used for this section of the study were New Zealand White rabbits.

In the main study, 4 unexpected abortions were produced in 2 dams each in all 3 treated groups and a premature birth in 0.1 mg/kg group, while none of these occurred in the control group. No other toxic effects were produced in the icatibant treated dams. At autopsy, light-grey depression was found on the surface of kidneys in 5-7 dams each in all 3 icatibant treated groups but only 1 dam in the control group. To determine if these effects were drug induced, the study was repeated but with 2 lower doses only and the vehicle group. The abortions or premature births were not reproduced in this study, and incidence of light grey colored kidneys in the treated groups was not increased, but it was also reported in 1 dam each in treated groups only. The depression on the kidney was due to chronic interstitial nephritis, and it was attributed to the strain of rabbits by the sponsor.

Morphological examination of fetuses showed no increase in incidence of major malformations in the treated groups. In fact, the control fetuses had greater multiple malformations or variation of skeletons including limbs. Minor anomalies or variations of liver or kidneys were reported in some fetuses, but they were reported as within the normal ranges. Among the dead fetuses examined (6 in control, 4 in 1 mg/kg and 15 in the 10 mg/kg groups), 10 mg/kg had significantly more stunted (8/15) or retarded fetuses (7/15) over the control group (2/6 and 1/6, respectively). Icatibant was not teratogenic under the test conditions. All 3 doses of icatibant did not have significant toxic effects on the dams. However, 10 mg/kg was toxic to the embryo reducing the litter sizes as a result of increased intrauterine embryofetal death rate. Icatibant treatment resulted in dose-related decreases for total implantations and total number of live fetuses as well as dose-related increases for percent of pre-implantation loss when compared to the control values.

Under the conditions tested in the rats and rabbits, icatibant did not induce major terata. However, icatibant has a potential to produce abortions/premature births, intrauterine death or resorptions.

Included in this NDA submission, were 2 reproductive toxicology studies to complete the reproductive toxicology battery. The sponsor combined the 13-week repeat dose toxicity study and the effects on fertility and early embryonic development to implantation study in rats. Male Sprague-Dawley rats were given 0 (vehicle control- 0.9% NaCl), 1, 3 and 10 mg/kg/day of icatibant SC doses for 10 weeks prior to mating, during and after mating. Female rats were given 0 (vehicle control- 0.9% NaCl), 1, 3 and 10 mg/kg/day of icatibant SC doses daily for 2 weeks prior to mating, during mating and until 7 days post-coitum. HD treated females (10 mg/kg/day) had an increase in pre-implantation loss compared to vehicle treated females. Untreated females paired with HD treated males showed no increase in pre-implantation loss compared to untreated females paired with vehicle control treated males, indicating that icatibant treatment of females and not males resulted in the pre-implantation loss. Icatibant had no effects on male sperm count,

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motility, or morphology. Based on no toxicity observed in male seminology and no effects on mating or fertility indices, the NOAEL for male fertility was 10 mg/kg/day. Based on an increase of pre-implantation loss observed in HD treated females, the NOAEL for female fertility was 3 mg/kg/day. These conclusions were in agreement with the sponsor's conclusions. This fertility and early embryonic study was conducted using current standard guidelines and fulfills the requirement for completion of a Segment 1 study.

In the second reproductive toxicity study submitted in this NDA, the effects of daily SC administration of icatibant on pre- and post-natal development in rats were evaluated. Sprague-Dawley rats were administered 0 (vehicle), 1, 3 and 10 mg/kg/day to mated females from Day 6 post-coitum until Day 21 post-partum. The effects of icatibant on the F_0 dams (maternal toxicity) and F_1 generation (systemic and developmental toxicity) were evaluated. Icatibant treatment delayed parturition at 1, 3 and 10 mg/kg/day, resulting in F_0 deaths (found dead or early sacrifice) in the 3 and 10 mg/kg/day groups. Clinical signs of toxicity (pallor of eyes and extremities, round back, piloerection and reddish discharge in vagina) were observed in 1 MD and in multiple HD F_0 females. After SC administration, icatibant and the M2 metabolite is found in rat milk. Based on the observed maternal toxicities including clinical signs, prolonged gestation periods for all icatibant dosage groups, F_0 spontaneous deaths and early sacrifice of icatibant treated dams, litter deaths, and necropsy findings in the MD and the HD groups, the maternal toxicity NOAEL could not be defined. The F_1 generation showed an increase in the number of pup deaths and cannabalism in the MD and HD compared to the VC groups. Therefore, the NOAEL for systemic toxicity in the F_1 generation was determined to be 1 mg/kg/day. The F_1 generation showed a slight decrease in air righting and hair growth compared to the VC control group. Therefore, the NOAEL for development of the F_1 generation was considered 3 mg/kg/day.

The sponsor has adequately completed the reproductive toxicity battery for icatibant. The relevant data indicate that the Pregnancy category for this drug should be C based on the the presence of increased pre-implantation loss, increased spontaneous abortions and increased pup deaths.

Special toxicology: Several special toxicology studies were previously reviewed under IND (b) (4) by Dr. Choi. Due to changes observed in male reproductive organs in IV and SC repeat dose rat toxicology studies, the sponsor conducted a 28-day repeat dose SC study to evaluate the effects of icatibant treatment on the pituitary-testicular axis. Male rats dosed with 1.25 mg/kg/day by SC injections or infusions for 28-days showed no significant effects on male reproductive organs or hormone levels during the dosing phase. The absence of male reproductive organs was likely due to the low doses of icatibant used. Interestingly, an unexpected activation significantly increased release and synthesis of testosterone in the serum and testes, after the 8-week recovery period was observed. Also noted after the recovery period were increased organ weights of testes, prostate, seminal vesicles and adrenal glands, especially after SC infusion. The increased testosterone in serum and testes and increased weights of testes, prostate and seminal vesicles after the recovery period suggested that these organs may have been suppressed during the 4-weeks of treatment period at a sub-threshold level and most likely due to low systemic drug exposure.

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Dr. Choi reviewed a local tolerance study in rabbits that were injected intra-articularly with single 10 or 60 µg/mL doses of icatibant in saline into the right knee joints of rabbits. Icatibant was well tolerated. In two local irritation studies (primary skin irritation study of rabbit skin and mucosal tolerance study in rabbit eye) icatibant was considered a non-irritant at 0.5 mg/mL icatibant solution. Antigenic potential of icatibant was studied in guinea pigs by homologous 4-hour PCA reactions and maximization tests. In the antigenicity study, when guinea pigs were sensitized with icatibant alone in intranasal or SC route, no anti-icatibant antibodies were produced. Thus, PCA test were negative. But, when guinea pigs were sensitized subcutaneously with icatibant plus Freund's adjuvant, antibody formation was observed when challenged with icatibant-BSA conjugated in one of 5 sera samples, with a titer of 23-25. Therefore, icatibant was a sensitizer under the conditions tested. In another study, a guinea pig maximization test, 8/10 icatibant treated guinea pigs plus Freund's adjuvant had slight erythema, while the control group showed no reaction. Therefore, icatibant was a sensitizer when administered with Freund's adjuvant in 2 studies.

Submitted to this NDA were 2 local toxicity studies conducted in rabbits using single dose SC administration in one study and intrarterial/intravenous/paravenous administrations in the second study. Icatibant administered via the SC route up to 40 mg in acetate buffer, pH 5.5 was comparable to the vehicle control for local irritation. In the second study, administration of icatibant up to 0.5 mg intra-arterially and intravenously and 0.1 mg paravenously resulted in little injection site irritation.

Two single-dose studies administering two unnatural, synthetic peptides that are both found in the icatibant drug substance, either (b) (4) at 1000 mg/kg, SC were conducted by the sponsor. Neither study was adequately designed with the absence of control groups. No animals died after (b) (4) administration. However, after (b) (4) (2/3) females died within 24-h post-dose.

An *in vitro* hemolysis test of icatibant showed that 0.1% of icatibant did not induce hemolysis of human whole blood after incubation for 30 min where incubation with the positive control (distilled water for injection) did induce hemolysis.

In an assessment of antibody responses in rat, dog and monkey, the sponsor concluded that hyperimmunization attempts, even with icatibant-protein conjugates in combination with adjuvant, did not elicit a sustained antibody response against icatibant. Based on the lack of information provided by the sponsor (icatibant lot and purity and detailed methods of study), no concrete conclusions can be drawn from this study report. However, based on the summary data provided by the sponsor, icatibant-thyroglobulin conjugate did not elicit a strong anti-icatibant antibody response.

For safety qualification of the (b) (4) impurity, the sponsor conducted 1 acute IV toxicity study, a bacterial reverse mutation study and a chromosome aberration study. (b) (4) was negative in the Ames test but positive in the chromosomal aberration test for inducing genotoxicity. Although positive for genotoxicity in the chromosome aberration test, (b) (4) (b) (4) is a member of the peptide drug class which is considered to have low genotoxic risk. The acute IV toxicity study dosed rats up to 100 mg/kg. No toxicity was observed in rats. However,

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the duration of this study is not considered acceptable to qualify this impurity for safety. Therefore, (b) (4) has not been adequately qualified for safety. A chemistry consult was completed to address issues related to drug substance and product impurities and comments to the sponsor will be addressed by the ONDQA reviewer, Dr. Eugenia Nashed.

2.6.6.2 Single-dose toxicity

No new data were submitted.

2.6.6.3 Repeat-dose toxicity

Study title: HOE 140: 14-day subcutaneous toxicity study in rats

Key study findings:

- Only 5 rats/sex/group were dosed in the main study. This study is considered inadequately powered to identify potential toxicities induced by icatibant.
- Clinical signs of toxicity were limited to injection site irritation.
- ↓ prostate wts in MD (-33%) and HD (-39) males; ↓ testes wts relative to brain weights in MD (-29%) and HD (-30%) groups, and ↑ female thymic wts in the HD group (30-38%)
- Gross findings included small male reproductive organs (testes, epididymides and prostate) and injection site irritation.
- Histopathological findings included: epididymides (oligospermia and aspermia), testes (hypospermia) were observed in all icatibant treat groups and injection site irritation was observed in the HD group.
- No NOAEL could be defined by the reviewer due to male reproductive effects of icatibant at all doses. Local effects were observed at the injection site at the HD. The sponsor called the NOAEL 10 mg/kg.

Study no.: JE049-0127
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4) (b) (4)
Date of study initiation: February 3, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, lot # D081, and 96.2% pure

Methods

Doses: 0 (vehicle), 1, 10 or 50 mg/kg/day
Species/strain: Sprague Dawley rats
Number/sex/group or time point (main study): 5/sex/group
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in water for injection, administered in 1.0 mL/kg and infusion rate was not applicable.
Satellite groups used for toxicokinetics or recovery: 3 rats/sex/group were used for TK analysis.
Age: 6-7 weeks old

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Weight: males: 166-197 g and females: 136-168 g
Sampling times: See below
Unique study design or methodology (if any): None.

Observations, Times and Results

Mortality: All animals were checked for mortality at least twice a day.
There were no deaths prior to scheduled sacrifice throughout this study.

Clinical signs: All animals were observed daily for clinical signs of toxicity. The type of findings observed and the incidence of each finding from study Days 1 to 14 are summarized below. Injection site irritation with alopecia, scabbing and scales was observed in HD males and females with the presence of reddened skin.

Observation	**Icatibant Dose (mg/kg/day)**							
	0		**1**		**10**		**50**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Injection site alopecia								
No observations	-	-	-	-	-	-	9	22
Number of animals	-	-	-	-	-	-	1/5	4/5
Days from-to	-	-	-	-	-	-	7-15	8-15
Injection site scabbed								
No observations	-	-	-	-	-	-	23	33
Number of animals	-	-	-	-	-	-	4/5	5/5
Days from-to	-	-	-	-	-	-	7-15	6-15
Injection site wh scales								
No observations	-	-	-	-	-	-	4	20
Number of animals	-	-	-	-	-	-	1/5	3/5
Days from-to	-	-	-	-	-	-	12-15	8-15
Skin reddened								
No observations	-	-	-	-	-	-	7	6
Number of animals	-	-	-	-	-	-	2/5	2/5
Days from-to	-	-	-	-	-	-	9-12	8-10

Body weights: Animals were weighed once during pretest and on Days 1, 3, 5, 8 and 14 of study.
There were no statistically significant differences in group mean body weights or group mean body weight gains in males or females treated with icatibant compared to vehicle treated control animals.

Food consumption: Food consumption was determined once during pretest and on Days 1, 3, 5, 8 and 14.
There were no treatment related affects on food consumption in the icatibant treated groups compared to the vehicle treated group.

Ophthalmoscopy: Ophthalmic examinations were not performed.

EKG: EKG examinations were not performed.

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Hematology: Blood samples were taken from a retrobulbar venous plexus of fasted animals. A complete hematological battery was assessed.

There were no statistically significant changes in the hematology parameters for males or females treated with icatibant compared to the vehicle control treated animals.

Clinical chemistry: Blood samples were taken from a retrobulbar venous plexus of fasted animals. A complete clinical chemistry battery was assessed.

There were no statistically significant changes in clinical chemistry parameters for males or females treated with icatibant compared to the vehicle control treated animals.

Urinalysis: Urine was collected from all animals overnight on ice in metabolism cages prior to necropsy. A complete urinalysis was conducted.

There were no statistically significant changes in urine parameters in the icatibant treated animals compared to the vehicle treated animals.

Gross pathology: A complete necropsy was conducted on all study animals.

Macroscopic findings were observed at injection sites in males and females and the male reproductive organs (testes, epididymides and prostate). Observations in the reproductive organs were most likely related to drug due to the absence of these observations in the vehicle control group. However, the effects on the male reproductive organs did not follow a dose-response. Injection site irritation was observed primarily in the HD group.

Observation	**Icatibant Dose (mg/kg/day)**							
	0		**1**		**10**		**50**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Testis		NA		NA		NA		NA
Abnormal content, material, ill-defined	-		-		1/5		-	
Small	-		1/5		1/5		1/5	
Epididymis		NA		NA		NA		NA
Small	-		1/5		1/5		-	
Prostate		NA		NA		NA		NA
Small	-		-		1/5		-	
Injection site								
Focus/area, crateriform, red	-	-	-	-	-	-	-	1/5
Focus/area raised, firm/hard, dark, red	-	-	-	-	-	-	1/5	-
Focus/area, raised, firm/hard, red, focal	-	-	-	-	-	-	1/5	1/5
Focus/area, raised, red, multifocal	-	-	-	-	-	-	3/5	-
Focus/area, raised soft, red, multifocal	-	-	-	-	-	-	-	2/5

- No macroscopic finding observed
NA- Not applicable

Organ weights: The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary, prostate with seminal vesicles, spleen, testes, thymus and thyroid gland were weighed. The absolute organ weights and relative organ weights to body weights and to brain weights were determined.

Decreases in male reproductive organ weights (prostate and testes) and an increase in female thymic weights were observed in icatibant treated rats compared to the vehicle control treated animals. The decrease in prostate weight was observed in the MD and HD for absolute weight and prostate weight relative to brain weight. A slight decrease in prostate weight was

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observed in the LD when assessing prostate weight relative to body weight. An increase in thymus weights were observed in females alone mainly in the HD group.

Organ weight change relative to controls

Organ	Sex	Icatibant dose (mg/kg/day)		
		1	10	50
Prostate wt	M	-	-31%	-39%
	F	NA	NA	NA
Prostate wt rel BW	M	-17%	-33%	-39%
	F	NA	NA	NA
Prostate wt rel to BR	M	-	-35%	-42%
	F	NA	NA	NA
Testes wt	M	-	-	-
	F	NA	NA	NA
Testes rel to BW	M	-	-	-
	F	NA	NA	NA
Testes rel to BR	M	-	-29%	-30%
	F	NA	NA	NA
Thymus wt	M	-	-	-
	F	-	-	+37%
Thymus wt rel to BW	M	-	-	-
	F	-	+15%	+30%
Thymus wt rel to BR	M	-	-	-
	F	-	-	+38%

- No statistically significant difference compared to controls
NA- Not applicable

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

All study animals had tissues prepared for histopathological examination. However, tissues from the control and the 50 mg/kg/day group animals were examined. The testis, epididymis, prostate, seminal vesicles and injection site from all other animals were examined. Also, macroscopic findings from the main study animals were evaluated. A complete histological battery was examined with the exception of the cervix. However, the vagina, ovaries, fallopian tubes and uterus were examined.

Observation	Icatibant Dose (mg/kg/day)							
	0		1		10		50	
	M	F	M	F	M	F	M	F
Epididymis	N=5	NA	N=5	NA	N=5	NA	N=5	NA
Cellular debris: lumen	2 (2.0)		2 (2.5)		4 (2.3)		5 (2.8)	
Oligospermia	-		1 (2.0)		2 (3.5)		4 (3.3)	
Aspermia	-		1 (5.0)		1 (5.0)		-	
Testis	N=5	NA	N=5	NA	N=5	NA	N=5	NA
Hypospermia	-		1 (5.0)		3 (2.3)		3 (1.7)	
Giant cell: multinucleated	-		-		2 (2.0)		3 (1.7)	
Injection site	N=5	N=5	N=5	N=5	N=5	N=4	N=5	N=5
Acanthosis	-	-	-	-	-	-	5 (2.2)	5 (2.0)
Necrosis	-	1 (1.0)	-	-	-	-	3 (2.0)	2 (2.5)
Inflammation:exudative	-	-	-	-	-	-	2 (2.5)	4 (2.5)

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Observation	Icatibant Dose (mg/kg/day)							
	0		1		10		50	
	M	F	M	F	M	F	M	F
Hemorrhage	-	-	1 (2.0)	1 (1.0)	-		4 (2.5)	-

- No macroscopic finding observed
NA- Not applicable
(#)- mean severity: grade 1= minimal; grade 2= slight; grade 3= moderate; grade 4= marked; grade 5= massive

After repeated 14-day daily SC dosing of rats, the target organs of toxicity were the epididymides, testes and the injection site. Oligospermia and aspermia were observed in epididymides at all icatibant doses and increased in incidence and severity with increased icatibant dose. Hypospermia was observed at all icatibant doses in the testes with increased incidence and severity. Other than injection site irritation, there were no additional histopathology findings in females. No NOAEL could be determined in this study due to male reproductive affects observed at all doses.

Toxicokinetics: Blood samples were taken on Day 1 and Day 14 from the TK animals (3/sex/group) at 0.5, 1, 2, 3, 7 and 24 h post-dose. Plasma concentrations were determined. The TK data are summarized in the following table.

Sampling period	TK Parameter	Icatibant Dose (mg/kg/day)					
		1		10		50	
		M	F	M	F	M	F
Day 1	C_{max} (mcg/mL)	1.35	1.11	7.49	7.51	20.6	16.9
	T_{max} (h)	0.5	0.5	1.0	1.0	0.5	0.5
	AUC_{0-t} (mcg*h/mL)	1.36	1.0	17.4	15.4	85	94.1
Day 14	C_{max} (mcg/mL)	1.36	1.15	9.48	10.1	31.6	37.7
	T_{max} (h)	0.5	0.5	1.0	0.5	1.0	0.5
	AUC_{0-t} (mcg*h/mL)	1.47	0.98	22.1	16.9	93.7	80

There were no significant gender differences in the TK parameters evaluated in rats on Days 1 and 14 of study. The systemic exposure levels increased in an approximately dose proportionate manner for all doses on Day 1 and Day 14. The t_{max} was achieved from 0.5 to 1.0 h post-dose for both males and females. After repeated SC administration for 14-days, there was no accumulation of icatibant in either male or female rats.

Study title: HOE 140: 14-day subcutaneous toxicity study in male rats with an 8-week recovery period

Key study findings:

- This study focused on male reproductive organ toxicity
- ↓ creatinine levels (-18%) in the HD group
- ↓ testis and prostate wts in the HD groups
- Histopathological findings included injection site irritation in the HD group (observed macroscopically and microscopically), oligospermia and cellular debris in the lumen of the epididymis and hypospermia in the testes. After the 8-week recovery period, the epididymal findings were mostly reversible with 1/10 animals having cellular debris and

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oligospermia remaining and hypospremia in the testes remained in HD males with similar incidence and slightly higher severity than the VC group.

- The sponsor did not evaluate a full histopathological battery of tissues in this study.
- No NOAEL could be determined by the reviewer based on the incomplete Histopathological battery. However, for male reproductive organ findings alone the NOAEL is considered 1 mg/kg by the reviewer; this dose is associated with an AUC of 1 µg*h/mL. The sponsor considered the NOAEL to be 1 mg/kg/day.

Study no.: JE049-0128
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: September 16, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, lot # D081, and 96.5% pure

Methods

Doses: 0 (untreated, juvenile), 0 (vehicle), 0.1, 1 or 50 mg/kg/day
Species/strain: Sprague Dawley rats
Number/sex/group or time point (main study): 10/group
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in water for injection, administered in 1.0 mL/kg and infusion rate was not applicable.
Satellite groups used for toxicokinetics or recovery: 3 rats for the vehicle control group and 9 rats/icatibant dose group were used for TK analysis and 10 additional male rats/group in all dosage groups with the exception of the untreated controls to be observed as recovery animals.
Age: 10 weeks old
Weight: males: 150-208 g
Sampling times: See below
Unique study design or methodology (if any): This study examined the effects of repeated subcutaneous administration of icatibant to 10 week old (sexually mature) rats and examined its effects on male reproductive organs. In the previous 14-day study (JE049-0127) conducted on sexually immature rats (6-7 weeks old), males showed hypospermatogenesis and oligospermia which were associated with reduced accessory sex gland weights. These findings were observed at all doses examined (1-50 mg/kg) and were attributed by the sponsor to a delay in sexual maturation that the sponsor considered as species-specific. The sponsor addressed whether these same findings would be observed in sexually mature male rats in this current study. Untreated juvenile rats were maintained as a control group for histopathological examination only.

Observations, Times and Results

Mortality: All animals from the main study groups were checked for mortality at least twice a day.

There were no deaths prior to scheduled sacrifice throughout this study.

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Clinical signs: All animals were observed daily for clinical signs of toxicity. The type of findings observed and the incidence of each finding from study Days 1 to 14 are summarized below. There were no clinical signs of toxicity observed in the vehicle control group or the LD or MD icatibant groups. Skin lesions were observed in the HD icatibant treatment group from study Day 3 of treatment throughout the treatment phase and continued to be observed for 23 days into the recovery period in the HD recovery animals. No additional clinical signs of toxicity were observed in any of the treatment groups.

Observation	**Icatibant Dose (mg/kg/day)**			
	0	**0.1**	**1**	**50**
Skin lesion Number of animals (Days from-to)	- -	- -	- -	5/20 (Day 3) 15/20 (Day 4) 16/20 (Day 7) 17/20 (Day 9) 19/20 (Days 10-14) 10/10 (Recov Days 1-12) 9/10 (Recov. Day 13) 4/10 (Recov Day 14) 2/10 (Recov. Days 15-23)

Body weights: Animals were weighed weekly including pretest and recovery.

There were no statistically significant differences in group mean body weights or group mean body weight gains in males treated with icatibant compared to vehicle treated control animals.

Food consumption: Food consumption was determined weekly.

There were no treatment related affects on food consumption in the icatibant treated groups compared to the vehicle treated group.

Ophthalmoscopy: Ophthalmic examinations were not performed.

EKG: EKG examinations were not performed.

Hematology: Blood samples were taken from a retrobulbar venous plexus of fasted animals. In the untreated control group, animals were sacrificed Day 2 of study and blood was collected for hematological assessment. For the vehicle control group and the icatibant treated groups, animals were sacrificed the day after the last dose was administered or at the end of the recovery period. A complete hematological battery was assessed.

There were no statistically significant changes in the hematology parameters for males treated with icatibant compared to the vehicle control treated animals.

Clinical chemistry: Blood samples were taken from a retrobulbar venous plexus of fasted animals. In the untreated control group, animals were sacrificed Day 2 of study and blood was collected for clinical chemistry assessment. For the vehicle control group and the icatibant treated groups, animals were sacrificed the day after the last dose was administered or at the end of the recovery period. A complete clinical chemistry battery was assessed.

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There were no statistically significant changes in clinical chemistry parameters for males treated with icatibant compared to the vehicle control treated animals with the exception of a slight decrease in creatinine levels in the HD compared to the vehicle treated animals. This difference was not observed in the recovery animals.

Δ% relative to vehicle treated controls

Parameter	Icatibant (mg/kg/day)		
	0.1	1.0	50
Creatinine (mcmol/L)	-	-	-18%
Recovery Creatine (mcmol/L)	-	-	-

- No statistically significant difference.

Urinalysis: Urine was collected from all animals overnight on ice in metabolism cages prior to necropsy. A complete urinalysis was conducted.

There were no statistically significant changes in urine parameters in the icatibant treated animals compared to the vehicle treated animals.

Gross pathology: A complete necropsy was conducted on all study animals.

Macroscopic findings were observed at injection sites and the male (testes, epididymides and seminal vesicles) reproductive organs. The findings in the male reproductive organs were not observed immediately after 14-days of repeated subcutaneous dosing but were observed in 1/10 males after the 8-week recovery period. The macroscopic findings of small male reproductive organs were observed in a single HD male no. 96.

Observation	Icatibant (mg/kg/day)			
	0	0.1	1.0	50
Injection site, N=10				
Erosion/ulceration	-	-	-	1
Focus/area red	-	-	2	7
Scab/crust, crateriform, firm/hard, red	-	-	-	1
Scab/crust, red	-	-	-	1
Scab/crust	2	-	2	8
Recovery Injection site, N=10				
Erosion/ulceration	-	-	-	-
Focus/area red	-	-	-	-
Scab/crust, crateriform, firm/hard, red	-	-	-	-
Scab/crust, red	-	-	-	-
Scab/crust	-	-	-	-
Testis N=10				
Small	-	-	-	-
Recovery Testis N=10				
Small	-	-	-	1
Epididymis N=10				
Small	-	-	-	-
Recovery Epididymis N=10				
Small	-	-	-	1
Seminal vesicle N=10				
small	-	-	-	-
Recovery Seminal vesicle N=10				
small	-	-	-	1

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- No finding.

Organ weights: The adrenals, brain, epididymides, heart, kidneys, liver, pituitary, prostate with seminal vesicles, spleen, testes, thymus and thyroid gland were weighed. The absolute organ weights and relative organ weights to body weights and to brain weights were determined.

Minor decreases in male reproductive organ weights (prostate and testes) were observed in icatibant treated rats at the highest dose compared to the vehicle control treated animals. The decrease in prostate weight was observed in the HD for absolute weight, prostate weights relative to body weights and prostate weight relative to brain weight. After the 8-week recovery period there was no statistically significant difference in prostate gland weights compared to the control group. A slight decrease in testes weights relative to body weights and to brain weights were observed in the HD treated groups after the dosing phase of the study and the 8-week recovery period.

Organ weight change relative to controls

Organ	Icatibant (mg/kg/day)		
	0.1	1.0	50
Prostate Gland			
Absolute wt change	-	-	-21%
Relative to body wt	-	-	-19%
Relative to brain wt	-	-	-21%
Recovery Prostate Gland			
Absolute wt change	-	-	-
Relative to body wt	-	-	-
Relative to brain wt	-	-	-
Testis			
Absolute wt change	-	-	-
Relative to body wt	-	-	-8%
Relative to brain wt	-	-	-10%
Recovery Testis			
Absolute wt change	-	-	-
Relative to body wt	-	-	-13%
Relative to brain wt	-	-	-12%

- No statistically significant difference.

Histopathology: Adequate Battery: yes (), no (X)—explain
Peer review: yes (X), no ( )

All study animals had tissues prepared for histopathological examination. However, limited tissues from the untreated juvenile and the vehicle treated control groups and the HD group were examined. The tissues microscopically examined included the testes, epididymis, prostate, seminal vesicle, kidney, injection sites (for VC and treated HD groups) and macroscopic findings. The slides for the specified tissues examined in this study were peer reviewed by Dr. B. Durchfeld-Meyer. The following table shows the icatibant related findings observed in the male reproductive organs and at the injection sites compared to the untreated juvenile male rats (reproductive organ comparison only) and the vehicle control (VC) males (comparison of both injection sites and reproductive organs).

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Observation	Icatibant (mg/kg/day)				
	Juvenile untreated control 0	**VC 0**	**0.1**	**1.0**	**50**
Epididymis, Cellular debris: lumen	N=10	N=10	N=10	N=10	N=10
Grade 1	3	2	-	-	5
Grade 2	7	-	-	-	5
Oligospermia					
Grade 1	-	1	-	-	5
Grade 2	-	-	-	-	5
Grade 3	-	-	-	-	-
Grade 4	2	-	-	-	-
Grade 5	8	-	-	-	-
Recovery Epididymis, Cellular debris: lumen	NA	N=10	N=10	N=10	N=10
Grade 1		-	-	-	1
Grade 2		-	-	-	-
Oligospermia					
Grade 5		-	-	-	1
Testis, Hypospermia	N=10	N=10	N=10	N=10	N=10
Grade 1	-	-	-	-	1
Grade 2	2	1	-	-	2
Giant cell: multinucleated, grade 2	-	-	-	-	1
Recovery Testis, Hypospermia	NA	N=10	N=10	N=10	N=10
Grade 1		-	-	-	-
Grade 2		-	-	-	1
Grade 3		-	-	-	-
Grade 4		-	-	-	-
Grade 5		-	-	-	1
Injection site back Necrosis of epidermis	NA	N=10	N=10	N=10	N=10
Grade 2		-	-	1	2
Grade 3		-	-	-	2
Inflammation					
Grade 1		-	-	-	1
Grade 2		-	-	-	2
Grade 3		-	-	-	2
Edema					
Grade 1		1	-	-	-
Grade 2		-	-	-	1
Recovery Injection site back	NA	N=10	N=10	N=10	N=10
Necrosis of epidermis		-	-	-	-
Inflammation		-	-	-	-
Edema		-	-	-	-
Injection site neck Necrosis of epidermis	NA	N=10	N=10	N=10	N=10
Grade 1		1	-	-	-
Grade 2		1	1	2	1
Grade 3		1	1	1	7

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Observation	Icatibant (mg/kg/day)				
	Juvenile untreated control 0	**VC 0**	**0.1**	**1.0**	**50**
Grade 4		-	-	-	1
Necrosis: pannicular muscle					
Grade 2		-	1	-	-
Grade 3		-	1	1	1
Acanthosis					
Grade 1		-	-	1	3
Grade 2		-	-	2	6
Grade 3		-	-	-	1
Inflammation					
Grade 1		-	1	-	-
Grade 2		3	-	1	4
Grade 3		1	-	-	2
Edema					
Grade 2		1	3	2	3
Grade 3		-	-	-	2
Infiltration: mixed inflam cell					
Grade 1		3	2	-	-
Grade 2		-	1	3	3
Hemorrhage					
Grade 2		-	-	-	2
Grade 3		-	-	-	2
Recovery Injection site neck	NA	N=10	N=10	N=10	N=10
Necrosis of epidermis		-	-	-	-
Necrosis: pannicular muscle		-	-	-	-
Acanthosis		-	-	-	-
Inflammation		-	-	-	-
Edema		-	-	-	-
Infiltration: mixed inflame cell		-	-	-	-
Hemorrhage		-	-	-	-

- No finding.
NA- Not applicable.
(#)- mean severity: grade 1= minimal; grade 2= slight; grade 3= moderate; grade 4= marked; grade 5= massive

The untreated juvenile controls showed that the secretory content of the accessory sex glands and cellular debris and low numbers of sperm in the epididymis are characteristic of this immature rat population. Comparing the VC treated rats with the icatibant treated rats showed an increase in incidence and severity of oligospermia and cellular debris in the lumen of the epididymis in the HD group. The HD males showed an increase in incidence and severity hypospermia in the testes compared to the VC group animals. After the 8-week recovery period, the epididymal findings were mostly reversible with 1/10 animals having cellular debris and oligospermia remaining. Hypospremia in the testes remained in HD males after the recovery period with similar incidence and slightly higher severity than the VC group. Injection site irritation at the two sites of administration, the back and the neck, resulted in severe irritation at the HD of icatibant. Injection site irritation resolved after the 8-week recovery period.

The macroscopic findings of small male reproductive organs were observed in a single HD male no. 96. Oligospermia was observed in the epididymis at Grade 5 and hypospermatogenesis in the testes was observed with a Grade 5 severity as well. There were no microscopic findings in the seminal vesicle in this animal.

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Although the sponsor did not evaluate a full histopathological battery of tissues in this study, the previous study (JE049-0127) that was conducted in males and females at 1, 10 and 50 mg/kg/day showed no additional histopathological findings other than those observed in the male reproductive organs and the local injection site. However, this previous study was inadequately powered (n= 5/sex/group), potentially not missing drug-induced toxicities. The findings observed in the male reproductive organs in this study were limited to the HD group. Taking the data from both the 14-day study conducted in males and females and the 14-day study conducted in males alone, the combination of these 2 studies do not fulfill a complete histopathology assessment due to inadequate animal numbers in the first study and inadequate histopathology assessment in the second study.

The sponsor considered the NOAEL to be 1 mg/kg/day. Based on the study results with increased incidence and severity of epididymal and testes findings in the HD and injection site irritation, the reviewer agrees with the sponsor with a NOAEL of 1 mg/kg/day for the male reproductive findings and the injection site. However, no overall NOAEL can be determined for this study due to the incomplete histopathological battery.

Toxicokinetics: Blood samples were taken on Day 1 and Day 14 from the TK animals (3/sex/time point) at 0.5, 1, 2, 3, 7 and 24 h post-dose. Icatibant plasma concentrations were determined. The TK data are summarized in the following table.

Sampling period	**TK Parameter**	**Icatibant Dose (mg/kg/day)**		
		0.1	**1**	**50**
Day 1	C_{max} (µg/mL)	0.073	0.738	11.258
	T_{max} (h)	0.5	0.5	3.0
	AUC_{0-24}(µg*h/mL)	0.04	0.956	122.423
Day 14	C_{max} (µg/mL)	0.078	1.355	26.786
	T_{max} (h)	0.5	0.5	3.0
	AUC_{0-24}(µg*h/mL)	0.044	1.0	177.5

On Day 1 of dosing, the C_{max} and AUC increased in an approximately dose-proportionate manner from LD to MD. From MD to HD, the C_{max} increased but did not increase in a dose-proportional manner (increased by 15-fold instead of ~50-fold). The AUC increased from the MD to the HD by ~128-fold instead of ~50-fold, making it a super-proportional increase to dose. After repeated dosing for 14 days, the C_{max} increased in an approximately dose proportional manner from the LD to the MD. From the MD to the HD, the C_{max} increased in a sub-proportional manner by ~20-fold instead of the ~ 50-fold. The AUC increased in an ~ dose-proportional manner from the LD to the MD. However, from the MD to the HD the AUC increased in a supra-proportional manner by ~177-fold. Based on these data, after 14-day repeat dosing, icatibant does not accumulate in males. The T_{max} ranges from 0.5 h to 3.0 h on Day 1 of dosing and from 0.5 h to 1.0 h on Day 14 of dosing.

Study title: Combined study of 13-week toxicity and effects on fertility and early embryonic development to implantation in rats by subcutaneous administration

Key study findings:

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- Slight decreases in thyroid absolute weights (-6 to -18%) and weights relative to BWs (-3 to -16%) were observed in males but this was not associated with macroscopic or microscopic findings.
- There were no microscopic findings in this study with the exception of injection site irritation.
- The NOAEL for systemic toxicity was 10 mg/kg/day; this dose is associated with an AUC of 16.55 µg*h/mL. However, no NOAEL for local effects was identified due to injection site irritation and necrosis at all doses; these findings are considered clinically monitorable.

Study no.: JE049-0108
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: February 17, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batches 0565984 (98.1% pure) and 0565985 (98.1%)

Methods

Doses: 0 (vehicle), 1, 3 and 10 mg/kg/day
Species/strain: Sprague Dawley rats
Number/sex/group or time point (main study): 10/sex/group
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in isotonic saline solution (0.9% NaCl), administered in 1.0 mL/kg and infusion rate was not applicable.
Satellite groups used for toxicokinetics or recovery: 6 rats/sex/group for TK analyses
Age: 5-6 weeks old
Weight: males: 470 g and females: 260 g
Sampling times: See below
Unique study design or methodology (if any): The sponsor combined the 13-week repeat dose toxicity study and the effects on fertility and early embryonic development to implantation study in rats. Under this section, the 13-week repeat dose section of the study report is reviewed. See the reproductive toxicology section for the review of the effects on fertility and early embryonic development to implantation study in rats. Male and female rats were administered 0 (VC), 1, 3 or 10 mg/kg/day of icatibant for 13 weeks and a standard toxicology assessment was conducted as described below.

Observations, Times and Results

Mortality: All animals were checked for morbidity or mortality twice a day.
One LD male (no.H20778) was found dead Week 2 after anesthesia administration on Day 14. No other animals died prior to scheduled sacrifice. This death was deemed accidental due to anesthesia.

Clinical signs: All animals were observed at least once a day for clinical signs of toxicity.

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There were no drug-related clinical signs of toxicity observed throughout the study period.

Body weights: Body weights were recorded for each animal at least once during the pre-treatment period, on the first day of treatment and then twice a week until the end of the study.

There were no statistically significant differences in group mean body weights or group mean body weight gains in males or females treated with icatibant compared to vehicle treated control animals.

Food consumption: Food consumption was recorded for each animal once a week through sacrifice.

There were no treatment related affects on food consumption in the icatibant treated groups compared to the vehicle treated group.

Ophthalmoscopy: Ophthalmoscopy was not conducted in this study.

EKG: EKG was not measured in this study.

Hematology: Blood samples were taken from the orbital sinus from all animals. Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period and at the end of the 13-week treatment period. A complete hematological battery was assessed with the exception of measurement of hematocrit.

There were no statistically significant differences in the hematological parameters assessed in the icatibant treated animals compared to the vehicle treated animals.

Clinical chemistry: Blood samples were taken from the orbital sinus from all animals. Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period and at the end of the 13-week treatment period. A complete clinical chemistry battery was assessed.

There were no statistically significant differences in the hematological parameters assessed in the icatibant treated animals compared to the vehicle treated animals.

Urinalysis: Urine was collected from all animals individually placed in metabolism cages for an overnight period of 14 hours once before the treatment period and at the end of the 13-week treatment period. A complete urinalysis was conducted.

There were no statistically significant changes in urine parameters in the icatibant treated animals compared to the vehicle treated animals.

Gross pathology: A complete necropsy was conducted on all study animals found dead or that were sacrificed at the end of the 13-week study period.

There were no drug-related macroscopic changes in the icatibant treated animals compared to the VC animals.

Organ weights: The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus and thyroids with parathyroids were weighed from all animals. Absolute organ

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weights and organ weights relative to body weights were determined. No organ weights relative to brain weights were provided.

Slight decreases in male thyroid weights (absolute and relative to body weight) were observed. Female thyroid weights did not change compared to the vehicle control females. No other changes in organ weights were observed.

Organ weight change relative to controls

Organ	Sex	Icatibant dose (mg/kg/day)		
		1	3	10
Thyroid wt	M	-6.3%	-12.2%	-18%
	F	-	-	-
Thyroid rel BW	M	-2.6%	-12.2%	-16%
	F	-	-	-

- No statistically significant difference compared to controls

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

Histopathological evaluation was conducted on control and HD animals sacrificed at the end of the treatment period and on any animals that died prior to the scheduled sacrifice. All macroscopic lesions of all animals in the LD and MD were also examined microscopically. The injection sites, prostate, seminal vesicles, right epididymis, right testes, ovaries (with oviducts), mammary gland, vagina and uterus were examined in the LD and MD groups. At least 10% of the histological slides from control (group 1) and HD (Group 3) in each sex group and all slides from identified target organs were peer reviewed. A complete histological battery was microscopically examined with the exception of the lachrymal gland, pharynx and cervix (however, the uterus, ovaries and fallopian tubes were examined). All other tissues were examined. The following table summarizes the icatibant induced toxicities observed after repeated SC administration for 13 weeks.

Observation	Icatibant Dose (mg/kg/day)							
	0		1		3		10	
	M	F	M	F	M	F	M	F
Injection sites	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10
Acute inflam, level 2	-	-	-	-	-	-	1 (2)	-
Area necros, level 1-last site; muscle; multifl	-	-	-	-	-	-	2 (2)	-
Area necros; level 1-next to last site; muscle; multifocal	-	-	1 (1)	-	3 (2)	-	1 (3)	-
Area necros; level 2; muscle; focal	-	-	-	-	1 (1)	-	1 (3)	-
Area necros; level 2; muscle; multifocl	-	-	-	-	1 (1)	-	1 (3)	1 (2)
Ulceration; level 2	-	-	-	-	-	-	1 (3)	1 (1)
Inflam; level 1; subcutaneous; diffuse	2 (1)	1 (1)	1 (1)	(2)	3 (1)	3 (1.2)	4 (2)	6 (1.5)
Inflam; level 2; subcutaneous; diffuse	1 (1)	1 (1)	3 (1.5)	1 (1)	5 (2)	1 (1)	6 (2.0)	2 (1)
Inflam; level 2-next to last site, subcut diff	1 (1)	-	3 (1.3)	-	5 (1.8)	-	6 (1.8)	-

- No macroscopic finding observed
(#)- mean severity: grade 1= minimal; grade 2= slight; grade 3= moderate; grade 4= marked; grade 5= massive

After 13-weeks of daily SC administration of icatibant to male and female rats, the target organ of toxicity was local injection site. Injection site irritation was observed in both males and

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females with increased incidence and severity with increased icatibant doses. Some inflammation was also noted in the vehicle control group. Based on the absence of any systemic toxicity at all doses the systemic NOAEL was considered 10 mg/kg/day.

Toxicokinetics: Satellite males and females had blood samples collected on Day 1 and during Week 13 at 0.5, 1, 2 and 4 h post-dosing. Plasma was prepared from samples and assessed for icatibant and the metabolite M_2. TK data are summarized in the following table.

	Parameter	Icatibant Dose (mg/kg/day)											
		TK of Icatibant						TK of M_2 Metabolite					
		1		3		10		1		3		10	
		M	F	M	F	M	F	M	F	M	F	M	F
Day 1	C_{max} (mcg/mL)	0.96	0.81	2.1	2.4	5.4	6.1	0.14	0.06	0.20	0.17	0.47	0.25
	T_{max} (h)	0.5	0.5	0.5	1	1	1	1	1	1	1	2	2
	$AUC_{0\ 5\text{-}4\ h}$ (mcg*h/mL)	0.82	0.66	2.5	3.7	12.4	12.6	0.13	0.07	0.42	0.29	1.12	0.64
Wk 13	C_{max} (mcg/mL)	1.0	1.1	2.3	2.7	6.1	6.8	0.09	0.06	0.18	0.13	0.39	0.18
	T_{max} (h)	0.5	0.5	0.5	1	2	1	1	1	2	1	2	2
	$AUC_{0\ 5\text{-}4\ h}$ (mcg*h/mL)	1.7	1.2	5.6	5.0	15.9	17.2	0.15	0.08	0.48	0.31	0.88	0.59

NC- not calculated because individual values were invalid (QC rejected).

On Day 1 and Week 13 of dosing, icatibant C_{max} and AUC levels increased in an approximate dose proportional manner in both males and females. There were no gender differences and icatibant did not accumulate after 13 weeks of daily SC dosing. The M2 metabolite C_{max} increased with increased icatibant dose on Day 1 and Week 13 in a sub-proportional at all doses. AUC levels were proportional from LD to MD and sub-proportional from MD to HD. The metabolite formed between 1 and 2 hours post-icatibant dose.

Other: Epididymal sperm was sampled prior to sacrifice and assessed for motility, morphology and sperm count. The left testis of each male was weighed and then sperm heads were counted.

There were no icatibant related effects on epididymal sperm count, motility or morphology or on testicular sperm head count or daily sperm production compared to the vehicle treated males.

Study title: Two-phase dose range-finding study by subcutaneous administration of icatibant in Beagle dogs

Key study findings:

- No animals died in either the Phase 1 or the Phase 2 study.
- The maximum non-lethal dose was 150 mg/kg (highest dose tested) in males and females, at which females showed a decrease in body weight and food consumption.
- Injection site irritation (scabbing, hematomas and thickening at the injection site) was observed in males and females dosed with ≥75 mg/kg/day.
- The sponsor determined that 100 mg/kg/day was the maximum dose to be used for the 13-week repeat dose toxicology study.

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Study no.: JE049-0115
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: December 15, 2004 (Phase 1) and January 11, 2005 (Phase 2)
GLP compliance: No
QA report: yes () no (X)
Drug, lot #, and % purity: Icatibant, batch # E084, and 95.9% pure

Methods

Doses: Dogs received icatibant subcutaneous injections as follows:

Phase 1: Days 1 to 3: 10 mg/kg/day
Days 4 to 6: 25 mg/kg/day
Days 7 to 9: 75 mg/kg/day
Days 10 to 12: 100 mg/kg/day
Days 13 to 15: 150 mg/kg/day

Phase 2: 100 mg/kg/day for 10 days

Species/strain: Beagle dogs

Number/sex/group or time point (main study): 1 dog/sex was administered doses of icatibant following the previously described schedule as Phase 1 of the study. Phase 2 of the study used a different male and female dog (1/sex) to receive the test item by daily subcutaneous injection for 10 days at 100 mg/kg/day.

Route, formulation, volume, and infusion rate: Subcutaneous, formulated in isotonic saline solution (0.9% NaCl), administered in 0.4 mL/kg and infusion rate was not applicable.

Satellite groups used for toxicokinetics or recovery: None.

Age: 7 and 9 months old

Weight: males: 7.4 and 6.6 kg and females: 7.3 and 6.8 kg

Sampling times: See below

Unique study design or methodology (if any): This study escalates doses of icatibant in Beagle dogs to evaluate the potential systemic toxicity and local tolerance to icatibant after 3 days dosing (Phase 1) and 10 days dosing (Phase 2). The aim is to establish a maximum tolerated dose (MTD) in dogs and to use these data to select doses for the 13-week toxicity study. There were no treatment free days between successive dose-levels.

Observations, Times and Results

Mortality: Each animal was checked for morbidity or mortality twice a day.

There were no premature deaths in either Phase 1 or Phase 2 of this study.

Clinical signs: Each animal was observed at least twice a day for clinical signs of toxicity. The primary clinical sign observed in either Phase 1 or Phase 2 of this study was injection site irritation with thickening at the injection site and erythema. In the Phase 1 study, injection site irritation was observed to increase with increased icatibant dose.

Phase 1: Injection site reactions were observed in the male and female as described in the table.

Reference ID: 2977968

Injection Site	Male G50501	Female G50551
Thickening		
Site no. 6	-	Days 8-12, slight (75-100 mg/kg)
Site no. 7	Days 8 and 9, moderate (75 mg/kg)	From day 8, moderate (75 mg/kg)
Site no. 8	Day 9, moderate (75 mg/kg) Days 10 and 12, moderate (100 mg/kg)	Day 9, slight (75 mg/kg) From Day 10, moderate (100 mg/kg)
Site no. 9	Days 10 to 12, moderate (100 mg/kg)	Days 10 to 13, moderate (100 mg/kg)
Site no. 10	Days 10 to 11, moderate (100 mg/kg)	Days 10 to 13, moderate (100 mg/kg)
Site no. 13	From day 14, moderate (150 mg/kg)	Day 14, moderate From Day 15, marked (150 mg/kg)
Site no. 14	From Day 15, moderate (150 mg/kg)	From day 15, moderate (150 mg/kg)
Erythema		
Site no. 13	-	From Day 14, moderate to severe, before dosing (150 mg/kg)

Phase 2: Injection site reactions were observed in the male and female as described in the table. Each animal was dosed with 100 mg/kg/day

Injection Site	Male G50502	Female G50552
Thickening		
Site no. 1	-	Day 1, marked From day 3, moderate
Site no. 2	From Day 3, moderate	From Day 3, slight
Site no. 6	-	From Day 10, moderate
Site no. 7	-	From Day 10, moderate
Erythema		
Site no. 2	Day 3, very slight before dosing	-

Body weights: For Phase 1, body weight was recorded for each animal once before dosing, on the first day of treatment, on the day of each change of dose-levels, on Day 12 and prior to necropsy. For Phase 2, body weight was recorded for each animals twice before the beginning of treatment and then on Days 1, 4, 7 and 10.

Phase 1: The mean body weight in the male was unaffected by icatibant treatment. The females in Phase 1 lost 0.3 kg between Days 12 and 16 of dosing at 150 mg/kg/day.

Phase 2 (100 mg/kg/day SC for 10 days: The mean body weight in the male was not affected by icatibant treated over the 10 days of daily dosing. The female lost 0.4 kg between Days 1 and Day 4 of dosing.

Food consumption: Food consumption was recorded for each animal by noting the difference between the quantity given and the remaining food left the next morning. Food intake per animal and per day was calculated for 12 days (Phase 1) or 14 days (Phase 2) before the beginning of the treatment period and then throughout the study. Food consumption was expressed as a percentage of the quantity given.

Phase 1: Food consumption in the male was not affected by icatibant treatment but the female treated with 150 mg/kg/day reduced intake by 50%.

Phase 2 (100 mg/kg/day SC for 10 days): Food consumption in the male was not affected by icatibant treatment. The females reduced food intake by ~50% on days 1, 8 and 10 of dosing.

Reference ID: 2977968

Ophthalmoscopy: Ophthalmic examination was not conducted in this study.

EKG: EKG examinations were not performed in this study.

Hematology: Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period, and on Day 11 (Phase 2 animals). A complete hematological battery was assessed for Phase 2 animals.

There were no changes in the hematological parameters in the icatibant treated animals when comparing the pretreatment period to the Day 11. Due to the low number of animals used in this study (1/sex at a single dose) and the absence of a control group, no conclusions can be drawn about the affects of icatibant on hematology.

Clinical chemistry: Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period, and on Day 11 (Phase 2 animals). A complete clinical chemistry battery was assessed for Phase 2 animals.

There were no changes in clinical chemistry parameters in the icatibant treated animals when comparing the pretreatment period to the Day 11. Due to the low number of animals used in this study (1/sex at a single dose) and the absence of a control group, no conclusions can be drawn about the affects of icatibant on clinical chemistry.

Urinalysis: Urine was collected once before the treatment period, and on Day 11 (Phase 2 animals). A complete urinalysis was conducted for Phase 2 animals.

There were no changes in urine parameters in the icatibant treated animals when comparing the pretreatment period to the Day 11 urine. Due to the low number of animals used in this study (1/sex at a single dose) and the absence of a control group, no conclusions can be drawn about the affects of icatibant on urinalysis.

Gross pathology: Phase 1 animals were sacrificed on Day 16 of study and Phase 2 animals were sacrificed on Day 11 of their study period. Each dog was anesthetized by intravenous injection of thiopental sodium and sacrificed by exsanguination. A complete necropsy was conducted on all study animals from Phase 1 and Phase 2.

Macroscopic findings were observed at injection sites (hematomas and thickening at injection sites) in both the Phase 1 and Phase 2 studies. In the Phase 2 study, 1 male showed adhesions in the lung. No controls were used in this study.

Phase 1 animals

Finding	Male N=1	Female N=1
Injection site 10 Hematoma subcutaneous tissue	1	-
Injection site 11 Hematoma subcutaneous tissue	1	1
Injection site 12 Hematoma subcutaneous tissue	1	1
Injection site 13 Thickened subcutaneous tissue	1	1
Injection site 14 Thickened subcutaneous tissue	1	1

Reference ID: 2977968

Phase 2 animals

Finding	Male N=1	Female N=1
Injection site 1 Hematoma subcutaneous tissue	1	-
Injection site 1 Thickened subcutaneous tissue	-	1
Injection site 2 Thickened subcutaneous tissue	1	1
Injection site 4 Hematoma subcutaneous tissue	-	1
Injection site 5 Thickened subcutaneous tissue	1	-
Injection site 6 Thickened subcutaneous tissue	-	1
Injection site 7 Hematoma subcutaneous tissue	1	-
Injection site 7 Thickened subcutaneous tissue	-	1
Injection site 8 Hematoma subcutaneous tissue	1	-
Injection site 9 Thickened subcutaneous tissue	-	1
Injection site 10 Hematoma subcutaneous tissue	1	-
Lung, adhesions	1	-

Organ weights: No organs were weighed from Phase 1 animals. The adrenals, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thymus and thyroids with parathyroids were weighed. The brain and the prostate were no weighed in this study. Due to the low number of study animals (1/sex) at the single dose (100 mg/kg/day) and the absence of a control group, no conclusions can be made about organ weight in this study.

Histopathology: Adequate Battery: yes (), no (X)—explain
Peer review: yes (), no (X)

No animals from the Phase 1 study were evaluated microscopically. Only limited tissues were evaluated in this study from Phase 2 animals. The kidneys, injection sites showing macroscopic abnormalities and injection sites No. 8, 9 and 10 were microscopically examined. In the absence of any control animals in this study, the findings can not be determined to be icatibant related or considered background. Injection site irritation was observed in the male (injection sites 1, 2, 5, 7, 8, 9 and 10) and tubular epithelial degeneration/necrosis and proteinaceous casts were observed in the kidneys in males. Additionally, monocyte cellular aggregation in the lungs was observed in the male. Injection site irritation was observed in the icatibant treated females (injection sites 1, 2, 4, 6, 7, 8, 9 and 10) and interstitial fibrosis, mineralization, tubular basophilia, tubular epithelial degeneration/necrosis, proteinaceous cast and interstitial monocyte cellular aggregation was observed in the kidney.

Reference ID: 2977968

Toxicokinetics: Blood samples were taken on Day 1 and Day 10 from each animal from Phase 2 to determine icatibant levels and the metabolite M_2 (b) (4) levels. TK data are summarized in the following table.

Day	Parameter	Icatibant 100 mg/kg/day			
		TK for Icatibant		TK for M2 metabolite	
		M	F	M	F
Day 1	C_{max} (mcg/mL)	52.2	54.2	2.01	0.73
	T_{max} (h)	1	2	24	24
	AUC_{0-24}(mcg*h/mL)	458	507	19	10
Day 10	C_{max} (mcg/mL)	77.4	57.8	3.38	1.13
	T_{max} (h)	1	1	0	0
	AUC_{0-24}(mcg*h/mL)	415	522	6	13

Blood samples were taken from 1 dog/sex at the indicated time points at one dose (100 mg/kg/day). Based on the available data, icatibant C_{max} and AUC levels were similar between the male and the female at Day 1 and at day 10 of the study. There was no accumulation of icatibant over the 10 day dosing period in either the male or the female. The M2 metabolite was ~3-fold higher in males than in females for C_{max} on day 1 and day 10 of the study. The AUC of the M2 metabolite was approximately the same in males and females on Day 1 and Day 10.

Study title: 4-week toxicity study by subcutaneous route in Beagle dogs with twice weekly T.I.D. Dosing and an 8-week treatment free period.

Key study findings:

- Erythema was observed in low incidence in males and females at each dose.
- Gross and microscopic findings were limited to injection site irritation (hematomas) which did not result in a dose response and were reversible.
- Testosterone levels were decreased in males treated with icatibant at 1 or 3.3 mg/kg TID twice weekly on the days of icatibant dosing. However, testosterone levels rebound in males during dosing intervals.
- A NOAEL of 3.3 T.I.D. administered twice weekly was defined in males (corresponding AUC_{0-24} 43.4 mcg*h/mL) and females (corresponding AUC_{0-24} h 44.5 mcg*h/mL), which is in agreement with the sponsor's study conclusion.

Study no.: JE049-0116
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: November 23, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batch # E083, and 82.7% pure

Methods

Doses: 0 (vehicle), 3 and 10 mg/kg (administered as 1 and 3.3 mg/kg T.I.D. twice weekly)

Reference ID: 2977968

Species/strain: Beagle dogs
Number/sex/group or time point (main study): 3/sex/group
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in isotonic saline solution (0.9% NaCl), administered in 0.4 mL/kg and infusion rate was not applicable.
Satellite groups used for toxicokinetics or recovery: 2/sex/group for recovery
Age: 12 months old (sexually mature)
Weight: males: 6.5-9.1 kg and females: 5.3-7.6 kg
Sampling times: See below
Unique study design or methodology (if any): Beagle dogs were dosed twice weekly with 0, 3 or 10 mg/kg/day using the SC route of administration. For each of the 2 days/week of dosing, animals were dosed T.I.D with 0, 1 (low-dose) or 3.3 mg/kg TID (high-dose) to reach the respective 0, 3 or 10 mg/kg/day icatibant dose. All dogs (n=5/sex/group) were dosed for 4 weeks and then 3/sex/group were sacrificed for analyses. Two dogs/sex/group were observed for a further 8 weeks as the recovery group animals. In this study, sexually mature dogs were used to determine the effects of icatibant treatment on the reproductive organs. Other repeat dose toxicology studies using sexually immature dogs showed delayed reproductive organ maturity in both females and males.

Observations, Times and Results

Mortality: All animals were checked for morbidity or mortality twice a day.
There were no deaths prior to scheduled sacrifice throughout this study.

Clinical signs: All animals were observed at least once a day for clinical signs of toxicity. The type of findings observed and the incidence of each finding from study Days 1 to 27 are summarized below. Erythema was observed as potential drug-related clinical sign of toxicity. In a single HD male and female generalized erythema was observed on Day 18. In the LD females, erythema of the right and left ears was observed on Day 18 and in one HD females was observed earlier on Day 15. Erythema was well-defined or moderate to severe in these animals.

Observation	**Icatibant Dose (mg/kg)**					
	0		**1 TID**		**3.3 TID**	
	M	**F**	**M**	**F**	**M**	**F**
Erythema:						
Right ear	-	-	-	1 (Day 18)	-	1 (Day 15)
Left ear	-	-	-	1 (Day 18)	-	1 (Day 15)
Generalized	-	-	-	-	1 (Day 18)	1 (Day 18)

Body weights: Body weights were recorded for each animal at least twice during the pre-treatment period, on the first day of treatment and then once a week until the end of the study.
There were no statistically significant differences in group mean body weights or group mean body weight gains in males or females treated with icatibant compared to vehicle treated control animals.

Food consumption: Food consumption was recorded for each animal by noting the difference between the quantity given and the remaining food left the next morning. This was estimated

Reference ID: 2977968

daily for at least 5 days before the beginning of treatment and throughout the study period. Food consumption was expressed as a percentage of the quantity given.

There were no treatment related effects on food consumption in the icatibant treated groups compared to the vehicle treated group.

Ophthalmoscopy: Ophthalmoscopy was not conducted in this study.

EKG: Telemetry was carried out for all animals. EKG examinations were performed on Days 1, 15 and 25. Recording began at least 45 minutes before the first (morning) administration and ended at least 20 hours after the first administration. These recordings corresponded with the T_{max} of icatibant, which was achieved at ~ 1 to 2 hours post-dose. This examination was performed using leads I, II, III and Cardiovit AT-6 Schiller. Heart rate, PQ- and QT-intervals and QRS-complex duration were determined, and QT was corrected using Bazett and Fridericia formulae. Blood pressure was also evaluated on Day 18 of dosing.

There were no statistically significant differences in EKG parameters in the icatibant treated animals compared to the vehicle treated animals. There was no statistically significant difference in BP in the icatibant treated males and females compared to the vehicle treated males and females at Day 18 of dosing.

Hematology: Blood samples were taken from a jugular vein. Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period and Day 24 at the treatment period. A complete hematological battery was assessed with the exception of measurement of hematocrit.

There were no statistically significant differences in the hematological parameters assessed in the icatibant treated animals compared to the vehicle treated animals.

Clinical chemistry: Blood samples were taken from a jugular vein. Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period and Day 24 at the treatment period. A complete clinical chemistry battery was assessed.

There were no statistically significant differences in the hematological parameters assessed in the icatibant treated animals compared to the vehicle treated animals.

Urinalysis: Urine was collected from all animals from individual diuresis cages once before the treatment period and Day 24 at the treatment period.

There were no statistically significant changes in urine parameters in the icatibant treated animals compared to the vehicle treated animals.

Gross pathology: A complete necropsy was conducted on all study animals.

Macroscopic findings observed were limited to slight injection site irritation (hematomas), which were not observed after the 8-week recovery period.

Observation	**Icatibant (mg/kg)**					
	0		**1 TID**		**3.3 TID**	
	M	**F**	**M**	**F**	**M**	**F**
Injection site 23, N=3 Hematoma subcutaneous tissue, diffuse	-	1	-	-	1	-

Reference ID: 2977968

Observation	Icatibant (mg/kg)					
	0		1 TID		3.3 TID	
	M	F	M	F	M	F
Rec Injection site 23, N=2 Hematoma subcutaneous tissue, diffuse	-	-	-	-	-	-
Injection site 24, N=3 Hematoma subcutaneous tissue, diffuse	-	-	1	1	-	1
Rec Injection site 24, N=2 Hematoma subcutaneous tissue, diffuse	-	-	-	-	-	-

- No macroscopic finding observed

Organ weights: The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus and thyroids with parathyroids were weighed from all animals. Absolute organ weights and organ weights relative to body weights were determined. No organ weights relative to brain weights were provided.

Decreases in prostate weights relative to body weights (-32.6%) were observed in HD males treated with icatibant compared to the vehicle treated males. However, this decrease was not statistically significant due to high variability between the control group (0.0685 ± 0.045) and the HD group (0.04647 ± 0.012). HD males showed an increase in group mean thymic weights relative to body weights (+79.5%) compared to the vehicle control group. This was not observed in females treated with icatibant. During the treatment period, there were no other statistically significant organ weight changes compared to vehicle controls. However, after the 8-week recovery period, slight decreases in absolute thymic weights in MD (-58%) and HD (-64%) females and a decrease in thymic weights relative to body weights in MD (-53%) and HD (-61%) females were observed in the previously icatibant exposed animals compared to the vehicle control treated animals. There were no microscopic correlates to these findings and they are of unclear toxicological significance.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

Histopathological evaluation was conducted on all study animals in both the treatment group and the recovery group. At least 20% of the histological slides from control (group 1) and HD (Group 3) in each sex group and all slides from identified target organs were peer reviewed. A complete histological battery was microscopically examined with the exception of the lachrymal gland, pharynx and seminal vesicles. All other tissues were examined. The following table summarizes the potential icatibant related histopathology findings after 4 weeks treatment compared to the VC group.

Observation	Icatibant (mg/kg)					
	0		1 TID		3.3 TID	
	M	F	M	F	M	F
Injection site 21	N=3	N=3	N=3	N=3	N=3	N=3
Hemorrhage	-	-	-	-	-	1 (1)
Inflammation	-	-	-	-	-	1 (1)
Rec Injection site 21	N=2	N=2	N=2	N=2	N=2	N=2
Hemorrhage	-	-	-	-	-	-
Inflammation	-	-	-	-	-	-

- No macroscopic finding observed

(#)- mean severity: grade 1= minimal; grade 2= slight; grade 3= moderate; grade 4= marked; grade 5= massive

Reference ID: 2977968

After repeated administration of icatibant and vehicle, there were sporadic incidences of injection site irritation in dogs from the VC and the icatibant treated groups. At injection site 21, the HD female group showed a low incidence and severity of local irritation. There were no additional histopathological findings in this study. Local injection sites are easily monitorable. Therefore, the reviewer determined the NOAEL to be the HD (3.3 mg/kg TID) administered twice weekly.

Toxicokinetics: Blood samples were taken on Day 22 from all animals to determine icatibant levels and the metabolite M_2 [redacted] (b) (4) levels. TK data are summarized in the following table.

	TK Parameter	Icatibant Dose (mg/kg/day)			
		1 TID		3.3 TID	
		M	F	M	F
TK for Icatibant	AUC_{Tot}(mcg*h/mL)	18.2	10.9	43.4	44.5
TK for M_2	AUC_{Tot} (mcg*h/mL)	0.575	NC	0.735	0.851

NC- not calculated because individual values were invalid (QC rejected).

On Day 22, AUC levels of icatibant increased with increased dosage in both males and females in an approximately dose proportionate manner. The M_2 metabolite also increased with increased dose but in a sub-proportionate manner for males (no data were available for females at the low dose). The t_{max} was reached between 1 and 2 hours post-dose in both males and females. No gender differences were observed in systemic exposure levels on Day 22 of the study.

Other: Hormonal assays: Blood was sampled to determine hormone levels. Using radioimmunoassay, luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone (PROG), estradiol (ESTR) and testosterone (TESTO) levels were measured at predose and then before and 24 hours after the first dose on Days 15 and 25. Vaginal smears were taken from each female to determine estrus cycle immediately before blood sampling for hormonal assays.

There were no statistically significant effects of icatibant treatment on LH, estradiol and progesterone levels in females compared to vehicle control animals. FSH levels were highly variable in females and males throughout the study. LH levels in icatibant treated males were also highly variable. Therefore, no conclusive effect of icatibant treatment could be determined. On the days of icatibant treatment significant decreases to almost ablation of testosterone levels were observed in males compared to the vehicle control levels. However, prior to dosing on Day 15, there were no significant differences in testosterone levels compared to the VC group. On Day 25 prior to dosing, testosterone levels had rebound to levels beyond the VC levels.

Parameter	Week	Icatibant (mg/kg)	
		1 TID	3.3 TID
		Males	Males
Testo	Day 15 (before 1st injection)	-	-
	Day 15 24h post-dose	-96%	-97%
	Day 25 (before 1st injection)	+166%	+154
	Day 25 24h post-dose	-96%	-97%

- No statistically significant difference compared to vehicle controls.
NA- Not applicable.

Reference ID: 2977968

Vaginal smears: There were not differences in vaginal smears in the females treated with icatibant and those treated with the vehicle control.

Study title: 13-week toxicity study by subcutaneous route in beagle dogs

Key study findings:

- Clinical signs of toxicity included erythema of the face, injection site irritation and several incidences of gastrointestinal disturbances (diarrhea and/or vomiting).
- ↓ leukocyte counts in HD males and ↑ platelet counts in HD females
- ↑ triglycerides and ALP in HD males and ↓ CK, creatinine, LDH and ASAT levels in HD males compared to the vehicle control animals.
- ↓ reproductive organ wts (absolute and relative to BWs) in males and females, ↑ adrenal wts relative to BWs in males and females, ↑ liver wts relative to BW in MD and HD males, ↓ thymic wts (absolute and relative to BWs) in both males and females compared to respective vehicle control treated animals.
- Macroscopic findings were observed at injection sites (hematomas and edema), the male (testes, epididymides and prostate) and female (ovaries and uterus) reproductive organs, thymus, liver and adrenal gland.
- The target organs of toxicity included: male (testes, epididymides, and prostate) and female (mammary gland, ovaries, uterus and vagina) reproductive organs, the injection site, adrenal gland, liver and thymus.
- Based on the arrest of sexual maturity in all icatibant treated males and females, lymphoid depletion in the thymus and on injection site irritation, no NOAEL can be determined for this study. This is in agreement with the sponsor's conclusions. Systemic exposures to icatibant from 35 - 230 µg*h/mL and for M2 from 0.96 -3.28 µg*h/mL ranged from the low to high doses.

Study no.: JE049-0117
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: February 21, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batch # E083, and 82.7% pure

Methods

Doses: 0 (vehicle), 10, 30 and 100* mg/kg/day
Species/strain: Beagle dogs
Number/sex/group or time point (main study): 4/sex/group
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in isotonic saline solution (0.9% NaCl), administered in 0.4 mL/kg and infusion rate was not applicable.
Satellite groups used for toxicokinetics or recovery: None.
Age: 6.6 to 7.3 months old
Weight: males: 6.5-8.5 kg and females: 6.4-7.4 kg
Sampling times: See below

Reference ID: 2977968

Unique study design or methodology (if any): Beagle dogs were dosed once daily with 0, 10, 30 or 100 mg/kg/day using the SC route of administration. *The sponsor observed the HD animals (100 mg/kg/day) to not tolerate the drug and decided to split the dose-level starting Day 12 to 2 SC doses of 50 mg/kg to equal 100 mg/kg/day dosing. The sponsor miscalculated the dose on Days 17 to 18 resulting in a higher daily dosing (200 mg/kg/day) and again Days 19-29 (120 mg/kg/day). On Day 30 these animals were dosed with 60 mg/kg/day and allowed 1 day as a washout period. From Day 32 to the end of the study animals were dosed with 60 mg/kg/day as two SC doses of 30 mg/kg.

Observations, Times and Results

Mortality: All animals were checked for morbidity or mortality at least twice a day.
There were no deaths prior to scheduled sacrifice throughout this study.

Clinical signs: All animals were observed at least once a day for clinical signs of toxicity. The type of findings observed and the incidence of each finding from study Days 1 to 93 are summarized below. Injection site irritation and edema (increase size) of face and injection sites were observed. Injection site erythema was observed 3-5 weeks, 6-10 weeks and 10 weeks for the low-dose, mid-dose and high-dose, respectively. Edema was observed starting Day 1 of study until 2 weeks post study initiation. Vomiting was observed the first 3 days of dosing. The sponsor reported abnormal vocalization in HD animals, hypoactivity in females on days 1 and 2, loss of balance and tremors in a single female Day 1 at Day 18, resulting in a reduction of dose-levels. The sponsor was notified by the CRO of abnormal vocalization and itching in HD females post-dose. Strong reactions to icatibant administration was observed on day 11 of dosing with abnormal vocalization, 1 male and 3 females rolled on the ground and 3 females licked themselves immediately after injection at the injection site. The doses were subsequently reduced. It was concluded by the CRO that SC injection of icatibant at 250 mg/mL and at 140 mg/mL was not tolerated in males or females.

Observation	**Icatibant Dose (mg/kg/day)**							
	0		**10**		**30**		**100/2x30**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Thickening at injection site:								
Site 1	0	0	0	0	0	0	1	0
Site 2	0	0	0	0	0	1	2	2
Site 12	0	0	0	0	0	0	1	3
Site 7	0	0	0	0	0	0	0	1
Site 17	0	0	0	0	0	1	0	1
Site 18	0	0	0	0	0	0	0	3
Site 21	0	0	0	0	0	0	0	1
Erythema at injection site:								
Site 2	0	0	0	0	0	0	1	0
Site 13	0	0	0	0	0	0	1	0
Site 18	0	0	0	0	0	0	1	1
Site 21	0	0	0	0	0	0	1	0
Erythema:								
Tongue	0	0	0	0	0	0	1	0
Right ear	1	2	3	3	3	0	0	0
Left ear	1	2	3	3	3	0	0	0

Reference ID: 2977968

Observation	Icatibant Dose (mg/kg/day)							
	0		10		30		100/2x30	
	M	F	M	F	M	F	M	F
Neck	0	0	1	0	0	0	0	0
Generalized	0	0	4	4	4	4	4	4
Head	0	0	0	0	0	1	0	0
Abnormal vocalization	0	1	0	1	0	0	3	3
Feces								
Soft	0	1	0	1	0	3	2	3
liquid	0	0	0	1	0	0	3	3
Vomit	0	0	1	0	4	0	1	0
Vomit/whitish	0	0	0	1	1	1	4	4
Increase in size:								
Head	0	0	2	2	3	2	0	2
Muzzle	0	0	1	0	2	0	0	0
Right ear	0	0	3	4	4	4	4	4
Left ear	0	0	3	4	4	4	4	4
Right lower eyelid	0	0	1	0	3	0	0	0
Left lower eyelid	0	0	1	0	3	0	0	0
Right upper eyelid	0	0	1	0	3	0	0	0
Left upper eyelid	0	0	1	0	3	1	0	0
Around the right eye	0	0	2	4	4	4	4	4
Around the left eye	0	0	2	4	4	4	4	4
Injection site 1	0	0	0	0	0	1	3	4
Injection site 2	0	0	0	2	0	1	4	4
Injection site 7	0	0	0	2	0	3	4	4
Injection site 11	0	0	0	0	0	0	4	3
Injection site 12	0	0	0	0	3	3	3	4
Injection site 13	0	0	0	0	1	0	1	4
Injection site 14	0	0	0	0	0	0	1	1
Injection site 17	0	0	0	0	1	2	2	4
Injection site 18	0	0	0	0	0	0	1	3
Injection site 3	0	0	0	0	0	0	0	2
Injection site 4	0	0	0	0	0	0	0	1
Injection site 6	0	0	0	0	0	0	0	1
Injection site 10	0	0	0	0	0	0	0	2
Injection site 16	0	0	0	0	0	0	0	1
Injection site 20	0	0	0	0	0	0	0	1
Injection site 21	0	0	0	0	0	0	0	2

Body weights: Body weights were recorded for each animal at least twice during the pre-treatment period, on the first day of treatment and then once a week until the end of the study.

There were no statistically significant differences in group mean body weights or group mean body weight gains in males or females treated with icatibant compared to vehicle treated control animals.

Food consumption: Food consumption was recorded for each animal by noting the difference between the quantity given and the remaining food left the next morning. This was estimated daily for at least 7 days before the beginning of treatment and throughout the study period. Food consumption was expressed as a percentage of the quantity given.

There were no treatment related effects on food consumption in the icatibant treated groups compared to the vehicle treated group.

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Ophthalmoscopy: All animals were examined prior to treatment and on 1 occasion at the end of the treatment period. Pupillary light and blink reflexes were recorded. Pupils were dilated with tropicamide and appendages, optic media and fundus were examined by indirect ophthalmoscopy. Slit-lamp was used to evaluate any abnormalities.

There were no treatment related observations in icatibant treated animals compared to the vehicle treated animals.

EKG: EKG examinations were performed on all animals before the treatment period, at the end of Week 1 at predose, 1 and 4 hours post-dose, and during Weeks 12 or 13 at predose and 1 and 4 hours post-dose. This examination was performed using leads I, II, III and Cardiovit AT-6 Schiller. Heart rate, PQ- and QT-intervals and QRS-complex duration were determined, and QT was corrected using Bazett and Fridericia formulae. Blood pressure was also evaluated.

There were no statistically significant differences in heart rate, PQ- QRS- or QT-intervals in males or females treated with icatibant compared to the vehicle control treated animals at Week 1 of dosing prior to dosing and 1 hours after dosing. However, a slight but statistically significant decrease in heart rate was observed in HD males (-29%) and HD females (-21%) 6 hours post dose during Week 1 of dosing compared to the vehicle control groups. A decrease in diastolic BP was noted in HD males (-39%) 6 h post-dose on Week 1 of dosing compared to the vehicle control group.

At Week 13 of dosing, there were no statistically significant differences in heart rate, PQ-QRS- or QT-intervals evaluated in the icatibant treated males and females compared to the vehicle control animals. There were no statistically significant changes in QT corrected for heart rate using either Bazett or Fridericia formulae in the icatibant treated animals compared to the vehicle treated animals. However, a statistically significant decrease in systolic BP (-19%) was observed in HD males 1 h post-dose in Week 12 and (-22%) was observed in HD females before dosing in Week 13, respectively.

Hematology: Blood samples were taken from a jugular vein. Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period, during Week 5 and then at Week 13 of the treatment period. A complete hematological battery was assessed.

A slight decrease in leukocyte number was observed in HD males at Weeks 5 and 13 compared to vehicle control animals. There was no exacerbation of this finding from Week 5 to 13 and this was not observed in females. HD females had a slight increase in platelet counts Weeks 5 and 13 of dosing compared to the vehicle control animals. There was no exacerbation of this finding in females and this finding was not observed in males.

Parameter	**Week**	**Sex**	**Icatibant Dose (mg/kg/day)**		
			10	**30**	**100/2x30**
Leukocytes (g/L)	5	M	-	-	- 41%
	13	M	-	-	-42%
	5	F	-	-	-
	13	F	-	-	-
Plat (g/L)	5	M	-	-	-
	13	M	-	-	-
	5	F	-	-	+40%

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	13	F	-	-	+31%

Clinical chemistry: Blood samples were taken from a jugular vein. Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period, during Week 5 and then at Week 13 of the treatment period. A complete clinical chemistry battery was assessed.

Changes in clinical chemistry parameters in the icatibant treated animals compared to the vehicle control treated animals were limited to males. Most changes in clinical chemistry parameters were observed in HD males with occasional changes in the LD or MD male groups.

Parameter	**Week**	**Sex**	**Icatibant Dose (mg/kg/day)**		
			10	**30**	**100/2x30**
Triglycerides (mmol/L)	5	M	-	-	+111%
	13	M	+67%	+97%	+161%
	5	F	-	-	-
	13	F			
ALP (IU/L)	5	M	-	-	+190%
	13	M	-	-	+143%
	5	F	-	-	-
	13	F			
ASAT (IU/L)	5	M	-	-29%	-32%
	13	M	-	-	-68%
	5	F	-	-	-
	13	F	-	-	-
LDH (IU/L)	5	M	-	-	- 40%
	13	M	-	-	-39%
	5	F	-	-	-
	13	F	-	-	-
Creatinine (umol/L)	5	M	-	-	-
	13	M	-	-	-25%
	5	F	-	-	-
	13	F	-	-	-
CK (IU/L)	5	M	-	-	-
	13	M	-36%	-26%	-48%
	5	F	-	-	-
	13	F	-	-	-

Urinalysis: Urine was collected from all animals once before the treatment period, during Week 5 and then at Week 13 of the treatment period. A complete urinalysis was conducted.

There were no statistically significant changes in urine parameters in the icatibant treated animals compared to the vehicle treated animals.

Gross pathology: A complete necropsy was conducted on all study animals.

Macroscopic findings were observed at injection sites (hematomas and edema), the male (testes, epididymides and prostate) and female (ovaries and uterus) reproductive organs (reduced size), thymus (reduced size), liver (enlarged) and adrenal gland (enlarged).

Observation	**Icatibant Dose (mg/kg/day)**							
	0		**10**		**30**		**100/2x30**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**

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Observation	Icatibant Dose (mg/kg/day)							
	0		10		30		100/2x30	
	M	F	M	F	M	F	M	F
Adrenal Glands enlarged	-	-	-	-	-	-	1	-
Epididymides reduced in size	-	NA	4	NA	4	NA	4	NA
Injection site 1								
Hematoma subcutaneous tissue	-	-	-	-	-	-	1	-
Hematoma subcutaneous tissue, diffuse	-	-	-	-	-	-	2	1
Injection site 10								
Hematoma subcutaneous tissue	-	-	-	-	-	-	1	-
Hematoma subcutaneous tissue, diffuse	-	-	-	-	-	-	1	2
Injection site 13								
Hematoma subcutaneous tissue	-	-	-	-	-	-	1	-
Hematoma subcutaneous tissue, diffuse	1	1	-	-	2	1	2	4
Injection site 15								
Hematoma subcutaneous tissue, diffuse	-	-	-	-	-	-	1	-
Injection site 16								
Hematoma, diffuse	2	-	-	2	3	-	3	2
Injection site 18								
Hematoma subcutaneous tissue, diffuse	-	-	-	-	-	1	1	1
Injection site 19								
Diffuse	-	-	-	-	-	-	1	-
Edema	-	-	-	-	2	-	1	1
Hematoma diffuse	2	1	1	1	2	2	2	2
Injection site 21								
Hematoma	-	-	-	-	-	-	1	-
Thickened	-	-	-	-	-	-	1	-
Injection site 2								
Edema	-	-	-	-	-	2	-	2
Hematoma, diffuse	-	1	-	-	-	2	-	3
Injection site 3								
Hematoma, diffuse	-	-	-	-	-	-	1	-
Injection site 4								
Hematoma, diffuse	-	-	-	-	-	-	2	-
Injection site 5								
Hematoma, diffuse	-	-	-	-	-	1	1	-
Injection site 7								
Thickened tissue	-	-	-	-	-	-	1	-
Edema	-	-	-	-	-	-	-	1
Hematoma	-	-	-	-	-	-	-	1
Hematoma diffuse	-	-	-	-	-	1	-	2
Injection site 12								
Hematoma, diffuse	-	-	-	-	-	-	-	1
Liver								
Enlarged	-	-	-	-	-	-	1	-
Prostate reduced in size	-	NA	4	NA	4	NA	4	NA
Testes reduced in size	-	NA	4	NA	4	NA	4	NA
Thymus reduced in size	-	-	1	1	2	2	2	2
Ovaries, reduced in size	NA	-	NA	3	NA	2	NA	2
Uterus, reduced in size	NA	-	NA	4	NA	4	NA	4

- No macroscopic finding observed
NA- Not applicable

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Organ weights: The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus and thyroids with parathyroids were weighed. Absolute organ weights and organ weights relative to body weights were determined. No organ weights relative to brain weights were provided.

Decreases in reproductive organ weights (absolute and relative to body weights) were observed in males and females compared to the vehicle control treated animals. Adrenal weights relative to body weights were increased in males and females compared to the vehicle control treated animals. Liver weights relative to body weights were increased in MD and HD males compared to the vehicle control treated animals. This was not observed in females. Thymic weights (absolute and relative to body weights) were decreased in both males and females compared to respective vehicle control treated animals. No organ weights relative to brain weights were provided.

Organ weight change relative to controls

Organ	Sex	Icatibant dose (mg/kg/day)		
		10	30	100/2x 30
Adrenals wt	M	-	-	-
	F	-	-	+35%
Adrenals rel to BW	M	-	-	+46%
	F	+14%	+24%	+36%
epididymides wt	M	-59%	-65%	-60.5%
	F	NA	NA	NA
epididymides rel BW	M	-57%	-64%	-58%
	F	NA	NA	NA
Liver wt	M	-	-	+46.5%
	F	-	-	-
Liver rel BW	M	-	+34%	+60%
	F	-	-	-
Prostate wt	M	-75%	-78%	-73%
	F	NA	NA	NA
Prostate rel BW	M	-73%	-76%	-70%
	F	NA	NA	NA
Testes wt	M	-81%	-79%	-80%
	F	NA	NA	NA
Testes rel BW	M	-81%	-79%	-78%
	F	NA	NA	NA
Thymus wt	M	-25%	-42%	-43%
	F	-	-	-
Thymus rel BW	M	-22%	-41%	-39%
	F	-9.0%	-34%	-22%
Ovary wt	M	NA	NA	NA
	F	-76%	-77%	-72%
Ovary rel BW	M	NA	NA	NA
	F	-74%	-77%	-74%

- No statistically significant difference compared to controls
NA- Not applicable

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

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All study animals had tissues prepared for histopathological examination. All macroscopic lesions from all animals were microscopically examined, All tissues from Groups 1 to 4 were microscopically evaluated and the least recent and most recent injection sites from all animals were examined from Groups 1 to 4. At least 10% of the histological slides from control (group 1) and HD (Group 4) in each sex group and all slides from identified target organs were peer reviewed by Dr. James Armstrong. A complete histological battery was microscopically examined with the exception of the lachrymal gland, pharynx and seminal vesicles. All other tissues were examined.

Observation	**Icatibant Dose (mg/kg/day)**							
	0		**10**		**30**		**100/2x30**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Injection site 1 n=4								
Subcut hemorrhage	-	-	-	-	-	-	2 (3.5)	1 (2.0)
Subcut inflam cells	-	-	-	-	-	-	3 (1.7)	-
Subcut collagen degener	-	-	-	-	-	-	1 (3.0)	-
Subcut hemosider	-	-	-	-	-	-	1 (2.0)	-
Subcut fibrosis	-	-	-	-	-	-	3 (2.0)	1 (2.0)
Injection site 10 n=4								
Subcut hemorrhage	-	-	-	-	-	-	2 (3.5)	2 (3.0)
Subcut collagen degener	-	-	-	-	-	-	2 (3.0)	2 (3.0)
Subcut inflam cells	-	-	-	-	-	-	3 (3.0)	2 (2.5)
Subcut fibrosis	-	-	-	-	-	-	2 (2.0)	1 (2.0)
Subcut hemosider	-	-	-	-	-	-	2 (2.0)	-
Deg./nec. Myop. Sub. m	-	-	-	-	-	-	3 (2.0)	1 (3.0)
Injection site 11 n=4								
Subcut inflam cells	-	-	-	-	-	-	1 (3.0)	-
Subcut hemorrhage	-	-	-	-	-	-	1 (1.0)	-
Subcut hemosider	-	-	-	-	-	-	1 (3.0)	-
Subcut fibrosis	-	-	-	-	-	-	1 (3.0)	-
Deg./nec. Myop. Sub. m	-	-	-	-	-	-	1 (1.0)	-
Injection site 13 n=4								
Subcut hemorrhage	1(2.0)	1(3.0)	-	-	2 (1.5)	1 (3.0)	3 (3.7)	4 (2.8)
Subcut inflam cells	1(1.0)	-	-	-	2 (3.0)	1 (3.0)	3 (2.7)	4 (3.0)
Subcut collagen degener	-	-	-	-	2 (2.5)	-	3 (2.7)	3 (3.0)
Subcut edema	-	-	-	-	-	-	1 (2.0)	-
Subcut fibrosis	-	-	-	-	2 (1.5)	1 (2.0)	2 (1.5)	2 (2.0)
Deg./nec. Myop. Sub. M	-	1 (1.0)	-	-	2 (3.0)	1 (3.0)	3 (3.0)	4 (3.8)
Subcut hemosider	-	-	-	-	-	-	-	1 (1.0)
Injection site 15 n=4								
Subcut inflam cells	-	-	-	-	1 (2.0)	-	1 (2.0)	-
Subcut collagen degener	-	-	-	-	1 (2.0)	-	1 (2.0)	-
Subcut fibrosis	-	-	-	-	1 (2.0)	-	1 (2.0)	-
Deg./nec. Myop. Sub. m	-	-	-	-	1 (2.0)	-	1 (2.0)	-
Injection site 16 n=4								
Subcut hemorrhage	2(2.0)	-	-	-	1 (2.0)	-	3 (3.0)	2 (3.0)
Subcut inflam cells	-	-	-	-	3 (2.3)	-	3 (3.3)	2 (3.0)
Subcut collagen degener	-	-	-	-	2 (2.5)	-	3 (3.0)	2 (2.5)
Subcut edema	-	-	-	-	1 (3.0)	-	3 (2.7)	-
Subcut hemosider	-	-	-	-	-	-	1 (2.0)	-
Deg./nec. Myop. Sub. m	-	-	-	-	3 (3.0)	-	3 (2.7)	2 (2.5)
Injection site 17 n=4								
Subcut inflam cells	-	-	-	-	-	-	1 (3.0)	-

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Observation	Icatibant Dose (mg/kg/day)							
	0		10		30		100/2x30	
	M	F	M	F	M	F	M	F
Subcut hemosider	-	-	-	-	-	-	1 (2.0)	1 (2.0)
Subcut fibrosis	-	-	-	-	-	-	1 (3.0)	1 (1.0)
Injection site 18 n=4								
Subcut hemorrhage	-	-	-	-	-	-	1 (4.0)	1 (3.0)
Subcut inflam cells	-	-	-	-	-	1 (2.0)	1 (2.0)	2 (2.5)
Subcut fibrosis	-	-	-	-	-	1 (2.0)	1 (2.0)	2 (2.0)
Deg./nec. Myop. Sub. M	-	-	-	-	-	1 (1.0)	2 (2.0)	2 (1.5)
Injection site 19 n=4								
Subcut collagen degener	2(1.5)	-	1 (3.0)	-	4 (3.0)	2 (3.0)	4 (3.8)	2 (3.5)
Subcut edema	-	-	-	-	2 (3.0)	2 (2.0)	4 (2.8)	2 (3.5)
Subcut hemosider	-	-	-	-	-	-	1 (1.0)	1 (2.0)
Subcut inflam cells	-	2 (1.0)	-	1 (2.0)	-	2 (3.0)	-	3 (2.7)
Subcut hemorrhage	-	-	-	1 (2.0)	-	2 (2.0)	-	2 (4.0)
Subcut fibrosis	-	-	-	1 (2.0)	-	2 (1.0)	-	4 (1.3)
Deg/nec. Myop.sub m.	-	1 (1.0)	-	1 (2.0)	-	2 (3.0)	-	3 (3.0)
Injection site 20 n=4								
Subcut hemorrhage	-	-	-	-	-	-	1 (1.0)	-
Subcut inflam cells	-	-	-	-	1 (2.0)	-	1 (3.0)	1 (2.0)
Subcut collagen degener	-	-	-	-	-	-	1 (3.0)	-
Subcut edema	-	-	-	-	-	-	1 (2.0)	-
Deg./nec. Myop. Sub. M	-	-	-	-	1 (3.0)	-	1 (2.0)	-
Subcut hemosider	-	-	-	-	-	-	-	1 (2.0)
Injection site 21 n=4								
Subcut inflam cells	-	-	-	-	-	-	4 (2.3)	1 (4.0)
Subcut collagen degener	-	-	-	-	-	-	2 (3.0)	1 (2.0)
Subcut edema	-	-	-	-	-	-	2 (3.0)	-
Subcut hemosider	-	-	-	-	-	-	3 (1.3)	2 (2.0)
Subcut fibrosis	-	-	-	-	-	-	3 (1.7)	2 (2.0)
Deg./nec. Myop. Sub. m	-	-	-	-	-	-	4 (2.5)	1 (3.0)
Injection site 2 n=4								
Subcut inflam cells	-	4 (1.5)	-	2 (2.0)	2 (1.0)	3 (3.3)	3 (1.7)	4 (3.3)
Subcut hemosider	-	-	-	-	-	-	2 (3.0)	1 (2.0
Subcut collagen degener	-	1 (2.0)	-	1 (2.0)	-	3 (3.0)	2 (2.0)	4 (3.3)
Subcut mineralization	-	-	-	-	-	-	2 (1.5)	-
Deg./nec. Myop. Sub. M	-	2 (1.0)	1 (1.0)	1 (1.0)	-	3 (3.3)	1 (1.0)	2 (2.5)
Subcut hemorrhage	-	2 (2.0)	-	1 (1.0)	-	3 (2.3)	-	3 (3.0)
Subcut edema	-	-	-	-	-	3 (2.7)	-	3 (3.7)
Injection site 3 n=4								
Subcut fibrosis	-	-	-	-	-	-	1 (2.0)	-
Subcut mineralization	-	-	-	-	-	-	1 (1.0)	-
Injection site 4 n=4								
Subcut hemorrhage	-	-	-	-	-	-	2 (2.5)	-
Subcut inflam cells	-	-	-	-	-	-	3 (1.7)	-
Subcut collagen degener	-	-	-	-	-	-	1 (3.0)	-
Subcut hemosider	-	-	-	-	-	-	2 (2.0)	-
Deg./nec. Myop. Sub. m	-	-	-	-	-	-	3 (2.7)	-
Injection site 5 n=4								
Subcut hemorrhage	-	-	-	-	-	-	1 (3.0)	-
Subcut inflam cells	-	-	-	-	-	-	2 (2.0)	-
Subcut collagen degener	-	-	-	-	-	-	1 (3.0)	-
Subcut edema	-	-	-	-	-	-	1 (3.0)	-
Subcut fibrosis	-	-	-	-	-	1 (1.0)	1 (2.0)	-

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Observation	Icatibant Dose (mg/kg/day)							
	0		10		30		100/2x30	
	M	F	M	F	M	F	M	F
Subcut hemosider	-	-	-	-	-	-	1 (2.0)	-
Deg./nec. Myop. Sub. m	-	-	-	-	-	-	2 (1.5)	-
Injection site 6 n=4								
Subcut inflam cells	-	-	-	-	-	-	1 (2.0)	-
Subcut hemosider	-	-	-	-	-	-	1 (2.0)	-
Subcut fibrosis	-	-	-	-	-	-	1 (3.0)	-
Deg./nec. Myop. Sub. m	-	-	-	-	-	-	1 (1.0)	-
Injection site 7 n=4								
Subcut inflam cells	-	-	-	-	-	1 (4.0)	3 (1.3)	3 (3.7)
Subcut fibrosis	-	-	-	-	-	-	3 (2.3)	1 (2.0)
Subcut hemosider	-	-	-	-	-	-	3 (1.7)	-
Subcut mineralization	-	-	-	-	-	-	1 (2.0)	-
Deg./nec. Myop. Sub. M	-	-	-	-	-	1 (1.0)	1 (3.0)	3 (3.7)
Subcut hemorrhage	-	-	-	-	-	1 (2.0)	-	4 (2.3)
Subcut collagen degener	-	-	-	-	-	1 (3.0)	-	3 (3.3)
Subcut edema	-	-	-	-	-	-	-	2 (3.0)
Injection site 9 n=4								
Subcut inflam cells	-	-	-	-	-	-	1 (1.0)	-
Subcut hemorrhage	-	-	-	-	-	-	1 (2.0)	-
Subcut hemosider	-	-	-	-	-	-	2 (2.0)	-
Subcut fibrosis	-	-	-	-	-	-	2 (2.5)	-
Deg./nec. Myop. Sub. m	-	-	-	-	-	-	2 (2.0)	-
Injection site 12 n=4								
Subcut hemorrhage	-	-	-	-	1 (2.0)	-	-	1 (2.0)
Subcut inflam cells	-	-	-	-	1 (3.0)	-	1 (2.0)	1 (2.0)
Subcut collagen degener	-	-	-	-	1 (3.0)	-	-	-
Subcut edema	-	-	-	-	1 (2.0)	-	-	-
Subcut hemosider	-	-	-	-	-	-	1 (2.0)	-
Subcut fibrosis	-	-	-	-	-	-	1 (2.0)	1 (2.0)
Deg./nec. Myop. Sub. m	-	-	-	-	1 (3.0)	-	1 (1.0)	-
Liver n=4								
Vacuolated hepatocy	-	-	-	-	-	-	1 (3.0)	-
Thymus n=4								
Lymphoid depletion	-	3 (1.7)	4 (2.5)	4 (2.5)	4 (2.5)	4 (3.5)	2 (4.0)	4 (4.5)
Adrenal Glands n=4								
Cort. Cell. Hypertrophy	-	-	-	-	-	-	1 (3.0)	-
Vacuol. Cortical cell	-	-	-	-	-	-	3 (2.7)	4 (2.8)
Prostate n=4								
Immature	-	NA	4 (NA)	NA	4(NA)	NA	4(NA)	NA
Testes n=4								
Immature	-	NA	4(NA)	NA	4(NA)	NA	4(NA)	NA
Epididymides n=4								
Immature	-	NA	4(NA)	NA	4(NA)	NA	4(NA)	NA
Mammary gland area								
No mammary development	NA	-	NA	4(NA)	NA	4(NA)	NA	4(NA)
Ovaries n=4								
Immature	NA	-	NA	4(NA)	NA	4(NA)	NA	4(NA)
Uterus n=4								
Immature	NA	-	NA	4(NA)	NA	4(NA)	NA	4(NA)
Vagina n=4								
Immature	NA	-	NA	4(NA)	NA	4(NA)	NA	4(NA)

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- No macroscopic finding observed
NA- Not applicable
(#)- mean severity: grade 1= minimal; grade 2= slight; grade 3= moderate; grade 4= marked; grade 5= massive

After daily SC administration of icatibant in males and female dogs, the primary target organs of toxicity were the reproductive organs (males- testes, epididymides and prostate and females- ovaries, uterus, vagina and mammary glands), adrenal glands, injection sites, thymus and liver. Male and female reproductive organs were immature in all animals at all doses of icatibant. Adrenal gland findings were observed only at the HD. Injection site effects were noted at multiple sites and were shown most frequently and with most severity at the HD. Lymphoid depletion was observed in the thymus and increased in incidence and severity with increased icatibant dose. Liver changes were low in incidence and only observed at the HD of icatibant.

Based on the immature reproductive organ findings in the male and female dogs and lymphoid depletion in the thymus at all doses of icatibant, no NOAEL could be defined for this study.

Toxicokinetics: Blood samples were taken on Day 1 and in week 13 from all animals to determine icatibant levels and the metabolite M_2 (b) (4) levels. TK data are summarized in the following table.

	Parameter	Icatibant Dose (mg/kg/day)											
		TK of Icatibant						TK of M2 Metabolite					
		10		30		100		10		30		100	
		M	F	M	F	M	F	M	F	M	F	M	F
Day 1	C_{max} (mcg/mL)	10.9	11.24	47.7	41.4	NC	74.4	0.40	0.32	1.16	1.01	NC	1.38
	T_{max} (h)	1.0	1.0	1.5	1.75	NC	2.0	1.75	2.00	3.00	2.50	NC	4.0
	AUC_{0-t} (mcg*h/mL)	24.6	22.9	190	164	NC	383	1.14	0.90	6.14	5.26	NC	7.65
Wk 13	C_{max} (mcg/mL)	17.6	17.7	34.4	41.6	82.3	71.0	0.39	0.31	NC	NC	0.63	0.77
	T_{max} (h)	0.75	0.75	1.75	1.50	0.88	0.75	2.0	2.0	NC	NC	2.0	2.0
	AUC_{0-t} (mcg*h/mL)	38.2	31.6	80.9	83	246	213	1.15	0.77	NC	NC	3.10	3.46

NC- not calculated because individual values were invalid (QC rejected).

On Day 1 and Week 13 of dosing, the C_{max} and AUCs of icatibant were similar between males and females, suggesting that there were no significant gender differences. This was also observed for the M2 metabolite. On Day 1 of dosing, the C_{max} increased in an approximately dose-proportional manner from the LD to the MD. However, from the MD to the HD, the C_{max} of icatibant was sub-proportional with an increase by ~1.8-fold instead of the expected ~ 3-fold increase. The AUCs for icatibant increased in a supra-proportional manner from LD to MD (8-10 fold increase) on Day 1. At week 13, the C_{max} and AUC increased in a dose proportional manner form the LD to the MD but C_{max} was sub-proportional from the MD to the HD (~1.7-fold increase instead of ~ 3-fold increase). AUC increased approximately dose-proportionately. However, the dosing schedule was modified throughout the study period in the HD group.

The M2 metabolite formed within ~ 2-4 hours post-icatibant administration. The M2 metabolite C_{max} and AUCs increased from the LD to the MD in an approximately dose related

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manner. From the MD to the HD, M2 only slightly increased in C_{max} and AUC levels for those levels that were quantifiable. This increase was sub-proportionate to dose.

Study title: 13-week toxicity study by subcutaneous route in sexually immature beagle dogs with daily or twice weekly T.I.D. dosing followed by a 14-week treatment-free period

Key study findings:

- Clinical signs included ↑ erythema and edema at multiple sites with ↑ dose of icatibant, which were reversible after cessation of treatment.
- The target organs of toxicity in the dog after daily SC treatment with icatibant are thymus and the male (testes, epididymides and prostate) and female (ovaries, oviducts, uterus and vagina) reproductive tissues as reflected by reduction in these organ wts relative to body weights, macroscopic findings of reduced size and microscopic findings of reduced size. After the 13-week recovery period, 1 of 2 males showed the testes and prostate as immature where 1 male had recovered and there were no findings in female reproductive organs suggesting these adverse effects were reversible.
- When males were treated twice weekly with icatibant, no effects were observed in the male reproductive organs when icatibant was administered SC twice weekly but females treated with SC administration of icatibant twice weekly showed delayed or impaired maturation of the reproductive organs and lymphoid depletion in the thymus was observed in males and females.
- Hormonal analysis showed that icatibant induced a decrease of testosterone, LH and FSH in male dogs, which secondarily induced sexual immaturity.
- No NOAEL was determined in this study based on the adverse effects on male and female reproductive organs and thymic lymphoid depletion. However, twice weekly administration of icatibant resulted in less severe toxicity in males than with daily dosing. Systemic exposures to icatibant ranged from 13.7 to 57.2 µg*h/mL for daily administration (no AUC was determined for TID dosing) and M2 ranged from 1.45 to 2.5 µg*h/mL in this study.

Study no.: JE049-0118
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: October 27, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batch # E083, and 82.7% pure

Methods

Doses: 0 (vehicle-Group 1), 3 (Group 2), 10 (Group 3) mg/kg daily and 10 mg/kg (Group 4) administered twice weekly at 3.3 mg/kg TID
Species/strain: Beagle dogs
Number/sex/group or time point (main study): 3/sex/group

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Route, formulation, volume, and infusion rate: Subcutaneous, formulated in isotonic saline solution (0.9% NaCl), administered in 0.4 mL/kg and infusion rate was not applicable.
Satellite groups used for toxicokinetics or recovery: An additional 2 dogs/sex/group were used as the recovery group
Age: 6.5 months old
Weight: males: 7.0-9.3 kg and females: 6.4-7.9 kg
Sampling times: See below
Unique study design or methodology (if any): For the VC, 3 and 10 mg/kg/day groups (Groups 1, 2 and 3, respectively), 5 dogs/sex/group were administered either the vehicle or icatibant via the subcutaneous route once daily. Group 4 was administered 10 mg/kg of icatibant as 3 SC injections which were delivered twice weekly. At the end of the treatment period (total of 13-weeks), animals were sacrificed, except for the last 2 surviving dogs of each sex and group, which were kept for a 14-week recovery period.

Observations, Times and Results

Mortality: All animals were checked for morbidity or mortality at least twice a day.
There were no deaths prior to scheduled sacrifice throughout this study.

Clinical signs: All animals were observed at least once a day for clinical signs of toxicity. The type of findings observed and the incidence of each finding during the treatment and the recovery periods are summarized below. Erythema and edema (increased size at multiple sites) increased in incidence with increase dose of icatibant. Gastrointestinal disturbances (vomiting and soft feces) were observed in the icatibant treated dogs and were not observed in the vehicle control treated animals. After cessation of treatment, all signs were reversible.

Observation	**Icatibant Dose (mg/kg/day)**							
	0		**3**		**10**		**3.3 TID**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Erythema: N=5								
Right ear	0	1	5	3	5	5	5	5
Left ear	0	1	4	3	5	4	4	5
Generalized	0	0	2	1	2	2	2	4
Recov Erythema: N=2								
Right ear	0	0	1	1	0	0	1	0
Left ear	0	0	1	0	0	0	1	0
Generalized	0	0	0	0	1	0	0	0
Feces, Soft N=5	0	0	0	1	3	0	0	0
Recov Feces, Soft N=2	0	0	0	0	0	0	0	0
Vomit/whitish N=5	0	0	0	0	1	0	0	0
Vomit/yellowish	0	0	0	0	1	0	0	0
Recov Vomit/whitish N=2	0	0	0	0	0	0	0	0
Vomit/yellowish	0	0	0	0	0	0	0	0
Increase in size: N=5								
Head	0	0	3	0	3	3	3	4
Right ear	0	0	5	5	5	4	4	4
Left ear	0	0	1	5	5	4	4	4
Around right eye	0	0	1	0	0	0	0	0
Right forelimb	0	0	0	0	1	0	1	1

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Observation	Icatibant Dose (mg/kg/day)							
	0		3		10		3.3 TID	
	M	F	M	F	M	F	M	F
Left forelimb	0	0	0	0	1	0	1	1
Right shoulder	0	0	0	0	1	0	0	0
Recov Increase in size: N=2								
Head	0	0	0	0	0	0	0	0
Right ear	0	0	0	0	0	0	0	0
Left ear	0	0	0	0	0	0	0	0
Around right eye	0	0	0	0	0	0	0	0
Right forelimb	0	0	0	0	0	0	0	0
Left forelimb	0	0	0	0	0	0	0	0
Right shoulder	0	0	0	0	0	0	0	0

Body weights: Body weights were recorded for each animal at least twice during the pre-treatment period, on the first day of treatment and then once a week until the end of the study.

There were no statistically significant differences in group mean body weights or group mean body weight gains in males or females treated with icatibant compared to vehicle treated control animals.

Food consumption: Food consumption was recorded for each animal by noting the difference between the quantity given and the remaining food left the next morning. This was estimated daily for at least 7 days before the beginning of treatment and throughout the study period. Food consumption was expressed as a percentage of the quantity given.

There were no treatment related effects on food consumption in the icatibant treated groups compared to the vehicle treated group.

Ophthalmoscopy: No ophthalmic examination was conducted in this study.

EKG: No EKG or blood pressure measurement was conducted in this study.

Hematology: Blood samples were taken from an appropriate vein. Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period, during Week 5, at Week 12 of the treatment period and at Week 21 and 27 during the recovery period. A complete hematological battery was assessed with the exception of hematocrit.

There were no statistically significant changes in the hematology parameters examined in the icatibant treated males or females compared to the vehicle control treated males.

Clinical chemistry: Blood samples were taken from a jugular vein. Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period, during Week 5, at Week 12 of the treatment period and at Week 21 and 27 during the recovery period. A complete clinical chemistry battery was assessed.

There were no statistically significant differences in the clinical chemistry parameters evaluated in males or females treated with icatibant compared to those treated with the vehicle control.

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Urinalysis: Urine was collected from all animals once before the treatment period, during Week 5 and then at Week 12 of the treatment period and during the recovery period. A complete urinalysis was conducted.

There were no statistically significant changes in urine parameters in the icatibant treated animals compared to the vehicle treated animals.

Gross pathology: A complete necropsy was conducted on all study animals.

Macroscopic findings were observed at injection sites (hematomas and edema), the male (testes, epididymides and prostate) and female (ovaries, oviducts and uterus) reproductive organs, thymus and liver. The findings at the injection site, thymus and liver were low in incidence and were not observed macroscopically in the recovery group animals. The findings in the male reproductive organs were not observed after the recovery period (epididymides and testes) or were low in incidence compared to the treatment period (prostate). Similarly, female reproductive organ findings were completely reversed in the ovaries and oviducts and were decreased in incidence in the uterus after the recovery period.

Observation	**Icatibant Dose (mg/kg/day)**							
	0		**3**		**10**		**3.3 TID**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Injection site 16 n=3								
Hematoma, diffuse	-	-	-	-	-	1	-	-
Rec. Injection site 16 n=2								
Hematoma, diffuse	-	-	-	-	-	-	-	-
Liver n=3								
Focus yellowish	-	-	-	-	-	-	1	-
Paleness	-	-	-	-	-	-	-	-
Rec Liver n=2								
Focus yellowish	-	-	-	-	-	-	-	-
Paleness	-	-	-	-	-	-	-	1
Epididymides reduced in size n=3	-	NA	2	NA	3	NA	-	NA
Rec. Epididymides reduced in size n=2	-	NA	-	NA	-	NA	-	NA
Prostate reduced in size n=3	-	NA	3	NA	3	NA	-	NA
Rec. prostate reduced in size n=2	-	NA	-	NA	1	NA	-	NA
Testes n=3		NA		NA		NA		NA
Reduced in size	-		3		3		-	
Soft	-		2		2		-	
Rec Testes n=2		NA		NA		NA		NA
Reduced in size	-		-		-		-	
Soft	-		-		-		-	
Thymus reduced in size n=3	1	-	-	-	1	1	1	-
Rec. Thymus reduced in size n=2	-	-	-	-	-	-	-	-
Ovaries, reduced in size n=3	NA	-	NA	-	NA	2	NA	1
Rec Ovaries, reduced in size n=2	NA	-	NA	-	NA	-	NA	-
Oviducts, reduced in size n=3	NA	-	NA	-	NA	2	NA	1
Rec Oviducts, reduced in size n=2	NA	-	NA	-	NA	-	NA	-
Uterus, reduced in size n=3	NA	-	NA	3	NA	3	NA	1
Rec. Uterus, reduced in size n=2	NA	-	NA	-	NA	1	NA	-

\- No macroscopic finding observed
NA- Not applicable

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Organ weights: The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus and thyroids with parathyroids were weighed. Absolute organ weights and organ weights relative to body weights were determined. No organ weights relative to brain weights were provided.

Decreases in reproductive organ weights (absolute and relative to body weights) were observed in males and females compared to the vehicle control treated animals through out the treatment period. After the 13-week recovery period, epididymal and prostate weights in males remained decreased but with less severity than the decreased weight during the dosing period. Testicular weights returned to normal compared to the vehicle control groups. Ovarian weights returned to normal after the 13-week recovery period as well. Adrenal weights relative to body weights were increased in males compared to the vehicle control treated animals but only slight. After the recovery period, there were no statistically significant differences in adrenal weights in the icatibant group compared to the vehicle control group.

Organ weight change relative to controls

Organ	Sex	Icatibant dose (mg/kg/day)		
		3	10	3.3 TID
epididymides wt	M	-71%	-70%	-
	F	NA	NA	NA
Rec epididymides wt	M	-32%	-29%	-
	F	NA	NA	NA
epididymides rel BW	M	-71%	-68%	-21%
	F	NA	NA	NA
Rec epididymides rel BW	M	-28%	-25%	-21%
	F	NA	NA	NA
Prostate wt	M	-87%	-80%	-
	F	NA	NA	NA
Rec prostate wt	M	-	-52%	-
	F	NA	NA	NA
Prostate rel BW	M	-86%	-79%	-49%
	F	NA	NA	NA
Rec Prostate rel BW	M	-20%	-49%	-
	F	NA	NA	NA
Testes wt	M	-79%	-79%	-
	F	NA	NA	NA
Rec testes wt	M	-	-	-
	F	NA	NA	NA
Testes rel BW	M	-79%	-78%	-
	F	NA	NA	NA
Rec Testes rel BW	M	-	-	-
	F	NA	NA	NA
Ovary wt	M	NA	NA	NA
	F	-58%	-66%	-57%
Rec ovary wt	M	NA	NA	NA
	F	-	-	-
Ovary rel BW	M	NA	NA	NA
	F	-55%	-63%	-51%
Rec ovary rel BW	M	NA	NA	NA
	F	-	-	-

- No statistically significant difference compared to controls
NA- Not applicable

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Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

All study animals had tissues prepared for histopathological examination at the end of the treatment period. All macroscopic lesions were from all animals were microscopically examined. Reproductive organs and accessory organs (Groups 1 to 4) were microscopically examined. At the end of the recovery period, all macroscopic lesions and reproductive organs and accessory organs were microscopically examined. The thymus was not examined at the end of the recovery period. Peer review was performed for at least 20% of the slides in the control and HD groups. A complete histological battery was microscopically examined with the exception of the lachrymal gland, pharynx and seminal vesicles. All other tissues were examined.

Observation	**Icatibant Dose (mg/kg/day)**							
	0		**3**		**10**		**3.3 TID**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Epididymides n=3								
Immature	-	NA	3(NA)	NA	3(NA)	NA	-	NA
Sloughed cells/lumen	-		-		-		-	
Oligospermia	-		-		-		-	
aspermia	-		-		-		-	
Rec.Epididymides n=2								
Immature	-	NA	-	NA	-	NA	-	NA
Sloughed cells/lumen	-		2 (1.0)		2 (1.5)		-	
Oligospermia	-		1 (4.0)		-		-	
aspermia	-		-		1 (NA)		-	
Prostate n=3								
Immature, reduced size	-	NA	3 (NA)	NA	3(NA)	NA	-	NA
Alveolar atrophy	-		-		-		-	
Rec Prostate n=2								
Immature, reduced size	-	NA	1 (NA)	NA	2 (NA)	NA	-	NA
Alveolar atrophy	-		-		1 (2.0)		-	
Testes n=3								
Immature	-	NA	3(NA)	NA	3(NA)	NA	-	NA
Peripubertal tests	-		-		-		-	
Tubuloar vacuolation	-		-		-		-	
Tubular degeneration	-		-		-		-	
Sloughed cells lumen	-		-		-		-	
Spermatid sumplasts	-		-		-		-	
Rec Testes n=2								
Immature, peripubertal	-	NA	1 (NA)	NA	1 (NA)	NA	-	NA
Tubuloar vacuolation	-		-		1 (2.0)		-	
Tubular degeneration	-		-		1 (2.0)		-	
Sloughed cells lumen	-		-		1 (2.0)		-	
Spermatid sumplasts	-		-		1 (1.0)		-	
Thymus n=3								
Lymphoid depletion	2(1.5)	1 (2.0)	1 (2.0)	-	3 (3.3)	3 (3.0)	2 (3.0)	2 (2.5)
Rec Thymus n=2								
Lymphoid depletion	-	-	-	-	-	-	-	-
Ovaries n=3								
Immature	NA	-	NA	3(NA)	NA	3(NA)	NA	3(NA)
Rec Ovaries n=2								

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Observation	Icatibant Dose (mg/kg/day)							
	0		3		10		3.3 TID	
	M	F	M	F	M	F	M	F
Immature	NA	-	NA	-	NA	-	NA	-
Uterus n=3 Immature	NA	-	NA	3(NA)	NA	3(NA)	NA	3(NA)
Rec Uterus n=2 Immature	NA	-	NA	-	NA	-	NA	-
Vagina n=3 Immature	NA	-	NA	2(NA)	NA	3(NA)	NA	3(NA)
Rec Vagina n=2 Immature	NA	-	NA	-	NA	-	NA	-

- No macroscopic finding observed
NA- Not applicable
(#)- mean severity: grade 1= minimal; grade 2= slight; grade 3= moderate; grade 4= marked; grade 5= massive

As summarize by the table above, the target organs of toxicity in the dog after treatment with icatibant are the male and female reproductive tissues and the thymus. Males treated with daily SC injections of icatibant showed immature epididymides, testes and prostate. After the 13-week recovery period, 1 of 2 males showed the testes and prostate as immature where 1 male had recovered. No sperm was present in this animal. When males were treated twice weekly with icatibant, no affects were observed in the male reproductive organs. Females treated with once daily SC icatibant also resulted in immature ovaries, oviducts, uterus and vagina. After the 13-week recovery period, no adverse histological findings were present in female organs. Unlike the males, females treated with SC administration of icatibant twice weekly showed delayed or impaired maturation of the reproductive organs. Thymus lymphoid depletion was observed in males and females during the icatibant treatment period with either daily or twice weekly dosing of icatibant. Daily SC administration of 10 mg/kg of icatibant showed more severe lymphoid depletion in the thymus than twice weekly administration. Males and females showed no signs of thymic toxicity after the 13-week recovery period.

No NOAEL could be determined in this study in females or in males after daily SC administration of icatibant due to immature reproductive effects. In females treated with twice weekly SC doses of icatibant, no NOAEL could be determined due to immature reproductive organs. These effects were reversible upon cessation of icatibant treatment. Males treated with twice weekly doses of icatibant showed no adverse effects on reproductive organs but did demonstrate thymic lymphoid depletion.

Toxicokinetics: Venous blood samples were taken on Day 1 and in week 13 from all animals to determine icatibant levels and the metabolite M_2 (b) (4) levels. TK data are summarized below.

	Parameter	Icatibant Dose (mg/kg/day)											
		TK of Icatibant						TK of M2 Metabolite					
		3		10		3.3 TID		3		10		3.3 TID	
		M	F	M	F	M	F	M	F	M	F	M	F
Day 1	C_{max} (mcg/mL)	5.1	4.4	14.6	16.1	-	-	0.16	0.14	0.28	NC	-	-
	T_{max} (h)	0.7	1.1	0.9	1.0	-	-	3.6	2.0	3.3	NC	-	-
	AUC_{0-t} (mcg*h/mL)	13.1	11.3	44.2	41.4	-	-	0.99	0.78	1.98	NC	-	-

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	AUC_{tot} (mcg*h/mL)	-	-	-	-	47.1	50.8	-	-	-	-	NC	1.52
Wk 13	C_{max} (mcg/mL)	5.1	4.8	21.8	20.6	-	-	0.19	0.24	0.23	0.32	-	-
	T_{max} (h)	0.6	0.8	0.8	0.9	-	-	3.3	3.2	4.0	3.2	-	-
	AUC_{0-t} (mcg*h/mL)	13.9	13.6	55.2	58.2	-	-	1.4	1.5	2.3	2.7	-	-
	AUC_{tot} (mcg*h/mL)	-	-	-	-	NC	NC	-	-	-	-	1.9	NC

NC- not calculated because individual values were invalid (QC rejected).
- Values not determined.

Exposure levels of icatibant increased with increased daily dose (3 to 10 mg/kg/day) in both males and females in an approximately dose-proportional manner. There was no accumulation after repeated daily SC administration of icatibant in males or females when comparing exposure levels at Day 1 to Week 13. The t_{max} for icatibant was achieved rapidly (0.6- 1 h post-dose) after SC administration in males and females. There were no PK gender differences observed after daily administration of icatibant to males and females. After twice weekly administration of icatibant, plasma concentrations and systemic exposure levels were below the level of detection in both males and females.

The M_2 metabolite of icatibant increased in systemic exposure with increased icatibant dose in supra-proportional manner. There was no accumulation of M_2 after repeat daily SC administration of icatibant and there were no significant PK gender differences in M_2. Systemic exposure to M_2 after twice daily administration of icatibant was not detectable.

Other: To assess the sexual maturity of dogs, the testicular size (cm^3) was measured at the end of the treatment period and once every 2 weeks during the recovery period.

As shown in the table below, icatibant treatment resulted in a decrease in testicular size compared to the vehicle control animals. Administration of icatibant as daily doses resulted in a greater decrease in testicular size compared to administration of icatibant twice weekly.

%Δ in testicular size compared to controls

Treatment Period	Icatibant dose (mg/kg/day)		
	3	10	3.3 TID
End of treatment period n= 5 dogs	-87%	-81%	-31%
Rec. wk 15 n=2 dogs	-80%	-65%	NSS
Rec. wk 17 n=2 dogs	-75%	-56%	NSS
Rec. wk 19 n=2 dogs	-46%	-44%	NSS
Rec. wk 21 n=2 dogs	-54%	-51%	NSS
Rec. wk 23 n=2 dogs	NSS	NSS	NSS
Rec. wk 25 n=2 dogs	-31%	NSS	NSS

NSS- No statistically significant difference compared to vehicle controls.

Blood was sampled to determine hormone levels. Using radioimmunoassay, luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone (PROG), estradiol (ESTR) and testosterone (TESTO) levels were measured at predose and then at Weeks 4, 8, 12, 21, 23, 25 and 27.

Treatment with icatibant resulted in significant decreases in testosterone, LH and FSH levels in males compared to the vehicle control levels. These decreases coincided with the

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decreases in testicular volumes. Testosterone and LH levels recovered after cessation of dosing. A rebound in FSH levels was observed in males during the recovery week 23. A decrease in FSH levels was observed in females during Week 4 of treatment with icatibant compared to the vehicle control groups. However, FSH was highly variable in females throughout the study period.

Parameter	Week	Icatibant Dose (mg/kg/day)					
		3	10	3.3 TID	3	10	3.3 TID
		Males			Females		
Testo	4	-93%	-95%	-76%	NA	NA	NA
	8	blq	blq	-87%	NA	NA	NA
	12	blq	blq	-82%	NA	NA	NA
	R21	-	-	-	NA	NA	NA
	R23	-	-	-	NA	NA	NA
	R25	-	-	-	NA	NA	NA
	R27	-	-	-	NA	NA	NA
LH	4	-	blq	-	-	-	-
	8	-81%	-78%	-	-	-	-
	12	-88%	-79%	-	blq	-	-
	R21	-	-	-	-	-	-
	R23	-	-	-	-	-	-
	R25	-	-	-	-	-	-
	R27	-	-	-	-	-	-
FSH	4	-93%	-86%	-72%	-97%	-95%	-85%
	8	-	blq	-	-	-	-
	12	-	-	-	-	blq	-
	R21	-	-	-			
	R23	+500%	+433%	-	-	-	-
	R25	-	-	-	-	-	-
	R27	-	-	-	-	-	-

- No statistically significant difference compared to vehicle controls.
NA- Not applicable.
Blq-below the level of quantitiation

2.6.6.4 Genetic toxicology

Study title: Bacterial reverse mutation test

Key findings:

- Icatibant did not induce bacterial mutagenicity under the conditions tested.

Study no.: JE049-0132

Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: April, 19, 2005
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batch no. 0565984, and 98.1% pure

Reference ID: 2977968

Methods

Strains/species/cell line: *Salmonella typhimurium* (*S. typhimurium*) strains TA1535, TA1537, TA98, TA100 and TA 102. Each strain was exposed to 5 dose-levels of icatibant (3 plates/dose-level).

Doses used in definitive study: Two definitive experiments were conducted. In the absence of metabolic activation (-S9 mix), icatibant was used at 39.06, 78.13, 156.25, 312.5, 625 and 1250 µg/plate for TA 1535, TA 100 (first and second experiment) and TA 102 (second experiment only) strains. For TA 1537, TA 98 (first and second experiment) and TA 102 (first experiment only) icatibant was assayed at 78.13, 156.25, 312.5, 625, 1250 and 2500 µg/plate in the absence of S9 mix.

In the presence of S9 mix, icatibant was used at 39.06, 78.13, 156.25, 312.5, 625 and 1250 µg/plate for TA 102 in the first experiment. For TA 1535 and TA 100 in the first experiment and TA 100 and TA 102 in the second experiment icatibant was assayed at 78.13, 156.25, 312.5, 625, 1250 and 2500 µg/plate in the presence of S9 mix. For TA 1537, TA 98 in both experiments and TA 1535 in the second experiment, icatibant was assayed at 156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate in the presence of S9 mix.

Basis of dose selection: A preliminary dose-range finding toxicity study was conducted to establish the icatibant doses to be used for the definitive experiments. Using the test strains TA98, TA 100 and TA 102 in the presence and absence of S9 mix, 6 concentrations of icatibant (10, 100, 500, 1000, 2500 and 5000 µg/plate) were assayed. No precipitation of the drug was observed up to 5000 µg/plate. Icatibant was toxic in the preliminary assay at dose levels ≥ 1000 µg/plate in the TA 100 strain and at ≥ 2500 µg/plate in TA98 and TA 102 strains in the absence of S9 mix. In the presence of S9 mix, toxicity was observe at ≥ 1000 µg/plate in TA 102, ≥ 2500 µg/plate for TA 100 and at 5000 µg/plate for TA 98 with a significant reduction of colony counts.

Negative controls: Vehicle (water for injection)

Positive controls:
Without S9 mix:
1 µg/plate of sodium azide for TA 1535 and TA 100 strains
50 µg/plate of 9-aminoacridine for TA 1537
0.5 µg/plate of 2-nitrofluorene for TA 98
0.5 µg/plate of mitomycin C for TA 102

With S9 mix:
2 µg/plate of 2 anthramine for TA 1535, TA 1537, TA 98 and TA 100
10 µg/plate of 2-anthramine for TA 102

Incubation and sampling times: In the two definitive studies, plates were exposed to either the negative or positive controls or drug treatment in the presence or absence of S9 metabolic activation for 48 to 72 h and incubated at 37°C. At the end of the treatment period, plates were counted with an automatic plate reader.

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Results

Study validity: Each concentration of icatibant, the positive controls and the negative control was assayed in triplicate for each bacterial strain tested. A complete battery of bacterial strains was examined in this study. Criteria for positive results included: a reproducible 2-fold increase for strains TA 98, TA 100 and TA 102 or 3-fold increase for strains TA 1535 and TA 1537 in the number of revertants compared with the vehicle controls, in any strain at any dose-level and/or evidence of a dose-relationship and reference to historical control data was taken into consideration when evaluating the study results. The assay methods, positive and negative controls, and the concentrations of drug used for the definitive study were adequate.

Study outcome: In the first definitive experiment, there was no increase in the number of revertants compared to the vehicle control groups for test strains TA1535, TA100, TA102, TA 1537 and TA 98 in the presence and absence of S9 mix. Icatibant did not precipitate out of media or interfere with colony counting. Slight toxicity was observed in TA 100 at concentrations ≥ 1250 µg/plate in the absence of S9 mix (reduction of cell count by 36%) and at ≥ 2500 µg/plate in the presence of S9 mix (reduction of cell count by ~60%), and for TA 102 in the absence of S9 mix at ≥ µg/plate (reduction of cell count by ~50%).

In the second definitive study, there was no increase in the number of revertants compared to the vehicle control groups for test strains TA1535, TA100, TA102, TA 1537 and TA 98 in the presence and absence of S9 mix. Icatibant did not precipitate out of media or interfere with colony counting. Slight toxicity was observed in TA 100 in the absence of S9 mix at icatibant concentrations ≥ 625 µg/plate (reduction in colony count by 23%), for TA 1537 at ≥ 2500 µg/plate (reduction of colony count by 50%), for TA 98 in the absence of S9 mix at ≥ 2500 µg/plate (reduction of colonies by ~ 50%) and for TA 102 at ≥ 1250 µg/plate (reduction of colony count by 50%).

Taking the 2 definitive study results together and considering that this study was performed at adequate doses and under valid study methods, icatibant did not induce bacterial mutagenicity under the conditions tested.

Study title: *In vitro* mammalian chromosome aberration test in cultured human lymphocytes

Key findings:

- Icatibant did not induce chromosomal aberration formation in human lymphocytes under the experimental conditions tested.

Study no.: JE049-0133

Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: September 22, 2004
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batch no. E084 and 96.2% pure

Reference ID: 2977968

Methods

Strains/species/cell line: Human lymphocytes

Doses used in definitive study: Two experiments were conducted. In the first experiment icatibant was assayed at 39.1, 78.1, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/mL in the presence and absence of S9 mix. In the second experiment, the icatibant concentrations were 156.3, 312.5, 625, 1250, 2500 and 5000 µg/mL in the presence and absence of S9 mix.

Basis of dose selection: The highest icatibant concentration level recommended for the assay was selected for the first experiment based on pH, osmolality and solubility. For selection of icatibant concentrations for the second experiment, any toxicity that was indicated by a reduction of the mitotic index in the first experiment was used to adjust the icatibant doses.

Negative controls: Vehicle (culture medium)

Positive controls:
Without S9 mix:
Mitomycin C was used at 3 µg/mL (3 h treatment) or 0.2 µg/mL (continuous treatment)

With S9 mix:
Cyclophosphamide was used at 12.5 or 25 µg/mL

Incubation and sampling times: In the first experiment, lymphocyte cultures were exposed to controls and icatibant in the presence and absence of S9 mix for 3 hours. Cells were harvested 20 h post-exposure to test item initiation. In the second experiment, in the absence of S9 mix cells were exposed to icatibant concentrations or controls continuously until cell harvest. In the presence of S9 mix, cells were exposed to drug or controls for 3 hours and then rinsed and then harvested 20 h and 44 h post-exposure to test item initiation. Cells were incubated at 37°C in 5.0% CO_2 and 90% relative humidity. After cell harvest, slides were prepared for evaluation of cytotoxicity (reduction of mitotic index) and for the induction of chromosomal aberrations.

Results

Study validity: Approximately 200 metaphase spreads per treatment were scored. The tests were considered positive for chromosomal aberrations if there were statistically significant increases ($p \leq 0.05$) over concurrent controls in the percentage of cells with chromosomal aberrations without greatly exceeding a 50% reduction in growth. Review of the study methodology including the concentrations used for the definitive study, the use of the appropriate positive and negative controls, the scoring criteria and the outcomes of the positive and negative controls showed that this study was adequate.

Study outcome: There was no precipitation of drug up to the highest concentration tested (5000 µg/mL). In the absence of S9 mix and after 3 h exposure to each icatibant concentration, cytotoxicity was observed at 5000 µg/mL with a reduction in mitotic index to 33% compared to the negative controls. After 20 h of treatment in the absence of S9 mix cytotoxicity was observed

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at icatibant concentrations ≥ 2500 µg/mL with mitotic indices decreasing to 39%. After 44 h of treatment the mitotic index was 44% compared to the negative controls at 5000 µg/mL. In the presence of S9 mix, there was no significant reduction of mitotic indices at any concentration assayed. Therefore, there was no cytotoxicity observed in the presence of S9 mix.

In the presence and absence of S9 mix there was no increase in the frequency of structural aberrations at any of the icatibant concentrations tested. For the negative and positive controls, the frequencies of chromosomal aberration formation were within the appropriate ranges. Under these study conditions, icatibant did not induce chromosomal aberrations in cultured human lymphocytes.

Study title: Bone marrow micronucleus test by intravenous route in rats

Key findings:

- Icatibant did not induced micronucleus formation under these study conditions.

Study no.: JE049-0135

Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: April 12, 2005
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batch no. 0565984, and 98.1% pure

Methods

Strains/species/cell line: Sprague Dawley rats; 5 rats/sex for the vehicle control, LD, MD and positive control groups and 8 rats/sex for the HD group. Rats were ~ 6 weeks old and ranged in body weight from 180.6 to 224.4 g for males and 119.6to 152.2 g for females.

Doses used in definitive study:
Males: Two IV administrations of 0 (vehicle), 3.75, 7.5, and 15 mg/kg of icatibant with a 24-hour interval between each dose
Females: Two IV administrations of 0 (vehicle), 2.5, 5, and 10 mg/kg of icatibant with a 24-hour interval between each dose

Basis of dose selection: A preliminary toxicity study was conducted to determine adequate doses of icatibant to be used for the definitive study. Three rats/sex were administered 20, 15 and/or 10 mg/kg of icatibant intravenously twice with a 24 h interval between doses. At 20 mg/kg (males only) 2/3 males died 2 hours after receiving the second dose. The remaining animal was hypoactive, showed sedation and piloerection. At 15 mg/kg (males and females), all treated animals were hypoactive and showed piloerection. Females also showed signs of sedation. One female (1/3) died 23 hours post the second treatment. In the 10 mg/kg group (females only), there were no clinical signs of toxicity and no mortality.

Negative controls: Vehicle control (0.9% NaCl, isotonic saline solution)

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Positive controls: Cyclophosphamide at 1.5 mg/mL

Incubation and sampling times: All animals were administered vehicle, the icatibant doses or cyclophosphamide. Animals from the vehicle control, treatment and cyclophosphamide groups had bone marrow harvested at 24 hours post-dosing. Slides were prepared from bone marrow cells that were harvested at sacrifice and cells were stained with Geimsa and microscopically assessed for micronuclei formation from 2000 polychromatic erythrocytes (PCEs) per animal.

Results

Study validity: Criteria for a result to be considered positive included a significant increase in the frequency of micronucleated PCE ($p < 0.05$) occurring at a minimum of 2 dose levels when compared to concurrent vehicle control and evidence of a positive dose-related response. Based on the review of the study methodology including the doses selected for male and female rats, the exposure time, the use of positive and negative controls and the outcome of these controls, this study was considered valid.

Study outcome: For males, dosed at 3.75 and 7.5 mg/kg, there were no clinical signs of toxicity observed. However, at 15 mg/kg, 2 animals died following icatibant administration. Females dosed up to 10 mg/kg IV showed no clinical signs of toxicity. However, the dose-ranging study showed that a dose of 15 mg/kg was lethal. Therefore, the dose of 10 mg/kg approximated the MTD. Males and females treated with icatibant showed similar mean values of micronucleated polychromatic erythrocytes (MPEs) and similar polychromatic erythrocyte/normochromatic erythrocytes ratios (PEs/NEs) as those observed from animals treated with the negative control. Male and female rats treated with cyclophosphamide significantly increased the MPEs compared to the vehicle controls. Vehicle treated and cyclophosphamide treated rats produced MPE counts and PE/NE ratios within the historical control ranges from the text facility.

Icatibant does not induced micronucleus formation under these study conditions.

2.6.6.5 Carcinogenicity

No carcinogenicity studies have been conducted by the sponsor to date.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Combined study of 13-week toxicity and effects on fertility and early embryonic development to implantation in rats by subcutaneous administration

Key study findings:

- HD treated females had an increase in pre-implantation loss compared to vehicle treated females.
- Untreated females paired with HD treated males showed no increase in pre-implantation loss compared to untreated females paired with vehicle control treated males.

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- Icatibant had no effects on male sperm count, motility, or morphology.
- Based on no toxicity observed in male seminology and no effects on mating or fertility indices, the NOAEL for male fertility was 10 mg/kg/day. Based on an increase of pre-implantation loss observed in HD treated females, the NOAEL for female fertility was 3 mg/kg/day. These conclusions were in agreement with the sponsor's conclusions.

Study no.: JE049-0108
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: February 17, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batches 0565984 (98.1% pure) and E084 (95.9%)

Methods

Doses: 0 (vehicle), 1, 3 and 10 mg/kg/day
Species/strain: Sprague Dawley rats
Number/sex/group or time point (main study): 24/sex/group
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in isotonic saline solution (0.9% NaCl), administered in 1.0 mL/kg and infusion rate was not applicable.
Satellite groups used for toxicokinetics or recovery: None.
Age: Males for the fertility study: 5-6 weeks old; Females: ~ 10 weeks old
Weight: males: 185 g and females: 224 g
Sampling times: None.

Unique study design or methodology (if any): The sponsor combined the 13-week repeat dose toxicity study and the effects on fertility and early embryonic development to implantation study in rats. Under this section, the reproductive toxicology study is reviewed. Male rats were given daily subcutaneous doses of icatibant for 10 weeks prior to mating, during and after mating. Female rats were given daily subcutaneous doses of icatibant for 2 weeks prior to mating, during mating and until 7 days post-coitum.

Parameters and endpoints evaluated: All animals were checked for morbidity and mortality at least twice a day and females were checked for gross BW on the first day of treatment, then twice a week until mated (or until sacrifice) and on Days 0, 4, 8, 11 and 15 post-coitum. The untreated females of the second pairing/mating were weighed on Days 0, 4, 8, 11 and 15 post-coitum. Food consumption was measured during the premating period weekly and during pregnancy over the periods of Days 0-8 and Days 8-15 post-coitum. The estrous cycle stage was assessed for each female during the pairing period. The mated females were sacrificed on Day 15 post-coitum and a macroscopic post-mortem examination of the principal thoracic and abdominal organs was performed. The ovaries and uteri were examined to determine the numbers of corpora lutea and implantations, classified as dead and live concepti and early and late resorptions. Following the observation that there were higher pre-implantation losses at 10 mg/kg/day at the day of hysterectomies, untreated, virgin females were paired with treated males from the VC and the HD groups to further assess effects of treatment. These females were sacrificed on Day 15 post-coitum and a hysterectomy was performed. The males in the LD and MD groups were sacrificed after all hysterectomies of the females had been performed.

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Following the results of the second pairing/mating period, the males from the LD and HD groups were sacrificed. For all males in the fertility assessment, macroscopic post-mortem examinations of the principal thoracic and abdominal organs were performed.

Results

Mortality: All icatibant treated males survived until scheduled sacrifice. Females treated with 0, 1, 3 and 10 mg/kg/day of icatibant were sacrificed with 1/24 (sacrificed day 40), 2/24 (sacrificed days 39 and 40), 4/24 (sacrificed days 39 and 40) and 0/24 rats, respective to dose. The decision to sacrifice was based on no observed mating and not due to clinical signs of toxicity. However, each female that was sacrifice was determined to be pregnant. All remaining icatibant treated females, after the pregnancy period, survived until the scheduled sacrifice.

Following the observation that there were higher pre-implantation losses (see below) at 10 mg/kg/day at the day of hysterectomies, untreated, virgin females were paired with treated males from the VC and the HD groups to further assess effects of treatment. All untreated females that were paired with the HD treated males survived until scheduled sacrifice.

Clinical signs: Males and females treated with icatibant had no clinical signs of toxicity compared to the vehicle treated males.

Body weight: There were no statistically significant changes in group mean body weight or group mean body weight change in males treated with icatibant compared to males treated with the vehicle control. There were no statistically significant changes in group mean body weight or group mean body weight change in females treated with icatibant during the pre-mating phase or during the pregnancy period compared the vehicle control treated females. Additionally, there were no statistically significant changes in group mean body weight or group mean body weight change in the untreated females that were mated with icatibant treated males compared to untreated females mated to the vehicle control treated males.

Food consumption: There were no statistically significant differences in icatibant treated males and females in food consumption compared to the vehicle control treated animals throughout the study period.

Toxicokinetics: TK assessment was not conducted in the fertility and early embryonic development phase of this combined study.

Necropsy: One LD and 1 MD male rat showed reduced testes size. Two LD rats showed reduced epididymis size. These findings did not follow a dose-response. One female in the MD group and 1 female in the HD group had deformed kidneys. The effects observed on the kidneys were not observed in males and did not increase with dose.

Fertility parameters: There were 1, 2, 4 and 0 pairs for the dose groups 0, 1, 3 or 10 mg/kg/day, respectively, with no evidence of mating during the pairing period. However, the females delivered before sacrifice so mating had occurred. There was no effect on the estrous cycle of the females at any dose.

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All control females were pregnant. There was 1 female paired with a male treated at 10 mg/kg/day that was not pregnant. There were 24, 23, 22 and 23 pregnant females in the groups administered 0, 1, 3 of 10 mg/kg/day, respectively, giving fertility indices of 100%, 96%, 92% and 100%, respectively. The mean number of corpora lutea was comparable to controls for all groups. However, the mean pre-implantation loss was higher than the controls at the HD (65 more corpora lutea than implantations vs 25 in the control group), and there were lower numbers of mean implantations and concepti. The post-implantation loss was also slightly higher in the HD group (24 implantations lost vs. 16 in the control group). There was no effects observed at 1 or 3 mg/kg/day compared to the vehicle control group. All concepti were alive.

Hysterectomy Data of Icatibant Treated Females

Parameter	**Icatibant dose mg/kg/day**			
	0	**1**	**3**	**10**
Pregnant females at term, n=24	23	21	18	23
With all dead concepti	0	0	0	0
With live concepti	23	21	18	23
Corpora lutea	393	354	301	406
No. per animal	17.1	16.9	16.7	17.7
Implantation sites	368	328	269	341
No.per animal	16.0	15.6	14.9	14.8
Preimplantation loss, total (%)	**25 (6.4)**	**26 (7.3)**	**32 (10.6)**	**65 (16.0)**
Concepti	352	308	259	317
No.per animal	15.3	14.7	14.4	13.8
Alive	100	100	100	100
Dead	0	0	0	0
Live concepti %	352	308	259	317
implantation sites	95.7	93.9	96.3	93.0
No. per animal	15.3	14.7	14.4	13.8
Postimplantation loss	16	20	10	24
% of implantation sites	4.3	6.11	3.7	7.0
No. per animal	0.7	1.0	0.6	1.0

Following the observation that there were higher pre-implantation losses at 10 mg/kg/day at the day of hysterectomies, untreated, virgin females were paired with treated males from the VC and the HD groups to further assess effects of icatibant treatment. Two untreated females paired with the treated males had no evidence of mating. However, 1 female was pregnant while the 2nd female was not. The treated males took an average of 1 day longer to mate with the untreated females than did the control males. The mean numbers of corpora lutea and implantations and the extent of pre- and post-implantation losses of the untreated females paired with males treated at 10 mg/kg/day were comparable to controls. As part of the 13-week toxicology study, icatibant treated males were evaluated for changes in sperm number, motility, and morphology. There were no effects on these seminological parameters after daily subcutaneous treatment with icatibant for 13-weeks.

Embryofetal development

No new embryofetal development studies were submitted to the NDA. Studies conducted in the rat and the rabbit were previously reviewed under IND (b) (4)

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Prenatal and postnatal development

Study title: Study for effects on pre- and post-natal development in rats by subcutaneous administration

Key study findings:

- Icatibant treatment delayed parturition at 1, 3 and 10 mg/kg/day, resulting in F_0 deaths (found dead or early sacrifice) in the 3 and 10 mg/kg/day groups.
- Clinical signs of toxicity (pallor of eyes and extremities, round back, piloerection and reddish discharge in vagina) were observed in 1 MD and in multiple HD F_0 females.
- After SC administration, icatibant and the M2 metabolite is found in rat milk.
- Based on the observed maternal toxicities including clinical signs, prolonged gestation periods for all icatibant dosage groups, F_0 spontaneous deaths and early sacrifice of icatibant treated dams, litter deaths, and necropsy findings in the MD and the HD groups, the maternal toxicity NOAEL could not be defined (< 1 mg/kg).
- The F_1 generation showed an increase in the number of pup deaths and cannabalism in the MD and HD compared to the VC groups. Therefore, the NOAEL for systemic toxicity in the F_1 generation was determined to be 1 mg/kg/day.
- The F_1 generation showed a slight decrease in air righting and hair growth at the HD compared to the VC control group. Therefore, the NOAEL for development of the F_1 generation was considered 3 mg/kg/day.

Study no.: JE049-0141
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: February 13, 2005
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batch no. E084, and 95.9% pure

Methods

Doses: 0 (vehicle), 1, 3 and 10 mg/kg/day
Species/strain: Sprague-Dawley rats
Number/sex/group: 24 mated females/group for the VC, LD and MD groups and 30 mated females/group for the HD group
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in sterile isotonic saline solution (NaCl 0.9%), 1 mL/kg and infusion rate is not applicable.
Satellite groups used for toxicokinetics: 6 mated females/group were used for TK and milk sampling
Study design: Mated female Sprague-Dawley rats were subcutaneously dosed from Day 6 post-coitum until Day 21 post-partum. The dams were allowed to litter and rear their progeny until weaning. The HD group (10 mg/kg/day) had difficulties delivering or did not deliver at which time dosing was stopped on Day 21 post-coitum until the females delivered. Pups from F_0 dams were evaluated and then culled. After weaning, these pups (now referred to as the F_1 generation) were evaluated through sexual maturity and then

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mated. Evaluation of fertility and necropsy after hysterectomy of the F_1 generation was conducted.

Parameters and endpoints evaluated: For the F_0 females, morbidity and mortality, clinical signs of toxicity, BW changes, food consumption, and complete hematology, clinical biochemistry and urinalysis batteries were compared between the icatibant treated females and the VC treated females. The effects of icatibant treatment on parturition were also observed. Blood and milk were sampled and evaluated for icatibant levels in the F_0 dams. The number of implantation sites was recorded in F_0 females. Once dams had delivered, litter size and pup observations (BWs, clinical signs, pup physical and reflex development) were recorded. Litters were culled as nearly as possible to 4 pups/sex/litter. After weaning, these pups (now referred to as F_1 generation) were checked at least twice a day for mortality and morbidity, clinical signs of toxicity, changes in BW and food consumption and for sexual maturity. The animals were tested for auditory function, pupil constriction, learning and memory, spontaneous locomotor activity and the day that sexual maturity was reached was recorded. Blood and urine samples were collected from all F_1 animals before pairing, for hematological and blood biochemical investigations and urinalysis was conducted as well. Between 12 and 14 weeks, F_1 animals were paired for mating. The F_1 generation was sacrificed on Day 14 post-coitum. A macroscopic post-mortem examination was performed on all animals and for pregnant females. The numbers of corpora lutea and implantations, classification of concepti (dead or live), early and late resorptions or implantation scars were recorded.

Results

F_0 in-life: HD F_0 females and 1 MD F_0 female were either found dead of were sacrificed early due to clinical signs of toxicity or litter death. The following table summarizes the deaths, the time of death and the clinical sings of toxicity associated with these F_0 females. The sponsor stated that these clinical signs of toxicity were not directly related to icatibant administration but a result of the drug-induced delayed parturition and drug-induced difficulties with delivery, as most of the clinical signs were not observed until time of expected delivery.

F_0 dam animal no.	Death/Sacrifice	Day of Death	Observations
HD G28869	Found dead	Day 23 p.c.	• No clinical signs; 14 dead fetuses and 1 early resorption
HD G28851	Found dead	Day 24 p.c.	• Reddish vaginal discharge on days 23 and 24; 13 dead fetuses in the uterine horns
HD G28863	Early sacrifice	Day 24 p.c.	• Difficulties to deliver; pallor of extremities, round back, cold to touch, piloerection, hypokinesia, abdominal breathing and pallor of eye • 11 dead fetuses in the uterine horns
HD G28868	Early sacrifice	Day 24 p.c.	• 11 pups in bedding and 1 in vagina and 5 dead fetuses in uterine horns. • Pallor of extremities, round back, cold to touch, piloerection, hypokinesia, abdominal breathing and pallor of eye
HD G28875	Early sacrifice	Day 24 p.c.	• Difficulties to deliver. • Pallor of extremities, round back, piloerection and abdominal breathing

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F_0 dam animal no.	Death/Sacrifice	Day of Death	Observations
			• 6 dead pups in bedding and 1 dead fetus in the uterus
HD G28878	Early sacrifice	Day 25 p.c.	• Difficulties to deliver • Pallor of extremities, round back, piloerection, abdominal breathing and pallor of eye • 13 dead pups in the bedding and 1 dead fetus in the uterus
HD G28880	Early sacrifice	Day 27 p.c.	• Pallor of extremities, round back and piloerection • On the day prior to sacrifice, live pups in bedding but they were no longer there on day of sacrifice (cannibalism).
HD G28852	Found dead	Day 1 p.p.	• Gave birth to 7 live pups • F_0 female had pallor of extremities, round back, cold to touch, pallor of eye and eyes half-closed prior to death.
HD G28859	Found dead	Day 2 p.p.	• Gave birth to 9 live pups • No clinical signs prior to death. At necropsy, serous content in thoracic cavity, reddish contents in the vagina and 1 dead fetus in uterus
MD G28833	Early sacrifice	Day 25 p.c.	• F_0 female had pallor of extremities, round back, and piloerection • No delivery • Implantation scars were observed.

p.c.-post-coitum; p.p.- post-partum

The duration of gestation was statistically significantly longer for all groups treated with icatibant than VC treated females. Females in the LD and MD groups delivered slightly later than VC groups but survived delivery and reared their progeny until weaning. The mean number of pups born per female was comparable to the VC group. These data are provided in the following table.

During the pregnancy period, F_0 females in the HD group were found dead (2/30 females), were sacrificed due to the absence of delivery (1/30 females) or were sacrificed due to difficulties to deliver (4/30). During the lactation period, HD F_0 females were found dead (2/30) or were sacrificed during the first few days of the lactation period because of death of their litter (11/30 females). The sponsor stopped dosing of the remaining HD F_0 females to allow for delivery. However, the same delays and difficulties with delivery were observed. A total of 9 HD F_0 females delivered and reared their progeny in the HD group. No F_0 females treated with 0 or 1 mg/kg/day died or were sacrificed prior to scheduled sacrifice.

The HD F_0 females showed marked clinical signs of toxicity that were closely related to the timing of delivery. The sponsor considered these clinical signs to be associated with the late onset of delivery. Clinical signs of toxicity in HD F_0 females included pallor of eyes and pallor of extremities, reddish vaginal discharge, round back, cold to touch, piloerection and abdominal breathing. One MD F_0 female also showed signs of clinical toxicity and was not observed to

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deliver but implantation scars were observed at necropsy, indicating delivery had taken place. Clinical signs of toxicity were observed in several HD females during the lactation period. These females were sacrificed early.

Summary of Clinical Signs (no. during pregnancy period/no. during lactation period)

	Icatibant Dose (mg/kg/day)			
	0	**1**	**3**	**10**
Gestation				
Gestation duration (days)	21.3	21.8	22.0	23.0
Mortality				
Found dead	0/0	0/0	0/0	2/2
Decision of sacrifice	0/1	0/0	1/0	1/11
Decision to sacrifice (difficulties to deliver)	0/NA	0/NA	0/NA	4/NA
General				
Pallor of extremities	0/0	0/0	1/0	5/4
Round back	0/0	0/0	1/0	5/2
Cold to touch	0/0	0/0	0/0	2/2
Piloerection	0/0	0/0	1/0	5/0
Hypokinesia	0/0	0/0	0/0	2/0
Abdominal breathing	0/0	0/0	0/0	4/0
Reddish vaginal discharge	0/0	0/0	0/0	3/4
Pallor of eye	0/0	0/0	0/0	3/4
Eyes half-closed	0/0	0/0	0/0	0/1

NA- not applicable to lactation period.

There were no statistically significant differences between icatibant treated dams and the VC control treated dams for BWs and BW gains, food consumption, hematology, clinical biochemistry and urinalysis parameters.

Blood samples for plasma levels of the test item were collected from all groups on Day 6 and Day 17 post-coitum at 0.5, 1, 2 and 4 hours post-dose. Icatibant and M2 levels were determined using HPLC/MS MS methods. The limit of quantification of icatibant from plasma was between 27.1-38.2 ng/L.

TK Parameter	**Icatibant TK**			**M2 TK**		
	1	**3**	**10**	**1**	**3**	**10**
C_{max} (µg/mL)						
Day 6 p.c.	1.25	2.74	7.88	0.0884	0.218	0.406
Day 17 p.c.	0.981	3.16	7.78	0.0847	0.140	0.414
Day 4 p.p.	1.49	3.77	nm	0.113	0.160	nm
T_{max} (h)						
Day 6 p.c.	0.5	1	2	2	1	1
Day 17 p.c.	0.5	0.5	1	1	2	2
Day 4 p.p.	1	1	nm	1	1	nm
AUC (0.5-4 h) (µg*h/mL)						
Day 6 p.c.	1.08	6.21	21.3	0.117	0.423	0.941
Day 17 p.c.	1.62	5.65	20.8	0.103	0.391	1.14
Day 4 p.p.	nc	nc	nm	nc	nc	nm

p.c.: post-coitum; p.p.: post-partum, nc: not calculated, nm: not measured due to early deaths

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On Day 6 post-coitum, the C_{max} for icatibant increased with increased dose in a sub-proportionate manner for all doses. However, on Day 17, C_{max} for icatibant increased in an approximately dose proportionate manner from the LD to the MD and sub-proportionately from the MD to the HD. The M2 metabolite C_{max} increased approximately in a dose proportionate manner from the LD to the MD but was sub-proportionate from the MD to the HD on Day 6. Day 17, the M2 increase was sub-proportionate for all doses. AUC levels of icatibant increased in supra-proportionately with increased dose Days 6 and 17 post-coitum. The M2 metabolite increased in an approximately dose proportionate manner on Day 6 and Day 17 and was observed at levels approximately 5-10% of the parent compound. There was no evidence of accumulation of icatibant after repeated SC administration.

For the LD, MD and HD groups, milk samples for levels of test item were planned to be collected on Day 4 post-partum at time point 0.5 and 4 hours after dosing. However, HD females either were found dead or were sacrificed prior to the scheduled milk sampling.

TK Parameter	Icatibant TK			M2 TK		
	1	3	10	1	3	10
C (µg/mL)						
1 h post-dose.	0.729	4.53	nm	Blq	0.239	nm
4.5 h post-dose.	0.216	1.11			0.084	

nm: not measured due to early deaths
blq- below the level of quantification

With increased icatibant dose, an increase in icatibant levels and M2 levels were observed in milk from lactating rats. These data show that after SC administration of icatibant, icatibant is excreted in milk.

F_0 necropsy: The pregnancy status of principle and satellite females is summarized in the follow table. All mated females in all dosage groups successfully mated. SC icatibant treatment did not adversely affect fertility indices at any dose. However, the number of HD F_0 females that successfully delivered was decreased compared to the VC control group. Only 9 HD F_0 females successfully delivered and reared their litter. At necropsy, one HD F_0 female still had fetuses in the uterine horns, even though it had delivered one or two days earlier and had pale liver and kidneys.

	Icatibant Dose (mg/kg/day)			
	0	1	3	10
Number of mated females (Mating index, %)	29 (100)	30 (100)	30 (100)	30 (100)
Number of non-pregnant females	2	3	2	0
Number of pregnant females (fertility index, %)	27 (93.1)	27 (90.0)	28 (93.3)	30 (100)
Delivered	27	25	27	22
Pregnant but did not deliver	0	2*	1*	1*
Sacrificed due to difficulties to deliver	0	0	0	4
Found dead	0	0	0	4
With dead litter	1	0	0	12**
Number of principle females with a litter on day 21 post-partum	22	20	21	9
Females w/ entire liveborn litter dying and/or				

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missing, cannibalized, culled				
Days 0-4	5	5	6	13
Days 0-21	5	5	6	13

*- marked BW gain during gestation period followed by BW loss Day 25 post-coitum and presence of only implantation scars in uterine horns suggest unnoticed delivery
**- includes 1 female which had live pups on Day 24 post-coitum but had not finished delivery, and no pups on day 24 post-coitum so could not be given a delivery date

At necropsy, the HD F_0 females showed an increase in findings compared to LD and MD groups and the VC controls. One MD female had an enlarged spleen. After inspection of the uterus, all icatibant treated females showed an increase in implantation sites with the HD F_0 having the highest increase and also showed reddish contents in uterine horns. Macroscopic findings of the vagina were observed in the HD F_0 females with fetuses inserted still inserted and reddish contents.

Summary of F_0 Necropsy Observations

	Icatibant Dose (mg/kg/day)			
	0	**1**	**3**	**10**
F_0 Females	24	24	24	30
Thoracic cavity: serous content	0	0	0	3 (10%)
Spleen: enlarged	0	0	1 (4.2%)	1 (3.3%)
Liver: Paleness	0	0	0	7 (23.3%)
Stomach: brownish focus	0	0	0	1 (3.3%)
Kidney: Paleness	0	0	0	6 (20%)
Uterus:				
Implantation sites	0	1 (4.2%)	1 (4.2%)	13 (43.3%)
Reddish contents in uterine horn	0	0	0	2 (6.7%)
Vagina:				
Fetuses inserted in vagina	0	0	0	1 (3.3%)
Reddish contents	0	0	0	4 (13.3%)

Based on the observed maternal toxicities including clinical signs(pallor of eyes and extremities, round back, piloerection), prolonged gestation periods for all icatibant dosage groups, F_0 spontaneous deaths and early sacrifice of icatibant treated dams, litter deaths, and necropsy findings in the MD and the HD groups, the maternal toxicity NOAEL could not be defined.

F_1 physical development: Litters with live pups showed a slight decrease in the HD group compared to the VC group. At Day 4 (post-partum), pups were culled to achieve as close to 4 pups/sex/litter as possible. Due to the decrease in the number of pups in the HD group as a result of F0 deaths and losses of litters, only 12 pups were culled compared to 107 in the VC control group. A significant increase in pup deaths or missing and/or cannibalized was observed in the HD group. Additionally, pups surviving until Day 4 post-partum and then until Day 21 were significantly reduced compared to the VC group. These data are summarized in the following table.

	Icatibant Dose (mg/kg/day)			
	0	**1**	**3**	**10**
Litters w/ Live born pups	27	25	27	22
Pups delivered (total) live born Live born index (%)	338 (100)	309 (100)	337 (100)	207 (100)

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Culled (total) Day 4 Cannibalized Missing Died	107 10 0 2	86 1 0 3	85 8 0 12	12 48 0 107
Pups dying, missing and/or cannibalized Day 1-4	12 (3.6%)	3 (1.0%)	20 (5.9%)	155 (74.9%)
Pups surviving 4 days (viability index, %)	326 (96.4)	306 (99.0)	317 (94.1)	51 (24.6)
Pups surviving 21 days (lactation index, %)	176 (100)	156 (99.4)	156 (100)	39 (100)

There were no macroscopic findings in pups from dams exposed to icatibant compared to the pups from dams exposed to VC. There were no statistically significant differences in pup weights/litter at Day 1 or Day 21 of litters from drug-treated dams to litters from VC treated dams. There was no difference in sex ratio between pups exposed to icatibant and pups exposed to the VC group. There were no differences in BWs, hematology, clinical biochemistry and urinalysis between F_1 animals exposed to icatibant and the VC exposed animals.

Pups exposed to icatibant showed similar reflex and physical development patterns as VC treated animals for the following: Pinna folding, tooth eruption, eye opening, auditory canal opening, surface righting and cliff avoidance. Slight decreases in the MD and HD exposed pups were observed in hair growth at Day 5 and air righting on Day 17 compared to the VC exposed pups.

	Icatibant Dose (mg/kg/day)			
	0	**1**	**3**	**10**
Live pups/litter (day1)	12.7	12.2	11.8	4.9
Hair growth day 5 (positive response, %)	100	100	98.7	94.9
Air righting day 17 (positive response, %)	92.0	93.6	91.7	82.1

F_1 behavioral evaluation: There were no differences in the F_1 generation that was exposed to icatibant and the VC exposed F_1 generation for mean age of appearance of sexual maturity, for response to acoustic startle, pupil constriction reflex, water T-maze test or motor activity.

F_1 reproduction: The F_1 generation that was exposed to icatibant had comparable reproductive data to the VC exposed F_1 generation. These data included pairs mated, mating index, mean number of days of pairing before mating, pregnant females, fertility index, females with live concepti and gestation index.

Hysterectomy data from the F_1 generation for animals exposed to icatibant were comparable to the VC exposed F_1 generation. These data included: pregnant females alive at term, number of corpora lutea, number of implantation sites, preimplantation site loss, number of concepti, number of live concepti, number of dead concepti and post-implantation loss. There was no difference in necropsy observations made in the F_1 generation exposed to icatibant and the F_1 generation exposed to VC.

F_2 findings: The F_2 generation was not evaluated.

2.6.6.7 Local tolerance

Reference ID: 2977968

Two local toxicity studies were conducted in rabbits using single dose administration via the subcutaneous route (study no. JE049-0142) and intrarterial/intravenous/paravenous routes (study no. JE049-0143).

Study no. JE046-0142 (non-GLP, initiated September 19, 2003) administered icatibant acetate (lot no. E084 and 96.2% pure) as a single 1.0 mL subcutaneous dose at 5, 10, 20 and 40 mg to 3 male New Zealand White rabbits/dose group (age 2-4 months old and mean BW 2.3 kg). The treatment was administered to the left flank of each animal and the vehicle (acetate buffer 0.5 M at pH 5.5) was administered to the right flank of each animal to serve as a control. Local reactions and clinical signs were checked daily for each animal. Local reactions were scored as follows:

Erythema and eshcar formation:
No erythema-0
Very slight erythema (barely perceptible)-1
Well defined erythema-2
Moderate to severe erythema-3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)-4

Edema:
No edema-0
Very slight edema (barely perceptible)-1
Slight edema (edges of area well-defined by definite raising)-2
Moderate edema (raised ~ 1mm)-3
Severe edema (raised >1mm and extending beyond area of exposure)-4

Body weight was recorded at predose and then on days 1 and 4. At the end of day 4, all rabbits were sacrificed and macroscopically examined. Skin samples were taken from the test site (left flank) and the control site (right flank) and histopathological examination was conducted.

After subcutaneous injection up to 40 mg, there were no premature animal deaths and no clinical signs of toxicity. No changes in BW gain were observed between all dosage groups. No edema was observed at the vehicle injection site or at the icatibant treated injection sites up to the highest dose injected (40 mg). Erythema and hematoma formation were observed with similar frequency and severity at the vehicle treated injection sites (up to 3/3 with erythema score of 2 and 1/3 had a hematoma) and the 5 mg (1/3 erythema with a score of 1 and 1/3 had a hematoma), 10 mg (1/3 erythema with a score of 2 and 1/3 had a hematoma), 20 mg (1/3 had erythema with a score of 1 and no hematomas), and 40 mg (2/3 had erythema with a score of 1.5 and no hematomas) icatibant injection sites. Therefore, this finding is most likely route and vehicle dependent and not drug-related. There were no icatibant-related histopathological findings at the injections sites that showed increased incidence compared to the vehicle sites in all animals from all dosage groups.

The second study (study no. JE049-143, GLP and initiated May 4, 1995) administered a single dose of icatibant (batch no. 102) at 0.5 mg, 0.5 mg and 0.1 mg as intra-arterial (central artery of ear), intravenous (marginal vein of the ear) or paravenous (beside the marginal vein of the ear) doses, respectively, to the right ear of female albino New Zealand rabbits (4 rabbits/group; age ~

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6 months and BW ranged from 3.5 to 4.6 kg). Isotonic saline solution was administered to the left ear of each rabbit as a control via each administration route. All animals were observed for clinical signs of toxicity and the injection sites were examined 24 h post dose and 2 of the rabbits were followed for 120 h post-dose. After 24 h post dose 2/4 rabbits per group were sacrificed and the injected area was histopathologically examined. After 120 h the remaining2/4 rabbits/group were sacrificed and the injection sites were examined.

After injection of icatibant via each route of administration, no rabbits showed any clinical signs of toxicity at 24 h or 120 h post-dose. Rabbits administered a single 0.5 mg intra-arterial dose of icatibant showed similar findings as the isotonic saline administered control animals. After intravenous injection of 0.5 mg icatibant, 1/4 animals showed slight reddening at the injection site but all other animals showed no irritation at the injection site. IV injection of isotonic saline showed no injection site irritation. After paravenous injection of 0.1 mg of icatibant, there were no signs of irritation at the injection site. Based on these data, icatibant was well tolerated after intra-arterial, intravenous and paravenous administration up to 0.5, 0.5 and 0.1 mg, respectively.

Taking the two local tolerance studies together, icatibant administration was well tolerated and contributed to little injection site irritation after intra-arterial, intravenous and paravenous administration. After subcutaneous administration to rabbits, slight irritation was observed at the injection site but these findings were considered route of administration and/or vehicle dependent and not related to icatibant administration, as similar findings were observed at the vehicle control injected sites.

2.6.6.8 Special toxicology studies

Study title: H-Oic-OH: Single dose subcutaneous toxicity study in mice

(b) (4)

Key study findings:

- The sponsor stated that there were no macroscopic changes observed at the end of the 3-week observation period and the maximum tolerated dose was considered >1000 mg/kg.
- Due to the absence of a control group, the limited number of animals tested and overall poor study, no conclusions can be made on the impact of H-Oic-OH SC administration.

Study no.: JE049-0151 (92.0453)
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)
(b) (4)
Date of study initiation: July 9, 1992
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: H-Oic-OH, batch no. H006, and ~ 98.0% pure

Reference ID: 2977968

Formulation/vehicle: H-Oic-OH dissolved in distilled water; administered SC in 10 mL/kg

Methods

Doses: 1000 mg/kg of H-Oic-OH via subcutaneous injection as a single dose. No control was used for this study.

Study design: 2 mice/sex (male BW ranged from 18-23 grams and female BW was 19 g) were administered H-Oic-OH and observed for 3 weeks post-administration for changes in behavior, changes in body weights, and then sacrificed and observed for macroscopic changes at the end of the 3-week observation period. No control group was used in this study.

Results: All animals survived after subcutaneous injection of H-Oic-OH up to 1000 mg/kg and no clinical signs of toxicity were observed throughout the 3-week post-dose follow-up period. There was observed BW loss after injection of H-Oic-OH. The sponsor stated that there were no macroscopic changes observed at the end of the 3-week observation period and the maximum tolerated dose was considered >1000 mg/kg.

Due to the absence of a control group, the limited animals tested and the poor design of this study, no conclusions can be made on the impact of H-Oic-OH SC administration. Although no animals died in this study, it is not known whether administration of H-Oic-OH causes any macroscopic or microscopic changes compared to controls due to the absence of controls in this study and due to the long observation period post-dose that may allow for recovery from any drug-induced insults.

Study title: H-D-Tic-OH: Single-dose subcutaneous toxicity study in mice

(b) (4)

Key study findings:

- The 2 female mice dosed with 1000 mg/kg of H-D-Tic-OH via SC died within 24 hours post-dose.
- Clinical signs of toxicity included reduced motility, transiently ataxic gait, marked flank respiration and abdominal position.
- There were no clinical signs of toxicity observed in males.
- Due to the absence of a control group, the limited number of animals tested and the poor design of this study, no conclusions can be made on the impact of H-D-Tic-OH administration.

Study no.: JE049-0152 (92.0454)
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: July 9, 1992

Reference ID: 2977968

GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: H-D-Tic-OH; Batch no. 009, and ~100% pure
Formulation/vehicle: H-D-Tic-OH formulated in potato starch mucilage and administered in 10.9 mL/kg vol

Methods

Doses: 1000 mg/kg of H-D-Tic-OH via subcutaneous injection as a single dose. No control was used for this study.

Study design: 2 mice/sex (male BW ranged from 18-23 grams and female BW was 19 g) were administered H-D-Tic-OH and observed for 3 weeks post-administration for changes in behavior and changes in body weights. Those that survived until 3-weeks post-dose were sacrificed and observed for macroscopic changes. No control group was used in this study.

Results: The 2 female mice dosed with H-D-Tic-OH died within 24 hours post-dose. Clinical signs of toxicity in the 2 females that died included reduced motility, transiently ataxic gait, marked flank respiration and abdominal position, which started between 1 and 4 hours post-dose and persisted for at least 5 hours. There were no clinical signs of toxicity observed in males. The sponsor stated that there were no changes in BW development for the surviving males throughout the 3-week observation period. Additionally, the sponsor stated that there were no macroscopic changes observed at the end of the 3-week observation period or in the animals that died within 24hours post-dose. However, there were no controls in this study with which to compared BWs or macroscopic examination of tissues.

Due to the absence of a control group, the limited number of animals tested and the poor design of this study, no conclusions can be made on the impact of H-D-Tic-OH administration. Administration of H-D-Tic-OH was toxic at 1000 mg/kg in females.

Study title: *In vitro* hemolysis test of HOE 140

Key study findings:

- This study was conducted under GLP conditions that were current as of 1982 and was not QA'd.
- Under the study conditions tested, 0.1% HOE 140 (icatibant) did not induce hemolysis of human whole blood.

Study no.: JE049-0150
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: February 26, 1993
GLP compliance: Yes- By 1982 standards
QA reports: yes () no (X)
Drug, lot #, and % purity: HOE 140, lot #66 and the purity of the compound is unknown
Formulation/vehicle: NA

Reference ID: 2977968

Methods

Doses: Negative control (physiological saline), 0.1% Icatibant and positive control (distilled water for injection)

Study design: Human normal blood from 3 donors (0.1 mL) was incubated with 1.0 mL of either negative or positive controls or icatibant at 37°C for 30 minutes. The mixture was then centrifuged and observed macroscopically for hemolytic reactions. Samples were scored as (-) no hemolysis, +1 (slight hemolysis), +2 (hemolysis red and clear), +3 (hemolysis red and turbid) or +4 (hemolysis discolored). In a second assay, whole blood was washed 3 times in saline and then the red blood cells were incubated with either negative or positive controls or icatibant at 37°C for 30 minutes. The optical density of each reaction mixture was determined.

Results: The positive and negative controls produced the expected results. The positive control induced hemolysis and the negative control did not. HOE 140 at 0.1% did not induced hemolysis under these study conditions.

Study title: Assessment of antibody responses towards icatibant in hyperimmunization studies

Key study findings:

- The sponsor concluded that hyperimmunization attempts in rat, dog and monkey, even with icatibant-protein conjugates in combination with adjuvant, did not elicit a sustained antibody response against icatibant.
- Based on the lack of information provided by the sponsor (icatibant lot and purity and detailed methods of study), no concrete conclusions can be drawn from this study report. However, based on the summary data provided by the sponsor, icatibant-thyroglobulin conjugate did not elicit a strong anti-icatibant antibody response.

Study no.: JE049-0166
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: Unknown
GLP compliance: No
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Icatibant conjugated with thyroglobulin, lot no. unknown and purity was unknown.
Formulation/vehicle: Icatibant conjugated with thyroglobulin and in adjuvant that consisted of 95% (V/V) paraffin oil, 2.4% (v/v) Tween 40, 0.1% (V/v) cholesterol and 0.01% (v/v) LPS.

Methods

Doses: NA (See under study design.)

Reference ID: 2977968

Study design: The sponsor conducted hyperimmunization studies in the rat, dog and monkey following standard immunization protocols and in the presence of adjuvant, the animals were repeatedly exposed to icatibant which was conjugated to a carrier protein. The presence of anti-icatibant antibodies was assessed by ELISA and Biacore assays.

For rats, 6 rats were immunized with icatibant-thyroglobulin conjugate. Antigen was injected intramuscularly. The initial immunization dosed 0.12 mg of conjugate followed by 4 boosts with 0.06 mg of conjugate. A single dog was immunized with 200 µg of icatibant thyroglobulin and then followed by 5 boosts. Two Cynomolgus monkeys were hyperimmunized with icatibant-thyroglobulin at 250 µg and followed with 5 boost. One monkey showed very low titers at all test bleeds and therefore, the immunization was stopped in that animal. The second animal had blood drawn and tested for anti-icatibant antibody formation. Rabbits (15 animals) were used as a positive control due to their ability to generate antibodies against protein conjugates of small molecular entities and hyperimmunized. Blood samples were taken from each species and assayed using ELISA for anti-icatibant IgG. Serum samples from each species were analyzed using Biacore.

Results: Hyperimmunization of rats, dogs and monkeys with icatibant-protein conjugates resulted in such a low antibody response that no specific anti-icatibant antibodies could be isolated when using the ELISA method. A single monkey showed a weak antibody response with a titer of 1:2048. The sponsor stated that rabbits produced significant amounts of anti-icatibant antibodies but these data were not provided in the study report. Using the Biacore method resulted in only a low content of icatibant-binding molecules was detected in the antiserum from rats, dog and monkey in contrast with rabbits that showed a high response. The following two summary tables of the results from the ELISA and the Biacore assays were provided by the sponsor.

Best Available Copy

Table 6: Results of hyperimmunization studies in rat, dog, monkey and rabbit (ELISA data)

Species	No. of animals	Icatibant conjugate	Titer obtained (1:n)	Purifiable icatibant-specific antibodies?	Study/animal
Rat	6	Thyr	512	-	18931 (chapter 4.1)
	10	Thyr	2048	-	20365#
Dog	1	Thyr	512	-	8459 (chapter 4.2)
Monkey	1	Thyr	512	-	8460#
	1	Thyr	none	-	9836 (chapter 4.3)
	1	Thyr	2048	-	9837 (chapter 4.3)
Rabbit	15	Thyr	150000	+	22946 (chapter 4.4)

Data details not shown in this report.

Table 7: Results of hyperimmunization studies in rat, dog, monkey and rabbit (Biacore data)

Species	Type of study	Dilution applied	Relative response	Study/animal reference
Monkey	Hyperimmunization	1:100	350	8460#
Rat	Hyperimmunization	1:100	750	18931 (chapter 4.1)
Dog	Hyperimmunization	1:100	25	8459 (chapter 4.2)
Rabbit	Hyperimmunization	1:100	3700	22030#

Data details not shown in this report.

Study title: Acute intravenous toxicity in rats

Key study findings:

Reference ID: 2977968

- There were no premature deaths, clinical signs of toxicity, statistically significant changes in BW gains and no macroscopic changes in the (b) (4) treated animals compared to the vehicle control treated animals.
- The minimal lethal dose was > 100 mg/kg of (b) (4) in rats.

Study no.: JE049-0155
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: August 8, 2006
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: (b) (4) batch no. 1005323 and 78.4% pure
Formulation/vehicle: (b) was formulated in 0.9% NaCl for intravenous administration with a 5 mL/kg vol

Methods

Doses: 0 (vehicle control) and 100 mg/kg of (b) (4) were intravenously administered to rats as a single dose

Study design: Five Sprague-Dawley rats/sex/group were administered a single IV dose of either vehicle as the control or 100 mg/kg of the impurity. All animals were observed for clinical signs of toxicity, mortality, and changes in BW for a period of 14-days post-dose. After the 14-day period, all animals were macroscopically examined.

Results: There were no premature deaths, no clinical signs of toxicity and no statistically significant changes in BW gains in the (b) (4) treated animals compared to the vehicle control treated animals. Compared to the control treated animals there were no macroscopic changes in the (b) (4) group. The minimal lethal dose was > 100 mg/kg of (b) (4) in rats.

Study title: Bacterial reverse mutation test

Key study findings:
- The study was considered valid based on the methodology used.
- Under the experimental conditions in this study, (b) (4) up to 5000 µg/plate did not induce an increase in the number of revertants compared to the negative controls.

Study no.: JE049-0154
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: August 11, 2006
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: (b) (4) batch no. 1005323 and 78.4% pure
Formulation/vehicle: (b) (4) was dissolved in water

Reference ID: 2977968

Methods

Doses: For the first mutagenicity study with and without S9 mix and using the plate incorporation method the following concentrations of (b) (4) was used: 0, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/plate. In the second study using the preincubation method the following concentrations were used with and without S9 mix: 625, 1250, 2500, 3750 and 5000 µg/plate. The maximum concentrations achieve the recommended limit concentration for this assay.

Study design: (b) (4) was tested in a dose-ranging test and then followed by 2 independent experiments in the presence and absence of metabolic activation (S9 mix). Experiments were performed using the direct plate incorporation method except for the 2nd test with S9 mix, which was performed using the preincubation method. Five strains of *Salmonella typhimurium*: TA 1535, TA 1537, TA98, TA 100 and TA102 were assayed in the 2 definitive experiments, where strains TA98, TA 100 and TA 102 were assayed in the dose-ranging study. In the dose-ranging study, single plates/concentration were assessed. In the 2 definitive studies, 3 plates/concentration with and without S9 mix were evaluated. After 48 h at 37°C, the revertant colonies were scored using an automatic counter. The potential for cytotoxicity was evaluated by the decrease in the number of revertants and or the thinning of the bacterial lawn.

Negative control was water for injection.

The positive controls without S9 mix:
1 µg/plate of sodium azide for TA 1535 and TA100 strains
50 µg/plate of 9-aminoacridine for TA 1537 strain
0.5 µg/plate of 2-nitrofluorene for TA 98 strain
0.5 µg/plate of mitomycin C for TA 102 strain

Positive controls with S9 mix:
2 µg/plate of 2-anthramine for TA 1535, TA 1537, TA98 and TA 100 strains
10 µg/plate of 2-anthramine for TA 102 strain

(b) (4) was freely soluble in water and was non-toxic in the dose-ranging assay. Therefore, the highest concentration (5000 µg/plate) was selected for the definitive studies. There was no precipitation of the drug at the doses examined.

Results: The study was considered valid based on the methodology used for each study, the criteria for evaluating the plates and the expected outcomes for the negative and the positive controls. The number of revertants in the negative control group was within the historical control data as were the positive control revertant numbers. The sponsor examined doses up to 5000 µg/plate in the 2 definitive studies, which was adequate due to the products free solubility in water, its lack of toxicity on the bacterial strains and the absence of precipitation that potentially would interfere with plate counting. Under the experimental conditions in this study, (b) (4) did not induce an increase in the number of revertants compared to the negative controls.

Reference ID: 2977968

Study title: *In vitro* mammalian chromosome aberration test in cultured human lymphocytes

Key study findings:

- (b) (4) induced chromosome aberrations in human lymphocytes under the study conditions assayed. This is in agreement with the sponsor's conclusions.

Study no.: JE049-0156
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: August 9, 2006
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: (b) (4) batch no. 1005323 and 78.4% pure
Formulation/vehicle: (b) (4) was dissolved in water

Methods

Doses: In the definitive study (the second experiment), the (b) (4) concentrations examined were: 0, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/mL in the absence and presence of S9 mix. The maximum concentration achieves the recommended limit concentration for this assay.

Study design: Two independent experiments (using duplicate cultures in each experiment for each concentration tested) in the presence and absence of metabolic activation (+ or –S9 mix) were conducted with different concentrations of (b) (4) to evaluate whether (b) (4) induces chromosome aberration formation in cultured human lymphocytes. The highest concentration of (b) (4) used for the first experiment was selected based on pH, osmolality and solubility. The test item was freely soluble in the vehicle (culture media) and showed no precipitation from media at 5000 µg/mL. In the first experiment, lymphocytes were exposed to controls and (b) (4) concentrations in the presence and absence of S9 mix for 3 hours and then rinsed. Cells were harvested 20 h after the beginning of treatment. In the first experiment, the (b) (4) concentrations examined were: 39.06, 78.13, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/mL.

The second experiment exposed lymphocytes to test article and controls without S9 mix continuously until harvesting of cells at 20 or 44 h post initiation of treatment. In the presence of S9 mix, cells were exposed to controls and test article for 3 h and then rinsed and incubated for 20 h post initiation of treatment and then had the cells harvested. Cells were fixed and then spread onto glass slides and scored for aberration formation. The cytotoxicity of icatibant was determined using the mitotic index (number of cells in mitosis/1000 cells examined). Analysis of 200 metaphases/concentration of test article was made with 100 metaphases/culture when possible. Only 50 metaphase cultures were analyzed when at least 10% cells with structural chromosome aberrations were observed. All metaphase analyses were performed blinded.

Negative controls: Vehicle (culture medium)

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Positive controls: In the absence of S9 mix, mitomycin C was used at a final concentration of 3 µg/mL for the 3 h incubation and at 0.2 µg/mL for the continuous incubation. In the presence of S9 mix, cyclophosphamide was used at a final concentration of 12.5 or 25 µg/mL.

Results: Based on the study methodology and the negative and positive controls providing results within their respective historical control data, and based on the (b) (4) concentrations tested, the 2 studies were considered valid. Criteria for a positive result were a reproducible and statistically significant increase in the frequency of cells with structural aberrations for at least one of the dose-levels and one of the two harvest times. Reference to historical control data was also compared.

In the first experiment after the 3 h treatment period and in the absence of S9 mix, there was little reduction in the mitotic index at 5000 µg/mL (MI 96% compared to the vehicle control) and there was no increase in structural chromosome aberrations. In the presence of S9 mix, there was no reduction in MI and no increase in structural chromosomes aberration frequency compared to the vehicle control group. The positive controls induced an increase in structural chromosome aberration formation in the presence and absence of S9 mix.

After 20 and 44 h treatments, in the absence of S9 mix, there was a slight to marked decrease in mitotic index [20 hr: MI at 53% and 37% at 2500 and 5000 µg/mL of (b) (4) respectively, compared to the vehicle controls [44 h: MI at 59%, 60% and 42% at 1250, 2500 and 5000 µg/mL of (b) (4) respectively, compared to controls]. A slight, but not statistically significant, increase in the frequency of cells with structural chromosomal aberrations (- gaps) was noted at 2500 µg/mL [mean % (- gaps) 2.0] and 5000 µg/mL [mean % (- gaps) 4.0] after the 20 h treatment.

After 44 h of treatment in the absence of S9 mix, a statistically significant increase in the mean number of cells (%) with structural aberrations (-gaps) was observed at 2500 and 5000 µg/mL. However, cytotoxicity levels significantly exceeded 50% (actual value is 42%) at 5000 µg/mL. At this level of cytotoxicity, structural aberration formation may be observed as a result of indirect mechanisms unrelated to test article. At 2500 µg/mL the cytotoxicity was at 60%. Therefore, (b) (4) did increase chromosome aberration formation in this study.

Second experiment without S9 mix: Chromosome Aberration (44-h treatment)

Concentrations (µg/mL)	No. of cells scored	Structural aberrations		Cells w/ structural aberrations	
		Total no. (+ gaps)	Total no. (– gaps)	Mean % (+ gaps)	Mean % (– gaps)
0	200	8	1	4.0	0.5
1500*	200	2	2	0.5	0.5
2500[A]	200	15	15	6.5	6.5
5000[A]	200	34	21	25.0	19.0
[B]MMC (0.2 µg/mL)	100	34	28	29	26

*-The study protocol indicates that 1250 µg/mL was tested, which differs from the concentration provided in the sponsor's table.
[A]-The reviewer questions these data due to no difference between the total number of structural aberrations observed when including gaps and excluding gaps.
[B]- No control was used for the 44 hour treatment period. After 20 hours continuous treatment with MMC, an increase in structural aberrations was observed.

In the presence of S9 mix and after 3 h of treatment and 20 h harvest and after 3 h of treatment and 44 h harvest, there was no reduction of mitotic index up to 5000 µg/mL and no increase in the frequency of structural aberration formation compared to the vehicle control group. The

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positive control (cyclophosphamide) reduced the MI to 35% and induced structural chromosome aberrations by 21% compared to the vehicle controls.

Based on the increased frequency of structural chromosome aberration formation observed in the absence of S9 mix at 44 h of treatment, the reviewer concludes the (b) (4) has mutagenic potential under these experimental conditions. While these data show that (b) (4) is positive for genotoxicity, the peptide class of drugs has a low genotoxic risk.

2.6.6.9 Discussion and Conclusions

In support of the proposed SC route of administration for this NDA, the sponsor conducted two 14-day SC toxicity studies in the rat, one 13-week SC toxicity study in the rat (submitted as a combined 13-week toxicity and fertility and early embryonic development study), a SC dose-ranging study in the dog, a 4-week SC toxicity study in the dog (twice weekly dosing), and two 13-week SC toxicity studies in the dog (1 with daily SC dosing and 1 with daily and TID administered twice weekly SC dosing). All studies demonstrated local injection site irritation. After 13-weeks repeat SC dosing in rats up to 10 mg/kg/day using a batch of icatibant synthesized by a new manufacturer, no systemic toxicities were observed. However, in two 14-day repeat dose SC studies using a batch of icatibant synthesized by a different (old) manufacturer, icatibant administration from 0.1 to 50 mg/kg/day resulted in significant injection site irritation at 50 mg/kg/day, decreases in prostate weights and testes weights in the 10 mg/kg/day and 50 mg/kg/day and increases in thymic weights in the HD (50 mg/kg/day) females. Histopathological findings included: epididymides (oligospermia and aspermia) and testes (hypospermia) were observed in icatibant treated groups and injection site irritation was observed in the 50 mg/kg/day group. These findings were not observed in the male reproductive organs at 1 mg/kg. The identification of a NOAEL of 10 mg/kg after 13 weeks while none was identified at 14 days may be due to adaptive responses to lower drug doses over time or differences in the impurity profiles of the differently manufactured batches. Of note, the 13 weeks study dosed to only 10 mg/kg while the 14-day study dosed to 50 mg/kg.

In dogs, SC administration of icatibant for 13-weeks (10, 30 and 100 mg/kg/day), resulted in clinical signs of toxicity included erythema of the face, injection site irritation, abnormal vocalization and several incidences of gastrointestinal disturbances (diarrhea and/or vomiting) at all doses. Macroscopic findings were observed at injection sites (hematomas and edema), the male (testes, epididymides and prostate) and female (ovaries and uterus) reproductive organs (organs all immature at all doses), thymus, liver and adrenal gland. Microscopic changes in the male (testes, epididymides, and prostate) and female (mammary gland, ovaries, uterus and vagina) reproductive organs, at the injection site (mainly HD groups but sporadic in LD and MD groups), in the adrenal gland (vacuolated cortical cell in HD males and females), liver (vacuolated hepatocyte in 1/4 males) and thymus (all doses) were observed. No NOAEL was determined for this study due to reproductive organ effects in all icatibant treated males and females, lymphoid depletion in the thymus and on injection site irritation. When altering the dosing schedule from daily administration to twice weekly administration of TID doses of icatibant in dogs (3.3 mg/kg TID twice weekly) for 13-weeks, no affects were observed in the male reproductive organs but females treated with SC administration of icatibant twice weekly showed delayed or impaired maturation of the reproductive organs. Lymphoid depletion in the thymus was observed in both males and females after twice weekly dosing. Observed clinical

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signs after daily dosing of icatibant at 3 and 10 mg/kg/day included increased erythema and edema at multiple sites that increased with increased dose. With cessation of treatment erythema abated. After daily administration of icatibant at 3 and 10 mg/kg/day, the thymus (lymphoid depletion) and the male (immature testes, epididymides and prostate) and female (immature ovaries, oviducts, uterus and vagina) reproductive tissues showed toxicity as reflected by reduction in these organ weights relative to body weights, macroscopic findings of reduced size and microscopic findings of reduced size. After the 13-week recovery period, 1/2 males showed the testes and prostate as immature where 1/2 males had recovered and there were no findings in female reproductive organs suggesting these adverse effects were reversible. Hormonal analysis showed that icatibant induced a decrease of testosterone, LF and FSH in male dogs, which secondarily induced sexual immaturity.

When dosing dogs for a shorter duration (4-weeks) at 1 and 3.3 mg/kg TID as twice weekly dosing erythema was observed in low incidence in males and females at each dose and gross and microscopic findings were limited to injection site irritation (hematomas) which did not follow a dose response. Testosterone levels were significantly decreased in males treated with icatibant at 1 or 3.3 mg/kg TID twice weekly but these levels rebounded between dosing intervals. A NOAEL of 3.3 T.I.D. (10 mg/kg) administered twice weekly was determined by the reviewer based on no microscopic drug-related findings.

At 13-weeks daily SC dosing of rats showed no toxicity after administration of a newly manufactured batch of icatibant from a different site manufacturing site. However, after IV and SC administration of icatibant for 1-month or 14-days, respectively, the target organs of toxicity were the male reproductive organs, kidneys, stomach and the injection sites (using old icatibant manufacturer). Dogs administered icatibant SC daily for 13 weeks showed erythema of the face (histamine reaction), male and female reproductive organ immaturity, thymus (lymphoid depletion), adrenal (cortical cellular vacuolation), liver (vacuolated hepatocyte) and injection site irritation. After 1-month IV administration, erythema and chronic gastritis were observed in dogs. When altering the dosing schedule in dogs from daily administration to twice weekly dosing, the effects on the male reproductive organs were reduced. However, females showed immature reproductive organs.

The sponsor submitted three genetic toxicology studies in this NDA. These studies were: a bacterial reverse mutation assay (a complete bacterial strain battery), an *in vitro* mammalian chromosome aberration test, and an *in vivo* bone marrow micronucleus assay in Sprague-Dawley rats using the icatibant. The bacterial reverse mutation assay and the *in vivo* micronucleus study in rats were studies used to bridge the change in manufacturer for the drug substance. Icatibant did not induce genetic toxicity in these three studies under the conditions assayed. These results are consistent with numerous other studies previously reviewed under INDs (b) (4)

Two reproductive toxicology studies were included in this NDA to complete the reproductive toxicology battery. The sponsor combined the 13-week repeat dose toxicity study and the effects on fertility and early embryonic development to implantation study in rats. Male and female Sprague-Dawley rats were administered 1, 3 and 10 mg/kg/day of icatibant SC. HD treated females (10 mg/kg/day) had an increase in pre-implantation loss compared to vehicle treated females. Icatibant had no effects on male sperm count, motility, or morphology. Based on no

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toxicity observed in male seminology and no effects on mating or fertility indices, the NOAEL for male fertility was 10 mg/kg/day. Based on an increase of pre-implantation loss observed in HD treated females, the NOAEL for female fertility was 3 mg/kg/day.

In the second reproductive toxicity study submitted in this NDA, the effects of daily SC administration of icatibant on pre- and post-natal development in pregnant rats at 1, 3 and 10 mg/kg/day. Icatibant treatment delayed partuition at all doses, resulting in F_0 deaths (found dead or early sacrifice) in the 3 and 10 mg/kg/day groups. Clinical signs of toxicity (pallor of eyes and extremities, round back, piloerection and reddish discharge in vagina) were observed in 1 MD and in multiple HD F_0 females. After SC administration, icatibant and the M2 metabolite is found in rat milk. Based on the observed maternal toxicities including clinical signs, prolonged gestation periods for all icatibant dosage groups, F_0 spontaneous deaths and early sacrifice of icatibant treated dams, litter deaths, and necropsy findings in the MD and the HD groups, the maternal toxicity NOAEL could not be defined. The F_1 generation showed an increase in the number of pup deaths and cannabalism in the MD and HD compared to the VC groups. Therefore, the NOAEL for systemic toxicity in the F_1 generation was determined to be 1 mg/kg/day. The F_1 generation showed a slight decrease in air righting and hair growth compared to the VC control group. Therefore, the NOAEL for development of the F_1 generation was considered 3 mg/kg/day. The sponsor has adequately completed the reproductive toxicity battery for icatibant based on the 2 new studies submitted to the NDA and previously reviewed studies under IND (b) (4) Based on the reproductive toxicity data, icatibant should be classified as Pregnancy Category C based on the presence of embryotoxicity, increased spontaneous abortions and increased pup deaths.

Two local toxicity studies were conducted in rabbits using single dose SC administration in one study and intrarterial/intravenous/paravenous administrations in the second study. Icatibant administered via the SC route up to 40 mg in acetate buffer, pH 5.5 was comparable to the vehicle control for local irritation. In the second study, administration of icatibant up to 0.5 mg intra-arterially and intravenously and 0.1 mg paravenously resulted in little injection site irritation.

Two single-dose studies administering two unnatural, synthetic peptides that are both found in the icatibant drug substance, (b) (4) at 1000 mg/kg, SC were conducted by the sponsor. Neither study was adequately designed with the absence of control groups. No animals died after (b) (4) administration. However, after (b) (4) (2/3) females died within 24-h post-dose.

An *in vitro* hemolysis test of icatibant showed that 0.1% of icatibant did not induce hemolysis of human whole blood after incubation for 30 min where incubation with the positive control (distilled water for injection) did induce hemolysis.

In an assessment of antibody responses in rat, dog and monkey, the sponsor concluded that hyperimmunization attempts, even with icatibant-protein conjugates in combination with adjuvant, did not elicit a sustained antibody response against icatibant. Based on the lack of information provided by the sponsor (icatibant lot and purity and detailed methods of study), no concrete conclusions can be drawn from this study report. However, based on the summary data

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provided by the sponsor, icatibant-thyroglobulin conjugate did not elicit a strong anti-icatibant antibody response.

For safety qualification of the (b) (4) impurity, the sponsor conducted 1 acute IV toxicity study, a bacterial reverse mutation study and a chromosome aberration study. (b) (4) was negative in the Ames test but positive in the chromosomal aberration test for inducing genotoxicity. Although positive for genotoxicity in the chromosome aberration test, (b) (4) (b) (4) is a member of the peptide drug class which is considered to have low genotoxic risk. The acute IV toxicity study dosed rats up to 100 mg/kg. No toxicity was observed in rats. However, the duration of this study is not considered acceptable to qualify this impurity for safety. Therefore, (b) (4) has not been adequately qualified for safety.

2.6.6.10 Tables and Figures

Not applicable because no new data were submitted.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Not applicable because no new data were submitted.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Jerini US, Inc. (Jerini) submitted this NDA 22-150 for Firazyr (icatibant) 30 mg, SC for the treatment of adults (≥ 18 years old) with hereditary angioedema (HAE). The proposed dosing regimen is a single SC dose of 30 mg icatibant (0.6 mg/kg for a 50 kg human) which is associated with a systemic exposure of 4107 ng*h/mL. The potential maximum human dose is 90 mg (1.8 mg/kg for a 50 kg human) which is associated with systemic exposures of 12.3 mcg*h/mL in humans.

HAE patients have acute episodes of swelling of the hands, feet, trunk, face, gut and/or airways. Swelling of the upper airways can be fatal if not treated promptly. In Type I (quantitative deficiency) and Type II (qualitative deficiency) HAE a deficiency of the C1-esterase inhibitor (C1-INH) has been identified. C1-INH is a serine protease inhibitor that inhibits components of the complement, coagulation, fibrinolytic and kinin-releasing systems. C1-INH inhibits kallikrein, a protease that contributes to the formation of bradykinin (BK) from the cleavage of kininogen. HAE patients have increased levels of BK. BK binds to the BK type 1 (B_1) and type 2 (B_2) receptors to trigger activation of many biochemical cascades. Two such cascades are coupled by the $G_{\alpha q/11}$ and $G_{\alpha i}$ proteins, leading to calcium mobilization and MAPK activation, respectively. Calcium mobilization and MAPK activation induces additional cellular events including cellular proliferation, excitation of neurons, vasodilatation, increased vascular permeability and smooth muscle cell contraction. These events are believed to contribute to the acute inflammation and edema observed in HAE patients.

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Icatibant is a synthetic decapeptide with a similar structure to endogenous BK but contains 5 non-proteinogenic amino acids. Icatibant was shown to be a competitive B_2 receptor antagonist with similar affinity as BK in numerous *in vitro* receptor binding assays and in cell and tissue culture models. The sponsor has proposed that treatment of HAE patients with icatibant may decrease BK-induced downstream events, including vascular permeability, vasodilation and inflammation. As shown by *Han et al.*[1], C1-INH deficient mice show increased vascular permeability, similar symptomology as HAE patients, which was reversed after treatment with icatibant.

Safety pharmacology studies that were conducted under IND (b) (4) and reviewed by Dr. Choi showed effects on CNS (decreased motor activity, assumption of prone position, reduced respiration, ptosis, piloerection and mydriasis at 1-10 mg/kg IV), CVS (icatibant decreased coronary blood flow in isolated guinea pig heart, aggravated the duration of post-ischemic reperfusion arrhythmias in isolated working hearts in Wistar rats and impaired cardiodynamics) and renal systems (decreased urine output, sodium and potassium excretion, to a lesser extent for GFR, renal plasma flow and osmolar clearance in beagle dogs at 0.3 mg/kg, IV). These effects were considered the result of the partial agonistic property of icatibant on B-receptors. These effects were not observed in rats or dogs in 13-week repeat dose toxicity studies. In safety pharmacology studies submitted to this NDA, icatibant did not inhibit HERG channels up to 300 µM icatibant concentrations, did not have an effect on respiratory function or GI transit in rats after IV administration up to 10 mg/kg. Gastric acid volume and acidity were decreased in rats compared to the vehicle control treatment in rats at 10 mg/kg, IV.

Icatibant is rapidly metabolized to M1 (b) (4) and M2 (b) (4) which have been identified in mice, rats, dog, monkey and man. The major metabolite in man is M1 (>90%). M1 is formed in rat (~70%), dog (~60%) and mouse (~50%). After SC administration, the M2 metabolite (b) (4) appears rapidly (0.2 to 3 h post-dose) in rats and dogs. In rats and dogs, increases in icatibant doses from LD to MD doses (0.1 to 3 mg/kg in rats and 10 to 30 mg/kg/day in dogs) produced approximately doses proportionate increases in systemic exposures. Increases in icatibant doses from MDs to HDs resulted in super-proportionate increases in systemic exposures in both rats (increased ~128-fold instead of the expected ~50-fold) and approximately dose proportionate in dogs. Using an alternate dosing schedule in dogs (1 or 3.3 mg/kg TID of icatibant twice weekly), AUC levels of icatibant and M2 increased in an approximate dose proportionate manner. The t_{max} was achieved from 0.5 to 3.0 h post-dose in rats and dogs. Neither icatibant nor M2 accumulated after repeat dosing in males and females up to 13-weeks dosing. There were no gender differences observed in rats or dogs in the systemic exposures of males and females.

Icatibant is rapidly absorbed with 100% bioavailability after subcutaneous administration. Protein binding was low in all species examined: 49% in rat, 44% in man, and ~38% in dog and monkey. Distribution studies showed that icatibant (or its metabolites) is distributed into the kidneys, liver, urinary bladder, lungs and spleen 1 hour and it still detectable in the liver, kidneys, spleen, bone marrow, adrenals and injection sites at 24-hours post SC dose in rats. Concentrations of radioactivity was detected in the milk of nursing rats with peak levels

[1] Han *et al.*, J. Clin. Invest. 109: 1057-1063, 2002 (cited by the sponsor)

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observed 7 h after administration of radiolabeled drug. Icatibant is not degraded the two main bradykinin-cleaving enzymes, angiotensin converting enzyme (ACE) and neutral endopeptidase. Neither icatibant nor the M1 or M2 metabolites are metabolized by human hepatocytes or microsomes. Additionally, icatibant is not an inducer of CYP450 isozymes. Therefore, icatibant is metabolically more stable than BK. Based on excretion studies, the primary route of elimination after SC administration of icatibant is via the kidneys in mice (~97.1%), rats ~61%) and dogs (~63%) with small amounts excreted in the feces (8.0% to 33%). Excretion was mainly renal, regardless of species and route of administration

Under IND (b) (4) acute toxicology studies conducted in the mouse, rat and dog were reviewed (Dr. Choi's review). The highest non-lethal IV dose was 30.7 mg/kg and the minimum lethal dose (MLD) was 48.3 mg/kg for mice. For rats, the highest non-lethal IV dose was 12.3 mg/kg and the MLD was 19.2 mg/kg. After IV dosing, rapid respiration, twitching and convulsions were observed prior to death. In an acute IV toxicity study, male dogs (n=2/group) were administered 0.4, 2 and 10 mg/kg icatibant. No mortality was observed in this study. However, numerous clinical signs, many suggestive of massive histamine release, were observed in the MD and HD groups. In a separate study, male dogs (n= 3/group) were administered single IV doses (infused over 5 h period) of 15, 30 and 60 mg/kg. An increase in serum CPK levels were above historical background (153 mU/mL) was noted in 1 MD animal (406 mU/mL) and two HD dogs (843 and389 mU/mL, respectively. The NOAEL in this study was 15 mg/kg. In acute SC toxicity studies, the highest non-lethal dose was 614 mg/kg and the MLD was 767 mg/kg for mice. In rats, no mortality was observed after a single SC injection of 1227 mg/kg icatibant. After SC injection, injection sites showed bluish discoloration that progressed to necrosis.

During icatibant's development, intranasal (IN), inhalation (IH), IV and SC routes of administration have been explored. Under INDs (b) (4), the main repeat dose toxicology studies investigated the intranasal (IN), inhalation (IH) and IV routes of administration with a few studies conducted using the SC route. Due to low bioavailability, no toxicity was observed in most IN toxicity studies. However, reduced testicular weights and spermiogenesis in the testes were observed in a single 1-month IN toxicity study carried out in the rat at 125, 1250 or 12500 µg/kg. The NOAEL was considered 125 µg/kg based on these testicular findings.

In a comparative IV/SC/IN 1-month toxicity study, male rats were either intravenously (10 mg/kg/day), subcutaneously (10 mg/kg/day) or intranasally (2.5 mg/animal/day) administered icatibant. Renal tubular atrophy was observed in 7/10 rats and renal tubular necrosis in 4/10 rats treated via the IV route. Impairment in sperm maturation was observed in 8/10 rats and oligospermia in the epididymides in 7/10 rats after IV adminsitration. Tubular atrophy (4/10) and tubular necrosis (1/10) were also observed in SC treated rats. Despite similar exposure levels, oligospermia in the epididymides in 1/10 animals was the only male reproduction effect observed in SC treated animals. No control groups were included in this study. TK analysis showed the exposure ($AUC_{0\text{-}4h}$) to be similar between IV (20.33 µg/mL) and SC (17.3 µg*h/mL) treated animals. No adverse effects were reported in the intranasal group.

In a 1-month toxicity study, rats were intravenously administered 0.1, 1 and 10 mg/kg/day of icatibant. Renal toxicity was observed in 10/10 males and 5/10 females with areas of epithelial

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necrosis, areas of repair and foci of tubular atrophy at the 10 mg/kg/day dose. Irreversible tubular atrophy was present in 5/5 males and 1/5 females after a 28-day recovery period. The NOAEL was 1 mg/kg/day. In a 1-month study, dogs were intravenously administered 0.1, 0.25 and 0.8 mg/kg/day. All HD males 3/3 had swelling of flews that was attributed to histamine release. The major part of the glandular portions in the basal mucosa was missing from the stomach of 1 HD male that was reported as a relatively rare change. The NOAEL was 0.25 mg/kg/day.

Based on the previous reviews (b) (4) of the 1-month repeat dose toxicity studies in rats and dogs, the target organs toxicity in the rats were the male reproductive organs (decreased spermatogenesis and decreased weights of testes, epididymis, prostate, and/or seminal vesicles), kidneys (uneven color, increased weight, epithelial necrosis of proximal tubules, foci of tubular atrophy, increased blood urea, uric acid, creatinine, hemoglobin and epithelial cells in urine), stomach (taut and enlarged) and liver (increased wt). In the dogs, the target organs were swelling of flews (histamine release reaction) and the stomach (chronic gastritis).

In support of the proposed SC route of administration for this NDA, the sponsor conducted two 14-day SC toxicity studies in the rat, one 13-week SC toxicity study in the rat (submitted as a combined 13-week toxicity and fertility and early embryonic development study), a SC dose-ranging study in the dog, a 4-week SC toxicity study in the dog (twice weekly dosing), and two 13-week SC toxicity studies in the dog (1 with daily SC dosing and 1 with daily and TID administered twice weekly SC dosing). After 13-weeks repeat SC dosing in rats up to 10 mg/kg/day using a batch of icatibant synthesized by a new manufacturer, no systemic toxicities were observed. However, in two 14-day repeat dose SC studies using a batch of icatibant synthesized by a different (old) manufacturer, icatibant administration from 0.1 to 50 mg/kg/day resulted in significant injection site irritation at 50 mg/kg/day, decreases in prostate weights and testes weights in the 10 mg/kg/day and 50 mg/kg/day and increases in thymic weights in the HD (50 mg/kg/day) females. Histopathological findings included: epididymides (oligospermia and aspermia) and testes (hypospermia) were observed in icatibant treated groups and injection site irritation was observed in the 50 mg/kg/day group. These findings were not observed in the male reproductive organs at 1 mg/kg. The identification of a NOAEL of 10 mg/kg after 13 weeks while none was identified at 14 days may be due to adaptive responses to lower drug doses over time or differences in the impurity profiles of the differently manufactured batches. Of note, the 13 weeks study dosed to only 10 mg/kg while the 14-day study dosed to 50 mg/kg.

In dogs, SC administration of icatibant for 13-weeks (10, 30 and 100 mg/kg/day), resulted in clinical signs of toxicity included erythema of the face, injection site irritation, abnormal vocalization and several incidences of gastrointestinal disturbances (diarrhea and/or vomiting) at all doses. Macroscopic findings were observed at injection sites (hematomas and edema), the male (testes, epididymides and prostate) and female (ovaries and uterus) reproductive organs (organs all immature at all doses), thymus, liver and adrenal gland. Microscopic changes in the male (testes, epididymides, and prostate) and female (mammary gland, ovaries, uterus and vagina) reproductive organs, at the injection site (mainly HD groups but sporadic in LD and MD groups), in the adrenal gland (vacuolated cortical cell in HD males and females), liver (vacuolated hepatocyte in 1/4 males) and thymus (all doses) were observed. No NOAEL was determined for this study due to reproductive organ effects in all icatibant treated males and females, lymphoid depletion in the thymus and on injection site irritation. When altering the

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dosing schedule from daily administration to twice weekly administration of TID doses of icatibant in dogs (3.3 mg/kg TID twice weekly) for 13-weeks, no affects were observed in the male reproductive organs but females treated with SC administration of icatibant twice weekly showed delayed or impaired maturation of the reproductive organs. Lymphoid depletion in the thymus was observed in both males and females after twice weekly dosing. Observed clinical signs after daily dosing of icatibant at 3 and 10 mg/kg/day included increased erythema and edema at multiple sites that increased with increased dose. With cessation of treatment erythema abated. After daily administration of icatibant at 3 and 10 mg/kg/day, the thymus (lymphoid depletion) and the male (immature testes, epididymides and prostate) and female (immature ovaries, oviducts, uterus and vagina) reproductive tissues showed toxicity as reflected by reduction in these organ weights relative to body weights, macroscopic findings of reduced size and microscopic findings of reduced size. After the 13-week recovery period, 1/2 males showed the testes and prostate as immature where 1/2 males had recovered and there were no findings in female reproductive organs suggesting these adverse effects were reversible. Hormonal analysis showed that icatibant induced a decrease of testosterone, LF and FSH in male dogs, which secondarily induced sexual immaturity.

When dosing dogs for a shorter duration (4-weeks) at 1 and 3.3 mg/kg TID as twice weekly dosing erythema was observed in low incidence in males and females at each dose and gross and microscopic findings were limited to injection site irritation (hematomas) which did not follow a dose response. Testosterone levels were significantly decreased in males treated with icatibant at 1 or 3.3 mg/kg TID twice weekly but these levels rebounded between dosing intervals. A NOAEL of 3.3 T.I.D. (10 mg/kg) administered twice weekly was determined by the reviewer based on no microscopic drug-related findings.

At 13-weeks daily SC dosing of rats showed no toxicity after administration of a newly manufactured batch of icatibant from a different site manufacturing site. However, after IV and SC administration of icatibant for 1-month or 14-days, respectively, the target organs of toxicity were the male reproductive organs, kidneys, stomach and the injection sites (using old icatibant manufacturer). Dogs administered icatibant SC daily for 13 weeks showed erythema of the face (histamine reaction), male and female reproductive organ immaturity, thymus (lymphoid depletion), adrenal (cortical cellular vacuolation), liver (vacuolated hepatocyte) and injection site irritation. Clinical studies have shown local injection irritation effects. After 1-month IV administration, erythema and chronic gastritis were observed in dogs. When altering the dosing schedule in dogs from daily administration to twice weekly dosing, the effects on the male reproductive organs were reduced. However, females showed immature reproductive organs.

At a pre-NDA meeting held January 24, 2007 between Jerini and DPAP, Jerini was informed of the need for chronic toxicity studies (26-week in rat and 39-week in dog) to support the chronic intermittent HAE indication. Jerini indicated that these studies have been initiated as of June 2007 (dog study) and July 2007 (rat study). The final study reports are expected to be submitted to the Division in September 2008.

The sponsor submitted three new genetic toxicology studies in this NDA. These studies were: a bacterial reverse mutation assay (a complete bacterial strain battery), an *in vitro* mammalian chromosome aberration test, and an *in vivo* bone marrow micronucleus assay in Sprague-Dawley

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rats using the icatibant. The bacterial reverse mutation assay and the *in vivo* micronucleus study in rats were studies used to bridge the change in manufacturer for the drug substance. Icatibant did not induced genetic toxicity in these three studies under the conditions assayed. The results of these studies are consistent with numerous others that were previously reviewed under INDs (b) (4)

The sponsor has not submitted studies to evaluate icatibant's carcinogenic potential. Jerini was informed of the need for 2 carcinogenicity studies to address the carcinogenic potential of icatibant (pre-NDA meeting January 24, 2007). Jerini indicated that the carcinogenicity studies would be initiated April 8, 2008 and July 7, 2008 for mice and rats, respectively.

Under IND (b) (4) a 5-week fertility study in male mice was conducted up to 80.8 mg/kg/day SC. There were no effects on copulation rate in icatibant treated animals compared to the vehicle control group. This study did not follow current Segment 1 study guidelines. Two teratogenic studies were reviewed (1 in rats and 1 in rabbits), which were preceded by dose-ranging studies. In the dose-ranging study conducted in rats, 3 pregnant rats/group were treated with 25, 50 and 100 mg/kg/day (no vehicle control was used) of icatibant SC from Days 7 through 18 of pregnancy. Significant maternal toxicity was observed at 25 mg/kg/day and higher (slight swelling at the injection sites, blue discoloration of skin, panting, reduced activity, abdominal position and then death) but concepti from this group were considered unremarkable with no teratogenicity observed.

In the definitive rat study, the effects of icatibant SC administration on morphological and post-natal development including fertility in Wistar rats was examined. Female rats (30 pregnant rats/group) were treated with 0 (vehicle control- 0.9% NaCl), 0.25, 2.5 and 25 mg/kg/group of icatibant SC from Days 7 through 18 of pregnancy. There were no significant major external of internal anomalies reported in pups for F_1 or F_2 generations. Although 1 or 2 fetuses in the 0.25 and 2.5 mg/kg group had multiple minor skeletal malformations or variations. Post-natal development of treated fetuses and fertility of F_1 generation were comparable to the control group. No major terata were produced in the study although 1 dam in the 25 mg/kg/day group had empty implantation sites in the uterus, and fetuses in the 25 mg/kg/day group had slightly greater numbers with less ossification of skeletons and variations of liver and kidneys than the control group (4 fetuses had lobus dexter of liver and 3 fetuses had distended pelvises in the kidney, while 1 fetus in the control group had the same findings). Therefore, icatibant was not teratogenic in rats under the conditions studied.

In a preliminary dose-ranging in rabbits, 2 pregnant rabbits/group were treated daily with SC injections up to 50 mg/kg/day of icatibant from Days 6 through 18 (no vehicle control was used). Rabbits were sacrificed on Day 29 of pregnancy and their uteri were examined. One dam in the 10 mg/kg group had only empty implantation sites in the uterus, indicating a clinically unnoticed abortion, but the second dam had 8 normally developed live fetuses. All dams in the 25 and 50 mg/kg/ groups had empty implantation sites in the uterus. Based on this study, doses of 0.1, 1 and 10 mg/kg/day were selected.

In the definitive rabbits study, Himalayan rabbits were treated with 0 (vehicle control- 0.9% NaCl), 0.1, 1.0 and 10 mg/kg/day of icatibant, SC once daily from Days 7 through 18 of

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pregnancy. Four unexpected abortions were produced in 2 dams each in all 3 treated groups and a premature birth in 0.1 mg/kg group, while none of these occurred in the control group. No other toxic effects were produced in the icatibant treated dams. At autopsy, light-grey depression was found on the surface of kidneys in 5-7 dams each in all 3 icatibant treated groups but only 1 dam in the control group. To determine if these effects were drug induced, the study was repeated but with 2 lower doses only and the vehicle group. The abortions or premature births were not reproduced in this study, and incidence of light grey colored kidneys in the treated groups was not increased, but it was also reported in 1 dam each in treated groups only. The depression on the kidney was due to chronic interstitial nephritis, and it was attributed to the strain of rabbits by the sponsor. Morphological examination of fetuses showed no increase in incidence of major malformations in the treated groups. Minor anomalies or variations of liver or kidneys were reported in some fetuses, but they were reported as within the normal ranges. Among the dead fetuses examined (6 in control, 4 in 1 mg/kg and 15 in the 10 mg/kg groups), 10 mg/kg had significantly more stunted (8/15) or retarded fetuses (7/15) over the control group (2/6 and 1/6, respectively). Icatibant was not teratogenic under the test conditions. All 3 doses of icatibant did not have significant toxic effects on the dams. However, 10 mg/kg was toxic to the embryo reducing the litter sizes as a result of increased intrauterine embryofetal death rate. Icatibant treatment resulted in dose-related decreases for total implantations and total number of live fetuses as well as dose-related increases for percent of pre-implantation loss when compared to the control values.

Under the conditions tested in the rats and rabbits, icatibant did not induce major terata. However, icatibant has a potential to produce abortions/premature births, intrauterine death or resorptions.

Two reproductive toxicology studies were included in this NDA to complete the reproductive toxicology battery. The sponsor combined the 13-week repeat dose toxicity study and the effects on fertility and early embryonic development to implantation study in rats. Male and female Sprague-Dawley rats were administered 1, 3 and 10 mg/kg/day of icatibant SC. HD treated females (10 mg/kg/day) had an increase in pre-implantation loss compared to vehicle treated females. Icatibant had no effects on male sperm count, motility, or morphology. Based on no toxicity observed in male seminology and no effects on mating or fertility indices, the NOAEL for male fertility was 10 mg/kg/day. Based on an increase of pre-implantation loss observed in HD treated females, the NOAEL for female fertility was 3 mg/kg/day. These conclusions were in agreement with the sponsor's conclusions.

In the second reproductive toxicity study submitted in this NDA, the effects of daily SC administration of icatibant on pre- and post-natal development in pregnant rats at 1, 3 and 10 mg/kg/day. Icatibant treatment delayed partuition at all doses, resulting in F_0 deaths (found dead or early sacrifice) in the 3 and 10 mg/kg/day groups. Clinical signs of toxicity (pallor of eyes and extremities, round back, piloerection and reddish discharge in vagina) were observed in 1 MD and in multiple HD F_0 females. After SC administration, icatibant and the M2 metabolite is found in rat milk. Based on the observed maternal toxicities including clinical signs, prolonged gestation periods for all icatibant dosage groups, F_0 spontaneous deaths and early sacrifice of icatibant treated dams, litter deaths, and necropsy findings in the MD and the HD groups, the maternal toxicity NOAEL could not be defined. The F_1 generation showed an increase in the

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number of pup deaths and cannabalism in the MD and HD compared to the VC groups. Therefore, the NOAEL for systemic toxicity in the F_1 generation was determined to be 1 mg/kg/day. The F_1 generation showed a slight decrease in air righting and hair growth compared to the VC control group. Therefore, the NOAEL for development of the F_1 generation was considered 3 mg/kg/day. The sponsor has adequately completed the reproductive toxicity battery for icatibant and the data indicate that icatibant should be classified as a Pregnancy Category C based on the presence of embryotoxicity, increased spontaneous abortions and increased pups deaths.

Under IND (b) (4), icatibant treatment at 1.25 mg/kg SC for 28 days had no effect on the pituitary-testicular axis during the dosing phase (most likely due to the low icatibant dose). Interestingly, an unexpected activation, significantly increased release and synthesis of testosterone in the serum and testes, after the 8-week recovery period was observed, suggesting that these organs may have been suppressed during the 4-weeks of treatment period at a sub-threshold level.

Local tolerance studies in rabbits showed that intra-articular injection of icatibant was well tolerated. In two local irritation studies (primary skin irritation study of rabbit skin and mucosal tolerance study in rabbit eye) icatibant was considered a non-irritant at 0.5 mg/mL icatibant solution. Icatibant administered via the SC route up to 40 mg in acetate buffer, pH 5.5 was comparable to the vehicle control for local irritation. In an antigenicity study, no anti-icatibant antibodies were produced when guinea pigs were sensitized with icatibant alone in intranasal or SC route. However, when guinea pigs were sensitized subcutaneously with icatibant plus Freund's adjuvant, antibody formation was observed when challenged with icatibant-BSA conjugated. In another study, a guinea pig maximization test, 8/10 icatibant treated guinea pigs plus Freund's adjuvant had slight erythema, while the control group showed no reaction. Therefore, icatibant was a sensitizer when administered with Freund's adjuvant in 2 studies. An *in vitro* hemolysis test of icatibant showed that 0.1% of icatibant did not induce hemolysis of human whole blood after incubation for 30 min where incubation with the positive control (distilled water for injection) did induce hemolysis.

For safety qualification of the (b) (4) impurity, the sponsor conducted 1 acute IV toxicity study, a bacterial reverse mutation study and a chromosome aberration study. (b) (4) was negative in the Ames test but positive in the chromosomal aberration test for inducing genotoxicity. Although positive for genotoxicity in the chromosome aberration test, (b) (4) (b) (4) is a member of the peptide drug class which is considered to have low genotoxic risk. The acute IV toxicity study dosed rats up to 100 mg/kg. No toxicity was observed in rats. However, the duration of this study is not considered acceptable to qualify this impurity for safety. Therefore, (b) (4) has not been adequately qualified for safety. This impurity and others are the subject of a consult review for ONDQA and comments to the sponsor will be forthcoming from the ONDQA reviewer, Dr. Eugenia Nashed.

Based on the completion of repeat dose toxicity studies conducted in dogs and rats up to 13-weeks duration, the target organs of toxicity were the male and female reproductive organs, the injection site irritation (with possible histamine release), thymus (lymphoid depletion), adrenal (cortical cellular vacuolation) and liver (vacuolated hepatocyte). When altering the dosing

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schedule from daily administration to twice weekly dosing in dogs, the effects on the male reproductive organs were reduced. However, females still showed immature reproductive organs. The 13-wk SC repeat dose study in rats produced a NOAEL of 10 mg/kg using a batch of icatibant synthesized by a new manufacturer. In the 13-wk dog study, no NOAEL was defined. However, in the 4-wk dog study using an alternative dosing regimen a NOAEL of 3.3 mg/kg TID dosed twice weekly was determined (equals 10 mg/kg). The recommended clinical dose of icatibant is one 30 mg subcutaneous injection with no more than 3 injections (90 mg) per 24 hour period recommended. On an AUC basis, safety margins of 4 and 10 in the rat and dog, respectively, at a single human dose (30 mg) or 1.4 and 3.6 for the rat and the dog, respectively, at the maximum daily dose (90 mg) are observed for the parent compound at the NOAEL doses for the 13-wk rat and 4-wk dog studies, respectively. Due to the intermittent dosing (not more than 8 doses of icatibant per month) of adult HAE patients with icatibant, the potential to observe similar toxicity as observed in animal studies is considered low. Additionally, there are currently no other approved drugs for the treatment of HAE. Lastly, the potential adverse effects of icatibant are outweighed by the risk of having a fatal HAE attack. To date, adverse effects in humans have shown local injection site irritation, which is considered monitorable.

Reproductive toxicology studies have shown that icatibant treatment dose not effect male or female fertility, or produce teratogenic effects, but may induce pre-implantation loss, spontaneous abortions, increase pup deaths and induce delayed parturition and result in difficulties in delivery. Based on the reproductive toxicity data icatibant should be classified as Pregnancy Category C.

Unresolved toxicology issues (if any): At a pre-NDA meeting held January 24, 2007 between Jerini and DPAP, Jerini was informed of the need for chronic toxicity studies (26-week in rat and 39-week in dog) and the need for 2 carcinogenicity studies to address the carcinogenic potential of icatibant to support the chronic intermittent HAE indication. To date, studies of up to only 13 weeks are available. Jerini indicated that the chronic toxicity studies have been initiated as of June 2007 (dog study) and July 2007 (rat study) and the carcinogenicity studies would be initiated April 8, 2008 and July 7, 2008 for mice and rats, respectively. In the Division's Filing Letter dated January 8, 2008, the sponsor was informed that the icatibant labeling will reflect a clinical dosing regimen that is supported by the nonclinical toxicology studies in both dose and duration; currently the sponsor has nonclinical support for subcutaneous clinical dosing for up to 13-weeks duration.

Recommendations: The nonclinical discipline recommends an approval of the application (NDA 22-150). The nonclinical data currently support a clinical dosing regimen of up to 13 weeks duration.

The following comment should be forwarded to the sponsor:
The completion of nonclinical chronic repeat dose toxicity studies (6-month in rat and 9- month in dog) via the subcutaneous route of administration is required to support chronic intermittent clinical dosing. The icatibant labeling will reflect a clinical dosing regimen that is supported by the nonclinical toxicology studies in both dose and duration. At this time you have nonclinical support for subcutaneous clinical dosing for up to 13-weeks duration. We acknowledge your submission stating that chronic toxicity studies have been initiated as of June 2007 (dog study)

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and July 2007 (rat study) and the carcinogenicity studies will be initiated April 8, 2008 and July 7, 2008 for mice and rats, respectively.

Suggested labeling: The review team decided not to evaluate the proposed product label at this time since the application is deemed not approvable. Therefore, labeling recommendations will not be made at this time.

Appendix/attachments: None

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12.2 Appendix 2: Pharmacology and Toxicology Review IND 68,214 (Serial No. 000), April 21, 2005

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

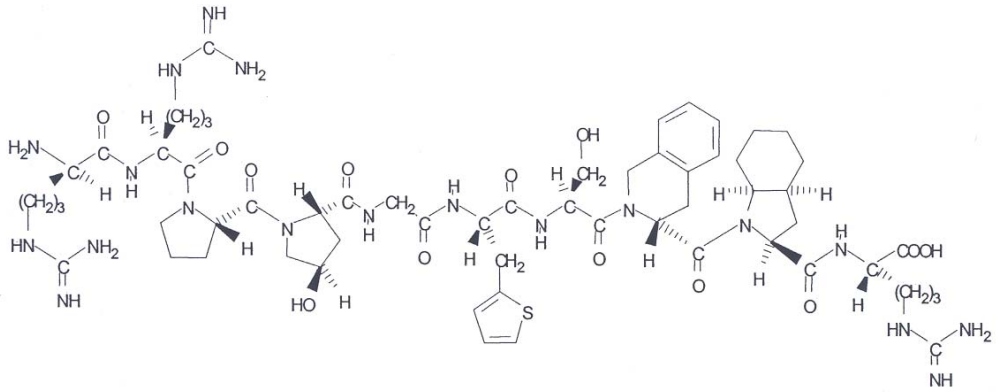
2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 68,214
Review number: 01
Sequence number/date/type of submission: 000/April 8, 2004/Initial submission
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Jerini AG, Berlin, Germany
Manufacturer for drug substance: (b) (4)

Reviewer name: Ronald Honchel, Ph.D.
Division name: GastroIntestinal and Coagulation Drug Products
HFD #: 180
Review completion date: April 21, 2005

Drug:

Trade name: None
Generic name: Icatibant acetate
Code name: JE 049/HOE 140
Chemical name: D-Arginyl-L-arginyl-L-prolyl-L[(4R)-4-hydroxyprolyl]-glycyl-L-[3-(2-thienyl)alanyl]-L-seryl-D-(1,2,3,4-tetrahydroisoquinolin-3-yl-carbonyl)-L-3aS,-7aS)-octahydroindol-2-yl-carbonyl]-L-arginine acetate.
CAS registry number: N/A
Short formula: D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK . nCH_3COOH
Amino acid sequence: H-D-Arg-L-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-OH. nCH_3COOH
Molecular formula/molecular weight: $C_{59}H_{89}N_{19}O_{13} \cdot C_2H_4O_2$ / 1304.6
Structure:



Relevant INDs/NDAs/DMFs: IND [redacted] (b) (4). Both INDs were submitted to HFD-570 by [redacted] (b) (4)

Drug class: Bradykinin type 2 (B_2) receptor antagonist

Intended clinical population: Treatment of Acute Attacks of Hereditary Angioedema

Clinical formulation: The drug will be supplied as a 10 mg/mL sterile, isotonic, acetate-buffered solution.

Route of administration: Subcutaneous

Proposed clinical protocol: The sponsor presented a clinical protocol to conduct a Phase III double-blind, placebo-controlled study to treat 56 (age ≥ 18 years), randomized hereditary angioedema patients (both male and female) with a single subcutaneous dose of 30 mg Icatibant at up to 15 investigator sites.

Previous clinical experience: A total of 580 subjects have been exposed to one or more Icatibant doses. There were no treatment-related serious adverse events reported when Icatibant was administered via the intranasal (201 subjects) or inhaled route (205 subjects) in Phase I and IIa studies. In a Phase IIa study, there were no serious adverse events reported when Icatibant was administered via the intra-articular route (a single dose of 90 μg) to 58 patients with symptomatic knee osteoarthritis. A total of 116 subjects have been administered Icatibant via the intravenous route in eight completed and two ongoing Phase I and IIa studies. In a recent Phase I study, there were no serious adverse events reported when Icatibant was administered to 26 healthy volunteers at intravenous doses of up to 0.8 mg/kg over 4 hours, up to 0.5 mg/kg over 1 hour t.i.d. (1.5 mg/kg/day) and 0.15 mg/kg for 3 consecutive days. In treatment groups receiving incrementally increasing doses of intravenous Icatibant, no serious adverse events were reported within subjects (n=4) or between subjects at doses up to 1.6 mg/kg/1hr with orthostatic hypotension reported in one patient at a 3.2 mg/kg/1hr dose. In a study investigating the subcutaneous route of administration, no serious adverse events were reported after administration of a single Icatibant dose of 0.05-0.4 mg/kg to 12 healthy volunteers.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Studies reviewed within this submission:

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Type of Study	Report/ Study #	Test Site	Lot #	Review Page #
		(b) (4)		
TOXICOLOGY				
Acute				
Rat, iv	JBC-92-RVAC-229		H032	9
Dog, iv	092-039-086-01		A062	11
Dog, iv infusion	JBC-92-DVAC-230		H032	13
REPRODUCTIVE TOXICITY				
Segment I				
Mouse, male 5-week iv	0-92-041-086-04		A062	29
Mouse, male 5-week sc	01-91-009-086-04		H032	31

The sponsor also submitted the following studies that had been previously reviewed under IND (b) (4) The pharmacology/toxicology review of these studies was incorporated into the appropriate sections of this review.

Type of Study	Report/ Study #	Test Site	Lot #	Review Page #
TOXICOLOGY		(b) (4)		
Acute				
Mouse, iv*	90.0487		C002	8
Mouse, sc*	90.0456		C002	8
Rat, iv*	90.0143		C002	8
Rat, sc*	90.0457		C002	8
Subchronic				
Rat, 1-month iv*	90.1386		C002	15
Dog, 1-month iv*	92.0315		C002	18
Rat, 1-month iv/sc/nasal*,**	90.0955		C002	21
GENETIC TOXICOLOGY				
Ames test*,**	Multiple studies		C002/ H032	25
Chromosome aberration test**	90.0750, Report #9847		H017	27
Mouse micronucleus test*	91.0662		C002	28
REPRODUCTIVE TOXICITY				
Segment II				
Rat, sc*	93.0541		A062	34

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Rabbit, sc**	93.0542	(b) (4)	A062	41

Studies not reviewed within this submission: None.

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2.6.2 PHARMACOLOGY

2.6.2.1 Brief Summary

Preclinical pharmacology studies were not included in this submission. A number of preclinical pharmacology studies were reviewed under IND (b) (4) Key points from the Pharmacology and Toxicology Review (dated November 4, 1991) of those studies are briefly summarized below. Using *in vitro* receptor binding studies measuring displacement of ^{3}H-bradykinin in guinea-pig ileum, guinea-pig trachea epithelium and human synovial cells, Icatibant was shown to be a competitive bradykinin type 2 (B_2) receptor antagonist with IC_{50} values in the 1-4 nM range. Selectivity for the B_2 receptor was originally based on the inability of Icatibant to inhibit contraction in the rabbit aorta which contain only B_1 receptors. The sponsor stated that in CHO cells expressing recombinant human B_1 or B_2 receptors, Icatibant was shown to have an IC_{50} value of 6.0 μM and 4.3 nM, respectively. However, that study was not included in this or IND (b) (4) submissions. Icatibant has the same B_2 receptor affinity as bradykinin. In addition, Icatibant was shown to inhibit bradykinin-induced effects in cultured endothelial cells, isolated guinea-pig hearts, bovine coronary arteries and a number of isolated organs. Icatibant was also shown to inhibit bradykinin-induced hypotension in rats *in vivo*.

2.6.2.4 Safety Pharmacology

Preclinical safety pharmacology studies were not included in this submission. A number of safety pharmacology studies were reviewed under IND (b) (4) and IND (b) (4) Key points from the Pharmacology and Toxicology Review of those studies (dated November 4, 1991 and March 22, 1993, respectively) are briefly summarized below.

Neurological effects: Icatibant produced no CNS effect in the following murine models:

Model	Dose	Route
Spontaneous motor activity	0.01 - 1 mg/kg	i.v.
Electroshock-induced seizures	1 mg/kg	i.v.
Antiepileptic/anxiolytic activity(*)	1 mg/kg	i.v.
Tetrabenazine-induced ptosis	1 mg/kg	i.v.
MAO inhibition test(**)	1 mg/kg	i.v.
Hexobarbital narcosis	1 mg/kg	i.v.
Apomorphine-induced climbing	1 mg/kg	i.v.
Survival time of sodium nitrate intoxication	1 mg/kg	s.c.
Inhibitory avoidance	3 - 30 μg/kg	s.c.

(*) : assessed by pentetrazole-induced convulsions
(**): assessed by D,L-5-HTP potentiation

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Intravenous injection of mice with 1-10 mg/kg Icatibant produced reduced motor activity, prone position, reduced respiration, piloerection, ptosis and mydriasis.

Cardiovascular effects: Icatibant at intravenous doses of 0.01 and 0.1 mg/kg injected over 1 minute did not affect blood pressure or heart rate in conscious dogs. At doses of 0.6 and 1 mg/kg, Icatibant markedly decreased blood pressure for 5 to 10 minutes and clinical signs included pain reaction, restlessness, mydriasis, and hypersalivation. In anesthetized rats, intravenous doses of 0.13 and 1.3 mg/kg Icatibant decreased mean arterial blood pressure for 3 minutes by 10% and 33%, respectively. By contrast, a subcutaneous dose as high as 13 mg/kg did not decrease blood pressure suggesting that bolus injection of the intravenous doses may be responsible for the hypotensive effect.

Renal effects: Normal glomerular filtration rate and renal plasma flow, but reduced urine, potassium and sodium excretion (approximately 40% for all three) were observed in conscious male dogs intravenously injected with a single dose of 0.3 mg/kg.

Analgesic effects: Icatibant at intravenous doses up to 0.5 mg/kg did not have any analgesic effect in the Randall-Selitto test (yeast-induced motor impairment in rats). Both intravenous and intraperitoneal doses of 3-10 mg/kg, but not 0.3 mg/kg, produced a mild analgesic effect in the mouse acetic acid writhing test model.

Other: Icatibant did not induce histamine release, a problem with many peptides, in rat or human skin mast cells. Symptoms consistent with histamine release have been observed in dogs after bolus intravenous administration. Intravenous doses of 0.1, 0.5 and 1.0 mg/kg Icatibant did not affect blood glucose levels in normal rats.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

Preclinical pharmacokinetic studies were not included in this submission. Pharmacokinetic studies were reviewed under IND (b) (4) The pharmacology/toxicology review of those studies dated November 4, 1991 is incorporated below.

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PHARMACOKINETICS :

Methods : SPF male (M) rats (180-190 g) were administered HOE 140-(-proyly-3,4-^{3}H) sc and iv. Specimens were dried before the radioactivity counting in order to be "free of tritium water of possible enzymatic origin".

RESULTS :

A. **ABSORPTION :**

After **0.01 mg/kg sc** : total radioactivity in the blood showed 2 peaks : 1 hour and 24 hours post dosing, with peak concentrations of 2.9 and 2.8 ng equivalents/g respectively (trough between 2 peaks was 0.9 ng equivalent/g). The **half-life of the first peak was 1.8 hours and the terminal half life was estimated as 6.3 days** (it was probably due to incorporation of tritium-labeled proline into the protein pool).The plasma levels were 1.5 -1.8 fold higher than blood levels.

B. **DISTRIBUTION :**

a. The body contained 18.7% of dose excluding GIT, 4 days after the sc dosing.

b. Radioactivity distribution was as follows :

Site	ng equivalents/g
Blood	1.2
Liver	7.1
Smooth muscle	6.7
Kidney	5.8
Spleen	4.3
Bone marrow	3.1
Site of injection	3.4

C. **METABOLISM :**

HOE was extensively metabolized to metabolite M 1 (H-D-Arg-Arg-Pro-Hyp-Gly-OH) and only 7% of in the urine was parent compound within 4 hours after 5 mg/kg iv.

D. **EXCRETION :**

The main excretion route is via urine, and 59.7% of sc dose was excreted in urine, and 3.6% in feces, and on the 4th day, about 1% was still detected in urine and feces. Majority of the drug in urine was metabolite M 1, and only 7% was excreted as parent compound within 4 hours after 5 mg/kg iv.

Addendum: Additional relevant information was provided by the sponsor that was either not included in previous pharmacology/ toxicology IND reviews or was not submitted by the sponsor. The two main bradykinin-cleaving enzymes, angiotensin converting enzyme (ACE) and neutral endopeptidase, do not degrade Icatibant. Therefore, Icatibant is metabolically more stable than bradykinin. Icatibant is extensively metabolized. Two major metabolites, M1 and

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M2 (the 4 carboxy-terminal amino acids), have been identified and both have been observed in all species examined. Excretion was mainly renal, regardless of species and route of administration.

2.6.6 TOXICOLOGY

2.6.6.2 Single-dose Toxicity

Single-dose toxicity studies in mice and rats were reviewed under IND (b) (4) The pharmacology/toxicology review of acute toxicity studies in mice and rats dated November 4, 1991 is incorporated below.

Acute toxicity studies were performed in mice and rats by iv and sc injections. After **iv** dosing, deaths occurred usually within minutes to hours except one rat after 2 days. They showed **rapid respiration, twitching and convulsions.** After **sc** dosing, similar but less severe signs were shown than after iv dosing. Injection sites showed bluish discoloration, which progressed to necrosis.

The LD_{50}s were as follows :

Model	Route	LD_{50} (mg/kg)
Rats	i.v.	12.3 - 19.2
Rats	s.c.	max. tol. > 1227
Mice	i.v.	48.3 - 76.6
Mice	s.c.	614 - 760

Summary of Acute Toxicity Study:

The LD_{50}s in mice and rats by the iv and sc routes were submitted. Mice were less sensitive than rats by iv route but more sensitive by sc route. HOE 140 (HOE) is **extremely toxic** in rats by iv route and **very toxic** in mice by iv route, **moderately** toxic in rats and mice by sc route, according to the rating system of Gleason et al.

Addendum: The highest non-lethal dose was 30.7 mg/kg, the minimum lethal dose was 48.3 mg/kg for mice. For rats, the highest non- lethal dose was 12.3 mg/kg, the minimum lethal dose was 19.2 mg/kg. In initial acute subcutaneous toxicity studies, the highest non-lethal dose was 614 mg/kg, the minimum lethal dose was 767 mg/kg for mice. In rats, no mortality was observed after a single subcutaneous injection of 1227 mg/kg Icatibant.

Study title: Single-Dose Intravenous Infusion Toxicity Study of HOE 140 in Rats

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Key study findings: The objective of this study was to determine the potential toxicity of single-dose Icatibant (0, 4, 20 or 100 mg/kg) administered intravenously to male rats (n = 10/group). Signs of toxicity in the 100 mg/kg group included one moribund animal and a 3-fold increase in BUN immediately after infusion. The NOAEL in this study was 20 mg/kg.

Study no.: JBC-92-RVAC-229
Volume #, and page #: Volume #3, page #39
Conducting laboratory and location: (b) (4)
Date of study initiation: November 20, 1992
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: HOE 140, lot # HO32, % purity not stated

Methods

Doses: 0, 4, 20 or 100 mg/kg (high dose was based on mortality observed in one of three 100 mg/kg and three of three 300 mg/kg HOE 140 treated animals in a preliminary study)
Species/strain: Rat/SD
Number/sex/group: n = 10/male/group
Route, formulation, volume, and infusion rate: HOE 140 solution was intravenously infused in a volume of 30 ml /kg at a speed of 10 ml/kg/hr (3-hour infusion)
Satellite groups used for toxicokinetics or recovery: None
Age: 6-Weeks at initiation of study
Weight: 115-169 g
Unique study design or methodology: Five animals per group were sacrificed after the 3-hour infusion period and the remaining five were sacrificed 7 days after the end of infusion

Observation and Times:

Clinical signs: All animals were observed for changes in the general health from the start of infusion to the end of infusion. Animals not sacrificed after the end of infusion were observed for an additional 3 hours after infusion and once a day thereafter.
Body weights: Body weights were recorded on the day of administration (Day 0) and on Days 1, 3, 5, and 7.
Clinical chemistry: Blood for clinical chemistry analysis was collected at sacrifice.
Urinalysis: Animals were placed in metabolism cages during the infusion period and urine for qualitative urinalysis was collected during this time period.
Gross pathology: All animals were autopsied for gross lesions in the organs and tissues after sacrifice.
Organ weights: Not recorded.
Histopathology: At autopsy, the heart, lung, liver, kidneys and pancreas were removed and fixed in 15% neutral buffered formalin. Paraffin sections were prepared and hematoxylin and eosin stained slides were examined microscopically.

Results:

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Mortality: No mortality was observed in any group, however, 1 animal in the 100 mg/kg group to be sacrificed immediately after infusion showed cyanosis and became moribund at the time of sacrifice.

Clinical signs: Decreased locomotor activity and crouching or tachypnea was observed 1 or 2 hours after the start of infusion in 20 and 100 mg/kg animals. These symptoms disappeared within 2 hours after the end of infusion. No notable differences in body weight changes were observed.

Body weight: There were no drug-related body weight changes.

Clinical chemistry: In groups sacrifice immediately after infusion, significant clinical chemistry findings included increased GPT (51 ± 13 mU/ml compared to 30 ± 4 mU/ml for control) in the 20 mg/kg group. Decreases in ALP (291 ± 36 mU/ml compared to 441 ± 82 mU/ml for control) and total protein (3.65 ± 0.17 mU/ml compared to 4.34 ± 0.22 mU/ml for control) were observed in 100 mg/kg groups sacrificed after infusion. Gamma-globulin ratio was significantly increased in the 4 and 100 mg/kg groups sacrificed after infusion, but not in the 20 mg/kg group (1.8 ± 0.2, 2.2 ± 0.3, 1.8 ± 0.6, and 2.4 ± 0.4 for 0, 4, 20 and 100 mg/kg groups, respectively). BUN (7.76 ± 1.31, 11.68 ± 2.54, and 24.75 ± 3.5 for 0, 20 and 100 mg/kg groups, respectively) was significantly increased, and total cholesterol (80 ± 9, 61 ± 9, and 66 ± 8 for 0, 20 and 100 mg/kg groups, respectively) and phospholipid (129 ± 8, 104 ± 17, and 103.5 ± 5 for 0, 20 and 100 mg/kg groups, respectively) decreased in 20 and 100 mg/kg groups sacrificed after infusion. Alpha 1-globulin ratio was decrease at all doses (19.0 ± 0.7, 16.7 ± 1.2, 17.1 ± 1.4, and 17.2 ± 1.1 for 0, 4, 20 and 100 mg/kg groups, respectively). No significant differences in clinical chemistry were observed in animals sacrificed 7 days after infusion

Urinalysis: There were no drug-related changes in urinalysis parameters.

Gross pathology: There were no drug-related autopsy findings.

Histopathology: There were no drug-related microscopic findings.

Study title: Single-Dose Intravenous Toxicity Study of HOE 140 in Dogs

Key study findings: The objective of this study was to determine the potential toxicity of single-dose Icatibant (0.4, 2.0 or 10 mg/kg) administered intravenously to male dogs (n = 2/group, no control group). Numerous clinical signs, decreased body temperature on the day of administration, and decreased serum sodium and potassium concentrations were observed in 2.0 and 10 mg/kg treated animals. A positive reaction for occult blood was observed in the feces of 1 animal from each treatment group between 60 min and 1 day after dosing. Many of the

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observed effects in this study were probably due to excessive histamine release due to the intravenous injection of drug. The NOAEL for this study was 0.4 mg/kg.

Study no.: 0-92-039-086-01
Volume #, and page #: Volume #3, page #126
Conducting laboratory and location: (b) (4) (b) (4)
Date of study initiation: January 20, 1993
GLP compliance: Not stated
QA report: yes ( ) no (X)
Drug, lot #, and % purity: HOE 140, Lot # A062, % purity not stated

Methods

Doses: 0.4, 2.0, and 10 mg/kg (no control animals, high dose was based on no mortality observed in a preliminary study at intravenous doses of 2, 4, and 8 mg/kg HOE 140)
Species/strain: Dog/beagle
Number/sex/group: 2/male/group
Route, formulation, volume, and infusion rate: Intravenous, solution, volume: 0.04, 0.2, or 1.0 of a 1% solution, injection rate of 10 mL/min
Age: 5-6 months old at initiation of study
Weight: 7.1-7.8 kg
Unique study design or methodology: Body temperature and pulse rate were measured at 0, 30 min, 2 hr, 6 hr and, thereafter, at the time of body weight measurement. Feces were examined for occult blood at the same time as body weight measurements plus at 6 hours after dosing.

Observation and Times:

Clinical signs: Animals were observed daily for mortality and changes in general health condition.
Body weights: Each animal was weighed at 0, 1, 2, 3, 7, 10, and 14 days after dosing.
Hematology: Blood for hematology and coagulation analysis was collected at 0, 1, 7 and 14 days after dosing.
Clinical chemistry: Blood for clinical chemistry analysis was collected at 0, 1, 7 and 14 days after dosing.

Results:

Mortality: No deaths were observed at any dose.

Clinical Signs: Transient soft feces was observed in one animal at 0.4 mg/kg. Decreased spontaneous activity, lying prone or recumbent, slow respiration, lacrimation, salivation, flushing of the skin, hyperemia of the conjunctiva, defecation, abdominal contraction, diarrhea and red feces were observed in 2 and 10 mg/kg groups. Additionally, staggering gait, tremor, vomiting, nasal discharge and swelling of the muzzle and paws were also observed in the 10 mg/kg group. Most of these changes disappeared within 4 hours after administration.

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Body Weight: There were no drug-related body weight changes

Hematology: Changes in hematological parameters were sporadic and not dose related. Therefore, the observed changes were considered incidental. Shortened APTT was observed sporadically and considered incidental.

Clinical Chemistry: Serum sodium and potassium remained near baseline levels in 0.4 mg/kg treated animals. However, decreases in baseline levels were observed in the 2 and 10 mg/kg group for serum sodium and the 10 mg/kg group for serum potassium (see table below). No controls were incorporated in this study.

	Na (mEq/L)				K (mEq/L)	
	Animal #1 (2 mg/kg)	Animal #2 (2 mg/kg)	Animal #1 (10 mg/kg)	Animal #2 (10 mg/kg)	Animal #1 (10 mg/kg)	Animal #2 (10 mg/kg)
Pre	137.5	147.4	150.0	152.4	6.61	7.36
1d	128.9	126.1	148.4	147.1	6.34	6.02
7d	126.6	124.9	147.5	143.3	6.30	4.15
14d	126.1	138.5	139.6	131.7	3.60	3.05

Other:

Body Temperature and Pulse Rate: A decrease in body temperature was noted in 2 and 10 mg/kg animals on the day of administration only. There were no drug-related changes in pulse rate.

Fecal Occult Blood: Positive fecal blood was observed in the feces discharged between 60 min and 1 day after administration in 1 animal each at all doses

Study title: Single-Dose Intravenous Infusion Toxicity Study of HOE 140 in Dogs

Key study findings: Male dogs (n = 3/group, no control group) were administered via intravenous infusion over a 5-hour period 15, 30, and 60 mg/kg HOE 140. Two animals per group were sacrificed after the 5-hour infusion period and the remaining animal was sacrificed 7 days after the end of infusion. Vomiting, altered ECG, and elevated serum CPK levels were noted in some of the mid and high dose animals. The NOAEL in this study was 15 mg/kg.

Study no.: JBC-92-DVAC-230
Volume #, and page #: Volume #3, page 159
Conducting laboratory and location: (b) (4)

Reference ID: 2977968

Date of study initiation: November 20, 1992
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: HOE 140, lot # H032, % purity not stated

Methods

Doses: 15, 30, and 60 mg/kg (no control group, high dose based on a preliminary study that showed no effects at 30 mg/kg, but vomiting and decreasing tendency of blood pressure at 60 mg/kg)
Species/strain: Dog/beagle
Number/sex/group: 3/male/group
Route, formulation, volume, and infusion rate: Intravenous, solution, 5 mL/kg, 1 mL/kg/hr for 5 hr
Age: approximately 8 months old at dosing
Weight: 8.5-10.0 kg
Unique study design or methodology: Two animals per group were sacrificed after the 5-hour infusion period and the remaining animal was sacrificed 7 days after the end of infusion.

Observation and Times:

Clinical signs: Animals were observed for mortality and clinical signs from the start of infusion to 3 hours after the end of infusion and at least once a day thereafter (note: n = 1/group after the end of infusion).
Body weights: Body weight was recorded just prior to administration (Day 0) and at 1, 3, 5 and 7 days after dosing.
ECG: ECG and blood pressure measurements were performed prior to Day 0 and 11 times at 30-min intervals after the start of infusion.
Clinical chemistry: Blood samples for clinical chemistry analysis were collected prior to Day 0, 30 min after the end of infusion, and on Day 7 prior to autopsy.
Urinalysis: Urinalysis was performed on samples collected prior to Day 0 and 30-40 min after the end of infusion.
Gross pathology: Animals were autopsied for gross lesions in the organs and tissues at the end of infusion or at the end of the 7-day observation period.
Organ weights: Liver, kidneys, heart, lung and pancreas were weighed at autopsy.
Histopathology: After weighing, organs were fixed in 15% neutral buffered formalin. Paraffin sections were prepared and hematoxylin and eosin stained slides were examined microscopically.

Results:

Mortality: No mortality was observed in any group.

Clinical signs: Vomiting was observed in one 30 mg/kg animal 30 min after the start of infusion, and slight salivation was observed in one 60 mg/kg animal for 40 min starting 1 hour after start of infusion. No other notable clinical signs were observed.

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Body weights: There were no drug-related changes in body weight.

ECG: Elevated P waves (0.45 mV; waves at 0.4 mV or more were diagnosed as "P-pulmonale") were observed 1 hour after the start of infusion in one 30 mg/kg animal, with an increase in ST segments (0.1 mV or more) observed during the same period. The sponsor stated that the changes in T waves were not apparently compound-related due to background data showing historically wide fluctuations in T waves. A slight degree of tachycardia (define as a heart rate of 170 or more) was observed in a 60 mg/kg animal 30 min after start of infusion. There were no drug-related changes in blood pressure.

Clinical chemistry: An increase in serum CPK levels above historical background (153 ± 70 mU/ml) was noted in one 30 mg/kg animal (227 mU/mL baseline, 406 mU/ml 3 hour after infusion) and two 60 mg/kg animals (baseline/3 hour after infusion for each animal = 200/843 and 227/389).

Urinalysis: An increased urine volume was observed in all animals at the end of infusion. Otherwise, there were no drug-related effects.

Gross pathology: There were no drug-related macroscopic findings at autopsy.

Organ weights: There were no drug-related effects on absolute or relative organ weights.

Histopathology: There were no drug-related histopathological changes observed in this study.

2.6.6.3 Repeat-dose Toxicity

Repeat-dose intravenous toxicity studies in rat and dog were reviewed under IND (b) (4). The pharmacology/toxicology review of repeat-dose toxicity studies in rat and dog dated November 4, 1991 is incorporated below.

ONE MONTH INTRAVENOUS TOXICITY STUDY OF HOE 140 (S 89 0140) IN RATS : Report No. 90.1386 : Study No. 90.0111:

Methods : Fifteen M + 15 F/group of immature Hoe:WISKf (SPF71) rats (mean weight of 103 g for M and 106 g for F, about 5 weeks old) were injected iv (2 mL/min), into a lateral caudal vein, once daily at **0.1 mg/kg** (0.04 mg/mL), **1 mg/kg** (0.4 mg/mL), or **10 mg/kg** (4 mg/mL) HOE 140 (HOE, Batch No. C 002) in a volume of **2.5 mL/kg**, along with a control group receiving 2.5 mL/kg saline (9 mg/ML), for 30 consecutive days. Ten M + 10 F/group were killed after ip injection of 50 mg/kg pentobarbital sodium, and the remaining 5 M + 5 F/group were kept an additional 28 days as recovery groups.

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Dose selection was made as following : 0.1 mg/kg as multiple of the intended therapeutic dose, 1 mg/kg as geometric mean of 0.1 and 10 mg/kg that was found to be the toxic dose in the preliminary study (not specified).

First dose (M/F) : 2/26/27, 1990
Last dose (M/F) : 3/27/28/1990
Killing (M/F) : 3/28/29/1990 and 4/24/25/1990

Measurements and Observations:

Daily : General behavior.
Weekly : Body weights (2x), food consumption (2x), eyes, teeth, visible mucous membrane, neurological condition
Day 30 : Hematology, clinical chemistry, urinalysis
Termination : Hematology, clinical chemistry, urinalysis, organ weights, gross and

showed a trend of dose-related decrease.

Clinical Chemistry : Group mean **urea** values in both M and F HD showed marked increase, with a dose-relation in F treated group, and HD also showed increased **uric acid** and **creatinine** values, especially in M with a trend of dose-relation for uric acid. Group mean values for **inorganic phosphate** (IP) and **total bilirubin** (TB) were increased in M of **MD and HD** (21% for IP and 28% for TB in HD), but with a trend of dose-relation for IP, although both increases were not significant ($p<0.05$).

After **recovery period**, all values were similar to the control values.

Urinalysis : HD group M had irregular epithelial cells in the sediment, and increased frequency of hemoglobin in F HD.

After **recovery** period, no abnormalities were reported. See pages 57-58, Vol. 1.5.

Organ Weight : **Kidney** weights, both absolute (A) and relative (R), in both M and F in HD were increased 11-19%), but none were significant ($p<0.05$), and after **recovery** period, no increase was apparent.

Liver weights (R) in HD M were also increased significantly ($p<0.05$) and after the recovery period, the liver weights (A in M and R in M and F) were also larger than respective control values, although all were **less than** 8% changes from the control values.

Relative **ovary weight** in HD was significantly ($p<0.05$) increased, but after recovery no increase was apparent.

Gross Pathology : Drug-related change was reported for **kidneys** in 6 HD rats, described as light or unevenly colored.

Renal pelvic dilatation reported in several rats in **all** groups was considered genetic in origin.

Histopathology : **Epithelial necrosis in proximal tubules** in kidneys were reported in 10/10 M + 5/10 F in HD, extensive in "some" rats, and occasionally with dilatation of the tubules, some repair and some with tubular atrophy, more in M than F.

Secretory deficiency in seminal vesicle was reported in 2 M of HD only.

After the **recovery** period, kidney tubules showed **"irreversible tubular atrophy"** in all 5 M and 1 F of HD, along with 1 F in MD, 2 M in LD and 1 M + 1 F in the control group. Two M in HD also had **foci of atrophy in pancreas** when none was reported in other groups.

Injection sites in the region of the **tail veins** had **perivenous fibroses** due to repeated iv injection. In addition, "circumscribed foreign body reactions around particles of transported skin" were reported in the lungs in small pulmonary arterial branches in all groups. See the pathologist's report on pages 295-300, Vol. 1.5.

COMMENTS: Irritation at the injection sites was partly due to vehicle, saline.

Summary of Intravenous Rat Toxicity Study :

Four groups of 15 m + 15 F/group of rats were injected 2.5 mL/kg iv at 0 (vehicle-saline), **0.1, 1, or 10 mg/kg/day** HOE for 30 consecutive days, then 10 M + 10 F/group were killed and the remaining 5 M + 5 F/group were kept an additional 4 weeks (28 days) as the recovery groups.

No rats died. No significant drug-related changes were reported for general health, behavior, eyes, neurological testing, body weight, and food consumption. Group mean values in **HD** for **RBC, Hb, and Hct** (M > F) were slightly decreased (as in the **one month intranasal toxicity study**). Group mean values for **urea** showed marked increase in M and F (dose-relate), and **uric acid (M>F), IP (M),** and **creatinine** (M>F) were increased . Urine in M of HD had **irregular epithelial cells** and increased frequency of hemoglobin in F HD. All these findings were **not** reported after

the 28 days of recovery period.

Both absolute and relative **kidney** weights in HD were increased (11-19%), but not significantly, and **liver** weights in HD were also increased in M and F, significantly ($p<0.05$) for relative **weight for** M, but all changes were less than 8%. Relative **ovary** weight in HD showed significant increase (18%). However, after the recovery period, none of above changes were significant.

Gross and histopathological findings were mainly limited to **kidneys**. Grossly 6 HD rats had light and/or marbled colored kidneys. Histopathology report showed **epithelial necroses of proximal tubules in 10/10 M and 5/10 F in HD only** while none were reported in other groups. **Tubular atrophy** and **dilated renal pelvis** were reported in some rats, but in all 4 groups. After the recovery period, **all 5 M and 1 F** in HD had **irreversible tubular atrophy**, along with 1 F in MD, 2 M in LD, and 1 M+ 1 F in control group.

Secretory deficiency in seminal vesicle was reported in 2/10 M in HD only. No foci of decreased spermatogenesis were reported in this study. **Foci of atrophy in pancreas** was also reported in 2/5 M in HD only.

In addition, **perivenous fibroses** for injection sites in tail vein were reported for histopathology, up to 2-6/sex/group, in all 4 groups, but after **the recovery** period, they were reported in 1 F control and 1 M in MD only. This finding indicated that repeated injection of **physiological saline** (vehicle) can induce necrosis, and the lesion can be reversible.

0.1 mg/kg/day iv may be considered NOEL.

ONE MONTH INTRAVENOUS TOXICITY STUDY OF HOE 140 (S 89 0140) IN DOGS : Report No. 90.0955 : Study No. 90.0125 :

Methods: Four groups of 3 M + 3 F/group of Hoe:BEAK strain of beagle dogs were injected iv "directly before feeding", once a day alternately into the right or left antebrachial cephalic vein at a rate of 5 mL/min at 0 **(physiological saline), 0.1, 0.25, or 0.8 mg/kg/day HOE** (1 mg/mL, batch No. C002), 7 days/week for one month, then 2 M + 2 F/group of dogs were killed after iv injection of T-61-Hoechst (see page 241, Vol. 1.7.), and the

remaining 1 M + 1 F/group of dogs were kept an additional 4 weeks as recovery groups without any treatment.

Justification of dose selection was that 0.1 mg/kg as a multiples of maximal daily dose planned in man, 0.25 mg/kg as the logarithmic mean dose between 0.1 and 0.8 mg/kg, and 0.8 mg/kg as anticipated toxic dose from a dose-ranging study (Report No. 89.1193).

First Dosing : 2/19/90
Last Dosing : 3/22/90 for groups I and II
3/21/90 for groups III and IV
Killing : 3/22/90-3/23/90 and 4/19/90.

Measurements and Observations :
Daily : General condition and behavior, feed
Weekly : Body weight
Day : -1, on days 30 and 58 : Neurological exam. eyes, hearing, teeth, blood chemistry, urinalysis.
Termination : Days 31-32 and 57-58

RESULTS :

Mortality : None.

General Condition : No drug-related impairment of the general condition was reported.

One M in HD had slight to marked **swelling of the flews** (the loose, hanging parts of the upper lip of dogs) right after injection from the 2nd day onward that lasted 1-4 hours, and the other 2 M in this group had a milder form of swelling only in the first week of study. This effect was attributed to drug-induced histamine release.

Body Weight : Group mean values were similar to the control group.

Food Consumption : No drug-related changes.

Behavior, Eyes, Hearing, Teeth, Neurological Examination : No drug-related changes were reported.

Electrocardiography : No drug-related changes were reported for EKG pattern or heart rate.

Hematology : No significant drug-related significant changes were reported or methemoglobin was present.

Coagulation Time : Prothrombin time showed variable changes in all 4 groups, including the control.

Clinical Chemistry : Only significant ($p<0.05$) change was decrease in **blood glucose** in MD and HD (M<F), but without clear cut dose-relation.

Urinalysis : No drug related significant changes were reported.

Organ Weight : No drug-related significant changes were reported.

COMMENTS: No summary tables were submitted, and individual value for each dog was submitted.

Gross Pathology : No drug-related changes were reported. Injection sites in many of dogs showed "bloody imbibition" in all 4 groups.

Histopathology : No drug-related findings were reported including spermatogenesis or iron metabolism. But the following changes were reported by the pathologist's report.

One M control and 1 M + 1 F in HD had "**chronic gastritis** in the gastric fundus mucosa, connected with round cell infiltrates and lesion to basal chief and parietal cells". "The major part of glandular portions in the basal mucosa" disappeared in 1 of 2 HD dogs (n = 4). It was stated that "there is no explanation as to these relatively **rare** changes." One F/2 in HD only reported foreign body reaction in the **lung**. Injection **sites** reported "vascular wall lesions and/or hemorrhage" with a trend of dose-relation for incidence, but after recovery, vascular lesions were

reported only the control and LD.

Summary of Intravenous Dog Toxicity study :

Four groups of 3 M + 3 F/group of beagle dogs were injected iv, once/day, 7 days/week for one month at dose of 0 (saline), or 0.1, 0.25, or 0.8 mg/kg/day HOE, then 2 M + 2 F/group were killed, and the remaining 1 M + 1 F/group of dogs were kept an additional 4 weeks as recovery groups.

No dogs died during the study. All 3 M in HD had **swelling of flews**(1-4 hours from the 2nd day for a week in 2 but one up to end), and this was attributed to **histamine release**, and was considered species specific effect, and the clinical significance was not clear. Only significant ($p<0.05$) finding was decreased **blood glucose** in MD and HD (M<F), but without apparent dose-response. No drug-related significant changes were reported for other usual parameters including hearing, teeth, neurological exam., EKG, and gross and histopathology.

Most common histopathology finding was limited to the injection sites with "vascular wall lesions" with/without hemorrhage in all 4 groups at the end of dosing, with a trend of dose-related increase for incidence in the treated groups. This finding indicated that repeated saline injection produced local lesions, and HOE was probably slightly irritating.

Chronic gastritis was reported in 1/2 M in the control and 1/2 M + 1/2 F in HD. But the 1 M in HD showed the major part of glandular portions in the basal mucosa" missing. This was reported as "relatively rare change". In the long term studies, more attention should be paid to stomach histopathology.

The absolute NOEL in this study was 0.1 mg/kg/day HOE.

Addendum: While the NOEL was 0.1 mg/kg/day (low dose) in both the rat and dog studies, the more severe effects (renal toxicity in rats, swelling of flews in dogs) were observed in high dose groups only and effects in mid dose groups were relatively minor. Therefore, the NOAEL in the rat and dog 1-month intravenous toxicity studies was 1.0 and 0.25 mg/kg/day, respectively.

The repeat-dose 1-month comparative (nasal/intravenous/subcutaneous) toxicity study in rat was reviewed under IND (b) (4). The pharmacology/toxicology review of the comparative repeat-dose toxicity study in rat dated March 22, 2004 is incorporated below.

COMPARATIVE REPEATED-DOSE (1 MONTH) TOXICITY STUDY IN RATS (NASAL/INTRAVENOUS/SUBCUTANEOUS) : Study No. 91.0519 : Report No. 92.0315:

Methods : 10 M/group of Hoe:WISKf(SPF71) Wistar rats (5-6 weeks old and

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mean body weight of 101 g) were treated with either **2.5 mg/rat** of intranasal solution batch 22 intranasally (**in**) or **10 mg/kg** HOE (batch HO28) in saline by **iv** or **sc** injection for **30 consecutive days** in order to "clarify absorption question" (according to German testing guidelines and European community guidelines). At the end of dosing, blood was collected for compound determination from 2 rats/group at 0.5 or 1, 2, 3 or 4 hours after the final dosing by killing the rats by cardiac incision under pentobarbital anesthesia. Organ weights were determined (for 10 organs) and gross and histopathology were performed.

COMMENTS : No control group was included, as shown on page 156 Vol. 1.9 [groups 1 (**in**), 2 (**iv**) and 3 (**sc**)].

Measurements and Observations :

Clinical Signs : Daily.
Body Weight and Food consumption : weekly
Blood Collection : Day 30.
Necropsy and Organ weights : Day 30 (9/25/93).
Dosing Period : 8/28/91-9/25/91.

Mortality : None.

Clinical Signs : All rats in iv group showed reduced motility for a "short time" on the first 2 days.

COMMENT : "Local reactions at the sites of treatment were not observed".

Body Weight : No significant differences between groups. See pages 166 and 168, Vol. 1.9.

Food Consumption : No significant differences between groups. See page 167, Vol. 1.9.

Compound Level Determinations : The first 4 hours after sc injection, the profile of parent drug (radioimmuno assay with a limit of 3 ng/mL) was similar to iv injection, indicating very good absorption after sc injection. When AUC was calculated for 4 hours after dosing, the bioavailability after intranasal administration was reported as on the order of 1%. For actual data, see pages 187-194, Vol. 1.9.

Summary of PK data on day 30 is as follows:

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	in (≈25 mg/kg)	iv (10 mg/kg)	sc (10 mg/kg)
C_{max} (μg/mL)	0.18	24.09	12.77
T_{max} (h)	0.5	0.5	0.5
AUC_{0-4} (μg·h/mL)	0.13	20.33	17.30

Organ weights : Group mean values showed the most change in iv group followed by sc group then in group and **iv group** had ↑ **kidney weights** (9%↑ for AW and **20%↑** for RW) and ↓ **testicular weights** (17%↓ for AW and 9%↓ for RW) when compared to sc or in group. Kidney weight of **sc group** had 9%↑ for AW and 8%↑ for RW, and testes was only 3%↑ for AW and 1%↑ for RW over in group.

COMMENT : Organ weights are not complete for all 3 groups, and many are missing except body weight, kidney and testes. See pages 169 and 171, Vol. 1.9.

Gross Pathology : 7/10 in iv group had kidneys with light color and/or uneven surface. Some had renal pelvis dilation.

8/10 in sc group showed "punctiform" hematoma (reddish-blue areas) in the injection areas.

Histopathology : Chronic inflammation with fibrin, inflammatory reactions and granulation tissue at the sc injection sites.

7/10 in iv group had **renal** tubular atrophy, 4 with "fresh" renal tubular necrosis and 5 with dilated pelvis, plus one rat in sc group with few areas of fresh renal proximal tubular necrosis.

8/10 in iv group had varying degrees of **impairment of maturation in spermiogenesis**, affecting individual tubules or even small segments of tubules in testes with/without areas of "fresh" **necrosis** in testes plus one rat only in sc group with slight segmental arrest of maturation in testes. See pages 164 and 199, Vol. 1.9.

7/10 in iv group also had oligospermia in epididymis, along with 1/10 in sc group. See pages 205 and 208, Vol. 1.9.

Summary of One month Comparative Toxicity Study in Rats (in, iv, and sc) :

10 rats each were treated with HOE intranasally (intranasal solution) at **2.5 mg/rat** or HOE in saline solution at **10 mg/kg** by iv or sc injection for 30 consecutive days, but

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without a control group. **2.5 mg/rat** is approximately 25 mg/kg/day at the beginning of dosing, then gradually decreased to 9 mg/kg at the end of 30 day dosing, as the body weight changed from 100 g to 280 g.

Drug-related changes were limited to organ weight, gross and histopathology of kidneys and testes : **increased kidney weights and decreased testes weights, punctiform hematomas at the injection sites** after sc injection grossly with corresponding histopathological findings of chronic inflammation. Kidneys after iv injections showed **7/10** with light color and/or uneven surface, and renal pelvis dilation in some rats, and their histology showed **renal tubular atrophy and necrosis**, along with one rat in sc group with proximal tubular necrosis. Histopathology of testes of **8/10** in iv group had varying degrees of **maturation arrest in spermiogenesis as well as necrosis** in one rat, plus one rat in sc group having slight segmental arrest of maturation of spermatogenesis. Also, **7/10 in iv group had oligospermia in epididymis**, along with 1/10 in sc group. Therefore, HOE inhibited spermatogenesis by the iv route and had an equivocal effect sc due to one rat.

It is not surprising that the **in** group did not produce any effect in kidney or testes because of its very low (less than 1%) bioavailability intranasally, compared to iv dosing or sc. What is puzzling is that why the sc injection group at the same dose as iv group produced only **1/10th effect** of iv dosing on kidney and testes, when over 85%-100% is bioavailable when compared to iv dosing. It was reported that after sc injection, absorption is "complete" (presumably 100%) as iv dosing, and the absorption profile is similar to iv. $AUC_{(0\text{-}4\ hr)}$ of parent compound is **85%** of iv as shown on previous page, although C_{max} was approximately 1/2 of iv dosing. The latter may be a reason for the reduced toxicity after sc injection.

NOTE : **Foci of reduced spermatogenesis** was also reported in a previous one month intranasal toxicity study in rats, which was the basis for this comparative study. See attached original review of IND (b) (4)

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2.6.6.4 Genetic Toxicology:

Ames test reports were submitted and reviewed under both [redacted] (b) (4). The pharmacology/toxicology reviews of Ames test reports from [redacted] (b) (4) (dated November 4, 1991 and March 22, 1994, respectively) are incorporated below.

1. STUDY OF THE MUTAGENIC POTENTIAL IN STRAINS OF SALMONELLA TYPHIMURIUM (AMES TEST) Study No. 89.1177, Report No. 89.1683:

Methods: S 890140 (HOE 140, batch no. C 002) was tested in triplicate at 6 doses from **4 µg/plate to 4000 µg/plate** with **TA 100** and **TA 98** strains of Salmonella typhimurium with/without metabolic activation, along with a negative (deionized water) and 2 positive control groups (sodium azide and 2-nitrofluorene without S-9 and 2-aminoanthracen and benz(a)pyrene with S-9). Two experiments were performed.

Study Duration : 11/21/89-11/30/89.

RESULTS : 1. HOE was negative with/without metabolic activation, when the 2 positive control showed expected positive results.

2. HOE was not cytotoxic up to 4000 µg/plate. See the actual data on pages 353-358, Vol. 1.8.

COMMENTS: a. Usually 4 strains are tested up to 5000 µg/plate. These two strains are for point mutation assays.

b. Molecular formula given on page 348 ($C_{71}H_{95}F_{18}N_{19}O_{25}S$, Vol. 1.8 is different from that given in Chemistry section.

HOE 140 : STUDY OF THE MUTAGENIC POTENTIAL IN STRAINS OF SALMONELLA TYPHIMURIUM (AMES TEST) AND E. COLI : Document No. 011343: Study No. 91.0537 :

Methods : HOE (Batch No. H 032) was tested in triplicate for **7** different

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concentrations from **4 to 10,000 μg/plate** on Salmonella typhimurium strains of TA 100, TA 1535, TA 1537, TA 1538 and Escherichia coli WP2uvrA with/without metabolic activation by S-9, along with appropriate positive (2-aminoanthracene, benzo(p)pyrene, and N-methyl-N-nitro-N-nitrosoguanidine:MNNG) and vehicle (Aqua bidest) control groups. For detailed methodology, see pages 031-034, Vol. 1.26.

RESULTS :

1. Toxicity Test : HOE was toxic to most of the bacterial strains at doses of **2500** μg/plate and visible precipitate was formed at this concentration and above. For actual data, see Tables 1-6 on pages 037-042, Vol. 1.26.

2. Ames Test : HOE did not produce a dose-dependent increase in number of revertants in any of the strains tested with/without metabolic activation, when the positive control groups produced significant dose-related increases in revertant colonies. For the actual data, see Tables 7-14 on pages 043-052, Vol. 1.26.

Summary of Mutagenic Study of HOE 140:

Seven concentrations of HOE from 4 to 10,000 μg/plate were tested on 4 strains of Salmonella typhimurium and one E. coli strain, with/without metabolic activation, along with appropriate positive and negative control groups.

HOE was **not mutagenic up to 10,000 μg/plate** under the test conditions, although a precipitate was noted from 2500 μg/plate and up in all strains.

HOE 140 : STUDY OF THE MUTAGENIC POTENTIAL IN STRAINS OF SALMONELLA TYPHIMURIUM (AMES TEST) AND E. COLI : Document No. 011892: Study No. 92.0405 :

Methods : HOE (Batch No. A 062) was tested in triplicate for **6** different concentrations from **4 to 5,000 μg/plate** on Salmonella typhimurium strains of TA 98, TA 100, TA 1535, TA 1537, TA 1538 and Escherichia coli WP2uvrA in absence and presence of metabolic activation by S-9, along with appropriate positive (sodium azide, 9-aminoacridine, 2 Nitrofurene, and MNNG) and vehicle (Aqua bidest) control groups. For detailed methodology, see pages 062-066, Vol. 1.26.

RESULTS :

1. Toxicity Test : HOE was not toxic to most of the bacterial strains. For actual data, see Tables 1-6 on pages 068-073, Vol. 1.26.

2. Ames Test : HOE did not produce a dose-dependent increase in number of revertants in any of the strains tested with/without metabolic activation, while the positive control groups produced significant dose-related increases in revertant colonies. For the actual data, see Tables 7-14 on pages 074-083, Vol. 1.26.

Summary of Mutagenic Study of HOE 140:

Six concentrations of HOE from 4 to 5,000 μg/plate were tested on 5 strains of Salmonella typhimurium and one E. coli strain, with/without metabolic activation, along with appropriate positive and negative control groups.

HOE was not mutagenic up to 5,000 μg/plate under the test conditions

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The *in vitro* chromosome aberration test in Chinese hamster bone marrow cells reviewed under IND (b) (4) The pharmacology/toxicology review of the chromosome aberration test dated March 22, 1994 is incorporated below.

IN VITRO CYTOGENETIC TEST IN BONE MARROW CELLS OF THE CHINESE HAMSTER (CHROMOSOME ANALYSIS) : Document No. 010676 : Study No. 91.0123 :

Methods : Four group of 15 M + 15 F/group of Chinese hamster (10-14 weeks old; M: 24-38 g; F: 25-33 g) were treated with intraperitoneal (IP) injection, once only, with **10, 50, or 100 mg/kg** HOE ("Charge" No. H 017) in saline, along with a negative control group receiving 10 mL/kg saline and a positive control (5 M + 5 F only) receiving 20 mg/kg Endoxan^R. 5 M + 5 F/group from 3 treated groups and negative control group were killed by CO_2 at 12, 24, or 48 hours post injection, and the positive control group was killed at 24 hours. Bone marrow slide from femora was prepared from all animals, and **50 metaphases/animal** were evaluated for chromosomes and chromosomal aberrations. For detailed methodology, see pages 158-160 and 163, Vol. 1.26.

RESULTS :

Behavior and Mortality : All animals survived. The following signs of toxicity were reported : increased tonus of the "abdominal position", reduced spontaneous activity, **clonic convulsions, irregular breathing and ataxic gait.** After 12 hours after dosing, all animals were normal.

Toxicity and Mutation : No cytotoxicity (mitotic index) or significant increase in aberration rate exclusive and inclusive gaps were reported for any HOE treated groups, although slight increase in inclusive gaps (up to 1.6%) in M and F were noted at 48 hour samples, as shown in Table 1 below :

The **positive control group** produced a marked increase in aberration rate with gaps.

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Table 1: **Summary:** Percentage of metaphases with aberrations per trial group (5 animals per group; 50 metaphases per animal)

Trial group	Dose mg/kg bodyweight	Killing time hours after admin.	Metaphases with aberrations inclusive gaps (%) 5 male	5 female	Metaphases with aberrations exclusive gaps (%) 5 male	5 female	Mitotic index ($\bar{x}$) m	f
negative control	0	12	0.8	0.4	0.0	0.4	8.0	9.0
S 89 0140	10	12	0.8	0.8	0.8	0.0	6.3	6.5
S 89 0140	50	12	0.0	0.0	0.0	0.0	6.4	7.5
S 89 0140	100	12	0.8	0.8	0.0	0.0	5.7	8.3
negative control	0	24	0.8	0.0	0.0	0.0	6.4	7.5
S 89 0140	10	24	0.8	0.8	0.0	0.4	8.1	7.6
S 89 0140	50	24	0.0	0.0	0.0	0.0	6.3	6.9
S 89 0140	100	24	1.2	0.8	0.0	0.0	6.7	8.2
Endoxan^R	20	24	8.8	12.0	7.2	9.6	6.4	6.3
negative control	0	48	0.4	0.4	0.0	0.0	5.1	7.2
S 89 0140	10	48	0.8	0.0	0.4	0.0	7.3	6.3
S 89 0140	50	48	1.6	0.8	0.4	0.0	6.4	6.4
S 89 0140	100	48	0.4	0.4	0.0	0.0	5.4	8.1

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Summary of In Vitro Chromosomal Analysis of Chinese Hamster Bone Marrow Cells :

In order to determine proper doses for this study, a preliminary dose-ranging study was performed with 3 M + 3 F/group of hamsters at 20, 100, 200, 300 or 500 mg/kg IP injections. All doses produced dose-related toxic clinical signs (reduced activity, increased tonus of the abdominal position, clonic convulsions, irregular breathing, uncoordinated gait, increased startled reflex, etc.). One M + 1 F in 300 mg/kg and 2 M + all 3 F in 500 mg/kg groups died.

In the principal study, single doses of HOE injected IP at 10, 50, or 100 mg/kg in saline in Chinese hamsters did not induce significant increases of bone marrow cells with chromosomal aberrations, although animals showed various toxic signs including clonic convulsions and irregular breathing. The positive control did produce significant increase in chromosomal aberration inclusive gaps of 8.8-12% and exclusive gaps of 7.2-9.6% of the metaphases.

The *in vivo* mouse micronucleus test was reviewed under IND (b) (4) The pharmacology/toxicology review of the mouse micronucleus test dated November 4, 1991 is incorporated below.

2. MICRONUCLEUS TEST IN MALE AND FEMALE NMRI MICE AFTER INTRAVENOUS ADMINISTRATION : Study No. 89.1351, Report No. 90.0750 Dated 7/23/90 :

Methods: Five M + 5 F/group of NMRKf (SPF71) mice (21-35 g, 7 weeks old) were injected iv once only at 50 mg/kg HOE (batch No. C 002) in 10 mL/kg (= the maximum non-lethal dose from a preliminary study, see page 382, Vol. 1.8), or 20 mg/kg Endoxan(R) in 10 mL/kg (positive control), along with a vehicle (10 mL/kg deionized water) control group, then they were killed at 24, 48, or 72 hours post dosing, and erythrocytes containing micronuclei determined from the bone marrow smears: the number of polychromatic erythrocytes with micronuclei /1000 polychromatic erythrocytes and number of normocytes with micronuclei/1000 normocytes, and the numbers were compared to the control as well as historical controls.

Study Duration : 4/2/90-4/5/90.

RESULTS :

a. The results showed that the incidence of polychromatic and normochromatic erythrocytes with micronuclei in HOE treated group was similar to

the negative control group for **all 3 time points** except in F at 24 hours postdosing, but the value in F was not statistically significant. Also the increase was only in F at one time point out of 3 and not in M, this finding was considered chance finding. And the ratio of polychromatic erythrocytes to normocytes was not affected by HOE treatment. Endoxan (cyclophophamid), the positive control, produced statistically significant increase in the number of polychromatic erythrocytes with micronuclei in both M and F as expected.

b. All mice survived after 50 mg/kg HOE. The signs of toxicity were reduced spontaneous activity, pink discoloration of the skin and piloerection, and they returned normal 2 hours post dosing.

Summary of Mutagenic Studies :

Both **Ames test** on TA 98 and TA 100 (4-4000 µg/plate) and **micronucleus test** in NMRI mice (50 mg/kg iv) showed that **HOE was not mutagenic** under the conditions tested.

Ames assays usually test 4-5 strains of Salmonella typhimurium and doses up to 5000 µg/plate. More studies (ex. Mouse Lymphoma and Chinese Hamster Ovary Assays) are needed for completion of mutagenic testing.

Addendum: The following Genetic Toxicology studies were performed under IND (b) (4) and (b) (4): 1) Ames test (*S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, TA 1538 and *E. coli* strain WP2uvrA); 2) *in vivo* chromosomal aberration test in Chinese hamster bone marrow cells; and 3) *in vivo* mouse micronucleus test. The conclusions from these studies were: 1) Icatibant, with and without metabolic activation, was negative in all Ames test assays; and 2) Icatibant was negative in the Chinese hamster chromosomal aberration and mouse micronucleus assays.

2.6.6.5 Carcinogenicity

No carcinogenicity studies have been performed or were submitted.

2.6.6.6 Reproductive and Developmental Toxicology

Fertility and early embryonic development

Study title: Effects of 5-Week Intravenous Administration of HOE 140 on Fertility of Male Mice

Key study findings: No adverse effects on male fertility were observed in male mice administered intravenously 0, 5, or 30 mg/kg HOE 140 for 35 consecutive days. This study was performed in 1993 (pre-Guidance) and does not follow current Segment I guidelines.

Reference ID: 2977968

Study no.: 0-92-041-086-04
Volume #, and page #: Volume #6, page #6
Conducting laboratory and location: (b) (4) (b) (4)
Date of study initiation: February 5, 1993
GLP compliance: Not stated
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: HOE 140, lot # A062, % purity not stated

Methods

Doses: 0, 5, and 30 mg/kg/day for 35 consecutive days (high dose was based on a preliminary study were mortality and clinical signs of toxicity were observed in mice treated with 40 mg/kg/day for 2 weeks, but no toxicity was observed in mice treated with 4 mg/kg/day)
Species/strain: Mouse/Jcl:ICR
Number/sex/group: 14/sex/group for saline controls (0 mg/kg) and 7/sex/group for treatment groups; females were not treated in this study
Route, formulation, volume, and infusion rate: Intravenous, solution, 0.1 mL/10 g b.w., 1 mL/min
Satellite groups used for toxicokinetics: None
Study design: Males (10 weeks old at the start of treatment) were administered HOE 140 for 35 consecutive days. After 35 days of compound administration, the males were mated for one week with untreated females (10 weeks old at start of mating) in proestrus or estrus. A male that did not show successful copulation was mated with another untreated female for one more week, but the results of the second mating were not included in the calculation of copulation or conception rate. At 3 days after successful copulation or at the end of the mating period, males were sacrificed and autopsied. A female in which a vaginal plug was not confirmed was observed for its estrous cycle for one week after the end of mating period were sacrificed and autopsied.
Parameters and endpoints evaluated: Animals were observed for mortality and clinical signs of toxicity daily. Animals were weighed and food consumption was measured once weekly. Males were sacrificed at 3 days after successful copulation or at the end of the mating period. Blood was collected at necropsy for GOT, GPT, ALP, LDH, BUN and creatinine determination. Organ weights were recorded. These organs and samples from reproductive organs were collected for microscopic examination. In addition, the epididymis was excised bilaterally at autopsy and the spermatozoa analyzed. All females with successful copulation were sacrificed on Day 13 of gestation. Laparotomies were performed, and the status of reproductive organs was evaluated. The number and types of implantation were counted and the internal organs macroscopically assessed.

Results

Mortality: A 30 mg/kg/day mouse died after the first dose. No other mortality was observed in this study.

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Clinical signs: Decreased spontaneous activity, lying prone, deep respiration, decreased body temperature and flushing of the skin were observed in the 30 mg/kg group. All but flushing of the skin disappeared by the end of the dosing period. The same clinical signs except for decreased body temperature were noted in the 5 mg/kg group. All clinical signs disappeared by Day 30 of dosing in this group.

Body weight: There were no drug-related effects on body weight gain.

Food consumption: There were no drug-related effects on food consumption.

Clinical chemistry: There were no drug-related effects on the clinical chemistry parameters measured.

Necropsy: Irregular surface of the kidney was observed in the 30mg/kg animal that died. Otherwise, there were no drug-related effects on macroscopic findings at autopsy.

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

Histopathology: There were no drug-related histopathological changes.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): There were no drug-related effects on copulation or conception rate. There were no drug-related effects on spermatozoal number, motility or morphology. There were no drug-related effects on the numbers of implantations, placental remnants, resorptions, and live/dead embryos.

Study title: Effects of 5-Week Subcutaneous Administration of HOE 140 on Fertility of Male Mice

Key study findings: No adverse effects on male fertility were observed in male mice administered subcutaneously 0, 16.2, or 80.8 mg/kg HOE 140 for 35 consecutive days. This study was performed in 1993 (pre-Guidance) and does not follow current Segment I guidelines.

Study no.: 0-91-009-086-04
Volume #, and page #: Volume #6, page #69
Conducting laboratory and location (b) (4)
Date of study initiation: January 30, 1992
GLP compliance: Not stated
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: HOE 140, lot #H032, % purity not stated

Methods

Doses: 0, 16.2, and 80.8 mg/kg/day for 35 consecutive days (high dose was based on a preliminary 2-week study were mortality was observed in 1/3 animals administered 242.4

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mg/kg/day HOE 140 and increased kidney weight was observed at both the 24.2 and 242.4 mg/kg/day dose)
Species/strain: Mouse/Jcl:ICR
Number/sex/group: 7/sex/group; females were not treated in this study
Route, formulation, and volume: Subcutaneous, solution, 0.1 mL/10 g b.w.
Satellite groups used for toxicokinetics: None
Study design: Males (10 weeks old at the start of treatment) were administered HOE 140 for 35 consecutive days. After 35 days of compound administration, the males were mated for one week with untreated females (9 weeks old at start of mating) in proestrus or estrus. A male that did not show successful copulation was mated with another untreated female for one more week, but the results of the second mating were not included in the calculation of copulation or conception rate. At 3 days after successful copulation or at the end of the mating period, males were sacrificed and autopsied. A female in which a vaginal plug was not confirmed was observed for its estrous cycle for one week after the end of mating period were sacrificed and autopsied.
Parameters and endpoints evaluated: Animals were observed daily for mortality and clinical signs of toxicity. Animals were weighed and food consumption was measured once weekly. Organ weights were recorded. Only samples from reproductive organs were prepared for microscopic examination. In addition, the epididymis was excised bilaterally at autopsy and the spermatozoa analyzed. All females with successful copulation were sacrificed on Day 13 of gestation. Laparotomies were performed, and the status of reproductive organs was evaluated. The number and types of implantation were counted and the internal organs macroscopically assessed.

Results

Mortality: A 16.2 mg/kg/day mouse died on Day 29 of administration. No other mortality was observed in this study.

Clinical signs: Crust formation was observed on the back near the injection site of 3 high dose animals.

Body weight: There were no drug-related effects on body weight gain.

Food consumption: Food Consumption: Increases over control were observed at weeks 3 and 4 in the 16.2 mg/kg/day group and at weeks 2, 4, and 5 in the 80.8 mg/kg/day group (see Figure below).

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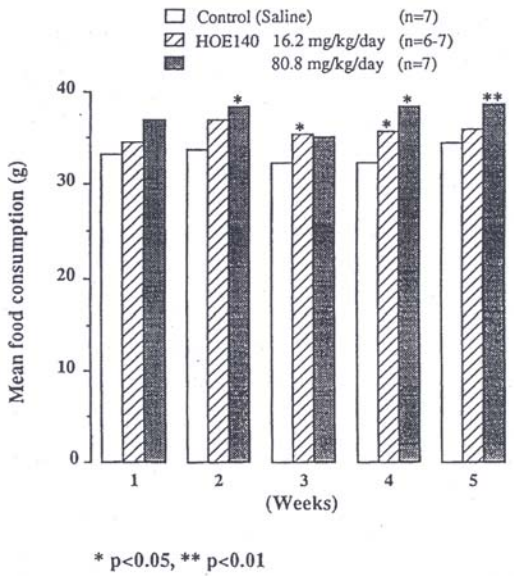

Fig. 2 Food consumption of male mice.

Necropsy: There were no drug-related effects on macroscopic findings at autopsy.

Organ weights: The heart weight was significantly (p<0.01) heavier for the 16.2 mg/kg/day group (232.8 g vs 179.7 g for controls), but heart weight for the 80.8 mg/kg/day group (188.1 g) was similar to controls. Otherwise, there were no drug-related effects on organ weights.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): There were no drug-related effects on copulation or conception rate. There were no drug-related effects on spermatozoal number, motility or morphology. There were no drug-related effects on the numbers of implantations, placental remnants, resorptions, and live/dead embryos.

Embryofetal development

Segment II studies in rat and rabbit were reviewed under IND (b) (4) The pharmacology/toxicology review of the segment II studies dated March 22, 1994 is incorporated below.

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groups while M fetuses were slightly greater in 25 mg/kg group.

Dead Embryofetal Primordia : Early and late intrauterine deaths occurred in all groups, and the total numbers/group in treated groups were similar to the control group. One dead fetus each in the control and 2.5 mg/kg groups were markedly stunted and weighed only 0.23 and 0.29 g (<3.0 g is defined as slightly retarded).

Placentas : Placentas of the fetuses from the treated groups were unremarkable macroscopically, and their weights were comparable to the control group, but the placentas of the dead fetuses were smaller than those of the live fetuses in 2 dams, although one was as large those of the live fetuses.

Morphological Examination of Live Fetuses : One fetus each in 0.25 and 2.5 mg/kg group had malformation in head and fore limbs (pages 32-33, Vol. 24). For summary of findings, see pages 119-120, Vol. 1.24.

Autopsy and Body Section Examination : One fetus in 0.25 mg/kg had a hematoma in the right cerebrum, and another one had the stomach filled with a soft mass.

One fetus in 25 mg/kg had displacement of the heart in a cranial direction and a light red soft mass in the thoracic cavity, and another fetus had bifurcated right medial lobe of the lung.

Some fetuses in all groups apart from 0.25 mg/kg group had blood in the abdominal cavity or in the vicinity of the stomach, a hematoma in a hepatic lobe, and distended renal pelvis and ureter.

Skeletons of Fetuses : 0.25 and 25 mg/kg groups showed increased incidence of fetuses with poorly ossified cranial bones and 5th metatarsal bone when compared to the control group, but without apparent dose-response.

One or two fetuses in various groups had variation of 12, 13, or 14th ribs as shown in the Tables on pages 123-124, Vol. 24.

Two fetuses found dead in utero in 2.5 mg/kg group were not dissected due to their small size, and their skeletons showed poor ossification, but otherwise normal. The actual data, see page 91, Vol. 1.24.

Body Weight : Group mean body weight (page 91) and body weight gain (page 101) values during pregnancy and lactation (pages 106 and 107) showed no statistically significant differences between groups.

COMMENT : Group mean body weight gain in 0.25 and 25 mg/kg groups was depressed 28 and 41% compared to the control group during lactation days 7-14, and all groups including the control group actually **lost body weight** during lactation days **14-21**. The order of loss was control > LD > MD > HD. For actual data, see page 107, Vol. 1.24.

Data from C section : All 20 dams in 0.25 and 2.5 mg/kg groups and 19 dams in 25 mg/kg group carried live fetuses until C section on day 21 of pregnancy. One dam in 25 mg/kg group had no live fetuses but only empty implantation sites in the uterus, showing **resorption**.

Other data show no significant differences between the control and treated groups as shown on pages 112-113, Vol. 1.24.

Examination of the Fetuses : The live fetuses delivered by C section in all treated groups developed normally, and their mean body weight and lengths were similar to the control group. F fetuses were slightly greater in number in 0.25 and 2.5 mg/kg

fully developed dead fetus in 25 mg/kg group had edema in the subcutis, a poorly ossified skeleton and fragmented and longitudinally displaced sternebrae and a short additional rib bilaterally on the first lumber vertebra.

Examinations After Birth : All 10 dams/group had live pups except one in 25 mg/kg group that was killed on the **27**th day of pregnancy, and this one had 4 autolyzed dead fetuses in the uterus. Group mean **duration of pregnancy** was 22.3 to 22.7 days (within normal for this strain) for all 4 groups, but with a trend of dose-related increases. Summary of findings were presented on pages 36 and 245, Vol. 24.

The number of implants and live pups, and survival up to day 21 after birth showed no significant differences between groups. For actual data, see page 246, Vol. 1.24.

Pups from Normal Delivery : Pups from all treated groups developed normally as those of the control group, and 0.25 and 25 mg/kg groups had equal number of M and F, but 2.5 mg/kg group had slightly more M, as shown in a summary table on page 246, Vol. 1.24.

No external or skeletal abnormalities were reported in the any of pups. Four dead fetuses from one dam in 25 mg/kg group were not examined for visceral anomalies due to autolysis.

Examination During the Lactation Period : Body weight gain of the treated pups during lactation was similar to the control group and their previous control values (page 376).

General behavior and **physical condition** of treated pups showed no differences from the control pups during this period except for the following :

One pup each in 2 litters and all pups in another litter in 0.25 mg/kg group and one pup in one litter in 25 mg/kg group were reported to have **insufficient suckling** during first 3 weeks of raising.

All pups in one litter of 0.25 and 2.5 mg/kg groups and in 2 litters of 25 mg/kg group had **impaired coat growth** in the 2nd or 3rd week.

Viability and raising rate of the pups were high in all groups and showed no differences between treated and the control groups.

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All dams raised their pups to weaning.

Physical development of pups were normal in all groups : **pinna separation, start of coat growth, incisor eruption and opening of the eyes** occurred at about the same time as in the control pups. For actual data, see page 296, Vol. 1.24.

Functional tests (hearing, vision, and righting reflex) and **behavior tests** (trainability, memory, retrainability) showed no significant differences between groups. See page 297, Vol. 1.24.

Motor activity, coordination and **sense of balance** were not impaired in any pups.

Determination of Fertility of the F_1 Generation (10 M + 10 F/group):

Clinical Examination : All F_1 rats showed no impairment of their **behavior** and they survived until the scheduled end of the study, except one M in 2.5 mg/kg group that died during mating period without any apparent cause.

Some rats in all but 25 mg/kg group had impaired coat growth or circumscribed loss of hair that was also seen in parent generation (blamed on strain of rats). For incidence of clinical findings, see pages 332 and 333, Vol. 1.24.

Body Weights : Weekly group mean body weight and body weight gain values showed no significant differences between groups. For actual data, see pages 349 and 350, Vol. 1.24.

Mating : All F showed intact estrus, and number of matings required by F until pregnancy showed no differences between groups, and all became pregnant by 4th mating, as shown in tables on pages 42 and 43, Vol. 1.24.

All M were fertile (except one M that died during mating) and one M in 0.25 mg/kg group required a 2nd F, and the fertility rate was 100% for all 4 groups.

Examination After Birth : All produced live pups.

Duration of pregnancy was not affected by drug treatment, and all dams littered between the 22nd and 24th day of pregnancy without any complication, with mean duration of **22.6** days for all groups including the control group. For actual duration of pregnancy, see page 45, Vol. 1.24.

All pups developed normally. One dam each in 2.5 and control groups and 2 dams each in 0.25 and 25 mg/kg group had dead pups. Although mean litter size of 25

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mg/kg group was smaller than other groups, it was not statistically significantly different from the control group. The size of litters was within normal range. For actual data, see page 355, Vol. 1.24.

No external anomalies were reported.

Autopsy of the Dams (F_0 and F_1) and Pups :

F_0 Dams : 1-2 dams/group including the control group had kidneys with moderate to severe uni- or bilateral pelvic dilatation.

The liver, kidney and spleen weights in dams delivered by C section were similar to the control group, but the **heart weight** in the dams from **25 mg/kg** group were slightly but statistically significantly larger than the control value (**7%↑** for AW and **9%↑** for RW, but with trend of dose-relationship). The higher **heart** weight was reported as above the "upper limit of our last 67 control values". The organ weights of dams delivered naturally were similar to the control group. For actual organ weights, see pages 322-325, Vol. 1.24.

COMMENT : Also, the actual relative weight (RW) of the **liver** in 25 mg/kg group was **9% larger** than the control group, and the absolute weight (AW) was only 3% larger than the control group.

Pups of the F_1 generation : No anomalies were reported for internal organs for pups killed after weaning, except for distention of renal pelvis in one F each of 2.5 mg/kg group and 25 mg/kg group. For summary findings, see page 299, Vol. 1.24.

Dams of F_1 : One M in 2.5 mg/kg group had moderate distension of renal pelvis. One F in 25 mg/kg group had a kink in the tip of the tail, the 19th and 20th coccygeal vertebrae were dysplastic, resulting in the tail being bent to the left, and 23rd and 24th coccygeal vertebrae were fused and the coccygeal vertebral column was bent between the 24th and 25th vertebra. Others were normal. For summary findings, see pages 365 and 366, Vol. 1.24.

Summary of Teratology and Fertility Study in Wistar Rats :

A **dose-ranging** study was performed with 3 pregnant F/group by injecting sc with 25, 50, and 100 mg/kg/day from days 7 through 18 of pregnancy. All 3 doses were too toxic to dams, as reported on page 40 of this review.

In the **principal** study, 30 pregnant F/group of rats were treated with sc injection, once daily

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with **0.25, 2.5 and 25** mg/kg/day HOE in 0.9% NaCl from days **7 through 18** of pregnancy (day 1 = day with spermatozoa in vaginal smear), along with a control group receiving 5 mL/kg 0.9% NaCl. 20 F/group were killed and delivered by **C section on day 21** of pregnancy, and the remaining 10 F/group were kept until they littered naturally and then raised their young. The fetuses were examined for viability, physical development and functional and behavioral disorders. One M and one F from each litter of F_1 generation (total 10 pairs) were raised to maturity for mating, and **their fertility** checked. After the birth of F_2 generation, the parent and pups were killed. Examination of dams included uterus status, body weight, macroscopic examination and organ weighs.

SC injection of HOE did not produce any significant effects on dams for the following: general physical condition, food consumption, body weight gain, organ weight change (liver, spleen, kidney), pregnancy rate, duration of pregnancy in F_0 or F_1 generation, live or dead pups or sex distribution.

Only significant finding for treated dams was slight but significant increase in **heart weight** in 25 mg/kg/day of C section group.

No significant major external or internal anomalies were reported for pups for F_1 or F_2 generations, especially in 25 mg/kg group, although one or two fetuses in 0.25 and 2.5 mg/kg group had multiple minor skeletal malformations or variations.

Post natal development (physical, behavioral, functional, and motor, etc.) of treated fetuses and fertility of F_1 generation were comparable to the control group.

Besides a kinky tail, especially one F fetus in 25 mg/kg group, **no major terata** were produced in the study although one dam in 25 mg/kg group had **empty implantation sites** in the uterus, and fetuses in 25 mg/kg group had slightly greater numbers with less ossification of skeletons and variations of liver and kidneys than the control group (4 fetuses had lobus dexter of liver and 3 fetuses had distended pelvises in the kidney, while only one fetus in the control had the same findings).

Therefore, HOE was not teratogenic in rats under the conditions studied.

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SUBCUTANEOUS EMBRYOTOXICITY STUDY IN HIMALAYAN RABBITS (SEGMENT II): Report No. 93.0542 : Document No.011771 :

Methods : Four groups of 15 pregnant F/group of Himalayan rabbits [Hoe:HIMK(SPFWiga, 8-11 months old, 2,591 ± 172 g) were treated by sc injection with **0.1, 1.0, and 10 mg/kg/day** HOE (Batch No. A 062) in 0.9% NaCl, once daily from days **7 through 18** of pregnancy, along with a control group receiving 2.0 mL/kg of 0.9% NaCl. On day **29** of pregnancy, surviving dams were killed and fetuses delivered by C section. Fetuses were kept in an incubator for 24 hours to determine survival rate and then examined morphologically for developmental disorders.

Due to the occurrence of abortions, premature births, increased light-grey depression on the surface of kidneys in HOE treated groups, this study was **repeated using 0.1 and 1.0 mg/kg** HOE and 0.9% NaCl groups. Rabbits used in this study were Chbb:NZW(SPF) with mean body weight of 2,673 ± 190 g and 10-11 months of age. For detailed information, see pages 14-21, Vol. 1.25.

RESULTS : Results of the principal study will be followed by the results from the repeated study.

Clinical Examination : **Marked vaginal hemorrhaging** was reported for one dam in 0.1 mg/kg group on the night of 28th to 29th day of pregnancy, one in 1 mg/kg group on 19th day of pregnancy, and one in 10 mg/kg group on the night of the 15th to 16th day of pregnancy, and they were killed. **Vaginal hemorrhaging** was indicative of **impending premature** birth in the former and **abortion** in latter two. Vaginal hemorrhaging was also noted in a second dam in 1 mg/kg group on the 19th and 20th days of pregnancy, but it was not severe and the dam was kept

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and not killed. Other dams showed no abnormal behavior or physical signs and survived to the scheduled end of the study. For other findings, see page 39, Vol. 1.25.

Repeated Study : No abnormality was reported.

Food Consumption : Group mean values showed no significant differences between groups, although the values were lower during the second week of pregnancy, and it was reported that some values were not accurately assessed. For actual mean data, see page 56, Vol. 1.25.

Repeated Study : No differences between groups were reported.

Body Weight Development : Group mean body weight gain in treated groups was **depressed**, especially during days **19th and 29th** with dose-relationship, although it was reported that statistical significance could not be determined due to large individual variations. For actual gain data, see page 74, Vol. 1.25.

Repeated Study : Group mean body weight gain showed no consistency due to large individual variations. See Table on page 79, Vol. 1.25.

Examinations Performed after C Section : In addition to the clinically detected **abortion**, one dam each in 1 and 10 mg/kg group also had clinically **undetected** abortions, since they had only **empty** implantation sites in the uterus. In **Two aborted dams each in all 3 treated groups** had 1 to 8 empty implantation sites, these being distributed evenly over the groups.

10 mg/kg group also had significantly **larger** late **intrauterine deaths** and total intrauterine deaths, but significantly **smaller** number of **live** fetuses from the control values. This group had significantly reduced number of live fetuses per litter, due to increased intrauterine death rate. **Total implantations** and total number of live fetuses showed **dose-related decreases** but percent of pre-implantation loss showed a **dose-related increase,** as excerpted in the Table below :

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		GROUP 1 0 MG/KG		GROUP 2 0.1 MG/KG		GROUP 3 1.0 MG/KG		GROUP 4 10 MG/KG	
NUMBER OF									
- PREGNANCIES	TOTAL	15		15		15		15	
- INTERCURRENT DEATHS	TOTAL	0		0		0		0	
- FEMALES WITH ABORTION	TOTAL	0		2		2		2	
- FEMALES WITH PREMATURE DELIVERY	TOTAL	0		1		0		0	
- CORPORA LUTEA	TOTAL	113	N	88	N	98	N	99	N
	MEAN	7.5		7.3		7.5		7.6	
	S.D	1.4		1.7		1.2		2.7	
- IMPLANTATIONS	TOTAL	99	N	79	N	76	N	71	A
	MEAN	6.6		6.6		5.8		5.5	
	S.D	2.2		2.5		1.4		2.4	
PRE-IMPLANTATION LOSS %	MEAN	12.83		12.94		20.33		24.52	
POST-IMPLANTATION LOSS %	MEAN	17.29		14.88		13.87		28.51	
- EARLY INTRAUTERINE DEATHS	TOTAL	9	N	8	N	8	N	10	N
	MEAN	0.60		0.67		0.62		0.77	
	S.D	0.74		0.89		0.96		1.24	
- LATE INTRAUTERINE DEATHS	TOTAL	6	N	0	N	4	N	15	* N
	MEAN	0.40		0.00		0.31		1.15	
	S.D	0.63		0.00		0.63		2.61	
- TOTAL INTRAUTERINE DEATHS	TOTAL	15	N	8	N	12	N	25	* A
	MEAN	1.00		0.67		0.92		1.92	
	S.D	0.85		0.89		0.95		2.66	
- LIVE FOETUSES	TOTAL	84	N	71	N	66	N	46	* A
	MEAN	5.6		5.9		4.9		3.5	
	S.D	2.3		2.9		1.8		2.3	
NUMBER OF FOETUSES	TOTAL	84	N	71	N	66	N	46	* A
% OF IMPLANTATIONS	MEAN	82.71		85.12		86.13		71.49	
MALES	%	58.33		59.15		46.88		50.00	

N: Normal range; A: Outside normal range; *: Significantly different from control.

One dam in 1 mg/kg group that had slight vaginal hemorrhage on 19th and 20th days of pregnancy had normally developed fetuses.

Repeated study: One dam in 0.1 mg/kg group had only dead embryo/fetuses undergoing resorption. All dams carried liver fetuses until delivery on day 29th by C-section, and number of corpora lutea, implants and live fetuses showed no differences between groups. For actual summary data, see pages 91-92, Vol. 1.25.

Examination of the Fetuses : Fetuses delivered by C section from treated group developed normally as the control fetuses. Their body weight and body length were similar also. The control and 0.1 mg/kg groups had more M fetuses but 1 mg/kg group had more F fetus and 10 mg/kg group had equal number of M and F fetuses.

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Dead embryos were reported in all 4 groups. Dams in 0.1 and 1 mg/kg groups had dead embryo/fetuses undergoing resorption, but dams in **10 mg/kg** group had more **dead** fetuses than embryo/fetuses undergoing resorption, especially in **2 dams with 7 dead fetuses** each. This is the upper limit of their previous spontaneous rate.

Placenta of the live fetuses in the treated groups were macroscopically similar to the control group, and their weights also were similar to the control, with one exception : the placentas of dead fetuses in 1 mg/kg group were smaller than those of live fetuses in the respective litters and were anemic.

24 hour survival rate of fetuses from the treated groups were comparable to the control group, or within normal range. All litters survived in the control, 0.1 and 1 mg/kg groups, but a **single pup** born alive in one litter in **10** mg/kg group **died one hour** after delivery.

Repeated Study : Fetuses and placenta from treated dams were normally developed, and embryofetal death rate was not increased. 24 hour survival rate of fetuses were comparable to the control group, as shown on page 91-92, Vol. 1.25.

Morphological Examination of Live Fetuses : One fetus each from the control and 1 mg/kg group had one or both forefeet that were bent to ventral direction in the carpal joint. One fetus in 10 mg/kg group had **scoliosis** of the vertebral column, the convexity being to the left, in conjunction with partial **aplasia** of the 2nd and 5th thoracic vertebra, and the first, 2nd, 4th, and 5th coccygeal vertebra were fused also. None of fetus in 0.1 mg/kg group had malformations.

Autopsy and body cross section examinations of live fetuses showed a low incidence of findings such as fused lung lobe or hemorrhage in stomach, etc., but mostly only one fetus each in all 4 groups. **Skeletons** of live fetuses from all groups including control group showed poor ossification of 13th coccygeal vertebrae or sternebrae, or variations of others. In fact, the control fetuses had greater variations or abnormalities than the treated groups. For summary findings, see pages 104-106, Vol. 1.25.

Among dead fetuses examined, "marked stunted fetus" was reported as **8**/15 in the **10** mg/kg group, but only 2/6 in the control and none in other groups, and "retarded fetus" was reported as **6**/15 in the **10** mg/kg group and 1/4 in 1 mg/kg group, but only 1/6 in the control group. But no major malformation was reported. **Skeletons** of dead fetuses were more poorly ossified and at a lower stage of development (page 29, Vol. 1.25). For summary data of morphological findings of dead fetuses, see page 107, Vol. 1.25.

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COMMENT : "Retarded" means a delay in skeletal ossification.

Repeated Study : One fetus each in the control and 0.1 mg/kg group had both forefeet bent in a ventral direction in the carpal joint and another fetus in the control had various malformations including cleft palate as described on page 30, Vol. 1.25.

Autopsy of the Dams : **Light-grey colored depression** of varying sizes on the surface of **kidneys** was reported for **7 dams** each in 0.1 and 1 mg/kg and **5** dams in 10 mg/kg group, along with one control dam. Mostly both kidneys were involved, and one dam each in 1 and 10 mg/kg group with dark-colored spots and right brown in color.

Several pale-reddish areas on the surface of **liver** were reported in one dam in the **1 mg/kg group**. The other dam that aborted on day 19 in this group had dark colored spots on the surface of both **kidneys** and light brown marbling of the margins of **all liver lobes**. For the summary report, see page 154, Vol. 1.25.

Two dams in **10 mg/kg** group had at least one enlarged kidney, and the third dam had a reduced heart weight.

Microscopic Examination of Kidneys of Dams : Depressions observed on the surface of kidneys were due to chronic interstitial nephritis. Tubular atrophy and iron and calcium deposits were reported in the kidneys of these dams and also some in the control group, thus the findings were reported by the sponsor as having a "spontaneous origin".

The mean **heart, liver, kidney and spleen** weights of other dams in treated groups were comparable to the control values. For actual mean values, see page 190, Vol. 1.25.

Repeated Study : **Light-grey depression** on the surface of both **kidneys** was reported in one dam each in two treated groups, while none in the control group. For the summary report, see pages 186-189, Vol. 1.25.

The mean **heart, liver, kidney and spleen** weights of other dams in treated groups were comparable to the control values. For actual mean values, see page 196, Vol. 1.25.

Summary of Teratogenic Study in Rabbits :

Preliminary dose-ranging study was conducted in two pregnant F/group of rabbits treated with sc injection of 10, 25, and 50 mg/kg/day HOE from days 6 through 18

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pregnancy, and necropsied on day 29 of pregnancy. All 3 doses produced **impairment of pregnancy** : Only **empty implantation sites** were noted in the uterus in one of 2 dams in 10 mg/kg group and **all** dams in 25 and 50 mg/kg group.

In the **principal** study, 4 groups of 15 pregnant F/group of rabbits were treated by sc injection with **0.1, 1.0, and 10 mg/kg/day** HOE in 0.9% NaCl, once daily from days **7 through 18** of pregnancy, along with a control group receiving 2.0 mL/kg of 0.9% NaCl. On day **29** of pregnancy, surviving dams were killed and fetuses delivered by C section. Fetuses were kept in an incubator for 24 hours to determine survival rate and then examined morphologically for developmental disorders.

Unexpected **abortions** were produced in 2 dams each in all 3 treated groups and a **premature birth** in 0.1 mg/kg group, while none of these occurred in the control group, although no other toxic effects were produced. At autopsy, **light-grey depression** was found on the surface of kidneys in 5-7 dams each in all 3 HOE treated groups, but only one dam in the control group.

To determine if these effects are drug induced, the study was **repeated, but with two low doses** only and vehicle control groups. **The abortions or premature births were not reproduced** in this study, and incidence of light grey colored kidneys in the treated groups was not increased, but it was also reported in one dam each in treated groups only. The depression on the kidney was due to chronic interstitial nephritis, and it was attributed to the strain of rabbits by the sponsor.

Although all 3 doses of HOE did not have significant toxic effects on the dams, **10** mg/kg was **toxic to the embryo**, by significantly **reducing** the litter sizes, as a result of an **increased intrauterine embryofetal death rate**. HOE treatment resulted in dose-related **decreases** for total implantations and total number of live fetuses as well as dose-related **increases** for percent of pre-implantation loss when compared to the control values, as shown on page 49 of this review.

Morphological examination of fetuses showed no increased incidence of major **malformations** in treated groups. In fact, the control fetuses had greater multiple malformations or variations of skeletons including limbs.

"Minor anomalies" or variations of liver or kidneys were reported in some fetuses, but they were reported as within the normal ranges.

Among the **dead fetuses** examined (6 in the control, 4 in 1 mg/kg and 15 in 10 mg/kg groups), 10 mg/kg had significantly more stunted (8/15) or retarded fetuses (7/15) over the control group (2/6 and 1/6 respectively).

Therefore, HOE was not a teratogen under the test conditions. **10 mg/kg** produced increased abortions or premature births and significant increases in intrauterine death rate or resorption rate, although dams tolerated up to 10 mg/kg.

Prenatal and postnatal development

No Segment III studies were submitted.

2.6.6.8 Special Toxicology Studies

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Special toxicology studies were not included in this submission. A number of relevant special toxicology studies in rabbit and guinea pig were reviewed under (b) (4). The IND (b) (4) pharmacology/toxicology review of skin and eye irritation studies in rabbit dated November 4, 1991 is incorporated below. The IND (b) (4) pharmacology/toxicology review of antigenic potential dated March 22, 1994 is also incorporated below.

. **PRIMARY SKIN IRRITATION STUD ON SHORN INTACT AND ABRADED RABBIT SKIN (PATCH TEST): Report No. : 91.0208 :**

Methods : A 2.5 x 2.5 cm patch with 0.5 mg/mL HOE solution (Batch #12, see the exact composition of the solution on page 321, Vol. 1.8) was applied to 2 adjacent sites of 3 x 3 cm; one **shorn** and one **abraded** on the flank skin of 3 M + 3 F New Zealand rabbits (2.83-4.35 kg, about 6 month old). Then the patches were covered and secured with a piece of impermeable PVC film and elastic bandage for 24 hours, according to **FDA guidelines** (see page 325, Vol. 1.8). The irritation score of the skin was determined immediately after removing the patches (24 hours) as well as 48 and 72 hours. (Study period : 2/25/91-2/28/91).

RESULTS: One M showed a "very slight erythema (score 1) in abraded skin at 48 and 72 hours, while none of other rabbits showed any sign of irritation. All rabbits appeared normal during the study. **The primary irritation score was reported as 0.04**, classified as "non-irritant". See actual scores on page 324, Vol. 1.8.

. **MUCOSAL TOLERANCE IN THE RABBIT EYE : Report No. 91.0275:**

Methods : A single dose of 0.1 mL HOE solution (see above study) was applied to the conjunctival sac of the left eye of 3 M + 3 F New Zealand rabbits, and the eyes were evaluated with a magnifying glass at 1, 7, 24, 48, and 72 hours post dosing, with the right eye as untreated control. The eyes were rinsed after 24 hours post dosing, and additional examinations were made at 48 and 72 hours after the instillation of a drop of sodium fluorescein (0.1 g/l), according to FDA guidelines (see pages 332-333, and 336-337, Vol. 1.8).

Study period : 2/26/91-3/1/91.

RESULTS : No changes were reported in the cornea, iris and conjunctiva, and the primary irritation score was 0 (**zero**), and all the rabbits were normal. See actual scores on page 335, Vol. 1.8.

Summary of Special Studies :

HOE solution (0.5 mg/mL) was "non-irritating" to shorn **skin** or **eyes** of the New Zealand rabbits under the conditions tested, although one of 6 rabbits showed a very slight erythema in abraded skin at 48 and 78 hours.

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ANTIGENICITY STUDY OF HOE 140 IN GUINEA PIGS - HOMOLOGOUS 4 HOUR PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) TEST : Document No. 011696 : Study No. 0-91-020-086-05 :

Lab Performing the Study : (b) (4)

Methods : Four groups of 5 M/group of Hartley guinea pigs (Japan SLC, Inc., 6 weeks old and 371-411 g) were sensitized intranasally (IN) or by sc injection of 2.5 mg/kg HOE (Lot No. H032) alone, once daily, 5 times/week for 3 weeks (total 15 doses), or HOE with Freund's adjuvant once/week for 4 weeks (total 4 doses) along with a control group receiving ovalbumin with Freund's adjuvant, once/week for 4 weeks as in the following table :

Sensitizing antigen	No. of animals	Dose (mg/body)	Concen-tration (%)	Volume (ml/body)	Route
HOE 140	5	2.5	25	0.01	i.n.
HOE 140	5	2.5	1.25	0.2	s.c.
HOE 140 (+FCA, FIA)	5	2.5	1.25*	0.2	s.c.
OVA (+FCA, FIA)	5	0.5	0.25*	0.2	s.c.

FCA: Freund's complete adjuvant
FIA: Freund's incomplete adjuvant
* As a 1:1 emulsion in FCA or FIA

14 days after the final sensitizing dose, blood was collected by heart puncture under sodium pentobarbital and serum collected from these samples.

For **homologous 4 hour PCA test**, 3 M/group were used. 0.1 mL of each serum from a sensitized guinea pig and its 2 fold diluted solution were injected intracutaneously on shaved back skin, along with a serum from untreated guinea pig that served as the negative control. Four hours later, they were challenged by injection into ear vein using HOE mixed with Evans blue (see below). Thirty minutes after the challenge,

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all were killed with intraperitoneal injection of sodium pentobarbital, and the sensitized sites of the back skin were examined for dye extravasation. The site with a dye spot of at least 5 mm in diameter was considered positive, and the highest dilution with a positive result was taken as the PCA titer.

Challenging antigen	Dose (mg/body)	Concentration (%)	Volume (ml/body)
HOE 140	0.2	0.04	0.5
HOE 140	0.06	0.012	0.5
HOE 140-BSA conjugate	2*	0.4	0.5
OVA	2	0.4	0.5

BSA: Bovine serum albumin

RESULTS : HOE was a sensitizer when administered with adjuvants as shown below :

Sensitizing antigen[a]	Route	Challenging antigen	Dose (mg/body)	No. positive/ No. examined (PCA titer)
HOE 140	i.n.	HOE 140	0.2	0/5
		HOE 140	0.06	0/5
		HOE 140-BSA	2	0/5
HOE 140	s.c.	HOE 140	0.2	0/5
		HOE 140	0.06	0/5
		HOE 140-BSA	2	0/5
HOE 140 (+FCA, FIA)	s.c.	HOE 140	0.2	0/5
		HOE 140	0.06	0/5
		HOE 140-BSA	2	1/5 (2^{3-25})
OVA (+FCA, FIA)	s.c.	OVA	2	5/5 (2^{12-216})

BSA: Bovine serum albumin
OVA: Ovalbumin
FCA: Freund's complete adjuvant
FIA: Freund's incomplete adjuva

Summary of Antigenicity Study (PCA) in Guinea Pigs :

Sera from sensitized guinea pigs with HOE alone by IN or sc were all negative for PCA reactions when challenged with HOE or HOE-BSA conjugate.

Using sera from sensitized guinea pigs with sc HOE plus Freund's adjuvant, **one of 5** sera samples gave a **positive PCA** reaction with titer 23-25 by challenge with HOE-BSA conjugate, although HOE alone was negative.

All sera from ovalbumin group were positive. See Table above.

Therefore, HOE is a slight sensitizer when given with adjuvant in PCA reaction under the conditions tested.

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OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Hereditary Angioedema (HAE) is an autosomal dominant trait. Mutations in the C1 esterase inhibitor (C1-INH) gene have been identified as the etiology for HAE. The time to first disease manifestation peaks in young adults in the first and second decade of life. Patients with HAE have episodes of swelling of the hands, feet, trunk, face, gut and airways. In addition, patients with HAE may have recurrent attacks of nausea, vomiting, and abdominal pain. Swelling of the upper airways can be fatal if not treated appropriately. Anxiety, stress, or minor trauma can "trigger" acute attacks. Acute attacks can also occur without any cause. Currently, there is no FDA-approved treatment for acute attacks of HAE.

Icatibant is a synthetic decapeptide with a similar structure to bradykinin, but contains five non-proteinogenic amino acids. Icatibant was shown to be a competitive bradykinin type 2 (B_2) receptor antagonist with the same affinity as bradykinin itself in numerous cell and tissue culture models. C1-INH is a serine protease inhibitor that can inhibit components of the complement, coagulation, fibrinolytic, and kinin-releasing systems. One of the proteases inhibited by C1-INH is kallikrein, the main enzyme responsible for bradykinin formation from high molecular weight kininogen. The sponsor hypothesized that in HAE patients, production of high molecular weight kininogen is "triggered" (see above). Lack of C1-INH then results in increased kallikrein activation that leads to excess formation of bradykinin that may be responsible for most of the clinical symptoms of HAE including increased vascular permeability, vasodilation and contraction of nonvascular smooth muscle. Therefore, treatment with a bradykinin antagonist, such as Icatibant, may ease some of the acute attack symptoms of HAE. In support of this hypothesis the increased vascular permeability in C1-INH deficient mice could be reversed by treatment with Icatibant (Han et al., *J. Clin. Invest.* 109:1057-1063, 2002 cited by the sponsor).

The sponsor presented a clinical protocol to treat 56 (age ≥ 18 years) randomized HAE patients (both male and female) with either a single subcutaneous injection of 30 mg Icatibant or placebo. The primary objective of this study is to assess the efficacy of Icatibant in the relief of symptoms resulting from moderate to severe cutaneous and abdominal edema attacks in subjects with HAE. The secondary objective will be to assess the following: rate of response, time to almost complete relief, global outcome, severity of each symptom, safety, and tolerability. The efficacy and safety in subjects experiencing laryngeal edema will be explored to the extent that subjects become available during the study period. The sponsor submitted the following preclinical studies with Icatibant: Toxicology – acute (mouse, rat and dog) and 1-month repeat dose (rat – i.v. and s.c., dog – i.v.); Genetic Toxicology – Ames test, mouse micronucleus test, and Chinese hamster chromosome aberration test; and Reproductive Toxicology – Segment I (male mouse – i.v. and s.c.) and Segment II (rat and rabbit – s.c.). In addition, Icatibant was previously evaluated for the treatment of allergic rhinitis by (b) (4) (b) (4)) under IND (b) (4) and the sponsored has received Right of Reference to this IND.

In safety pharmacology studies, Icatibant at intravenous doses of 0.01 and 0.1 mg/kg injected over 1 minute did not affect blood pressure or heart rate in conscious dogs. At doses of 0.6 and 1

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mg/kg, Icatibant markedly decreased blood pressure for 5 to 10 minutes and clinical signs included pain reaction, restlessness, mydriasis, and hypersalivation. In anesthetized rats, intravenous doses of 0.13 and 1.3 mg/kg Icatibant decreased mean arterial blood pressure for 3 minutes by 10% and 33%, respectively. By contrast, a subcutaneous dose as high as 13 mg/kg did not decrease blood pressure suggesting that bolus injection of the intravenous doses may be responsible for the hypotensive effect. The sponsor stated that Icatibant produced no serious adverse effects in various cardiovascular, renal or central nervous system safety pharmacology models. However, since Icatibant is a partial B_2 receptor agonist, cardiovascular, renal and central nervous system effects were observed in the above studies. Reduced urine and sodium excretion in dogs intravenously injected with a single dose of 0.3 mg/kg. Bradykinin exerted beneficial cardiac effects, including attenuation of arrhythmias and improvement in cardiodynamics, in various cardiac reperfusion/ischemia models. Icatibant administration usually enhanced the negative cardiac effects and mortality observed in these models (at high enough concentrations, Icatibant was a partial agonist in rats, but not dogs). These and other studies suggest Icatibant and other bradykinin antagonists may pose an increased risk in patients with cardiovascular and renal disease. In addition, numerous CNS effects such as decreased motor activity and reduced respiration were observed in mice intravenously injected 1-10 mg/kg Icatibant.

Most of the nonclinical pharmacokinetic studies were performed using radiolabeled Icatibant that allowed for the detection of radioactivity, but not the measurement of functional protein. Icatibant is rapidly absorbed with 100% bioavailability after subcutaneous administration. Bioavailability for Icatibant is low for the inhaled (2-3%) and the intranasal (1%) routes. The distribution of Icatibant appears to be biphasic in rats and dogs and triphasic in humans with the majority of drug removed in the first phase and a half-life of approximately 1-4 hours in all species (compared to a half-life of seconds for bradykinin). Protein binding was low in all species examined: 49% in rat, 44% in man, and 38% in monkey and dog. According to the sponsor (studies not yet submitted to the FDA) low levels of radioactivity (<1% of administered dose) were detected in the fetuses after intravenous administration of 1 mg/kg labeled Icatibant in both 14-day and 18-day pregnant rats. Concentrations of radioactivity in fetal blood corresponded to blood concentrations in the dams. Radioactivity was detected in the milk of nursing rats with peak levels observed 7 hours after administration of radiolabeled drug. Icatibant is not degraded by the two main bradykinin-cleaving enzymes, angiotensin converting enzyme (ACE) and neutral endopeptidase. Therefore, Icatibant is metabolically more stable than bradykinin. Two major metabolites, M1 and M2, have been identified and both have been observed in all species examined. Excretion was mainly renal, regardless of species and route of administration.

Many of the toxicology studies were performed via intranasal administration. In these intranasal studies, Icatibant blood levels were typically only 1-2% of what would have been obtained if the same dose had been administered intravenously or subcutaneously and animals generally did not receive high enough doses to produce toxicity. Therefore, only intravenous or subcutaneous studies were reviewed.

In initial acute intravenous (bolus) toxicity studies (n = 1-2 animals/sex/group), the highest non-lethal dose was 30.7 mg/kg, the minimum lethal dose was 48.3 mg/kg, and the LD_{50} was between

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48.3 and 76.7 mg/kg for mice. For rats, the highest non- lethal dose was 12.3 mg/kg, the minimum lethal dose was 19.2 mg/kg, and the LD_{50} was between 12.3 and 19.2 mg/kg. In initial acute subcutaneous toxicity studies, the highest non-lethal dose was 614 mg/kg, the minimum lethal dose was 767 mg/kg, and the LD_{50} was between 614 and 767 mg/kg for mice. In rats, no mortality was observed after a single subcutaneous injection of 1227 mg/kg Icatibant. After intravenous dosing, deaths occurred usually within minutes to hours. Rapid respiration, twitching and convulsions were observed prior to death. After subcutaneous injection, injection sites showed bluish discoloration that progressed to necrosis. In a separate study, male rats (n = 10/group) were administered single intravenous doses (infused over a 3-hour period) of 0, 4, 20, and 100 mg/kg Icatibant. Moribundity was observed in 1/10 high dose animals. BUN was significantly increased in mid and high dose groups (7.76 ± 1.31, 11.68 ± 2.54, and 24.75 ± 3.5 for 0, 20 and 100 mg/kg groups, respectively) immediately after infusion. No significant dose-related autopsy findings or microscopic findings were reported for either animals sacrificed immediately or 7 days after infusion. The NOAEL for this study was 20 mg/kg. In an initial acute intravenous (bolus) toxicity study, male dogs (n = 2/group) were administered 0.4, 2 and 10 mg/kg Icatibant. No mortality was observed in this study. However, numerous clinical signs, many suggestive of massive histamine release, were observed in mid and high dose groups. In a separate study, male dogs (n = 3/groups) were administered single intravenous doses (infused over a 5-hour period) of 15, 30, and 60 mg/kg. An increase in serum CPK levels above historical background (153 ± 70 mU/ml) was noted in one 30 mg/kg animal (227 mU/mL baseline, 406 mU/ml 3 hour after infusion) and two 60 mg/kg animals (baseline/3 hour after infusion for each animal = 200/843 and 227/389). The NOAEL in this study was 15 mg/kg.

In a 1-month toxicity study, rats were intravenously administered 0.1, 1 and 10 mg/kg/day Icatibant. Renal toxicity was observed in 10/10 males and 5/10 females with areas of epithelial necrosis, areas of repair and foci of tubular atrophy at the 10 mg/kg/day dose. Irreversible tubular atrophy was present in 5/5 males and 1/5 females after a 28-day recovery period. The NOAEL was 1 mg/kg/day.

In a comparative 1-month toxicity study, male rats were either intravenously (10 mg/kg/day), subcutaneously (10 mg/kg/day), or intranasally (2.5 mg/animal/day) administered Icatibant. Renal tubular atrophy was observed in 7/10 and renal tubular necroses in 4/10 intravenously treated animals. Impairment in sperm maturation was observed in 8/10 animals and oligospermia in the epididymes in 7/10 of the intravenously treated animals. Tubular atrophy (4/10) and tubular necrosis (1/10) were also observed in subcutaneously treated rats. However, no control groups were included in this study. Toxicokinetic analyses on Day showed the exposure ($AUC_{0\text{-}4h}$) to be similar between intravenous (20.33 μg·h/mL) and subcutaneous (17.30 μg·h/mL) treated animals (unable to compare to human subcutaneous exposure results, human results reported as $AUC_{0\text{-}\infty}$). C_{max} was higher in the intravenous treated animals (24.09 μg/mL) compared to subcutaneous treated animals ((12.77 μg/mL). No adverse effects were reported in the intranasal group.

In a 1-month toxicity study, dogs were intravenously administered 0.1, 0.25 and 0.8 mg/kg/day. All high dose males (3/3) had swelling of flews that was attributed to histamine release. The major part of glandular portions in the basal mucosa were missing from the stomach of one high dose male that was reported as a relatively rare change. The NOAEL was 0.25 mg/kg/day.

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Some repeat-dose studies in the rat had shown an apparent effect on male reproductive organs. Due to low bioavailability, no toxicity was observed in most intranasal toxicity studies. However, reduced testicular weight, spermiogenesis in the testes, and spermiogenesis were observed in a single 1-month intranasal toxicity study. In the comparative intravenous/subcutaneous/intranasal study, impairment in sperm maturation (7/10) and oligospermia in the epididymes (8/10) were common in intravenous treated animals. However, despite similar exposure levels, oligosperma in the epididymes in 1/10 animals was the only male reproduction effect observed in subcutaneously treated animals. Dose-related effects on male reproduction were not observed in any other toxicity study.

Icatibant was non-mutagenic in the Ames test, mouse micronucleus test and the *in vivo* chromosome aberration test in Chinese hamster bone marrow cells. Segment I reproductive toxicity studies were performed on male mice only. No adverse effects on fertility were observed at subcutaneous doses up to 80.8 mg/kg/day or intravenous doses up to 30 mg/kg/day. In Segment II studies, no teratogenic effects or maternal toxicity were observed at subcutaneous doses up to 25 mg/kg/day (high dose) in pregnant rats. Intrauterine embryofetal death rate was increased in rabbits subcutaneously treated with 10 mg/kg/day (high dose). No teratogenic effects were observed at doses up to 10 mg/kg/day in the rabbit. Segment III studies have not been performed. Icatibant is classified as a non-irritant to the skin or eye. Icatibant was found to be mildly antigenic when administered in conjunction with adjuvant and has been classified as a sensitizer.

In addition to the above nonclinical data, the sponsor stated their intention to perform the following studies: 1) 1-month repeat-dose intravenous toxicity study in dog; 2) reproductive toxicity studies in rats; and 3) respiratory function in accordance with ICH guideline S7A.

The highest proposed dose for the submitted protocol is 0.6 mg/kg (assuming a 50 kg body weight). The lowest reported NOAELs for subchronic (1-month) intravenous toxicity studies were 1 mg/kg/day (1.7-fold safety factor) for a 1-month rat study and 0.25 mg/kg/day (no safety factor) in a 1-month dog study. The subcutaneous route was evaluated only in a one 1-month comparative study (intavenous/subcutaneous/intranasal) performed in rat and renal toxicity was observed at the only dose (10 mg/kg/day) utilized. In a single-dose rat toxicity study where Icatibant was intravenously infused over a 3-hour period, the NOAEL was 20 mg/kg (33-fold safety factor). In a single-dose dog toxicity study where Icatibant was intravenously infused over a 5-hour period, the NOAEL was 15 mg/kg (25-fold safety factor). In healthy volunteers, the maximum tolerated single dose over a 1-hour intravenous infusion period was 1.6 mg/kg (erythema and/or pruritis was seen in all 4 subjects, and hypotension was observed in 1 subject, at the 3.3 mg/kg/hour dose). Therefore, a single subcutaneous dose of 0.6 mg/kg is safe to proceed with the clinical study.

Recommendations:

1. From a preclinical standpoint, the proposed clinical study is safe to proceed.

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Ronald Honchel, Ph.D. Date
Pharmacologist, HFD-180

Comment:

Jasti B. Choudary, B.V.Sc., Ph.D. Date
Supervisory Pharmacologist, HFD-180

cc:
IND
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Honchel
R/D Init. J. Choudary 2/11/05

APPENDIX/ATTACHMENTS

None.

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12.3 Appendix 3: Pharmacology and Toxicology Review IND 68,214 (Serial No. 52-53), May 12, 2008

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

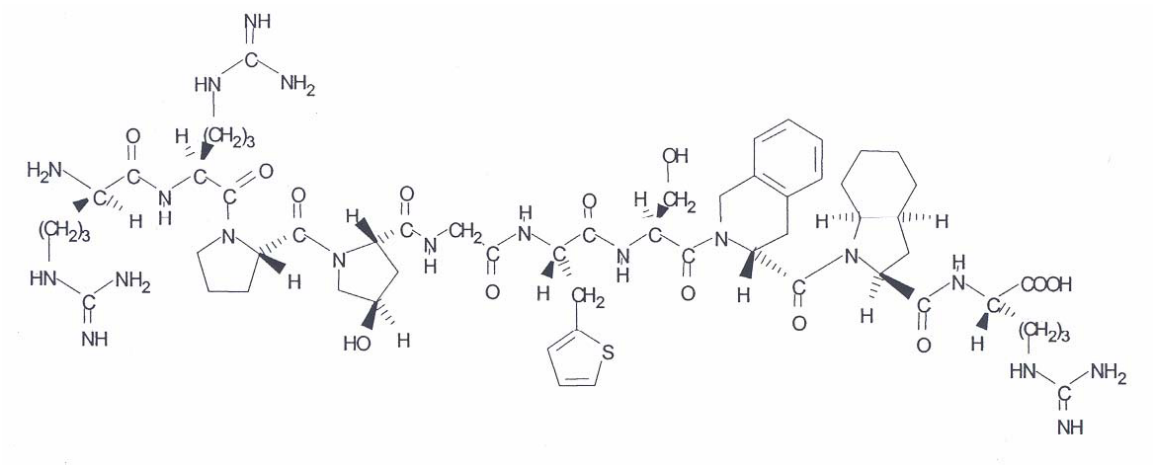
2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 68,214
Review number: 3
Sequence number/date/type of submission: SDN 52/April 30, 2008/IT-SPA (mouse)
SDN 53/May 7, 2008/IT-SPA (rat)
Information to sponsor: Yes () No (X)
Sponsor and/or agent: Jerini AG, Berlin Germany
Manufacturer for drug substance: (b) (4)

Reviewer name: Molly E. Shea, Ph.D.
Division name: Division of Pulmonary and Allergy Products (DPAP)
Review completion date: June 12, 2008

Drug:

Trade name: Firazyr
Generic name: Icatibant acetate
Code name: JE 049/HOE 140
Chemical name: D-arginyl-L-arginyl-L-prolyl-L[(4R)-4-hydroxyprolyl]-glycyl-L-[3-(2-thienyl)alanyl]-L-seryl-D-(1,2,3,4-tetrahydroisoquinolin-3-yl-carbonyl)-L-3aS,-7aS)-octahydroindol-2-yl-carbonyl]-L-arginine acetate
CAS registry number: NA
Amino acid sequence: H-D-Arg-L-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-OH· nCH_3COOH
Molecular formula/molecular weight: $C_{59}H_{89}N_{19}O_{13} \cdot C_2H_4O_2$/1304.6
Structure:



Relevant INDs/NDAs/DMFs: IND (b) (4) NDA 22-150 (icatibant subcutaneous injection for hereditary angioedema)

Drug class: Bradykinin type 2 (B_2) receptor antagonist

Intended clinical population: Patients with acute hereditary angioedema (HAE)

Clinical formulation: Icatibant will be delivered as a 10 mg/mL sterile, isotonic, acetate-buffered solution.

Route of administration: Subcutaneous injection (SC)

Proposed clinical protocol: None in this submission.

Previous clinical experience: A total of 756 subjects have been exposed to one or more doses of icatibant by intranasal, inhalation, intra-articular, intravenous or subcutaneous routes of administration during icatibant's development. The following table provides a summary of the types of studies explored with icatibant and the doses administered.

Study	Dosage	No of Subjects Exposed
11 intranasal allergic rhinitis studies	0.01 to 15 mg/day	201
4 inhalation studies for chronic bronchitis	0.1 to 12 mg/day	205
2 intra-articular studies for osteoarthritis of the knee	90 to 2000 µg/kg	234
7 single-dose IV studies	0.0005 to 4.5 mg/kg	97
4 PK IV studies in various populations (elderly, renally and hepatically impaired)	0.05 mg/kg to 30 mg/day	87
3 Phase 1 SC studies for HAE	0.05 mg/kg to 45 mg/day	58
Phase 2a for HAE	0.4 and 0.8 mg/kg, IV and 30 and 45 mg/day, SC	12, IV 8, SC
FAST-2/Phase 3	Active control or 30 mg, SC	36 + 3 open label

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

Studies reviewed within this submission:

JE049-0159: 13-week subcutaneous administration toxicity study in the mouse (audited draft report)

JE049-0160: 13-week subcutaneous administration toxicity study in the rat (audited draft report)

Studies not reviewed within this submission: None.

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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

In Type I (quantitative deficiency) and Type II (qualitative deficiency) hereditary angioedema (HAE) a deficiency of C1 esterase inhibitor (C1-INH) results in an increased release of bradykinin (BK). BK binds to the BK type-2 receptor (B_2) to trigger activation of many intracellular processes. Included in these processes are biochemical cascades, two of which are coupled by the $G_{\alpha q/11}$ and $G_{\alpha i}$ proteins, leading to calcium mobilization and MAPK activation, respectively. Calcium mobilization and MAPK activation induces many cellular events including cellular proliferation, excitation of neurons, vasodilatation, increased vascular permeability and smooth muscle cell contraction. These events are believed to contribute to the increased vascular permeability and acute inflammation observed in HAE patients.

Icatibant is a synthetic decapeptide with similar structure as endogenous BK. It is a potent, selective B_2 receptor antagonist that binds to B_2 with similar affinity as BK. Icatibant binding at the B_2 receptor inhibits BK induced processes and is hypothesized to contribute to the decrease of vascular permeability and acute inflammation observed in HAE patients. *In vitro* studies have shown that icatibant binds to both B_1 and B_2 receptors (K_i values of 2 nM and 1.2 µM, respectively) and inhibits BK with IC_{50} of 6 µM and 4.3 nM, respectively. These data show that icatibant is selective for the B_2 receptor subtype over the B_1 receptor. There are two major metabolites of icatibant, M1 (b) (4) and M2 (b) (4) These metabolites M1 and M2 also bind to B_2 receptors but with less inhibition of BK than the parent drug and M2 having greater inhibition of BK than the M1 metabolite.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Reference is made to the nonclinical review of IND (b) (4) (reviewed by Dr. Choi) and to the New Drug Application (NDA 22-150; date of review March 18, 2008) for a detailed description of the absorption, distribution, metabolism and elimination (ADME) studies conducted in the mouse and rat. These studies were limited for the mouse. Icatibant is metabolized to the active metabolites M1 (b) (4) and M2 (b) (4) by hydrolysis of icatibant's peptide backbone, thought to be induced by peptidases. No i*n vitro* serum protein data are available for the mouse. However, protein binding was observed as 38-49% in rat, dog, monkey and human. The major metabolite in man is M1 (>90%). M1 is formed in the mouse (~50%).

Like mice, rats metabolized icatibant to the active metabolites M1 (b) (4) and M2 (b) (4) by hydrolysis of icatibant's peptide backbone, thought to be induced by peptidases. M1 is formed in the rat (~70%). M2 is formed in rat (~9% of observed parent levels) and human (~75%). After SC administration in rats, distribution studies showed that icatibant (or its metabolites) is distributed into the kidneys, liver, urinary bladder, lungs and spleen 1 hour post-dose and is still detectable in the liver, kidneys, spleen, bone marrow, adrenals and injection sites at 24-hours post-dose. After repeat SC dosing in rats, icatibant is rapidly distributed and

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metabolized with the M2 metabolite [redacted] (b) (4) appearing from 0.2 to 3 hours post-dose in rats. Neither icatibant nor M2 accumulated after repeat dosing in males and females up to 13-weeks dosing as was observed in the NDA. The biological half-life was more than 5 hours after 26 µg/kg SC in rats and terminal half-life was over 6 days in rats after 10 µg/kg, SC. There were no gender differences observed in rats in the systemic exposures of males and females. *In vitro* serum protein binding of icatibant was low with 44 and 49% in man and rat, respectively. Icatibant is excreted in the milk of rats after IV administration. Neither icatibant nor the M1 or M2 metabolites is metabolized by human hepatocytes or microsomes. Additionally, icatibant is not an inducer of CYP450 isozymes. Based on excretion studies, the primary route of elimination after SC administration of icatibant is via the kidneys in mice (~97.1%) and rats (~61%) with small amounts excreted in the feces (8.0% to 33%).

Data extrapolated from the NDA (22-150) for icatibant provided potential systemic exposure comparisons. The proposed clinical dose of 30 mg icatibant SC (0.6 mg/kg for 50 kg human) is speculated to achieve an approximate systemic exposure of 4107 ng*h/mL and 3023 ng*h/mL for icatibant and the M2 metabolite, respectively. At the maximum recommended dose of 90 mg/day, the respective exposures project to 12.3 µg*h/ml and 9.1 µg*h/mL.

2.6.6 TOXICOLOGY

2.6.6.3 Repeat-dose toxicity

Study title: JE049-0159- Icatibant: 13-week subcutaneous administration toxicity study in the mouse (audited draft report)

The sponsor selected the dose levels for this 13-week mouse study based on previous repeat dose studies in Sprague-Dawley rats via the subcutaneous route. The observed effects in rats on sexual organs, resulting from blockade of the known role of bradykinin in regulation of pituitary secretion, was the most important dose limiting factor taken into consideration by the sponsor for this mouse study. At the dose levels selected, the clinical signs were expected to be limited to local injection site reactions and no adverse effects on body weight or food consumption.

Key study findings:

- MTD was determined to be 50 mg/kg/day for male and female mice.
- The 100 mg/kg/day dose exceeded the MTD with extreme injection site reaction considered the dose limiting toxicity.
- Clinical signs of toxicity included lesions and sores at the site injection and thinning of fur (males and females) and transient aggression (males only).
- Potential target organs of toxicity were injection sites (males and females), adrenal glands (males and females), female reproductive organs (vagina, uterus, and cervix), female mammary gland, thymus (males and females), skeletal muscle (males and females) and stomach (males only).
- No NOAEL could be determined in this study due to the absence of examination of the 10 and 25 mg/kg/day groups for the target organs of toxicity.

Study no.: JE049-0159

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Volume #, and page #: Volume 1 and page 34
Conducting laboratory and location: (b) (4)

Date of study initiation: July 26, 2007
GLP compliance: Yes- The study was conducted in compliance with GLP regulations but the report is an unsigned draft report.
QA report: yes () no (X)
Drug, lot #, and % purity: Icatibant acetate, batch no (b) (4) E 086 and 83.5% pure (content of free base)

Methods

Doses: 0 (sterile saline), 10, 25 and 50 mg/kg/day for weeks 1 to 8 and then added an additional group, 100 mg/kg/day from Weeks 9 to 13

Species/strain: Crl:CD1 (ICR) Mice

Number/sex/group or time point (main study): Weeks 1 to 8: 10 mice/sex/group for controls, 10, 25 or 50 mg/kg/day; Weeks 9 to 13: 10 mice/sex/group for the controls, 25 and 50 mg/kg/day, 5 mice/sex/group in the 10 mg/kg/day group and 10 males and 8 females in the 100 mg/kg/day group.

Route, formulation, volume, and infusion rate: Subcutaneous, icatibant in sterile 0.9% physiological saline, administered as 1.0 mL/kg from week 1 to week 8 and then in 2 mL/kg for the HD group (100 mg/kg) from week 9 to week 13 as a bolus injection. Three injection sites were used with daily rotation: injection site 1 at the scruff, injection site 2 at the left hind quarter and injection site 3 at the right hind quarter. Due to sores at injection site 1 in animal no. 33 (50 mg/kg/day) the animals were dosed at injection site 2 on Day 25.

Satellite groups used for toxicokinetics or recovery: For controls, 10, 25 and 50 mg/kg/day 6, 18, 18 and 18 mice/sex/group were allocated to the TK group from Week 1 to Week 8. From Week 9 to Week 13 6, 18, 18 and 18 mice/sex/group were allocated to controls, 25, 50 and 100 mg/kg/day with all animals from the 10 mg/kg/day group being transferred to the 100 mg/kg/day group.

Age: 24 to 27 days of age on arrival

Weight: Males: 31.3 to 43.2 g; Females: 23.5 to 31.8 g

Sampling times: See below

Unique study design or methodology (if any): During the first 8 weeks of dosing, 10 mice/sex/group were administered 0 (control), 10, 25 or 50 mg/kg/day of Icatibant via SC. The sponsor stated that there were few signs of toxicity observed in the mice. Therefore, as of Week 9 to the end of study (Week 13), the low dose animals (originally dosed with 10 mg/kg/day) were split to have 5 mice/sex/group to receive 10 mg/kg/day and the remaining 5 mice/sex/group to be administered 100 mg/kg/day to serve as a new high-dose group. Additional spare mice (5 males and 3 females) that were not dosed previously were included in this 100 mg/kg/day group for final HD animal numbers equal to 10 males and 8 females.

Observation, Times and Results:

Mortality: One icatibant treated male (50 mg/kg/day) was killed on Day 29 (animal no. 33) due to being found in a moribund state. This animal had skin lesions including a serious ear ulceration with superficial pustular dermatitis. A control male mouse (animal no. 1) was found dead on Day 53.

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Three TK mice, 1 female (no. 137) from the 50 mg/kg/day, 1 male (no. 90) and 1 female (no. 163) from the 100 mg/kg/day group died prior to the end of study. Animal nos. 137 and 90 showed no lesions to associate with a cause of death. Animal no. 163 had a moderately opaque eye and showed clinical signs of semi-closed eye prior to death. Due to the absence of histopathological examination of the TK animals, it is unclear that these deaths of related to icatibant treatment.

Clinical signs: All animals were observed daily for signs of ill health or overt toxicity. Each animal was given a detailed physical examination at weekly intervals up to the end of Week 5 and then twice weekly thereafter.

Sores and lesions were recorded in all animals which were related to injection sites. Mice administered icatibant at 50 and 100 mg/kg/day doses had an increased frequency of sores and lesions. In the 10 and 25 mg/kg/day group, males and females showed a low incidence of injection site lesions compared to the vehicle control group animals. A noticeable increase in incidence and duration of sores and/or lesions at the injection sites was observed in males at the 50 mg/kg/day group throughout the dosing phase and in both males and females treated with the 100 mg/kg/day group from weeks 9-13. Females in the 25 and 50 mg/kg/day groups showed a resolution of injection site irritation after 4 weeks of treatment which reappeared at week 11 through the end of study. Thinning fur was in males and females with increased frequency in the icatibant treated animals. Transient signs of aggression were observed in males alone in the 25 and 50 mg/kg/day groups.

Clinical Sign	**Sex**	**Icatibant Dose (mg/kg/day)**				
		0 (VC)	**10[B]**	**25**	**50**	**100[A]**
Sores/ lesion on neck or back	M	3/10 (wks 1-4) 2/10 (wks 5-8)	2/10 (wks 3-4) 1/10 (wks 5-8)	2/10 (wks 1-4) 1/10 (wks 5-8)	5/10 (wks 1-4) 4/10 (wks 5-8) 1/10 (wks 9-14)	5/10 (wks 9-12) 4/10 (wks 13-14)
	F	6/10 (wks 1-4)	2/10 (wks 1-4)	2/10 (wks 1-4) 2/10 (wks 11-14)	3/10 (wks 1-4) 4/10 (wks 11-14)	6/8 (wks 9-12) 4/8 (wks 13-14)
Thinning fur	M	2/10 (wks 5-8)	-	1/10 (wks-6-14)	3/10 (wks 5-8) 3/10 (wks 11-12) 5/10 (wks 13-14)	1/10 (wks 11-14)
	F	1/10 (wks 9-12)	-	1/10 (wks 9-12)	-	2/8 (wks 9-12) 3/8 (wks 13-14)
Aggressive	M	-	-	1/10 (wk 5)	2/10 (wks 5-8)	-
	F	-	-	-	-	-

A- The 100 mg/kg/day group was dosed from Weeks 9-13 only. The male group had an n=10 mice and the females group had an n= 8 mice.

B- Males and females in the 10 mg/kg/day group had an n=10/sex/group from weeks 1-8 and then n=5/sex/group from weeks 9-13 after reallocation of animals to make the 100 mg/kg/day HD group.

- No finding reported.

Body weights: Individual body weights were recorded on day -7, before treatment on the first day of dosing and twice weekly throughout the dosing phase and before necropsy.

There were no statistically significant differences in group mean body weights or group mean body weight gains in males and females treated with icatibant compared to their respective control groups.

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Food consumption: The amount of food consumed by each cage of main study animals was determined weekly. Consumption was calculated as g/animal/week. Food consumption was not recorded for animals allocated to the TK group.

There were no statistically significant differences in food consumption in males or females treated with icatibant compared to animals treated with controls. However, there was a general increase in food consumption (+12 to 16%) in males starting Week 2 of dosing through Week 5 in the 25 and 50 mg/kg/day groups compared to the control group. After Week 5 control animal food consumption was similar to icatibant treated males.

Ophthalmoscopy: Ophthalmic exam was not conducted in this study.

EKG: EKGs were not recorded in this study.

Hematology: Blood samples were taken on Week 13 for animals dosed with control, 10, 25 or 50 mg/kg/day and on Week 14 for animals dosed with 100 mg/kg/day. Blood samples were taken from the orbital sinus puncture under isoflurane anesthesia from non-fasted animals. A complete hematological battery was evaluated from blood samples.

Slight but statistically significant increases in platelet counts in icatibant treated males were observed at the end of the dosing period. MPV showed slight but statistically significant decreases in males and females treated with 100 mg/kg/day of icatibant. PDW was decreased in 100 mg/kg/day females alone and lymphocytes were slightly decreased in 100 mg/kg/day males alone. Neutrophil levels as percent of WBC were increased in males and females in the 100 mg/kg/day group compared to the vehicle control animals.

%Δ Hematological Parameters vs Controls

Parameter	Sex	Icatibant Dose (mg/kg/day)			
		10	25	50	100
Plat (10^9/L)	M	-	+23%	+21%	+33%
	F	-	-	-	-
MPV (fl)	M	-	-	-	-15%
	F	-	-	-	-14%
PDW (%)	M	-	-	-	-
	F	-	-	-	-12%
L (10^9/L)	M	-	-	-	-46%
	F	-	-	-	-
N (% WBC)	M	-	-	-	+69%
	F	-	-	-	+50%

- Not statistically significant

Clinical chemistry: Blood samples were taken on Week 13 for animals dosed with control, 10, 25 or 50 mg/kg/day and on Week 14 for animals dosed with 100 mg/kg/day. Blood samples were taken from the orbital sinus puncture under isoflurane anesthesia from non-fasted animals. A complete clinical chemistry battery was evaluated from blood samples.

There were no statistically significant or biologically relevant changes in serum clinical chemistry parameters in males or females treated with icatibant compared to their respective controls.

Urinalysis: Urinalysis was not assessed in this study.

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Gross pathology: All main study animals were subjected to necropsy. Each main study animal was given an IP injection of sodium pentobarbitone and then was exsanguinated. A full macroscopic examination was performed under the general supervision of a pathologist and all lesions were recorded.

Macroscopic examination showed eye opacity in one 50 mg/kg/day female which was moribund prior to sacrifice. Skin irritation in males and irritation at the injection sites including the scruff, hind left quarter, and hind right quarter was observed in both males and females treated with icatibant at doses of 25 mg/kg and above. Large liver and kidneys were observed in males and females at doses of ≥25 mg/kg icatibant but this finding did not show a dose relationship. The male reproductive organs (prostate, testes and seminal vesicle) were observed as small at doses of 25 mg/kg and above. The following table summarizes the icatibant related macroscopic findings observed in mice compared to controls.

Macroscopic Observations

Finding	Males					Females				
	0 N=10	**10 N=5**	**25 N=10**	**50 N=10**	**100 N=10**	**0 N=10**	**10 N=5**	**25 N=10**	**50 N=10**	**100 N=8**
Eye opacity	0	0	0	0	0	0	0	0	1	0
Skin + Subcutis, Sore	0	0	0	1	2	0	0	0	0	0
Liver										
Pale	0	0	0	1	1	0	0	1	0	1
Large	0	0	1	0	1	0	0	2	0	1
Kidney, Large	0	0	1	1	0	0	0	1	0	0
Prostate, small	0	0	0	0	1	NA	NA	NA	NA	NA
Testes, small	0	0	0	1	0	NA	NA	NA	NA	NA
Seminal vesicle,						NA	NA	NA	NA	NA
Small	0	0	1	0	0					
Dark	0	0	1	0	0					
Abnormal shaped	0	0	1	0	0					
IJS1 Scruff										
Red	0	0	0	2	1	0	0	0	0	0
Sore	0	0	1	8	9	0	0	1	4	4
Dark	0	0	0	0	0	0	0	0	0	1
Red area	0	0	0	0	2	0	0	0	0	2
IJS2 Hind left										
Red	0	0	0	1	2	0	0	0	0	1
Sore	0	0	0	1	4	0	0	0	0	0
Red area	0	0	0	0	5	0	0	0	0	2
IJS3 Hind right										
Sore	0	0	0	0	4	0	0	0	0	0
Red area	0	0	0	0	2	0	0	0	0	1
Red focus	0	0	0	0	2	0	0	0	0	0

NA- Not applicable
IJS- Injection site numbers 1, 2 or 3.

Organ weights: All animals were weighed before necropsy. Organ weights were taken for the following tissues: brain, heart, kidney, liver, prostate, spleen, testes, epididymides, and thymus. The testes and epididymides were weighed together.

Compared to the vehicle control groups, males and females showed slight increases in absolute kidney weights and kidney weights adjusted for body weights. Prostate weights in the

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100 mg/kg/day group were decreased compared to the VC group when comparing both the absolute organ weights and the organ weight adjusted to body weights. Absolute liver weights and liver weights adjusted for body weights were increased in a dose related manner in females treated with icatibant compared to the VC group.

%Δ Absolute Organ Weights vs Controls

Organ	Sex	Icatibant Dose (mg/kg/day)			
		10	25	50	100
Kidneys	M	-	+13%	+28%	+16%
	F	+4%	+14%	+16%	+10%
Prostate	M	-17%	-	+15%	-36%
	F	NA	NA	NA	NA
Liver	M	-	-	-	-
	F	+7%	+14%	+18%	+25%

- Not statistically significant
NA- Not applicable

%Δ Organ Weights Relative to Body Weights vs Controls

Organ	Sex	Icatibant Dose (mg/kg/day)			
		10	25	50	100
Kidneys	M	-	+6%	+19%	+10%
	F	-	+8%	+12%	+10%
Prostate	M	-19%	-	+11%	-35%
	F	NA	NA	NA	NA
Liver	M	-	-	-	-
	F	-	-	+7%	+22%

- Not statistically significant
NA- Not applicable

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

Gross lesions from all animals and a complete histopathological battery of tissues from the control and 50 and 100 mg/kg/day animals were examined by the study pathologist. The 10 and 25 mg/kg/day groups had the injection sites and thymus macroscopically examined in males and females and the adrenals, uterus, cervix and vagina macroscopically examined in females. No tissues from the 10 and 25 mg/kg/day groups were microscopically examined in males or females.

The following table summarizes incidences and severities of the icatibant related histopathological findings in males and females treated with 0 (VC), 50 and 100 mg/kg/day. Bracketed numbers () are the number of unscheduled deaths per group and the related incidence and severity of any finding in these animals is included in the table.

Microscopic Observations

Finding	Males					Females				
	0	10	25	50	100	0	10	25	50	100
Injection site 1 scruff	N=9 (1)	NE	NE	N=9 (1)	N=10	N=10	NE	NE	N=10	N=8
Focal muscle degeneration Min	0			1	3	0			0	0

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Finding	Males					Females				
	0	10	25	50	100	0	10	25	50	100
Mild	0 (0)			0 (1)	1	0			1	0
Moderate	0			0	1	0			0	1
Dermal hemorrhage										
Minimal	0			0	1	0			0	0
Deep dermal edema										
Moderate	0			0	1	0			0	0
Acanthosis										
Min	0 (0)			1 (1)	2	1			3	1
Mild	0			3	3	0			3	4
Moderate	0			1	3	0			0	1
Superficial pustular dermatitis										
Mild	0			3	2	0			2	4
Moderate	0			0	3	0			0	1
Marked	0			0	1	0			0	0
Ulceration										
Min	0			0	1	0			2	0
Mild	0			1	3	0			0	2
Moderate	0			0	1	0			0	1
Dermal acute inflammation										
Min	0			0	1	0			1	0
Mild	0			1	1	0			0	0
Dermals subacute inflammation										
Min	0			1	0	2			2	2
Mild	0			1	2	0			0	0
Moderate	0			0	1	0			0	0
Focal fat necrosis										
Min	0			1	0	0			1	0
Moderate	0			0	0	0			0	1
Injection site 2 L Hind Qtr	N=9 (1)	NE	NE	N=9	N=10 (1)	N=10	NE	NE	N=10	N=8
Dermal hemorrhage										
Min	0			0	0	0			0	1
Mild	1			0	3	0			0	0
Moderate	0			0	1	0			0	0
Focal muscle degeneration										
Min	0			4	2	0			0	2
Mild	0			0	2	0			3	0
Epidermal necrosis										
Min	0			0	2	0			0	0
Mild	0			0	1	0			0	0

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Finding	Males					Females				
	0	10	25	50	100	0	10	25	50	100
Moderate	0			0	1	0			0	0
Dermal necrosis										
Min	0			0	1	0			0	0
Mild	0			0	1	0			0	0
Deep dermal edema										
Mild	0			0	2	0			0	5
Acanthosis										
Min	0 (0)			2	1 (1)	0			1	2
Mild	0			1	1	0			0	0
Moderate	0			0	1	0			0	0
Marked	0			0	1	0			0	0
Superficial pustular dermatitis										
Mild	0			0	1	0			0	1
Moderate	0			0	1	0			0	0
Ulceration										
Min	0			0	0	0			1	0
Mild	0			0	2	0			0	2
Moderate	0			0	1	0			0	0
Marked	0			0	1	0			0	0
Dermal acute inflammation										
Min	1			0	0	0			0	0
Mild	0			0	5	0			0	0
Moderate	0			0	1	0			0	0
Dermal subacute inflammation										
Min	0			3	2	4			5	5
Mild	0			0	0	1			1	2
Focal fat necrosis										
Min	0			0	0	1			0	1
Degenerative arteritis										
mild	0 (0)			0 (1)	0	0			0	0
Injection site 3 R Hind Qtr	N=9 (1)	NE	NE	N=9 (1)	N=10	N=10	NE	NE	N=10	N=8
Focal muscle degeneration										
Min	0			0	2	0			4	1
Mild	0			2	1	0			4	3
Dermal hemorrhage										
Min	0			0	1	0			0	0
Mild	0			9	1	0			0	0
Deep dermal edema										

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Finding	Males					Females				
	0	10	25	50	100	0	10	25	50	100
Min	0			0	1	1			0	1
mild	0			0	0	0			0	1
Acanthosis										
Min	0			4	4	1			2	2
Mild	0			0	2	0			0	2
Moderate	0			0	4	0			0	0
Superficial pustular dermatitis										
Mild	0			1	3	0			0	2
Moderate	0			0	1	0			0	0
Ulceration										
Mild	0			0	1	0			0	0
Dermal acute inflammation										
Min	1			0	1	0			0	0
Mild	0			0	1	0			0	0
Dermal subacute inflammation										
Min	1			0	0	2			3	1
Mild	0			0	0	0			2	0
Adrenal	N=9 (1)	NE	NE	N=9 (1)	N=10	N=10	NE	NE	N=10	N=8
Zona fasciculatea hypertrophy										
Min	0 (0)			0 (0)	0	2			4	4
Mild	0 (0)			0 (0)	0	0			3	0
Mammary gland	N=9 (1)	NE	NE	N=9 (1)	N=10	N=10	NE	NE	N=10	N=8
Acinar development										
Min	0			0	0	2			3	1
Mild	0			0	0	0			1	1
Ductular dilatation										
Min	0			0	0	2			0	3
Periarteritis										
mild	0			0	0	0			1	0
Thymus	N=9 (1)	NE	NE	N=9 (1)	N=10	N=10	NE	NE	N=10	N=8
Atrophy										
Min	1 (1)			5	5	4			6	4
Mild	1			0 (1)	1	2			1	3
Moderate	0			0	1	0			0	1
Medullary lymphocytic hyperplasia										
Min	0			0	1	1			1	0
moderate	0			0	0	0			1	0

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Finding	Males					Females				
	0	10	25	50	100	0	10	25	50	100
Uterus	NA	NA	NA	NA	NA	N=10	NE	NE	N=10	N=8
Diminution in myometrial bulk										
min						1			6	8
Cervix	NA	NA	NA	NA	NA	N=10	NE	NE	N=10	N=8
Degenerating deep squamous epithelium, present						1			3	2
Squamous epithelium, present						0			1	0
Mucinous epithelium, present						1			4	6
Vagina	NA	NA	NA	NA	NA	N=10	NE	NE	N=9	N=8
Mucinous epithelium, present						1			6	3
Degenerating squamous epithelium, present						2			2	5
Eye	N=9	NE	NE	N=9	N=10	N=10	NE	NE	N=10	N=8
Loss of photoreceptor layer,										
mild	0			0	0	0			1	0
marked	0			0	0	0			0	1
Loss of outer nuclear layer,										
mild	0			0	0	0			1	0
moderate	0			0	0	0			0	1
Focal choroidal chronic inflam, mild	0			0	0	0			0	1
Cataract, mild	0			0	0	0			1	0
Loss of inner nuclear layer, marked	0			0	0	0			1	0
Skeletal muscle	N=9	NE	NE	N=9	N=10	N=10	NE	NE	N=10	N=8
Steatosis										
Min	0			0	0	0			0	0
Mild	0			0	1	0			1	0
Peripheral muscle degeneration, mild	0			1	1	0			0	0
Stomach	N=9	NE	NE	N=9	N=10	N=10	NE	NE	N=10	N=8
Glandular hyperplasia										
Min	0			1	0	0			0	0
mild	0			1	0	0			0	0

NE- Not examined
NA- Not applicable

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The target organs of toxicity in the mouse included injection sites (males and females), adrenal glands (males and females), female reproductive organs (vagina, uterus, and cervix), female mammary gland, thymus (males and females), skeletal muscle (males and females) and stomach (males only). The dose-limiting toxicity in this study was injection site reaction observed with increased incidence and severity compared to the vehicle control group animals at 100 mg/kg/day in males and females. Hemorrhage, ulceration and necrosis were observed at multiple injection sites in males and females with significant increases in incidence and severity at the 100 mg/kg/day dose. Additionally, this dose was administered to males and females for a duration of 4 weeks (Dosing Weeks 9-13). This short duration of dosing compared to the full 13-week dosing with the 50 mg/kg/day indicates that this toxicity is observed rapidly at the 100 mg/kg/day dose and may not allow for repeat SC administration for a duration > 4 weeks in mice. At 50 mg/kg/day doses, males and females showed an increased incidence of injection site hemorrhage, muscle degeneration and ulceration compared to their respective vehicle control groups. Although the incidence of ulceration at 50 mg/kg/day was lower than the 100 mg/kg/day group in males and females, chronic administration at 50 mg/kg/day may potentiate the ulceration at the injection sites limiting the dosing duration.

No NOAEL could be determined for this study as neither the 10 not the 25 mg/kg/day groups were evaluated in this study. However, the reviewer considers the MTD was exceeded with the 100 mg/kg/day based on the injection site toxicity. The MTD is considered 50 mg/kg/day based on tolerable levels of injection site reaction.

Toxicokinetics: Blood samples for TK analyses (0.2 mL, Day 1; 0.3 mL, Week 13/14) were taken at 0.5, 1, 2, 4, 8 and 24 hours post-dose. Samples were taken from the orbital sinus under an isofluorane anesthesia. Plasma samples were prepared and analyzed for levels of icatibant and metabolite M2 using LC-MS/MS after solid phase extraction. The lower limit of quantitation was 25 ng/mL for icatibant and 2.5 ng/mL for M2 metabolite, respectively.

Week	Parameter	Icatibant SC Dose (mg/kg/day)							
		10		25		50		100	
		M	F	M	F	M	F	M	F
1	C_{max} (ng/mL)	9640	14000	29600	24800	48900	42900	-	-
	t_{max} (h)	0.5	0.5	0.5	0.5	0.5	1.0	-	-
	AUC_{0-t} (ng*h/mL)	10100	13100	49700	34900	94500	86200	-	-
13 [A]	C_{max} (ng/mL)	-	-	32200	23400	50500	43500	47100	54600
	t_{max} (h)	-	-	0.5	0.5	0.5	1.0	1.0	0.5
	AUC_{0-t} (ng*h/mL)	-	-	41500	38500	61300	68400	101000	99900
M2 metabolite									
1	C_{max} (ng/mL)	620	710	2240	1840	3680	3440	-	-
	t_{max} (h)	0.5	1.0	1.0	1.0	2.0	1.0	-	-
	AUC_{0-t} (ng*h/mL)	1630	1420	4580	4120	8630	8510	-	-
	$AUC_{0-infin}$ (ng*h/mL)	-	1460	4690	4150	8690	8610	-	-
	$T_{1/2}$ (h)	-	7.89	8.73	4.42	4.13	4.94	-	-
13 [A]	C_{max} (ng/mL)	-	-	1280	1190	1130	3220	3300	3970
	t_{max} (h)	-	-	1.0	1.0	0.5	1.0	2.0	2.0
	AUC_{0-t} (ng*h/mL)	-	-	3570	3770	1780	8470	7860	9780
	$AUC_{0-infin}$ (ng*h/mL)	-	-	3600	3840	-	8610	8160	9990
	$T_{1/2}$ (h)	-	-	3.52	5.52	-	5.48	6.97	5.97

- Data not available at this time point
[A] Samples taken in Week 5 of dosing for animals dosed at 100 mg/kg/day

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The parent drug, icatibant, has C_{max} values that were comparable in males and females at all doses. There was no accumulation of drug after 13 weeks repeat dosing in either males or females as observed by similar AUC values at Week 1 as Week 13. At week 1, systemic exposure increased in a slightly less than dose proportionate manner from 10 mg/kg/day to 25 mg/kg/day but roughly proportional from 25 mg/kg/day to 50 mg/kg/day in males and females. At Week 13, systemic exposure increased in an approximate dose proportionate manner from 25 mg/kg/day to 50 mg/kg/day. From 50 mg/kg/day to 100 mg/kg/day, the systemic exposure was less than dose proportionate (increased by ~1.5 fold instead of the expected 2.0-fold). Icatibant plasma concentrations were ~13 to 20-fold greater than those of M2.

The M2 metabolite C_{max} increased with increased dose at Week 1. At Week 13, the C_{max} increased from 25 mg/kg/day to 50 mg/kg/day in females but did not show an increase in males. From 50 mg/kg/day to 100 mg/kg/day no increase of C_{max} was observed, suggesting a saturation of the receptor cleaving the parent compound at this dose. T_{max} of M2 was reached in males and females from 0.5 to 2.0 h post SC administration of the parent drug. The systemic exposure of M2 increased in a dose proportionate manner at Week 1 for both males and females. However, at Week 13 systemic exposure levels increased from 25 mg/kg/day to 50 mg/kg/day in an ~ dose proportionate manner (males could not be determined due to insufficient data points) but from 50 mg/kg/day to 100 mg/kg/day systemic exposure of M2 did not increase. These data suggest that saturation of the receptor (peptidase) to the parent drug resulting in similar exposure levels of M2 between these dosage groups.

Study title: JE049-0160-Icatibant: 13-week subcutaneous administration toxicity study in the rat (Audited draft report)

The sponsor selected the doses for this study based on previous repeat dose studies conducted in Sprague-Dawley rats via the subcutaneous routes. The observed effects on the sexual organs, resulting from blockade of the known role of bradykinin in regulation of pituitary hormone secretion, were the most important dose limiting factor taken into consideration by the sponsor. At the dose levels selected (10, 20, and 30 mg/kg/day), based on previous studies conducted by the sponsor, clinical signs were expected to be limited to local injection site reactions (if any) and no adverse effects on body weight or food consumption.

Key study findings:

- 1 HD male in the TK group died with clinical signs of toxicity including small male reproductive organs. However, this animal was not microscopically examined, therefore, it is not known whether this death is drug related or not.
- Clinical signs of toxicity included sores/lesions at the injection sites on the head, neck and back, thinning of fur and staining of fur in males and females
- Males showed a decrease in BW gains compared to controls, which coincided with decreased food consumption; HD females also show reduced BW gain
- Adrenal wts were increased and thymic, prostate and epididymal wts were decreased
- Potential target organs of toxicity were injection sites (males and females), adrenal glands (males and females), kidneys, liver, thymus, marrow, female reproductive organs (vagina, uterus, and mammary gland), male reproductive organs (epididymides, prostate, seminal vesicles and testes).

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- No NOAEL could be determined for this study as body weight effects, male and female reproductive organ, thymus and adrenal effects are observed at all doses
- The MTD was considered 10 mg/kg/day in females based on reduced body weight gains at the HD (30 mg/kg/day), injection irritation at doses ≥ 20 mg/kg/day and the target organs of toxicity including the female reproductive organs, thymus and adrenals at all icatibant doses.
- The MTD was not determined in males as male body weight gains exceeded 10% in all groups compared to the controls and males were losing weight at week 8-13.

Study no.: JE049-0160
Volume #, and page #: Volume 1 and page 458
Conducting laboratory and location: (b) (4)
Date of study initiation: August 1, 2007
GLP compliance: Yes- The study was conducted in compliance with GLP regulations but the report is an unsigned draft report.
QA report: yes () no (X)
Drug, lot #, and % purity: Icatibant acetate, batch no (b) (4) E 086 and 83.5% pure (content of free base)

Methods

Doses: 0 (vehicle control), 10, 20 and 30 mg/kg/day SC
Species/strain: CrL: WI (Han) rat
Number/sex/group or time point (main study): 10 rats/sex/group
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in 0.9% physiological saline, administered in 1.0 mL/kg initially and then on Day 22 the volume was increased to 5 mL/kg and then decreased to 3 mL/kg on Day 43; bolus injection. Changes in the dose volume were made since the concentration of the high dose (30 mg/ml at 1 ml) was potentially responsible for the rapid appearance of bald patches and scabbed areas. The initial adjustment allowed some healing and the volume was further adjusted to 3 ml/kg to provide a concentration (10 mg/ml) that was consistent with the clinical dose. Test article was administered as daily subcutaneous injections using a rotation of 7 injection sites. Injection site 1-left shoulder, injection site 2- right shoulder, injection site 3 – right mid-side, injection site 4- right hip, injection site 5- rump, injection site 6- left hip and injection site 7- left mid side.
Satellite groups used for toxicokinetics or recovery: Satellite TK animals were allocated to the control group (5 rats/sex) and all icatibant dose groups (10 rats/sex/group)
Age: 9 to 10 weeks old
Weight: Males: 228.3 to 315.6 g, Females: 142.3 to 223.8 g
Sampling times: See below.
Unique study design or methodology (if any): None.

Observation, Times and Results:

Mortality: No animals died prior to scheduled necropsy. One HD (30 mg/kg/day) male (no. 112) from the TK group died during study. This animal was observed with fur loss and slight sore at the injection sites. The testes were moderately small and pale, the epididymides and the seminal

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vesicles were moderately small and all lobes of the lungs were red. No histopathological exam was conducted for this animal. The potential cause of death of this study animal was not provided. Therefore, this death may be drug related.

Clinical signs: All animals were observed daily for signs of ill health or overt toxicity. Each animal was given a detailed physical examination at weekly intervals. From Day 29 detailed clinical observations were performed twice weekly on all main study animals. Post-dosing observations were performed at 1 to 2 h post-dose once daily during Week 1 and once weekly from Week 2 until the end of study.

Clinical signs of toxicity included sores/lesions on the head, neck and back, thinning of fur and staining of fur in males and females treated with icatibant. The sores and lesions and thinning of fur were observed mainly at injection sites in animals in the 30 mg/kg/day group. The sores and lesions healed with increased dose volume/decreased dose concentration on Day 22 from a volume of 1 mL/kg to 5 mL/kg and then decreased to 3 mL/kg on Day 43.

Clinical Sign	Sex	Icatibant Dose (mg/kg/day)			
		0 (VC)	10	20	30
Sores/lesion on head, neck or back	M	-	4/10 (wks 6-9) 4/10 (wks 10-14)	-	1/10 (wk 2) 1/10 (wk 10)
	F	1/10 (wk 2)	3/10 (wks 6-9) 4/10 (wks 10-14)	1/10 (wk 10)	1/10 (wk 2)
Thinning fur	M	1/10 (wk 2)	3/10 (wks 6-9) 8/10 (wks 10-14)	5/10 (wks 6-14)	2/10 (wk 9-14)
	F	1/10 (wks 10-14)	4/10 (wks 6-14)	1/10 (wks 6-9) 2/10 (wks 10-14)	-
staining	M	-	1/10 (wk 14)	-	-
	F	-	1/10 (wk 10)	-	1/10 (wk 5)

- No finding reported.

Body weights: Individual body weights were recorded on day -7, before treatment on the first day of dosing and twice weekly throughout the dosing phase and before necropsy.

Changes in icatibant treated male and female group mean body weights compared to control treated males and females did not reach statistical significance throughout the 13-week treatment period. However, males treated with icatibant showed a statistically significant decrease in group mean body weight gains (34-46%) compared to the control treated males. Between weeks 8 and 13, treated animals lost weight (group mean weight losses of 2.8, 4.4 and 5.1 g) compared to a group mean weight gain of 11.9 g by the controls. HD females treated with icatibant showed a trend of decreased group mean (-26%) body weight gains compared to the control treated females at week 13 and this group showed a mean weight loss of 5.3 grams vs a gain of 1.2 g in controls. The statistically significant changes in icatibant treated males compared to male controls are summarized in the following table.

%Δ Male Group Mean Body Weight Gains vs Controls

Week	Icatibant Dose (mg/kg/day)		
	10	20	30
Start- 4	-	-	-
4-8	-	-	-51%
Start-13	-34%	-40%	-46%

- No statistically significant difference.

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Food consumption: The amount of food consumed by each cage of main study animals was determined weekly. Consumption was calculated as g/animal/week. Food consumption was not recorded for animals allocated to the TK group.

Males treated with icatibant had a statistically significant decrease in food consumption compared to control treated males at Weeks 8, 11, 12 and 13. When assessing group mean food consumption over selected intervals, males showed a statistically significant decrease in food consumption compared to controls when evaluating over the whole treatment interval (weeks 1-13). Females treated with icatibant did not show a statistically significant change in food consumption compared to controls throughout the treatment period. The statistically significant changes in males are summarized in the following table.

%Δ Male Group Mean Food Consumption vs Controls

Week	Icatibant Dose (mg/kg/day)		
	10	**20**	**30**
8	-12%	-11	-12%
11	-11%	-10%	-8%
12	-8%	-8%	-8%
13	-8%	-10%	-10%
Interval weeks 1-13	-	-8%	-6%

- No statistically significant difference.

Ophthalmoscopy: Ophthalmic exam was not conducted in this study.

EKG: EKGs were not recorded in this study.

Hematology: Blood samples (1.0 mL) were taken on Week 13 from the lateral caudal vein after an overnight fast. A complete hematological battery was evaluated from blood samples.

Slight increases in prothrombin time were observed in females treated with icatibant compared to female controls. Fibrinogen levels were slightly increased in HD males and in all icatibant treated females compared to controls. Male and females treated with icatibant showed a decreased in lymphocyte counts and increased neutrophil counts compared control treated animals. These results are summarized in the following table.

%Δ Hematological Parameters vs Controls

Parameter	Sex	Icatibant Dose (mg/kg/day)		
		10	**20**	**30**
PT (s)	M	-	-	-
	F	+11%	+11%	+12%
FC (g/L)	M	-	-	+24%
	F	+20%	+24%	+32%
L (10^9/L)	M	-25%	-25%	-35%
	F	-55%	-48%	-43%
N (% WBC)	M	-	-	+94%
	F	+75%	+94%	+113%
L (% WBC)	M	-	-14%	-23%
	F	-18%	-22%	-24%

- Not statistically significant

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Clinical chemistry: Blood samples (1.2 mL) were taken on Week 13 from the lateral caudal vein after an overnight fast. A complete clinical chemistry battery was evaluated from blood samples.

There were no statistically significant changes in males treated with icatibant compared to males treated with controls. Females treated with icatibant at 10 and 30 mg/kg/day showed slight decreased in total serum protein levels by -9% and -30%, respectively compared to female controls. No additional changes in clinical chemistry parameters were observed in icatibant treated females compared to controls.

Urinalysis: Urinalysis samples were collected from all main study animals in Week 12. Samples were collected over a 6 h day time period. A complete urinalysis was conducted.

Urine volume in females treated with icatibant increased with statistical significance compared to control treated females. Male urine volume was comparable between the icatibant treated rats and control treated rats. Total sodium urine levels were substantially increased in icatibant treated female urine compared to controls. This was not observed in males treated with icatibant. Total potassium, chloride and calcium urine levels were increased in icatibant treated males and females compared to their respective controls. These increases were substantial but did not necessarily follow a dose response in males or females. When adjusting electrolyte levels for urine volume, none of the electrolytes evaluated reached statistical significance in males or females.

%Δ Urinalysis Parameters vs Controls

Parameter	Sex	Icatibant Dose (mg/kg/day)		
		10	20	30
Volume (mL)	M	-	-	-
	F	+222%	+211%	+183%
Total Na (mmol)	M	-	-	-
	F	+96%	+82%	+96%
Total K (mmol)	M	+65%	+72%	+86%
	F	+124%	+106%	+109%
Total Cl (mmol)	M	+100%	+100%	+124%
	F	+183%	+167%	+200%
Total Ca (mmol)	M	+96%	+70%	+126%
	F	+119%	+119%	+109%

- Not statistically significant

Gross pathology: All main study animals were subjected to necropsy. Each main study animal was given an IP injection of sodium pentobarbitone and then was exsanguinated. A full macroscopic examination was performed under the general supervision of a pathologist and all lesions were recorded.

Macroscopic findings were observed in the cecum (females only-injection site lesion), adrenal gland (males and females), male reproductive organs (testes, epididymides, seminal vesicle and prostate), uterus, thymus (males and females) and injection sites. Male reproductive organs, adrenals and thymic observations were made at all doses and increased with incidence with increased icatibant doses. Female reproductive organ findings were observed in the MD and HD animals with similar frequency. The following table summarizes the incidences of the macroscopic findings relate to drug.

Macroscopic Observations

Finding	Males	Females

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	0 N=10	10 N=10	20 N=10	30 N=10	0 N=10	10 N=10	20 N=10	30 N=10
Cecum, injection lesion	0	0	0	0	1	1	2	2
Adrenal Large	0	0	1	2	0	8	8	6
Testis Small Pale area	0 0	3 3	5 4	6 5	NA	NA	NA	NA
Epididymides, small	0	2	1	1	NA	NA	NA	NA
Seminal vesicle Small	0	4	5	8	NA	NA	NA	NA
Prostate, small	0	3	3	7	NA	NA	NA	NA
Uterus, thin	NA	NA	NA	NA	1	0	2	2
Thymus, small	0	2	1	2	0	6	2	5
IJS1 Left shoulder Fur loss Sore	0 0	0 0	8 2	7 6	0 0	0 0	4 3	10 7
IJS 2 Right shoulder Sore Fur loss	0 0	2 0	5 7	6 8	0 0	1 0	5 3	6 10
IJS 3 Right mid-side Sore Fur loss	0 0	1 0	7 5	3 7	0 0	1 0	6 1	7 8
IJS 4 Right hip Sore Fur loss	0 0	1 0	4 8	8 8	0 0	0 1	1 1	6 9
IJS 5 Rump Fur loss Sore	0 0	0 0	6 5	6 2	0 0	1 2	2 2	8 1
IJS 6 Left hip Fur loss sore	0 0	0 0	6 2	8 7	0 0	0 1	1 1	9 3
IJS 7 Left mid-side Fur loss Sore	0 0	0 0	6 7	6 6	0 0	0 2	1 4	9 6

NA- Not applicable
IJS- Injection site 1, 2, 3, 4, 5, 6, or 7

Organ weights: All animals were weighed before necropsy. Organ weights were taken for the following tissues: adrenals, brain, heart, kidney, liver, ovaries, pituitary, prostate, spleen, testes plus epididymides, and thymus.

Males and females treated with icatibant showed statistically significant increases in absolute adrenal weights and adrenals weights adjusted to body weights compared to controls. Absolute thymic weights and thymic weights adjusted for body weights were decreased in both males and females treated with icatibant compared to their respective controls. Males showed a statistically significant decrease in reproductive organ weights after icatibant treatment compared to controls including decreases in prostate and testes plus epididymide absolute weights and weights adjusted for body weights compared to control treated males. These changes in absolute organ weights and organ weights adjusted for body weights compared to controls are summarized the following tables.

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%Δ Absolute Organ Weights vs Controls

Organ	Sex	Icatibant Dose (mg/kg/day)		
		10	20	30
Adrenals	M	+19%	+30%	+35%
	F	+78%	+53%	+60%
Thymus	M	-20%	-22%	-37%
	F	-53%	-45%	-51%
Prostate	M	-41%	-49%	-58%
	F	NA	NA	NA
Testes/epididymides	M	-20%	-27%	-31%
	F	NA	NA	NA

NA- Not applicable

%Δ Organ Weights Relative to Body Weights vs Controls

Organ	Sex	Icatibant Dose (mg/kg/day)		
		10	20	30
Adrenals	M	+31%	+43%	+50%
	F	+88%	+66%	+79%
Thymus	M	-	-	-23%
	F	-50%	-42%	-45%
prostate	M	-42%	-50%	-59%
	F	NA	NA	NA
Testes/epididymides	M	-18%	-25%	-29%
	F	NA	NA	NA

- Not statistically significant
NA- Not applicable

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

Gross lesions from all animals and a complete Histopathological battery of tissues from the control and HD animals were examined by the study pathologist. In addition to the controls and the HD group, the following tissues were examined from the low and intermediate dose animals:

Males and females: adrenal glands, bone marrow, injection sites and thymus
Males: epididymides, prostate, testes and seminal vesicles
Females: mammary gland, ovaries, uterus and vagina.

The following table summarizes the icatibant related toxicity findings with incidence and severity compared to the VC group.

Microscopic Observations

Finding	Males				Females			
	0	10	20	30	0	10	20	30
Injection site 1 L. shoulder	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10
Epithelial necrosis and ulceration								
Min	0	0	1	1	0	0	1	0
Slight	0	0	0	1	0	0	0	2
Moderate	0	0	0	2	0	0	0	1
Focal epidermal								

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Finding	Males				Females			
	0	10	20	30	0	10	20	30
hyperplasia								
Min	0	0	2	2	0	2	0	3
Slight	0	0	4	5	0	0	4	7
Moderate	0	0	0	1	0	0	0	0
Epithelial crust formation								
Min	0	0	0	2	0	0	1	1
Slight	0	0	1	3	0	0	1	5
Moderate	0	0	0	2	0	0	0	0
Dermal fibrosis and inflammatory cell infiltr								
Min	0	1	6	5	0	2	4	7
Slight	0	0	1	4	0	0	0	3
Focal myodegeneration/regeneration								
Min	0	0	0	1	0	0	0	1
Slight	0	0	2	2	0	0	0	0
Focal subcutaneous inflammation								
min	0	0	1	1	0	0	0	2
Injection site 2 R. Shoulder	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10
Epithelial necrosis and ulceration								
Min	0	0	1	0	0	0	0	0
Slight	0	2	0	2	0	0	1	4
Moderate	0	0	0	2	0	0	0	1
Focal epidermal hyperplasia								
Min	0	1	2	3	0	2	1	3
Slight	0	1	4	5	0	0	4	7
Moderate	0	0	1	1	0	0	0	0
Epithelial crust formation								
Min	0	1	0	0	0	2	2	1
Slight	0	0	2	3	0	0	0	1
Moderate	0	0	0	1	0	0	0	1
Dermal fibrosis and inflammatory cell infiltr								
Min	0	1	3	4	0	2	5	4
Slight	0	0	3	4	0	0	1	5
moderate	0	0	1	1	0	0	0	1
Focal subcutaneous inflammation								
Min	0	1	0	1	0	0	1	1
Injection site 3 R. Mid-side	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10
Epithelial necrosis and								

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Finding	Males				Females			
	0	10	20	30	0	10	20	30
ulceration								
Min	0	0	0	0	0	0	0	0
Slight	0	0	1	3	0	0	0	0
Moderate	0	0	0	3	0	0	1	3
marked	0	0	1	0	0	0	1	3
Focal epidermal hyperplasia								
Min	0	0	1	5	0	0	4	1
Slight	0	1	4	3	0	0	2	7
Moderate	0	0	1	1	0	0	0	0
Epithelial crust formation								
Min	0	0	3	2	0	0	1	1
Slight	0	0	2	1	0	0	1	2
Moderate	0	0	0	2	0	0	0	3
Dermal fibrosis and inflammatory cell infiltr								
Min	0	0	0	1	0	1	4	5
Slight	0	1	4	5	0	0	2	2
moderate	0	0	1	0	0	0	0	1
Focal myodegeneration/regeneration								
Min	0	1	3	1	0	1	2	0
Slight	0	1	3	8	0	4	6	6
Focal subcutaneous inflammation								
Min	0	0	0	1	0	0	0	1
slight	0	0	1	0	0	0	0	0
Injection site 4 R. Hip	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10
Epithelial necrosis and ulceration								
Slight	0	0	0	1	0	0	0	2
Moderate	0	1	1	6	0	0	0	2
Focal epidermal hyperplasia								
Min	1	1	3	1	0	2	5	4
Slight	0	0	3	7	0	0	0	5
Moderate	0	0	0	2	0	0	0	0
Epithelial crust formation								
Min	1	1	2	0	0	0	0	1
Slight	0	0	3	4	0	0	0	3
Moderate	0	0	0	3	0	0	0	0
Dermal fibrosis and inflammatory cell infiltr								
Min	0	0	4	5	0	2	3	6

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Finding	Males				Females			
	0	10	20	30	0	10	20	30
Slight	0	0	0	3	0	0	0	1
Focal myodegeneration/regeneration								
Min	0	2	3	0	0	3	3	5
Slight	0	0	3	5	0	1	1	2
Intra-dermal hemorrhage								
Min	0	0	1	0	0	0	0	1
Injection site 5 rump	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10
Epithelial necrosis and ulceration								
Min	0	0	0	0	0	1	0	0
Slight	0	0	1	1	0	0	0	1
Moderate	0	0	0	1	0	0	0	0
Focal epidermal hyperplasia								
Min	0	1	3	5	0	1	2	6
Slight	0	0	2	2	0	1	1	4
Moderate	0	0	0	1	0	0	0	0
Epithelial crust formation								
Min	0	1	0	2	0	1	0	0
Slight	0	0	1	2	0	0	1	2
Dermal fibrosis and inflammatory cell infiltr								
Min	0	0	4	5	0	1	2	6
Slight	0	0	2	0	0	0	0	2
Focal myodegeneration/regeneration								
Min	1	1	0	3	0	0	1	6
Slight	0	0	2	2	0	0	1	2
Injection site 6 L. Hip	N=10	N=10	N=10	N=10	N=10	N=10	N=9	N=10
Epithelial necrosis and ulceration								
Min	0	1	0	0	0	1	0	0
Slight	0	0	0	3	0	0	0	0
Moderate	0	0	0	2	0	0	0	0
Focal epidermal hyperplasia								
Min	0	4	4	1	0	1	1	4
Slight	0	0	1	6	0	1	0	3
Moderate	0	0	0	2	0	0	0	1
Epithelial crust formation								
Min	0	1	0	0	0	1	2	1
Slight	0	0	0	4	0	0	0	1

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Finding	Males				Females			
	0	10	20	30	0	10	20	30
moderate	0	0	0	2	0	0	0	0
Dermal fibrosis and inflammatory cell infiltr								
Min	0	2	3	5	0	1	1	3
Slight	0	0	2	4	0	0	0	4
Focal myodegeneration/regeneration								
Min	0	2	1	2	0	2	4	5
Slight	0	2	1	1	0	0	0	0
moderate	0	0	1	0	0	0	0	0
Intra-dermal hemorrhage								
Min	0	0	0	1	0	0	0	3
slight	0	0	0	0	0	0	0	1
Injection site 7 L. Mid-side	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10
Epithelial necrosis and ulceration								
Min	0	0	1	0	0	0	1	0
Slight	0	0	3	1	0	0	0	0
Moderate	0	0	0	1	0	0	0	2
Focal epidermal hyperplasia								
Min	0	0	2	2	1	2	3	4
Slight	0	0	6	5	0	0	2	6
Epithelial crust formation								
Min	0	0	1	0	0	0	1	0
Slight	0	0	4	1	0	0	1	1
moderate	0	0	0	1	0	0	0	0
Dermal fibrosis and inflammatory cell infiltr								
Min	0	0	5	4	0	3	4	5
Slight	0	0	3	3	0	0	2	5
Focal myodegeneration/regeneration								
Min	0	0	2	0	0	1	0	4
Slight	0	0	2	1	0	0	1	1
Intra-dermal hemorrhage								
Slight	0	0	0	0	0	0	0	1
Adrenal cortex	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10
Diffuse hypertrophy of zona fasciculata								
Min	0	8	3	0	0	0	4	1
Slight	0	1	5	4	0	4	4	7
moderate	0	0	2	6	0	6	2	1

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Finding	Males				Females			
	0	10	20	30	0	10	20	30
Thymus	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10
Lymphocyte depletion								
Min	0	4	6	8	1	3	2	1
Slight	0	0	0	1	0	6	7	6
moderate	0	0	0	0	0	0	0	3
Femur with joint and marrow	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10
Decreased hematopoietic tissues								
Min	0	0	1	2	0	0	1	1
Slight	1	6	3	2	1	6	4	3
Moderate	1	1	0	4	0	1	4	6
Sternum and marrow	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10
Decreased hematopoietic tissue								
Min	3	5	4	3	1	3	2	0
Slight	1	1	1	5	1	6	5	6
moderate	0	0	0	0	0	0	2	2
Epididymides	N=10	N=10	N=10	N=10	NA	NA	NA	NA
Bilateral hypospermia								
Min	0	2	2	3				
Slight	0	0	1	2				
Intratubular degenerate spermatozoa/spermatids								
Min	0	4	6	6				
Slight	0	0	1	2				
Prostate gland	N=10	N=10	N=10	N=10	NA	NA	NA	NA
Reduced secretion								
Min	0	5	2	3				
Slight	0	3	6	6				
Moderate	0	0	2	1				
Seminal vesicles	N=10	N=10	N=10	N=10	NA	NA	NA	NA
Reduced secretion								
Min	0	8	3	1				
Slight	0	1	4	4				
moderate	0	0	3	5				
Testes	N=10	N=10	N=10	N=10	NA	NA	NA	NA
Unilateral germinal epithelial degeneration								
Min	0	2	0	1				
Slight	0	0	1	0				
Bilateral germinal epithelial degeneration								
Min	0	3	1	2				
Slight	0	3	3	1				
Moderate	0	1	5	5				
Dystrophic tubular mineralization present	0	1	8	6				
Mammary gland	NA	NA	NA	NA	N=10	N=10	N=10	N=10

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Finding	Males				Females			
	0	10	20	30	0	10	20	30
Masculinization of mammary tissue								
Min					0	5	5	2
Slight					0	1	3	5
Ovaries, no. of corpora lutea	NA	NA	NA	NA	N=10	N=10	N=10	N=10
0-49					10	0	1	1
40-99					0	4	7	6
100-149					0	6	2	3
Uterus	NA	NA	NA	NA	N=10	N=10	N=10	N=10
Atrophy of endometrial gland								
Min					0	1	0	0
Slight					0	5	4	4
moderate					0	3	5	6
Vagina	NA	NA	NA	NA	N=10	N=10	N=10	N=10
Degeneration of mucus layer								
Min					0	2	1	2
Slight					0	0	2	6
Epithelial atrophy								
Min					0	0	0	2
Slight					0	0	0	1
moderate					0	0	2	4
Kidneys	N=10	NE	NE	N=10	N=10	NE	NE	N=10
Mineral deposits-inner and outer medulla stripes								
Min	1			0	1			3
slight	0			0	0			1
Liver	N=10	NE	NE	N=10	N=10	NE	NE	N=10
Periportal fat vacuolation, min	1			4	3			6
Lungs	N=10	NE	NE	N=10	N=10	NE	NE	N=10
Aggregates of alveolar macrophages								
Min	3			6	2			6
Slight	1			2	1			0
moderate	0			0	0			1

NE- Not examined
NA- Not applicable

The target organs of toxicity in the rat included injection sites (males and females), adrenal glands (males and females), kidneys, liver, lungs, thymus, marrow, female reproductive organs (vagina, uterus, and mammary gland), male reproductive organs (epididymides, prostate, seminal vesicles and testes). The findings in the male and female reproductive organs are a result of the secondary pharmacodynamic action of icatibant and its blockade of bradykinin induced regulation of pituitary hormone secretion. In the rat, the MTD was considered the 10 mg/kg/day dose for females based on the decreased group mean body weight gains at the HD (30 mg/kg/day), injection site irritation at doses ≥ 20 mg/kg/day and on the decreased incidence of effects observed in reproductive organs (females), adrenals and thymus compared to doses >10

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mg/kg/day. For male rats, the MTD was exceeded as observed with a decrease in male group mean body weight gains exceeding 10% in all groups compared to the controls and males losing weight at week 8-13. No NOAEL was determined for the rat study due to findings in the reproductive organs, adrenals and thymus in the 10 mg/kg/day low dose group.

Toxicokinetics: Blood samples for TK analyses (0.5 mL on Day 1 and Week 13) were taken at 0.5, 1, 2, 4, 8 and 24 hours post-dose. Samples were taken from the lateral caudal vein. Plasma samples were prepared and analyzed for levels of icatibant and metabolite M2.

TK analysis was conducted but data were not included in the audited draft report.

2.6.6.4 Genetic toxicology

The sponsor submitted three new genetic toxicology studies in NDA 22-150. These studies were: a bacterial reverse mutation assay (a complete bacterial strain battery), an *in vitro* mammalian chromosome aberration test, and an *in vivo* bone marrow micronucleus assay in Sprague-Dawley rats using the icatibant. The bacterial reverse mutation assay and the *in vivo* micronucleus study in rats were studies used to bridge the change in manufacturer for the drug substance. Icatibant did not induced genetic toxicity in these three studies under the conditions assayed. The results of these studies are consistent with numerous other genetic toxicity assays that were previously reviewed under INDs (b) (4)

Route	Study	Concentration/Dose	Study Outcome
In vitro	Bacterial gene mutation	4 to 4000 µg/plate	Negative
		4 to 10,000 µg/plate	Negative
		4 to 5000 µg/plate	Negative
		39 to 5000 µg/plate	Negative
Intravenous	Mouse micronucleus	50 mg/kg	Negative
	Rat micronucleus	Males: 3.75, 7.5, 15 mg/kg Females: 2.5, 5, 10 mg/kg	Negative
Intraperitoneal	Hamster chromosome aberrations	10, 50, 100 mg/kg	Negative

For safety qualification of the (b) (4) impurity, the sponsor conducted 1 acute IV toxicity study, a bacterial reverse mutation study and a chromosome aberration study. (b) (4) was negative in the Ames test but positive in the chromosomal aberration test for inducing genotoxicity. Although positive for genotoxicity in the chromosome aberration test, (b) (4) (b) (4) is a member of the peptide drug class which is considered to have low genotoxic risk. The acute IV toxicity study dosed rats up to 100 mg/kg. No toxicity was observed in rats. However, the duration of this study is not considered acceptable to qualify this impurity for safety. Therefore, (b) (4) has not been adequately qualified for safety.

2.6.6.9 Discussion and Conclusions

In support of Jerini's proposed mouse and rat carcinogenicity protocols, Jerini submitted two 13-week repeat dose toxicity studies, 1 in the mouse and 1 in the rat. In the mouse study, mice were administered 0 (VC), 10, 25 or 50 mg/kg/day of icatibant, SC through Week 8. Due to the absence of clinical signs of toxicity observed in these dosage groups, Jerini re-allocated 5 mice/sex from the 10 mg/kg/day group to a new HD group of 100 mg/kg/day. Jerini also used

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remaining spare animals (5 males and 3 females) that had not been previously exposed to drug and included these animals in the 100 mg/kg/day group for a final n= 10 male mice and 8 female mice. The 100 mg/kg/day group was dosed from Week 9 to Week 13. The rat study subcutaneously administered 0 (VC), 10, 20 or 30 mg/kg/day of icatibant for 13 weeks. The target organs of toxicity common between mouse and rat included: adrenals, injection sites, female mammary gland, thymus, and female reproductive organs (uterus and vagina). Exclusive to the mouse at the icatibant doses examined, the eye, cervix, skeletal muscle and stomach were additional target organs of toxicity. In the rat, bone marrow (in femur and sternum), kidneys, liver and lung were observed as potential target organs of toxicity that were not observed in the mouse at the icatibant doses tested. In the mouse, the MTD was determined to be 50 mg/kg/day based on the dose limiting injection site reactions at doses > 50 mg/kg/day. No NOAEL could be determined for the mouse study due to the 10 and 25 mg/kg/day not being examined for injection site irritation. In the rat, the MTD was considered the 10 mg/kg/day dose for females based on the decreased group mean body weight gains at the HD (30 mg/kg/day), injection site irritation at doses ≥ 20 mg/kg/day and on the decreased incidence of effects observed in reproductive organs (females), adrenals and thymus compared to doses >10 mg/kg/day. For male rats, the MTD was exceeded as observed with a decrease in male body weight gains exceeding 10% in all groups compared to the controls and males losing weight at week 8-13. No NOAEL was determined for the rat study due to findings in the reproductive organs, adrenals and thymus in the 10 mg/kg/day low dose group.

Icatibant did not induce genetic toxicity in the presence or absence of metabolic activation in the bacterial reverse mutation assays or *in vitro* mammalian chromosomal aberration studies or micronuclei formation in *in vivo* assays under the conditions tested.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

Icatibant acetate is being developed for the treatment of patients with hereditary angioedema (HAE) who are 18 years old and older. In Type I (quantitative deficiency) and Type II (qualitative deficiency) HAE a deficiency of C1-esterase inhibitor (C1-INH) results in an increased release of bradykinin (BK). BK binds to the bradykinin type-2 receptor (B_2) to trigger activation of many cascades leading to vasodilatation, increased vascular permeability and smooth muscle cell contraction, resulting in the increased vascular permeability and acute inflammation observed in HAE patients. Icatibant is a synthetic decapeptide that is a selective B_2 receptor antagonist that binds to the B_2-receptor with similar affinity as BK. Icatibant binding at the B_2 receptor inhibits bradykinin induced processes and is hypothesized to contribute to the decrease of vascular permeability and acute inflammation observed in HAE patients.

Absorption, distribution, metabolism and elimination (ADME) studies were conducted in the mouse and rat. However, these studies were limited for the mouse. In both the mouse and the rat, Icatibant is metabolized to the active metabolites M1 (b) (4) and M2 (b) (4) by hydrolysis of icatibant's peptide backbone, thought to be induced by peptidases. The M1 is formed at >90%, ~50% and 70% in man, mouse and rat, respectively. M2 is formed in rat ~9% and in human ~75%. After SC administration in rats, distribution studies showed that icatibant

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(or its metabolites) is distributed into the kidneys, liver, urinary bladder, lungs and spleen 1 hour post-dose and is still detectable in the liver, kidneys, spleen, bone marrow, adrenals and injection sites at 24-hours post-dose. After repeat SC dosing in rats, icatibant is rapidly distributed and metabolized with the M2 metabolite appearing from 0.2 to 3 hours post-dose. Neither icatibant nor M2 accumulated after repeat dosing in male and female rats up to 13-weeks dosing as was observed in the NDA. The biological half-life was more than 5 hours after 26 µg/kg SC in rats and terminal half-life was over 6 days in rats after 10 µg/kg, SC. There were no gender differences observed in rats in the systemic exposures of males and females. Serum protein binding of icatibant was low with 44 and 49% in man and rat, respectively. No serum protein data are available for the mouse.

Neither icatibant nor the M1 or M2 metabolites is metabolized by human hepatocytes or microsomes. Additionally, icatibant is not an inducer of CYP450 isozymes. Based on excretion studies, the primary route of elimination after SC administration of icatibant is via the kidneys in mice (~97.1%) and rats (~61%) with small amounts excreted in the feces (8.0% to 33%).

Jerini completed a full genetic toxicity battery assessment for icatibant. Icatibant did not induce genetic toxicity in the presence or absence of metabolic activation in the bacterial reverse mutation assays or *in vitro* mammalian chromosomal aberration studies or micronuclei formation in *in vivo* assays under the conditions tested.

The sponsor proposed a 104-week CD-1 mouse carcinogenicity study at SC doses of 0 (VC), 10, 30 and 100 mg/kg/day. In support of their proposed doses, a 13-week repeat dose SC study was conducted in mice at 0 (VC), 10, 25, 50 (13-week dosing) and 100 (4-week dosing) mg/kg/day. MTD was achieved in this study at 50 mg/kg/day. There were no drug-related deaths in mice. Clinical signs of toxicity were sores and lesions at the injection sites and thinning of fur in males and females and aggressive behavior (males only). There were no significant changes in body weight gains in the icatibant treated mice compared to controls. The potential target organs of toxicity were injection site irritation (males and females), adrenal glands (males and females), female reproductive organs (vagina, uterus, and cervix), female mammary gland, thymus (males and females), skeletal muscle (males and females) and stomach (males only). The 100 mg/kg/day dose exceeded the MTD with extreme injection site reaction (hemorrhage, ulceration and necrosis) considered the dose limiting toxicity. No NOAEL could be determined in this study due to the absence of examination of the 10 and 25 mg/kg/day groups for the target organs of toxicity.

The sponsor's proposed doses for a standard 2-year carcinogenicity study in CD-1 mice were based on the MTD approach. The sponsor considered icatibant doses up to 100 mg/kg/day in mice as the maximum feasibly administrable dose without study compromising problems of local tolerance (*i.e.* MTD) and stated that this dose provides an exposure which is at least 20 times that reached in the clinic at 30 mg/day. The sponsor indicated that the daily dose in humans was 30 mg/day, when in fact the clinical doses of 30 mg/day up to 3 times daily SC are being sought for approval. Therefore the maximum recommended human dose (MRHD) of icatibant is 90 mg/day.

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Based on a detailed review of the 13-week mouse study, the target organs of toxicity were injection site (males and females), adrenal glands (males and females), female reproductive organs (vagina, uterus, and cervix), female mammary gland, thymus (males and females), skeletal muscle (males and females) and stomach (males only). The dose-limiting toxicity in this study was injection site reaction with hemorrhage, ulceration and necrosis observed at multiple injection sites in males and females that increased in incidence and severity at the 100 mg/kg/day dose. The reviewer considers that the MTD was exceeded with the 100 mg/kg/day based on the injection site toxicity. The MTD was determined to be 50 mg/kg/day based on tolerable levels of injection site reaction.

For the 104-week Wistar rat study, the sponsor proposed two dosing options for the Carcinogenicity Assessment Committee to consider. The two options were either:

Option A: 0 (0.9% physiological saline-VC), 3 mg/kg t.i.d (twice weekly), 10 mg/kg t.i.d (twice weekly) and 10 mg/kg/day

Option B: 0 (0.9% physiological saline-VC), 10 mg/kg t.i.d. (twice weekly), 3.3 mg/kg/day and 10 mg/kg/day

These alternative dosing regimens were proposed based on a MTD and pharmacodynamic (PD) approach by the sponsor. In support of their proposed doses, a 13-week repeat dose SC Wistar rat study was conducted at 0, 10, 20 and 30 mg/kg/day. One HD male TK animal died with macroscopic findings summarized by the sponsor as small testes, epididymides and seminal vesicles. Due to the lack of histopathological examination and no additional information on this animal's death, it is not known if this death is drug related. No main study animals died prior to scheduled sacrifice. Clinical signs of toxicity included sores and lesions at injection sites and on the head, neck and back, thinning of fur and staining of fur in males and females. Males showed a decreased in BW gains (-34 to -51%) compared to controls, which coincided with decreased food consumption (-6 to -12%). HD females also show reduced BW gain. Urinalysis showed a significant increase in electrolyte levels (+65% to +200%) in males and females. However, when evaluated based on urine volume, there was no statistically significant difference between controls and icatibant treated rats. The adrenal wts were increased (+19 to 60% in males and females) and thymic (- 20 to -51% in males and females), prostate (-41 to -58%) and testes plus epididymal wts (-20 to -31%) were decreased. The target organs of toxicity in the rat included injection site (males and females), adrenal glands (males and females), kidneys, liver, lungs, thymus, marrow, female reproductive organs (vagina, uterus, and mammary gland), male reproductive organs (epididymides, prostate, seminal vesicles and testes). The dose-limiting toxicities in the female were reduced body weight at 30 mg/kg/day, injection site irritation at doses ≥ 20 mg/kg/day and target organs of toxicity in the reproductive organs, thymic and adrenal findings at all icatibant doses. In the male, dose limiting toxicity was a decrease in body weight gain exceeding 10% at all doses compared to controls. Males were also observed with injection site irritation, male reproductive organ toxicities and toxicities in the adrenals and the thymus. The findings in the male and female reproductive organs are a result of the pharmacodynamic action of icatibant and its effects on the pituitary secretion. No NOAEL could be determined for this study as male and female reproductive organ, thymus and adrenal effects are observed at all doses. However, the reviewer considered the MTD in the female as 10

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mg/kg/day dose based on the injection site findings and decreased body weight gains and incidence of effects observed in the thymus, adrenals and female reproductive organs compared to doses >10 mg/kg/day. For males, the MTD was not determined (< 10 mg/kg) due to reductions in male body weight gains that exceeded 10% in all icatibant treated animals.

The sponsors proposed dosing regimens for the 104-week rat carcinogenicity study were based on a MTD approach and on the secondary PD actions of icatibant. The sponsor considered the 10 mg/kg/day as the practical limit dose based on local tolerance at the injection site. Additionally, the sponsor considered the effects on the reproductive organs, as well as the adrenals, would be expected at ≥10 mg/kg/day. Icatibant has been shown to delay sexual maturation in rats and dogs with repeated administration and has been shown to decrease of stimulating hormones from the pituitary to result in this sexual maturation delay (reference to NDA 22-150 review dated March 18, 2008). The sponsor states that the effects on these tissues as a result of secondary PD actions of icatibant could affect the general health of animals and compromise the study and its interpretation. The alternative dosing regimen was based on repeat dose SC toxicity studies conducted in the dog.

The reviewer considers the 10 mg/kg/day dose as the MTD in the female rat. The dose-limiting toxicities include the injection site toxicity, reduced body weight gain (HD only), adrenals, thymus and the female reproductive organs. For males, the MTD was not determined as male body weight gains exceeded 10% in all icatibant treated animals. Additional toxicities observed in males included the adrenals, thymus and the male reproductive organs. Although effects on these tissues were observed at 10 mg/kg/day they were decreased in incidence and severity compared to doses >10 mg/kg/day. Additionally, icatibant's secondary PD effects most likely will result in humans with repeat SC administration and this effect should be examined as it relates to clinical use. The sponsor did not evaluate their proposed alternative dosing regimens in rats in their 13-week dose-ranging study. Therefore, the reviewer can not conclude that these regimens would adequately support the doses proposed based on the rat data provided.

Internal comments: The Executive CAC meeting was held on June 10, 2008 to discuss the sponsor's proposed mouse and rat carcinogenicity protocols. Based on the discussion at this meeting the following recommendations were given. These recommendations were captured in the meeting minutes and were faxed to the sponsor.

Mouse:

- The Committee recommended daily doses of 0 (VC), 5, 15, and 50 mg/kg/day, by SC injection and injection site rotation in males and females, based on an MTD criterion (severe local toxicity).

Rat:

- The Committee concluded that the data were insufficient to make dose recommendations for male rats as all tested doses exceeded the MTD based on significant reductions in body weight gain in comparison to control animals. The proposed alternative dosing regimens (t.i.d dosing, 2 times per week) are not supported by the 13-week repeat dose

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rat study which administered drug on a daily basis. Due to the absence of TK data in the draft study report for the dose-ranging study, rat to human exposure comparisons can not be calculated.

- The Committee recommended daily doses of 0 (VC), 1, 3, and 10 mg/kg/day for female rats, by SC injection with site rotation, based on MTD (severe local toxicity).

- If a dosing regimen other than daily dosing is desired, the range-finding study should be conducted using that regimen.

General comments:

- The sponsor should contact the Division prior to terminating any groups or making any dose adjustments.
- The proposed hematological and urine measurements should be omitted.
- TK evaluations are not needed beyond 6 months.

External comments (to sponsor): None.

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12.4 Appendix 4: Pharmacology and Toxicology Review IND 68,214 (Serial No. 55-57), October 24, 2008

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

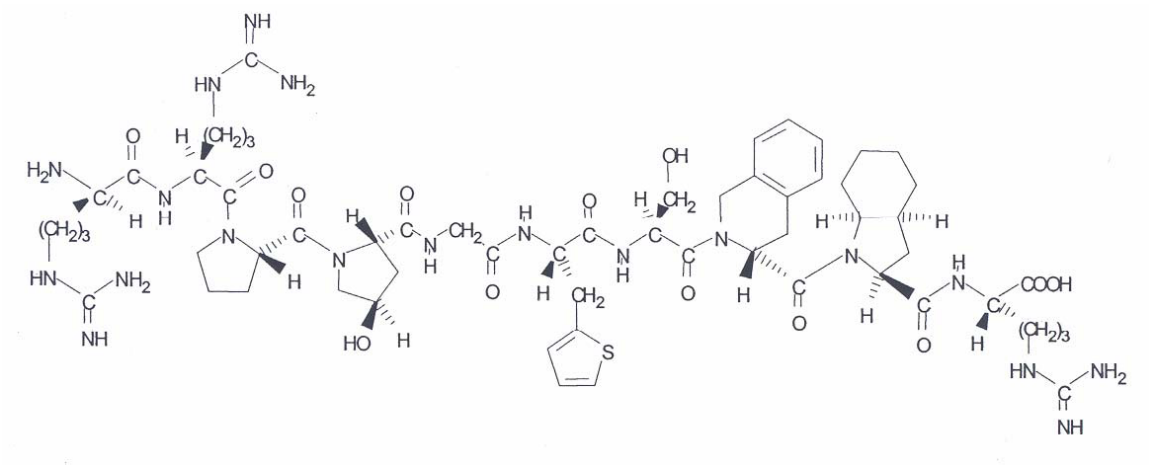
2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 68,214
Review number: 4
Sequence number/date/type of submission:
SDN 55/July 3, 2008/Response to carcinogenicity SPA
SDN 56/August 13, 2008/IT-26-week rat study
SDN 57/September 8, 2008/SPA (Male rat protocol)
Information to sponsor: Yes () No (X)
Sponsor and/or agent: Jerini AG, Berlin Germany
Manufacturer for drug substance: (b) (4)

Reviewer name: Molly E. Shea, Ph.D.
Division name: Division of Pulmonary and Allergy Products (DPAP)
Review completion date: October 24, 2008

Drug:

Trade name: Firazyr
Generic name: Icatibant acetate
Code name: JE 049/HOE 140
Chemical name: D-arginyl-L-arginyl-L-prolyl-L[(4R)-4-hydroxyprolyl]-glycyl-L-[3-(2-thienyl)alanyl]-L-seryl-D-(1,2,3,4-tetrahydroisoquinolin-3-yl-carbonyl)-L-3aS,-7aS)-octahydroindol-2-yl-carbonyl]-L-arginine acetate
CAS registry number: NA
Amino acid sequence: H-D-Arg-L-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-OH· nCH_3COOH
Molecular formula/molecular weight: $C_{59}H_{89}N_{19}O_{13} \cdot C_2H_4O_2$/1304.6
Structure:



Relevant INDs/NDAs/DMFs: IND (b) (4) NDA 22-150 (icatibant subcutaneous injection for hereditary angioedema)

Drug class: Bradykinin type 2 (B_2) receptor antagonist

Intended clinical population: Patients with acute hereditary angioedema (HAE)

Clinical formulation: Icatibant will be delivered as a 10 mg/mL sterile, isotonic, acetate-buffered solution.

Route of administration: Subcutaneous injection (SC)

Proposed clinical protocol: None in this submission.

Previous clinical experience: A total of 756 subjects have been exposed to one or more doses of icatibant by intranasal, inhalation, intra-articular, intravenous or subcutaneous routes of administration during icatibant's development. The following table provides a summary of the types of studies explored with icatibant and the doses administered.

Study	Dosage	No of Subjects Exposed
11 intranasal allergic rhinitis studies	0.01 to 15 mg/day	201
4 inhalation studies for chronic bronchitis	0.1 to 12 mg/day	205
2 intra-articular studies for osteoarthritis of the knee	90 to 2000 µg/kg	234
7 single-dose IV studies	0.0005 to 4.5 mg/kg	97
4 PK IV studies in various populations (elderly, renally and hepatically impaired)	0.05 mg/kg to 30 mg/day	87
3 Phase 1 SC studies for HAE	0.05 mg/kg to 45 mg/day	58
Phase 2a for HAE	0.4 and 0.8 mg/kg, IV and 30 and 45 mg/day, SC	12, IV 8, SC
FAST-2/Phase 3	Active control or 30 mg, SC	36 + 3 open label

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

Studies reviewed within this submission:

JE049-0163: 26-week subcutaneous administration toxicity study in the rat followed by a 4-week treatment free period (audited draft report)

Studies not reviewed within this submission: The 13-week rat SC study was submitted in draft form for review for the original carcinogenicity special protocol assessment (review number 3; dated of June 12, 2008). The toxicology findings in the final report were the same as previously reported in the audited draft study report. Included in the final report were the toxicokinetic data that were omitted in the draft study report. These data are included in the pharmacokinetic portion of this review.

JE049-0160: 13-week toxicity study in the rat (Final report)

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2.6.4 PHARMACOKINETICS/TOXICOKINETICS

The sponsor submitted an audited draft study report for the 13-week repeat dose SC rat study (number JE049-0160) which was reviewed (Dated June 12, 2008; Review number 3) for the original carcinogenicity special protocol assessment (SPA). Male and female rats were subcutaneously administered 0 (saline vehicle), 10, 20 or 30 mg/kg/day of icatibant. In the draft report the sponsor did not include the toxicokinetic (TK) data for this study. In the current carcinogenicity SPA (dated September 8, 2008) the sponsor included the final study report for this 13-week rat toxicity study that included the TK results. There were no changes in the toxicology data compared to the draft report data. For this study, blood samples (0.5 mL) were drawn from TK allocated animals on Day 1 and in Week 26 at the following time points: 0.5, 1, 2, 4, 8 and 24 hours post-dose. Samples were collected from the lateral caudal vein and plasma was separated. The levels of icatibant and its M2 metabolite were determined using LC-MS/MS after solid phase extraction. The lower limit of quantitation was 25.0 ng/mL for icatibant and 2.5 ng/mL for the M2 metabolite, respectively. From these data the T_{max}, C_{max}, AUC_{0-t} and AUC_{0-Inf} were calculated for icatibant and M2. The following table summarizes these data.

Time	Parameter	Icatibant SC Dose (mg/kg/day)					
		10		20		30	
		M	F	M	F	M	F
	Icatibant levels						
Week 1	C_{max} (ng/mL)	4550	4980	6240	9390	9790	12900
	t_{max} (h)	2.0	1.0	4.0	2.0	2.0	2.0
	AUC_{0-t} (ng*h/mL)	21100	18200	32800	34100	49200	52600
	AUC_{0-inf} (ng*h/mL)	21300	18200	-	34400	59600	55400
	$t_{1/2}$ (h)	1.04	0.796	-	1.06	2.84	1.65
Week 26	C_{max} (ng/mL)	4290	5720	7560	9730	13000	10900
	t_{max} (h)	2.0	2.0	2.0	2.0	2.0	1.0
	AUC_{0-t} (ng*h/mL)	23100	27000	42200	45100	72300	60800
	AUC_{0-inf} (ng*h/mL)	28300	30900	57900	56500	98500	77300
	$t_{1/2}$ (h)	3.0	2.45	3.83	3.06	3.76	3.43
	M2 Metabolite levels						
Day 1	C_{max} (ng/mL)	345	334	489	670	590	786
	t_{max} (h)	4.0	2.0	4.0	2.0	4.0	2.0
	AUC_{0-t} (ng*h/mL)	2160	1640	3840	3110	5930	5370
	$AUC_{0-infin}$ (ng*h/mL)	2180	1660	3880	3130	5990	5420
	$t_{1/2}$ (h)	3.53	3.72	3.60	3.54	3.45	3.50
Week 26	C_{max} (ng/mL)	362	270	456	566	616	654
	t_{max} (h)	2.0	2.0	2.0	2.0	4.0	4.0
	AUC_{0-t} (ng*h/mL)	3540	2420	5930	5060	8020	7050
	$AUC_{0-infin}$ (ng*h/mL)	3610	2460	6060	5150	8210	7200
	$t_{1/2}$ (h)	4.11	3.91	4.35	4.04	4.32	4.18

- Data not available at this time point

On Day 1 and Week 13 peak concentrations of icatibant for both male and female rats occurred 1 to 4 hours post-dose. The C_{max} and AUC values for icatibant were comparable between males and females throughout the study period. Additionally, the C_{max} and AUC levels for icatibant increased in an ~ proportional manner with increased dose in males and females. AUC values

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were comparable in males and females at all dose levels. Based on C_{max} values, plasma concentrations of icatibant were ~12 to 21-fold higher than those for the metabolite M2. Peak concentration of M2 in males and females occurred between 2 and 4 hours post-dose. C_{max} values were comparable between males and females for M2 formation.

2.6.6 TOXICOLOGY

2.6.6.3 Repeat-dose toxicity

Study title: 26-week subcutaneous administration toxicity study in the rat followed by a 4 week treatment-free period

The sponsor selected the doses for this study based on the previous 13-week repeat dose SC study (JE049-0160) conducted in the Crl:WI (Han) rats. The observed effects on the sexual organs, resulting from blockade of the known role of bradykinin in regulation of pituitary hormone secretion, were the most important dose limiting factors taken into consideration by the sponsor. The sponsor selected doses for the 26-week study (3, 10, and 30 mg/kg/day) with the expectation that clinical signs would be limited to local minor injection site reactions and no adverse effects on body weight or food consumption.

Key study findings:

- 22% of HD group (7/30 males and 6/30 females) were sacrificed between Weeks 13 and 25 due to severe injection site toxicity
- The remaining HD males (18) and females (19), with the exception of 5 rats/sex (selected for recovery), were sacrificed in week 26 but prior to scheduled necropsy of study due to severe injection site toxicity
- Clinical signs of toxicity included sores/lesions at injection sites (all doses), staining of fur, excessive activity and thin appearance in males and females
- Males showed a decrease in BW gains and BWs compared to controls (PD effect due to icatibant decreasing pituitary hormone secretion resulting in ablation of testosterone levels); female body weights were not affected in the same manner
- Adrenal and thymic wts were decreased in males and females; male prostate and epididymal wts were decreased and female ovarian wts were increased
- Potential target organs of toxicity in male and female rats included skin, injection sites, adrenal glands, aorta, bone marrow (femur joint and marrow from sternum), kidneys, liver, lungs, thymus and connective tissues. Female reproductive organs (vagina, ovaries, and uterus) and female mammary gland (mascularization) and male reproductive organs (prostate, epididymides, testes, and seminal vesicles) were target organs of toxicity.
- No NOAEL could be determined due to findings in the male and female reproductive organs, thymus, bone marrow (sternum and femur joint), aorta and adrenal gland were observed in the LD group (3 mg/kg/day)
- The MTD was considered 3 mg/kg/day in males and females based on severe injection site toxicity (hemorrhage, ulceration and focal muscle degeneration) at doses ≥10 mg/kg/day in males and females and moribund conditions in males and females at 30 mg/kg/day dose that resulted in early sacrifice.

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Study no.: JE049-0163
Volume #, and page #: Vol. 1, and page 5
Conducting laboratory and location: (b) (4)
Date of study initiation: July 18, 2007
GLP compliance: Yes- The study was conducted in compliance with GLP regulations but the report is an unsigned draft report.
QA report: yes ( ) no (X)
Drug, lot #, and % purity: Icatibant, batch no. (b) (4) E 086, and 98.3% pure

Methods

Doses: 0 (saline vehicle control), 3, 10 and 30 mg/kg/day, SC
Species/strain: Crl:WI (Han) rat
Number/sex/group or time point (main study): 20/sex/group
Route, formulation, volume, and infusion rate: SC injection was formulated in 0.9% physiological saline. The dosing volumes were 1 ml/kg for Weeks 1 through 5, 5 mL/kg for Weeks 6-12 and then 3 mL/kg for Weeks 13 through 26. Test article was administered as daily subcutaneous injections using a rotation of 7 injection sites. Injection site 1-left shoulder, injection site 2- right shoulder, injection site 3 – left flank, injection site 4- right flank, injection site 5- left hip, injection site 6- right hip and injection site 7- rump. Groups 1, 2 and 3 were dosed once daily for 26 weeks and Group 4 was dosed once daily for 25 weeks.
Satellite groups used for toxicokinetics or recovery: 10 rats/sex/group were used for recovery and an additional 10 rats/sex/group were used as satellite animals for blood draw.
Age: ~ 11 weeks old at dosing
Weight: Males: 266.1 to 376.1 g and Females: 171.8 to 241.5 g
Sampling times: See below
Unique study design or methodology: In Week 25 of study, the HD group (30 mg/kg/day) showed deterioration of health as a result of severe injection site lesions. Most of these animals in this group were terminated. Five males (3 from main study, 1 from recovery group and 1 from the satellite group) and 5 females (3 from main study, 1 from recovery group and 1 from the satellite group) were retained as the HD group animals for the recovery phase. Due to the changes in procedures for the HD, the satellite animals from LD(3 mg/kg/day) and MD (10 mg/kg/day) were subjected to full necropsy and post-life procedures as for the scheduled recovery animals.

Observation, Times and results:

Mortality: Seven males and 6 females from the HD group (30 mg/kg/day) were sacrificed prior to schedule necropsy due to severe injection site toxicity (between weeks 13 and 25). The findings included open sores and lesions of full skin thickness at the injection sites. Histopathology showed that death was considered a consequence of severe ulcerative skin lesions at the injection site. Additionally, one male in the LD group (3 mg/kg/day) died as a result from accidental injection of the dose into the thoracic cavity.

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In Week 26 of the study, the remaining HD group animals, with the exception of 5 rats/sex that were selected for recovery assessment, were sacrificed for humane reasons related to severe injection site toxicity. The recovery rats in the HD group were no longer dosed for the last week of the dosing phase. The following table summarizes the number of rats that were sacrificed, the Week that they were sacrificed and the basis for their sacrifice.

	Icatibant (mg/kg/day)			
	0	**3**	**10**	**30**
Males				
No. of deaths	0/20	1/20	0/20	7/20
Animal ID/Week of death (associated finding)		50/Week 25 (Found dead)		113/Week 13 (Moribund) 126/Week 15 (Ulcerated abdomen/moribund) 103/Week 23 (Moribund) 108/Week 24 (Moribund/Lesions abdomen) 115/Week 24 (Moribund) 122/Week 24 (Moribund/Sores on back) 124/Week 21 (Moribund) Remaining HD males (Week 26)
Females				
No. of deaths	0/20	0/20	0/20	6/20
Animal ID/Week of death (associated finding)				248/Week 14 (Ulcerated abdomen/moribund) 270/Week 15 (Moribund) 261/Week 18 (Ulcerated right hip/moribund) 268/Week 19 (Moribund) 266/Week 24 (Moribund) 250/Week 22 (Moribund) Remaining HD females (Week 26)

Clinical signs: All animals were observed daily for signs of ill health. A complete physical exam was conducted once weekly on all study animals. Post-dose observations were performed once daily at 1 to 2 hours until Week 6. From Week 7 onwards, clinical observations were performed twice weekly.

Sores and lesions were observed in males and females at the injection sites with increased incidence in icatibant treated rats compared to controls, particularly during Weeks 1-4 of dosing. For males, staining of fur increased in incidence with increased icatibant dose and with increased exposure time. Staining of fur in females did not show a dose relationship. From weeks 5 to 8, HD females showed excessive activity which was not observed in females for the remaining of the dosing period and was not observed in males. Males and females showed increased incidence over time of being thin in appearance.

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Observation	Sex	Icatibant (mg/kg/day)			
		0	3	10	30
Sores/lesions at injection site	M	0 (wk 1-24) 1 (wk 25-27)	1 (wk 1-4) 1 (wk 5-8) 1 (wk 9-12) 1 (wk 13-16) 0 (wk 17-27)	0 (wk 1-27)	23 (wk 1-4) 2 (wk 5-8) 2 (wk 9-12) 0 (wk 13-16) 0 (wk 17-20) 2 (wk 21-24) 0 (wk 25-27)
	F	0 (wk 1-4) 2 (wk 5-8) 1 (wk 9-12) 0 (wk 13-16) 1 (wk 17-20) 0 (wk 21-27)	0 (wk 1-27)	0 (wk 1-8) 1 (wk 9-12) 0 (wk 13-24) 1 (wk 25-27)	21 (wk 1-4) 0 (wk 5-8) 1 (wk 9-12) 0 (wk 13-20) 1 (wk 21-24) 0 (wk 25-27)
Staining of fur	M	0 (wk 1--8) 1 (wk 9-12) 1 (wk 13-16) 0 (wk 17-27)	0 (wk 1-27)	0 (wk 1-8) 1 (wk 9-12) 1 (wk 13-16) 2 (wk 17-20) 2 (wk 21-24) 3 (wk 25-27)	0 (wk 1-4) 2 (wk 5-8) 2 (wk 9-12) 1 (wk 13-16) 2 (wk 17-20) 1 (wk 21-24) 1 (wk 25-27)
	F	0 (wk 1-4) 0 (wk 5-8) 2 (wk 9-12) 6 (wk 13-16) 5 (wk 17-20) 1 (wk 21-24) 2 (wk 25-27)	0 (wk 1-4) 1 (wk 5-8) 2 (wk 9-12) 3 (wk 13-16) 4 (wk 17-20) 6 (wk 21-24) 8 (wk 25-27)	1 (wk 1-4) 3 (wk 5-8) 5 (wk 9-12) 6 (wk 13-16) 7 (wk 17-20) 9 (wk 21-24) 11 (wk 25-27)	0 (wk 1-27)
Excessively active	M	0 (wk 1-27)	0 (wk 1-27)	0 (wk 1-27)	0 (wk 1-27)
	F	0 (wk 1-27)	0 (wk 1-27)	0 (wk 1-27)	0 (wk 1-4) 26 (wk 5-8) 0 (wk 9-27)
Thin in appearance	M	0 (wk 1-27)	0 (wk 1-27)	0 (wk 1-16) 1 (wk 17-20) 0 (wk 21-27)	0 (wk 1-16) 1 (wk 17-20) 2 (wk 21-24) 3 (wk 25-27)
	F	0 (wk 1-16) 1 (wk 17-20) 0 (wk 21-27)	0 (wk 1-24) 2 (wk 25-27)	0 (wk 1-8) 1 (wk 9-12) 1 (wk 13-16) 1 (wk 17-20) 0 (wk 21-24) 2 (wk 25-27)	0 (wk 1--8) 1 (wk 9-12) 1 (wk 13-16) 1 (wk 17-20) 4 (wk 21-24) 6 (wk 25-27)

Body weights: Individual body weights were recorded on Day -7, before treatment on the first day of dosing, at twice weekly intervals for the first 13 weeks and then once weekly thereafter and before necropsy.

Starting Day 22 of the dosing phase, MD and HD icatibant treated male rats showed a reduction in body weights compared to control treated males. This slight decrease did not reach statistical significance until Day 81 for the HD (-15% BW vs control males) and did not reach statistical significance for the MD male group compared to the control males throughout the

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study. The LD group had a lower body weight compared to the control group starting Day 64 of treatment but this decrease was slight compared to controls. Males showed a dose dependent decrease in body weights with -6%, -17% and -26% for the LD, MD and HD groups, respectively, at the end of the dosing phase. There was a dose related decrease in group mean body weight gains in males treated with icatibant compared to controls.

Females treated with icatibant did not show body weight differences compared to the control treated females until Day 183 of study. HD females showed a slight decrease in body weights (-8%) compared to control females during the dosing phase. After the recovery period, the HD female group showed a decrease in body weights by (-9%) compared to control females. Changes in body weight gains in females treated with icatibant compared to control females were observed but were not related to dose. These results are summarized in the following table and the graphs provided by the sponsor.

Δ% in Body Weight Gains and Body Weights vs Controls

Week	Sex	Icatibant Dose (mg/kg/day)		
		3	10	30
Changes in Body Wt Gains				
Start to 25	M	-20%	-46%	-74%
Start to 25	F	-8%	+34%	-
Changes in Body Wts				
13	M	-	-12%	-17%
26		-6%	-17%	-26%
30 (R)		NE	NE	-19%
13	F	-	-	-
26		-	+11%	-8%
30 (R)		NE	NE	-9%

(R)- Recovery Group
- Did not reach statistical significance
NE- Not evaluated

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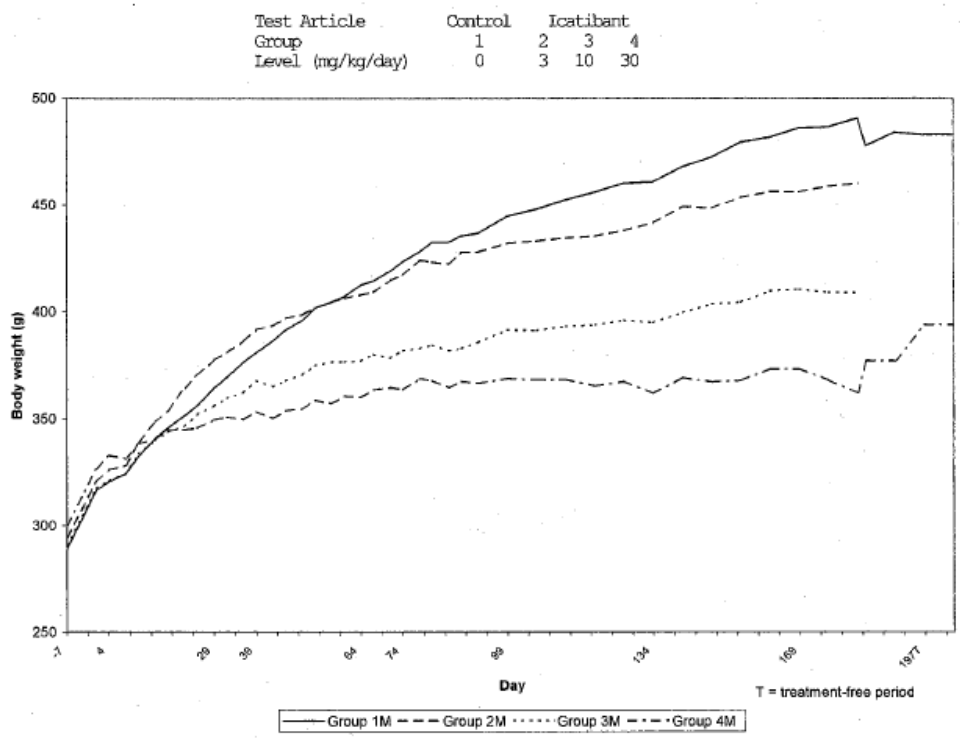
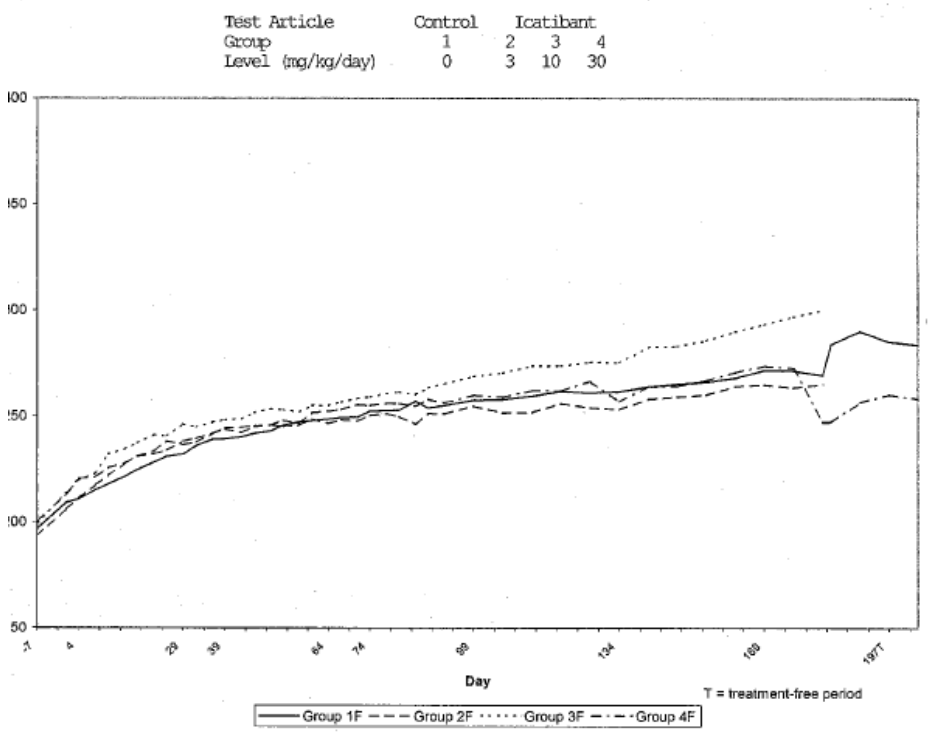
Group mean body weights – males



Best Available Copy

Group mean body weights – females



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Food consumption: The amount of food consumed by each cage of main study and recovery animals was determined at least once weekly. Consumption was calculated as g/animal/week.

There were no statistically significant differences in food intake in the icatibant treated animals compared to placebo.

Ophthalmoscopy: Ophthalmic examination was performed on all main study and recovery animals, pretreatment and on control and HD animals in Weeks 12 and 25. A mydriatic agent was instilled into the eyes prior to examinations.

There were no drug-related changes observed in males or females treated with 30 mg/kg/day of icatibant (the only examined icatibant treated group) compared to the control treated males and females.

EKG: NA

Hematology: Blood samples (2.6 mL) were drawn from the lateral caudal vein for 10 animals/sex/group after an overnight period of fasting for both hematology and clinical chemistry analyses. Where possible, samples were also taken from the abdominal aorta of all decedents at necropsy. A complete hematological battery was assessed.

Slight increases in white blood cell counts (WBC) were observed in MD and HD males and HD females at the end of the dosing phase. Additionally, prolongation of prothrombin time was observed in Week 26 for HD males and females compared to the control males and females, respectively. Neutrophils and leukocytes as percentages of WBCs changed in both males and females treated with icatibant compared to respective controls. Neutrophil percentage was increased in MD and HD males and in all treated females in Week 26. Leukocyte counts were slightly reduced in HD males and in all treated females in Week 13 and in MD and HD males and all treated females in Week 26. All changes observed in males and females during the dosing phase were completely reversible after the 30-day recovery period.

Δ% of Parameter in Icatibant Treated Group *vs.* Controls

Parameter	Week	Males (mg/kg/day)			Females (mg/kg/day)		
		3	10	30	3	10	30
WBC (10^9/L)	13	-	-	-	-	-	-
	26	-	-34%	-71%	-	-	-58%
	30 (R)	-	-	-	-	-	-
PT (s)	13	-	-	-	-	-	-
	26	-	-	+20%	-	-	+22%
	30 (R)	-	-	-	-	-	-
N (% WBC)	13	-	-	+88%	+77%	+192%	+138%
	26	-	+64%	+95%	+100%	+153%	+132%
	30 (R)	-	-	-	-	-	-
L (% WBC)	13	-	-	-22%	-13%	-33%	-23%
	26	-	-22%	-30%	-25%	-37%	-32%
	30 (R)	-	-	-	-	-	-

(R)- Recovery
- Not statistically significant.

Clinical chemistry: Blood samples (2.6 mL) were drawn from the lateral caudal vein for 10 animals/sex/group after an overnight period of fasting for both hematology and clinical

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chemistry analyses. Where possible, samples were also taken from the abdominal aorta of all decedents at necropsy. Due to a technical error, Week 13 clinical chemistry samples from males were not analyzed for ALP. Therefore, an additional 0.6 mL of blood were taken and analyzed in Week 19. A complete clinical chemistry battery was assessed.

Increases in liver enzymes (AST and ALP) were observed in HD males and females treated with icatibant compared to controls. These increases resolved after the 4-week treatment free period. Serum glucose levels were increased in HD males and females compared to controls Week 26 of the study. Each of these parameters was similar to controls after the 30 day recovery period. Creatinine levels were decreased in HD males and females during Week 26 compared to controls. Female creatinine levels returned to the normal range after the recovery period but the HD male levels remained elevated. These data are summarized in the following table.

Δ% of Parameter in Icatibant Treated Group *vs.* Controls

Parameter	Week	Males (mg/kg/day)			Females (mg/kg/day)		
		3	10	30	3	10	30
AST (IU/L)	13	-	-	+82%	-	-	+154%
	19	-	-	+68%	-	-	+111%
	26	-	-	+111%	-	-	+96%
	30 (R)	-	-	-	-	-	-
ALP (IU/L)	13	-	-	-	-	-	+100%
	19	-	-	-	-	-	-
	26	-	-	+26%	-	+92%	+135%
	30 (R)	-	-	-	-	-	-
Glucose (mmol/L)	13	-	-	-	-	-	-
	19	-	-	-	-	-	-
	26	-	-	+98%	-	-	+106%
	30 (R)	-	-	-	-	-	-
Creatinine (µmol/L)	13	-	-	-	-	-	-
	19	-	-	-	-	-	-
	26	-	-	-29%	-	-	-40%
	30 (R)	-	-	-24%	-	-	-

(R)- Recovery
- Not statistically significant.

Urinalysis: Urine samples were collected from 10 animals/sex/group with the highest identification numbers in Weeks 12 and 25 and from all recovery animals in Week 29. Samples were collected over a six hour daytime period. Food and water were removed during collection. A complete urinalysis was assessed.

Urine volume was higher in the icatibant treated animals compared to controls but this increase did not show a dose related effect. The total excretion of electrolytes (Na^+, K^+, Ca^{++} and Cl^-) was also significantly higher than controls. At the end of the recovery period the levels of urinary parameters in the 30 mg/kg/day group were similar to controls.

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Δ% of Parameter in Icatibant Treated Group *vs.* Controls

Parameter	Week	Males (mg/kg/day)			Females (mg/kg/day)		
		3	10	30	3	10	30
Volume (mL)	12	+58%	+104%	+58%	+150%	+311%	+228%
	25	-	-	-	-	+140%	-
	29	NE	NE	-	NE	NE	-
Total Na (mmol)	12	-	-	-	+60%	+176%	+100%
	25	-	-	-	-	+135%	-
	29	NE	NE	-	NE	NE	-
Total K (mmol)	12	-	-	-	-	-	-
	25	-	+77%	+60%	-	+136%	+128%
	29	NE	NE	-	NE	NE	-
Total Cl (mmol)	12	-	-	-	-	+246%	+171%
	25	-	-	-	-	+213%	+157%
	29	NE	NE	-	NE	NE	-
Total Ca (mmol)	12	-	-	-	-	+218%	+206%
	25	+113%	+219%	+131%	+113%	+104%	-
	29	NE	NE	-	NE	NE	-

(R)- Recovery
- Not statistically significant
NE- Not evaluated

Gross pathology: All main study and recovery animals, including decedents, were subject to necropsy. Satellite animals from Group 2 and 3 and those reallocated to the main study from Group 4, were also subject to necropsy following completion of blood sampling for hormone analysis. Each animal was injected with sodium pentobarbitone and then exsanguinated. A full macroscopic examination was conducted.

The following table is a summary of the gross pathology from main study and recovery animals. The main study animals included early decedents. One male in the LD group and 7 HD males and 6 HD females were sacrificed prior to schedule termination.

In brief, macroscopic findings included thin appearance at the end of study for males and females and findings at necropsy in the adrenal glands, liver, lung, stomach and thymus in males and females. Males (testes, epididymides, prostate and seminal vesicles) and females (uterus and ovary) showed changes in reproductive organs. Additional macroscopic observations were related to injection site changes in both males and females (connective tissues, skin, and injection sites).

Macroscopic Observations

Finding	Icatibant dose (mg/kg/day)							
	Males				Females			
	0	3	10	30	0	3	10	30
Adrenal	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Large	0	0	2	6	0	15	16	6
Soft	0	0	0	1	0	0	0	1
Recov. Adrenal	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Pale	0	0	0	1	0	0	0	0
Large	0	0	0	0	0	1	2	0
Thin	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
	0	0	0	3	0	2	1	4
Recov. Thin	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
	0	0	0	0	0	1	0	2

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Finding	Icatibant dose (mg/kg/day)							
	Males				Females			
	0	3	10	30	0	3	10	30
Liver	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Pale	3	1	0	7	0	0	0	1
Large	0	0	0	3	0	0	0	1
Thick	0	0	0	2	0	0	0	0
Mass	0	0	0	0	0	1	0	0
Recov. Liver	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Pale	0	1	0	1	0	0	0	0
Thick	0	0	0	0	0	0	0	0
Large	0	0	0	0	0	0	0	0
Mass	0	0	0	0	0	0	0	0
Lung	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Mottled	0	0	0	0	0	0	0	1
Recov. Lung	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Mottled	0	0	0	0	0	0	0	0
Stomach	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Pale	0	0	0	2	0	0	0	1
Thick	0	0	0	0	0	0	0	1
Recov. Stomach	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Pale	0	0	1	0	0	0	0	0
Thick	0	0	0	0	0	0	0	0
Thymus	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Small	0	0	1	4	0	9	8	5
Dark	0	0	0	0	0	1	0	1
Recov. Thymus	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Small	0	0	0	0	0	0	0	0
Dark	0	0	0	0	0	0	0	0
Ovary	NA	NA	NA	NA	N=20	N=20	N=20	N=26
Red					0	0	0	1
Large					0	5	15	5
Dark					0	0	3	3
Small					0	0	0	3
Recov. Ovary	NA	NA	NA	NA	N=10	N=9	N=10	N=5
Red					0	0	0	0
Large					0	1	1	1
Dark					0	0	0	0
Small					0	0	0	0
Uterus	NA	NA	NA	NA	N=20	N=20	N=20	N=26
Thin					0	0	5	24
Recov. Uterus	NA	NA	NA	NA	N=10	N=9	N=10	N=5
Thin					0	0	1	0
Epididymides	N=20	N=20	N=20	N=26	NA	NA	NA	NA
Small	0	0	2	6				
Recov. Epididymides	N=10	N=10	N=10	N=5	NA	NA	NA	NA
Small	0	0	1	0				
Prostate	N=20	N=20	N=20	N=26	NA	NA	NA	NA
Small	0	0	4	19				
Recov. Prostate	N=10	N=10	N=10	N=5	NA	NA	NA	NA
Small	0	0	1	0				
Seminal vesicle	N=20	N=20	N=20	N=26	NA	NA	NA	NA
Small	0	0	12	24				
Recov. Seminal vesicle	N=10	N=10	N=10	N=5	NA	NA	NA	NA

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Finding	Icatibant dose (mg/kg/day)							
	Males				Females			
	0	3	10	30	0	3	10	30
Small	1	0	1	0				
Testes	N=20	N=20	N=20	N=26	NA	NA	NA	NA
Soft	0	1	2	3				
Small	1	1	5	20				
Dark	0	1	0	3				
Pale area	0	1	1	4				
Recov. Testes	N=10	N=10	N=10	N=5	NA	NA	NA	NA
Small	0	0	1	0				
Soft	0	0	0	0				
Dark	0	0	0	0				
Pale area	0	0	0	1				
Abnormal appearance	0	0	1	0				
Connective tissue	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Thick	0	0	1	3	0	0	2	5
Recov Connect tissue	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Thick	0	0	0	0	0	0	0	0
Skin + Subcutis	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Sore	0	0	4	16	0	0	2	8
Lesion	0	0	0	1	0	0	0	0
Fur loss	0	0	1	11	0	3	2	6
Mass 1	0	0	0	0	0	0	1	0
Mass 2	0	0	0	0	0	0	1	0
Recov. Skin + Subcutis	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Sore	0	0	1	1	0	0	0	0
Lesion	0	0	0	0	0	0	0	0
Fur loss	1	0	1	0	4	0	0	1
Mass 1	0	0	0	1	0	1	0	0
Mass 2	0	0	0	0	0	0	0	0
Right hip	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Sore	0	0	7	25	0	0	2	20
Fur loss	0	0	3	22	0	1	4	19
Red	0	0	0	1	0	0	0	0
Recov. Right hip	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Sore	0	0	0	3	0	0	0	1
Fur loss	0	0	1	2	0	0	0	0
Red	0	0	0	0	0	0	0	0
Right Mid Flank	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Sore	0	0	5	25	0	0	9	21
Fur Loss	0	0	2	21	0	0	3	18
Lesion	0	0	0	0	0	0	0	1
Red	0	0	0	0	0	1	0	1
Recov. Right Mid Flank	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Sore	0	0	0	2	0	0	0	0
Fur Loss	0	0	0	1	0	0	0	0
Red	0	0	0	0	0	0	0	0
Lesion	0	0	0	0	0	0	0	0
Right shoulder	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Sore	0	0	6	26	0	0	2	19
Fur loss	0	0	1	21	5	6	2	19
Recov Right shoulder	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Sore	0	0	1	2	0	0	0	0

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Finding	Icatibant dose (mg/kg/day)							
	Males				Females			
	0	3	10	30	0	3	10	30
Fur loss	2	0	0	0	4	3	0	0
Rump	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Sore	0	0	8	21	0	0	5	10
Fur loss	0	0	3	22	0	0	4	16
Recov. Rump	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Sore	0	0	2	2	0	0	0	0
Fur loss	0	0	0	2	0	0	0	0
Left hip	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Sore	0	0	10	26	0	0	3	20
Fur loss	0	0	3	22	0	0	3	18
Recov. Left hip	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Sore	0	0	0	5	0	0	0	0
Fur loss	0	0	0	1	0	0	0	1
Left mid flank	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Sore	0	0	6	22	0	0	5	16
Fur loss	0	1	0	18	0	0	3	16
Red	0	0	0	0	0	0	0	2
Red area	0	0	0	1	0	0	0	0
Recov. Left mid flank	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Fur loss	0	0	0	0	1	0	0	0
Sore	0	0	0	0	0	0	0	0
Red area	0	0	0	0	0	0	0	0
Left shoulder,	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Sore	0	0	2	21	0	1	3	24
Fur loss	0	0	2	20	4	4	3	17
Red	0	0	1	0	0	0	0	1
Recov. Left shoulder	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Fur loss	0	0	0	0	4	1	0	0
Sore	0	0	0	1	0	0	0	1
Red	0	0	0	0	0	0	0	0

NA- Not applicable

Organ weights: The adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes + epididymides, thyroid + parathyroids were weighed at the scheduled necropsy for all animals.

Increases in absolute liver weights for MD and HD females and liver weights adjusted for body weights were increased in HD males compared to their respective controls. Adrenal weights (absolute and adjusted for body weight) were increased in all males and females treated with icatibant compared to controls. In males, this increase was dose related and in females this increase did not show a dose relationship. Thymic weights (absolute and adjusted to body weights) were decreased in a dose related manner in males and were decreased in a non-dose dependent manner in females. Male reproductive organs (prostate, testes, and epididymides) showed a dose related decrease in absolute weight and adjusted for body weight. Whereas in females, the ovaries were increased in the icatibant treated groups compared to the control treated females. These data are summarized in the following table.

Reference ID: 2977968

Δ% of Parameter in Icatibant Treated Group *vs.* Controls

Organ	Males (mg/kg/day)			Females (mg/kg/day)		
	3	10	30	3	10	30
Liver						
Absolute wt	-	-	-	-	+25%	+35%
Rel to BW	-	-	+27%	NP	NP	NP
Adrenals						
Absolute wt	+18%	+59%	+68%	+77%	+118%	+76%
Rel to BW	+23%	+70%	+83%	+76%	+92%	+56%
Thymus						
Absolute wt	-15%	-25%	-35%	-35%	-6%	-11%
Rel to BW	-8%	-6%	-11%	-36%	-19%	-20%
Prostate				NA	NA	NA
Absolute wt	-30%	-47%	-62%			
Rel to BW	-30%	-47%	-63%			
Testes/epididymides				NA	NA	NA
Absolute wt	-12%	-24%	-44%			
Rel to BW	-10%	-19%	-38%			
Ovaries	NA	NA	NA			
Absolute wt				+38%	+187%	+32%
Rel to BW				+37%	+158%	+18%

(R)- Recovery
- Not statistically significant
NA- Not applicable
NP- Data not provided

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

From the main study animals, a complete histopathological battery of tissues was examined for controls, HD and any early decedents. Organs that were identified in the 13-week repeat dose SC rat study or organs identified as potential target organs in the high-dose group were also microscopically examined for the low dose and mid-dose groups. These tissues included: adrenals glands (males and females), bone marrow (males and females), injection sites (males and females), thymus (males and females), epididymides (males), prostates (males), testes (males), seminal vesicles (males), mammary glands (females), ovaries(females), uteri(females), and vagina (females), liver (males and females), brown fat (males and females), kidneys (males) and spleens (females).

From the recovery animals, a completed battery of tissues was examined from the control, HD and early decedents groups. From the LD and MD groups the adrenal glands, injection sites and brown fat were examined in males and females. The epididymides and testes were examined for LD and MD males. For females, the ovaries and spleen were examined in the LD and MD groups. The following table summarizes incidences and severities of the icatibant related histopathological findings in males and females treated with 0 (VC), 3, 10 and 30 mg/kg/day.

Reference ID: 2977968

Microscopic Observations

Finding	Icatibant dose (mg/kg/day)							
	Males				Females			
	0	3	10	30	0	3	10	30
Adrenal cortex	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Diff hypertroph zona fasc								
Min	0	14	3	2	0	5	1	0
Slight	0	1	14	5	0	6	2	7
Mod	0	0	3	19	0	5	6	6
Marked	0	0	0	0	0	3	11	13
Recov. Adrenal cortex	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Diff hypertroph zona fasc								
Min	0	0	0	2	1	0	0	4
Aorta	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Macrovesicular vacuolation of brown fat								
Min	0	0	3	4	0	6	2	2
Slight	0	2	8	8	0	6	4	6
Mod	0	1	0	5	0	1	9	13
Marked	0	0	0	0	0	0	5	5
Recov. Aorta	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Macrovesicular vacuolation of brown fat								
Min	0	0	0	0	0	0	1	0
Bone sternum w marrow	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Decreased hematopoietic tissue								
Min	1	2	2	10	1	2	1	2
Slight	0	0	4	3	0	10	10	9
Mod	0	0	0	0	0	4	9	15
Recov Bone sternum w marrow	N=10	-	-	N=5	N=10	-	-	N=5
Decreased hematopoietic tissue, Min	0			0	0			0
Connective tissue (abdominal fat)			N=1	N=3			N=3	N=5
Macrovesicular vacuolation of brown fat								
Slight	-	-	0	1	-	-	-	-
Moderate	-	-	1	2	-	-	1	5
Recov. Connective tissue (abdominal fat)								
Macrovesicular vacuolation of brown fat	-	-	-	-	-	-	-	-
Femur joint	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Decrease hematopoietic tissue								
Min	0	4	3	5	0	7	4	2
Slight	0	3	3	6	0	6	9	5
Mod	0	0	3	3	0	1	7	19
Recov. Femur joint	N=10	-	-	N=5	N=10	-	-	N=5
Decrease hematopoietic tissue, Min	0			0	0			0
Kidneys	N=20	N=20	N=20	N=26	N=20		N=1	N=26
Unilateral hydronephrosis								

Reference ID: 2977968

Finding	Icatibant dose (mg/kg/day)							
	Males				Females			
	0	3	10	30	0	3	10	30
Min	2	1	2	6	2	-	0	3
Slight	1	2	4	0	1	-	0	2
Bilateral hydronephrosis								
Min	0	0	0	1	0	-	0	3
Slight	1	1	1	1	0	-	1	3
Focal urothelial hyperplasia								
Min	2	5	4	6	1	-	0	4
Slight	0	0	0	1	0	-	0	0
Bilateral urothelial inflam								
Min	0	0	4	4	0	-	0	0
Recov. Kidneys	N=10	-	-	N=5	N=10	-	-	N=5
Bilateral hydronephrosis								
Min	0			0	0			1
Focal urothelial hyperplasia								
Min	3			0	2			0
Liver	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Hypertrophy of centrilobular hepatocytes								
Min	0	0	1	11	0	0	0	13
Slight	0	0	0	6	0	0	0	8
Recov. Liver	N=10	-	-	N=5	N=10	-	-	N=5
Hypertrophy of centrilobular hepatocytes	0			0	0			0
Lungs	N=20	N=13	N=12	N=26	N=20	N=12	N=12	N=26
Aggregates of alveolar macrophages								
Min	6	4	5	10	3	5	4	4
Slight	2	0	5	2	2	2	0	6
Agonal congestion present	0	0	0	3	0	0	0	2
Recov. Lungs	N=10	-	-	N=5	N=10	-	-	N=5
Aggregates of alveolar macrophages								
Min	0			1	3			2
Slight	0			1	1			1
Thymus	N=20	N=19	N=20	N=23	N=20	N=20	N=19	N=25
Lymphocyte depletion								
Min	1	13	9	7	0	5	5	6
Slight	0	0	6	6	0	13	8	15
Mod	0	0	1	5	0	1	6	3
Recov. Thymus	N=10	-	-	N=5	N=10	-	-	N=5
Lymphocyte depletion								
Min	3			1	1			0
Epididymides	N=20	N=20	N=20	N=26	NA	NA	NA	NA
Bilateral hypospermia								
Min	0	0	0	5				

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Finding	Icatibant dose (mg/kg/day)							
	Males				Females			
	0	3	10	30	0	3	10	30
Slight	0	0	0	6				
Moderate	0	0	0	1				
Marked	0	0	0	1				
Severe	0	1	0	0				
Intratubular degenerate spermatozoa/spermatids								
Min	0	2	0	11				
Slight	0	0	0	1				
Epididymides	N=10	N=10	N=10	N=5	NA	NA	NA	NA
Bilateral hypospermia								
Min	0	0	0	0				
Intratubular degenerate spermatozoa/spermatids								
Min	0	0	0	1				
Prostate gland	N=20	N=20	N=20	N=26	NA	NA	NA	NA
Reduced secretion								
Min	0	9	7	1				
Slight	0	0	7	8				
Mod	0	0	3	17				
Recov. Prostate gland	N=10	-	-	N=5	NA	NA	NA	NA
Reduced secretion								
Min	0			0				
Seminal Vesicles	N=20	N=20	N=20	N=26	NA	NA	NA	NA
Reduced secretion								
Min	0	7	6	0				
Slight	0	0	8	7				
Moderate	0	0	3	17				
Marked	0	0	0	2				
Recov. Seminal Vesicles	N=10	-	-	N=5	NA	NA	NA	NA
Reduced secretion								
Min	1			0				
Testes	N=20	N=20	N=20	N=26	NA	NA	NA	NA
Bilateral germinal epithelial degeneration								
Min	0	3	3	5				
Slight	0	3	3	7				
Mod	0	0	1	4				
Marked	0	1	0	1				
Dystrophic tubular mineralization present	0	4	5	8				
Intra-tubular sperm stasis								
Min	0	1	1	1				
Spermatid retention, Min	0	0	0	8				
Recov. Testes	N=10	N=10	N=10	N=5	NA	NA	NA	NA
Bilateral germinal epithelial degeneration								
Min	0	5	0	0				

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Finding	Icatibant dose (mg/kg/day)							
	Males				Females			
	0	3	10	30	0	3	10	30
Slight	0	0	1	1				
Mod	0	0	1	1				
Dystrophic tubular mineralization present	0	0	1	1				
Mammary Gland	NA	NA	NA	NA	N=20	N=20	N=20	N=25
Mascularization of mammary tissue, present					0	7	18	25
Recov. Mammary Gland	NA	NA	NA	NA	N=9	-	-	N=5
Mascularization of mammary tissue, present					0			0
Ovaries	NA	NA	NA	NA	N=20	N=20	N=20	N=26
Number of corpora lutea								
0-49					19	6	0	9
50-99					1	13	9	13
100-149					0	1	10	3
150-199					0	0	1	1
Number developing follicles								
1-5					2	1	8	13
6-10					5	6	6	10
11-15					5	10	6	0
16-20					5	2	0	1
21-25					1	1	0	0
26-30					2	0	0	0
Sex cord stromal hyperplasia, min					0	0	0	1
Degenerate corpora lutea present					0	2	14	16
Recov. Ovaries	NA	NA	NA	NA	N=10	N=9	N=10	N=5
Number of corpora lutea								
0-49					9	8	8	5
50-99					1	1	2	0
100-149					0	0	0	0
150-199					0	0	0	0
Number developing follicles								
1-5					3	3	7	0
6-10					2	6	1	0
11-15					4	0	1	0
16-20					0	0	1	0
26-30					0	0	0	1
36-40					0	0	0	2
41-45					0	0	0	1
46-50					0	0	0	1
61-65					9	9	10	5
Degenerate corpora lutea								

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Finding	Icatibant dose (mg/kg/day)							
	Males				Females			
	0	3	10	30	0	3	10	30
present					0	0	4	0
Uterus	NA	NA	NA	NA	N=20	N=20	N=20	N=26
Atrophy								
Min					0	0	7	0
Slight					0	0	4	0
Mod					0	0	9	0
Marked					0	0	0	26
Uterus	NA	NA	NA	NA	N=10	-	-	N=5
Atrophy					0			0
Vagina	NA	NA	NA	NA	N=20	N=20	N=20	N=26
Atrophic/anoestrus, present					0	0	16	24
Vagina	NA	NA	NA	NA	N=10	-	-	N=5
Atrophic/anoestrus, present					0			0
Skin	N=20	N=20	N=20	N=26	N=20	N=3	N=4	N=26
Focal fibrosing dermatitis								
Min	0	0	0	2	0	1	2	0
Slight	0	0	1	0	0	0	1	2
Mod	0	0	0	6	0	0	0	4
Marked	0	0	0	0	0	0	0	1
Focal ulceration					N=20	N=20	N=20	N=26
Slight	0	0	2	0	0	0	0	0
Moderate	0	0	0	6	0	0	0	5
Marked	0	0	1	1	0	0	0	2
Severe	0	0	0	2	0	0	0	1
Focal degeneration of muscle, slight	0	0	0	0	0	0	0	2
Recov. Skin	N=10	-	N=1	N=5	N=10	-	-	N=5
Focal fibrosing dermatitis								
Mod	0		1	1	0			0
Focal ulceration								
Moderate	0		0	1	0			0
Marked	0		1	0	0			0
Focal degeneration of muscle, slight	0		0	0	0			0
Skin Tumors,								
Keratoacanthoma	0	1	0	0	0			0
Squamous papilloma	0	-	0	1	0			0
Injection Sites	N=20	N=20	N=20	N=26	N=20	N=20	N=20	N=26
Focal fibrosing dermatitis								
Min	0	1	1	0	3	2	3	0
Slight	0	0	3	0	0	1	8	0
Moderate	0	0	10	8	0	1	8	8
Marked	0	0	4	17	0	0	0	18
Severe	0	0	0	1	0	0	0	0

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Finding	Icatibant dose (mg/kg/day)							
	Males				Females			
	0	3	10	30	0	3	10	30
Focal ulcerations								
Min	0	0	0	0	1	0	1	0
Slight	0	0	1	0	0	0	2	0
Mod	0	0	7	7	0	1	6	7
Marked	0	0	4	16	0	0	0	18
Severe	0	0	0	3	0	0	0	0
Focal degeneration of muscle								
Min	0	0	3	0	0	0	10	2
Slight	0	1	6	3	0	0	2	6
Mod	0	0	1	7	0	0	0	2
Subcutaneous inflam cell infiltration								
Min	0	3	1	3	1	0	4	1
Slight	0	0	1	1	0	0	0	0
Subcutaneous edema								
Min	0	0	0	2	0	0	0	0
Slight	0	0	0	1	0	0	0	0
Dermal hemorrhage								
Min	0	0	1	0	0	0	0	0
Slight	1	0	0	3	0	0	1	3
Mod	0	0	0	0	0	0	0	1
Abscessation, Mod	0	0	0	0	0	0	0	1
Recov. **Injection Sites**	N=10	N=10	N=10	N=5	N=10	N=9	N=10	N=5
Focal fibrosing dermatitis								
Min	2	1	3	0	1	2	6	0
Slight	0	1	2	1	0	0	1	5
Moderate	0	0	3	4	0	0	0	0
Focal ulcerations								
Min	0	0	0	1	0	0	0	0
Slight	0	0	0	2	0	0	0	0
Mod	0	0	0	1	0	0	0	0

- Not examined
NA- Not applicable

The target organs of toxicity in the male and female rats included skin, injection sites, adrenal glands, aorta, bone marrow (femur joint and marrow from sternum), kidneys, liver, lungs, thymus, connective tissues, and the reproductive system. Reproductive organs included vagina, ovaries, uterus, mammary glands (mascularization) in females and prostate, epididymides, testes, and seminal vesicles in males. The dose-limiting toxicity in this study was severe injection site irritation observed with increased incidence and severity compared to the vehicle control group animals at 10 mg/kg/day dose in males and females. Hemorrhage, ulceration and focal muscle degeneration were observed in males and females with significant increases in incidence and

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severity at doses ≥10 mg/kg/day. Additionally, the 30 mg/kg/day dose resulted in early sacrifice of males and females for humane reasons due to extreme local injection site toxicity.

No NOAEL could be determined for this study as findings in the male and female reproductive organs, thymus, bone marrow (sternum and femur joint), aorta and adrenal gland were observed in the LD group (3 mg/kg/day). However, the reviewer considered the MTD to be the 3 mg/kg/day dose based on tolerable levels of injection site reaction and decreases in male body weights did not exceed 10% of the controls. The 10 mg/kg/day dose exceeded the MTD based on significant local injection site toxicity in males and females and on a decrease in male body weights (-17%) that exceeded 10%.

Toxicokinetics: Blood samples (0.5 mL) were drawn from satellite animals on Day 1 and in Week 26 at the following time points: 0.5, 1, 2, 4, 8 and 24 hours post-dose. Samples were collected from the lateral caudal vein and plasma was separated. The levels of icatibant and its M2 metabolite were determined using LC-MS/MS after solid phase extraction. The lower limit of quantitation was 25.0 ng/mL for icatibant and 2.5 ng/mL for the M2 metabolite, respectively. From these data the T_{max}, C_{max} and AUC_{0-t} were calculated for icatibant and M2. These data are summarized in the following table.

Time	Parameter	Icatibant SC Dose (mg/kg/day)					
		3		10		30	
		M	F	M	F	M	F
	Icatibant levels						
Day 1	C_{max} (ng/mL)	1580	1620	4110	5540	9080	11800
	t_{max} (h)	2.0	1.0	2.0	2.0	2.0	2.0
	AUC_{0-t} (ng*h/mL)	4360	4250	16100	15700	45300	41100
	AUC_{0-inf} (ng*h/mL)	-	4640	16700	23800	60500	43200
	$t_{1/2}$ (h)	-	1.03	1.47	2.36	3.59	1.61
Week 26	C_{max} (ng/mL)	2160	1840	6160	6190	10200	15200
	t_{max} (h)	2.0	1.0	2.0	2.0	1.0	2.0
	AUC_{0-t} (ng*h/mL)	7270	5170	25100	18100	53800	61300
	AUC_{0-inf} (ng*h/mL)	7380	6340	25300	29300	64000	62400
	$t_{1/2}$ (h)	1.13	1.49	0.992	2.57	2.81	1.23
	M2 Metabolite levels						
Day 1	C_{max} (ng/mL)	144	138	295	383	614	652
	t_{max} (h)	2	2	2	2	2	2
	AUC_{0-t} (ng*h/mL)	579	359	1850	1610	6070	4530
	$AUC_{0-infin}$ (ng*h/mL)	589	-	1870	1630	6110	4570
	$t_{1/2}$ (h)	1.19	-	3.55	1.07	3.14	3.47
Week 26	C_{max} (ng/mL)	134	80.4	259	219	510	624
	t_{max} (h)	2	2	2	2	4	2
	AUC_{0-t} (ng*h/mL)	788	363	1560	1200	4840	3830
	$AUC_{0-infin}$ (ng*h/mL)	800	370	1600	1210	4920	3930
	$t_{1/2}$ (h)	3.86	1.22	4.27	3.90	3.91	4.46

- Data not available at this time point

Icatibant plasma concentrations were approximately 11 to 15-fold higher than those to M2. Exposure to icatibant was comparable between males and females with M2 levels higher (~47%) in males than females at all doses. There was an increase in icatibant systemic exposure over 26 week dosing period at all dose levels. M2 levels did not show this increase. The dose response

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for both icatibant and M2 systemic exposures were ~ proportional across 3 to 30 mg/kg/day on day 1.

Other: Hormone analysis was conducted by sampling blood from all satellite animals in Weeks 13, 26 and from all satellite animals and surviving HD animals Week 30. Males were assessed for changes in levels of testosterone, lutenizing hormone (LH), and follicle stimulating hormone (FSH). Females were assessed for changes in LH and FSH.

All icatibant treated males showed a statistically significant decrease in testosterone and FSH levels compared to control males in Weeks 13 and 26 of the study. After the 30-day recovery period the MD and HD group testosterone levels remained decreased compared to control but not as severely as during the dosing phase of the study. The FSH levels in all treated males were significantly greater than controls after the 30 day recovery period which is considered a rebound effect.

All females treated with icatibant showed a decrease in LH compared to control females during Weeks 13 and 26 of the study. After the 30 day recovery period these levels remained reduced compared to control females. There were no changes observed in male LH levels throughout the study. The following table summarizes these results.

Δ% of Parameter in Icatibant Treated Group *vs.* Controls

Parameter	Week	Males (mg/kg/day)			Females (mg/kg/day)		
		3	10	30	3	10	30
Testosterone (mmol/L)	13	-82%	-90%	-94%	NA	NA	NA
	26	-82%	-93%	-93%	NA	NA	NA
	30 (R)	-	-45%	-12%	NA	NA	NA
FSH (ng/mL)	13	-17%	-26%	-39%	-	-	-
	26	-34%	-54%	-46%	-30%	-	-15%
	30 (R)	+63%	+93%	+70%	-	-	-
LH (ng/mL)	13	-	-	-	-69%	-69%	-69%
	26	-	-	-	-33%	-33%	-33%
	30 (R)	-	-	-	-56%	-56%	-56%

(R)- Recovery
- Not statistically significant.
NA- Not applicable.

2.6.6.9 Discussion and Conclusions

In support of the proposed male rat carcinogenicity protocol, Jerini submitted a 26-week repeat dose toxicity study conducted in male and female rats. Rats were subcutaneously administered icatibant at 0 (saline vehicle), 3, 10 and 30 mg/kg/day. The target organs of toxicity in male and female rats were skin, injection sites, adrenal glands, aorta (brown fat), bone marrow (femur joint and marrow from sternum), kidneys, liver, lungs, thymus, connective tissues, and the reproductive system. Reproductive organs included vagina, ovaries, uterus, mammary glands (mascularization) in females and prostate, epididymides, testes, and seminal vesicles in males. These target organs of toxicity were previously identified in the 13-week repeat dose SC rat study with the exception of skin, connective tissue and the aorta. In the 26 week study, no NOAEL could be determined due to findings in the male and female reproductive organs, thymus, bone marrow (sternum and femur joint), aorta and adrenal gland that were observed in the LD group (3 mg/kg/day). The MTD was considered 3 mg/kg/day in males and females based

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on severe injection site toxicity (hemorrhage, ulceration and focal muscle degeneration) at ≥10 mg/kg/day in males and females; male body weight loss exceeding 10% a doses ≥10 mg/kg/day; and moribund conditions in males and females at 30 mg/kg/day dose that resulted in early sacrifice. Similar to the 13-week study, males showed a significant decrease in body weight gains that exceeded the 10% compared to controls at all doses. This effect is a result of icatibant's pharmacodynamic activity resulting in the inhibition of testosterone secretion which in turn reduces muscle mass. The LD of 3 mg/kg/day resulted in a slight decrease in male body weight (-6% compared to controls) and all male rats in this group were viable and in relatively good health at the end of the 26-week study.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

Icatibant acetate is being developed for the treatment of patients with hereditary angioedema (HAE) who are 18 years old and older. In Type I (quantitative deficiency) and Type II (qualitative deficiency) HAE a deficiency of C1-esterase inhibitor (C1-INH) results in an increased release of bradykinin (BK). BK binds to the bradykinin type-2 receptor (B_2) to trigger activation of many cascades leading to vasodilatation, increased vascular permeability and smooth muscle cell contraction, resulting in the increased vascular permeability and acute inflammation observed in HAE patients. Icatibant is a synthetic decapeptide that is a selective B_2 receptor antagonist that binds to the B_2-receptor with similar affinity as BK. Icatibant binding at the B_2 receptor inhibits bradykinin induced processes and is hypothesized to contribute to the decrease of vascular permeability and acute inflammation observed in HAE patients. The anticipated clinical dosing of icatibant in patients having an HAE attack is 30 mg/day up to 3 times daily SC. Therefore, the maximum recommended human dose (MRHD) of icatibant is 90 mg/day, which is associated with AUC levels of 14.27 mcg*h/mL (extrapolated AUC levels observed in elderly females treated with 30 mg/day SC dosing).

Absorption, distribution, metabolism and elimination (ADME) studies have been conducted in the rat. Icatibant is metabolized to the active metabolites M1 (b) (4) and M2 (b) (4) by hydrolysis of icatibant's peptide backbone, thought to be induced by peptidases. The M1 is formed at >90% and 70% in man and rat, respectively. M2 is formed in rat ~9% and in human ~75%. After SC administration in rats, distribution studies showed that icatibant (or its metabolites) is distributed into the kidneys, liver, urinary bladder, lungs and spleen 1 hour post-dose and is still detectable in the liver, kidneys, spleen, bone marrow, adrenals and injection sites at 24-hours post-dose. After repeat SC dosing in rats, icatibant is rapidly distributed and metabolized with the M2 metabolite appearing from 0.2 to 3 hours post-dose. Neither icatibant nor M2 accumulated after repeat dosing in male and female rats up to 13-weeks dosing based on the currently submitted TK data. The half-life was more than 5 hours after the 26 µg/kg SC dose in rats and terminal half-life was over 6 days in rats after the 10 µg/kg, SC dose. There were no

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gender differences observed in rats in the systemic exposures of males and females. Serum protein binding of icatibant was low with 44 and 49% in man and rat, respectively.

Neither icatibant nor the M1 or M2 metabolites is metabolized by human hepatocytes or microsomes. Additionally, icatibant is not an inducer of CYP450 isozymes. Based on excretion studies, the primary route of elimination after SC administration of icatibant is via the kidneys in rats (~61%) with small amounts excreted in the feces (8.0% to 33%).

Jerini completed a full genetic toxicity battery assessment for icatibant (Reviewed number 4, dated June 12, 2008). Icatibant did not induce genetic toxicity in the presence or absence of metabolic activation in the bacterial reverse mutation assays or *in vitro* mammalian chromosomal aberration studies or micronuclei formation in *in vivo* assays under the conditions tested.

In support of the carcinogenicity doses, a 26-week repeat dose SC rat study was evaluated. Male and female rats were subcutaneously administered icatibant at 0, 3, 10 and 30 mg/kg/day. Seven HD males and 6 HD females were sacrificed between weeks 13 and 26 due to severe injection site toxicity. At week 26, the remaining HD animals with the exception of 5 rats/sex kept for recovery assessment were sacrificed for humane reasons related to injection site toxicity. Clinical signs of toxicity included sores and lesions, staining of fur, excessive activity and thin appearance in males and females. Males showed a decreased in BW gains (-20 to -74%) and body weights (-6 to -26%) compared to controls. Females treated with icatibant did not show this same effect on BW gain. This effect on male body weight is a result of icatibant's mechanism of action. Icatibant inhibits bradykinin binding which regulates hormone secretion of the pituitary. As a result of bradykinin inhibition, testosterone secretion is significantly reduced at all dose levels administered to male rats resulting in a decrease in muscle mass in the males. Urinalysis showed a significant increase in electrolyte levels (+670% to +246%) in males and females, which coincided with an increase in urine excretion volume. The adrenal wts (+18 to 118%) and liver wts (+25 to +35%) were increased in males and females and thymic wts (- 6 to -35%) were decreased in males and females. In males, prostate (-30 to -63%) and testes plus epididymal wts (-10 to -44%) were decreased. In females, ovarian wts were increased (+18 to +187%). The target organs of toxicity in male and female rats included skin, injection sites, adrenal glands, aorta (macrovesicular vacuolation of brown fat), bone marrow (femur joint and marrow from sternum), kidneys, liver, lungs, thymus and connective tissues. Female reproductive organs (vagina, ovaries, and uterus) and female mammary gland (mascularization) and male reproductive organs (prostate, epididymides, testes, and seminal vesicles) were also target organs of toxicity. The dose limiting toxicity in males and females were injection site irritation (hemorrhage, ulceration and focal muscle degeneration) at doses ≥ 10 mg/kg/day. Ulcerations at injection sites and moribund conditions in males and females at 30 mg/kg/day dose resulted in early sacrifice. In the females, the target organs of toxicity were the reproductive organs (MD and HD for vagina and uterus and all doses for ovaries), mammary glands (all doses), thymus (all doses), liver and kidneys (HD), and bone marrow, aorta and adrenal findings at all icatibant doses. In the male, dose limiting toxicity was severe injection toxicity at doses ≥ 10 mg/kg/day. Males were also observed with reproductive organ toxicities (all doses) that coincided with the drastic decreases in testosterone levels (-82% to -94%) and toxicities in the adrenals (all doses), aorta (all doses), bone marrow (all doses), kidneys (mainly HD), liver (HD), lungs (HD) and the thymus (all doses). The findings in the male and female reproductive organs are a result of the

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pharmacodynamic action of icatibant and its effects on the pituitary secretion. No NOAEL could be determined due to findings in the male and female reproductive organs, thymus, bone marrow (sternum and femur joint), aorta and adrenal gland that were observed in the LD group (3 mg/kg/day). The MTD was considered 3 mg/kg/day in males and females based on severe injection site toxicity (hemorrhage, ulceration and focal muscle degeneration) at doses ≥10 mg/kg/day in males and females, decreases in male body weights exceeding 10% at doses ≥10 mg/kg/day and moribund conditions in males and females at 30 mg/kg/day dose that resulted in early sacrifice. At the MTD, the $AUC_{0\text{-}Inf}$ levels were 7380 ng*h/mL and 6340 ng*h/mL in males and females, respectively.

The sponsor proposed doses [0 (vehicle), 1, 3 and 6 mg/kg/day] in male rats for the 104-week carcinogenicity study based on a MTD approach and on the secondary PD actions of icatibant. The sponsor considered the study results from the 13-week repeat dose SC rat study, the 26-week repeat dose rat study and the combined rat toxicity and fertility study to support their proposed doses. The 13-week repeat dose rat SC study (Study no. JE049-0160; doses of vehicle, 10, 20 or 30 mg/kg/day) resulted in treatment-related changes in adrenal glands, injection sites and thymus in males and females, epididymides, prostate glands, seminal vesicles and testes of males and mammary glands, ovaries, uterus and vagina of females. A significant decrease in body weight gains in males was also observed. In the combined rat toxicity and fertility study (JE049-0108) at daily doses of 0, 1, 3 and 10 mg/kg/day SC body weights and body weight gains in males were equal to or greater than control males throughout the 13-Week treatment period. Data from the 26-week rat study showed that the decrease in male body weight were related to the pharmacological effects of icatibant reducing testosterone levels. The sponsor stated that the 10 mg/kg/day icatibant dose in male rats will result in a significant reduction in circulating plasma testosterone and hence may be unacceptable for selection for administration for the duration of a carcinogenicity study. Furthermore the sponsor stated that although a dose dependent reduction in circulating testosterone will occur, selection of dose levels of 1, 3 and 6 mg/kg/day will have less negative impact on animal body weight. Based on these data, the sponsor selected doses at 1, 3 and 6 mg/kg/day in males.

The reviewer considers the 10 mg/kg/day dose to exceed the MTD in male rats based on injection site toxicity observed in the 26-week repeat dose SC study. Additional toxicities in males included male reproductive organs, adrenals, aorta (macrovesicular vacuolation of brown fat), bone marrow, connective tissue, kidneys, liver, lungs and thymus. Male body weights were reduced at all doses (≥6%), decreasing muscles mass. This decrease in BW (-6%) was tolerable for males through the 26-week dosing period at 3 mg/kg/day. Therefore, the reviewer does not consider this decrease in body weight a dose limiting toxicity. The sponsor did not evaluate the 6 mg/kg/day dose of icatibant in any of their rat studies. Based on extrapolation of the data, at 6 mg/kg/day a decrease of body weight would be approximately -10 to -12% after 26 weeks of SC administration. At the 6 mg/kg/day dose, it is not known whether injection toxicity would be substantial. At the proposed carcinogenicity doses of 1, 3 and 6 mg/kg/day in male rats, the expected exposure margins would be 0.17-, 0.52- and 1.03-fold, respectively, at the MRHD. Due to the very low potential exposure margin at the MTD of 3 mg/kg/day, the potential body weight decrease of ~10% at 26-weeks, and the need for relevant information related to clinical exposure, the reviewer considered the sponsors proposal of a HD of 6 mg/kg/day, SC to be reasonable.

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Internal comments: The Executive CAC meeting was held on October 21, 2008 to discuss the sponsor's proposed male rat carcinogenicity protocol. Based on the discussion at this meeting the following recommendations were given. These recommendations were captured in the meeting minutes and were faxed to the sponsor.

Male Rat:

- The Committee concurred with the proposed doses of 0 (VC), 1, 3, and 6 mg/kg/day for male rats, by SC injection and with injection site rotation, based on MTD (severe local toxicity).
- If a survival problem occurs during the study, the sponsor should immediately contact the reviewing division at the FDA prior to terminating any animals or changing any dosing.

Female Rat:

- The Committee noted that the June10, 2008 recommendation of the top dose of 10 mg/kg/day Icatibant may be revised to 6 mg/kg/day, SC in female rats. This revised recommendation was based on the significant injection site toxicity observed at the 10 mg/kg/day dose in females in the 26-week repeat-dose rat study submitted in support of the proposed male rat doses,

General comments:

- The potential carcinogenicity of the M2 metabolite will not be addressed by the proposed study. The sponsor should conduct and submit for review a dose ranging study for M2 for FDA concurrence on dose selection and consider adding an arm to the proposed carcinogenicity study. If a separate arm is not added, another carcinogenicity study on the M2 metabolite will be needed.
- The sponsor should omit ophthalmic exam and urine measurements.

External comments (to sponsor): None.

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12.5 Appendix 5: Pharmacology and Toxicology Review IND 68,214 (Serial No. 66), May 15, 2009

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

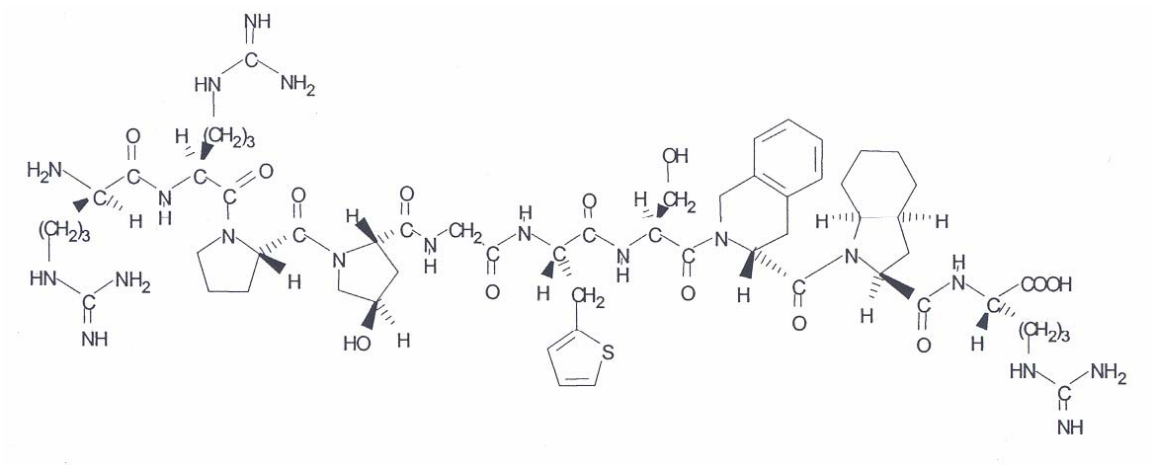
2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 68,214
Review number: 4
Sequence number/date/type of submission:
SDN 66/March 20, 2009/ (b) (4) qualification studies
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Jerini AG, Berlin Germany
Manufacturer for drug substance: (b) (4)

Reviewer name: Molly E. Shea, Ph.D.
Division name: Division of Pulmonary and Allergy Products (DPAP)
Review completion date: May 15, 2009

Drug:

Trade name: Firazyr
Generic name: Icatibant acetate
Code name: JE 049/HOE 140
Chemical name: D-arginyl-L-arginyl-L-prolyl-L[(4R)-4-hydroxyprolyl]-glycyl-L-[3-(2-thienyl)alanyl]-L-seryl-D-(1,2,3,4-tetrahydroisoquinolin-3-yl-carbonyl)-L-3aS,-7aS)-octahydroindol-2-yl-carbonyl]-L-arginine acetate
CAS registry number: NA
Amino acid sequence: H-D-Arg-L-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-OH· nCH_3COOH
Molecular formula/molecular weight: $C_{59}H_{89}N_{19}O_{13} \cdot C_2H_4O_2$/1304.6
Structure:



Relevant INDs/NDAs/DMFs: [redacted] (b) (4) NDA 22-150 (Complete Response Issued)

Drug class: Bradykinin type 2 (B_2) receptor antagonist

Intended clinical population: Patients with acute hereditary angioedema (HAE)

Clinical formulation: Icatibant will be delivered as a 10 mg/mL sterile, isotonic, acetate-buffered solution.

Route of administration: Subcutaneous injection (SC)

Proposed clinical protocol: None in this submission.

Previous clinical experience: Not applicable

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

Studies reviewed within this submission:

JE049-0167	Induction of micronuclei in the bone marrow of treated mice
Draft Protocol v5	[redacted] (b) (4) 14-day subcutaneous administration toxicity study in the rat

Studies <u>not</u> reviewed within this submission: None.

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Introduction and Drug History:

Jerini US, Inc. is developing their product icatibant acetate for the treatment of hereditary angioedema (HAE). The original Investigational New Drug application (IND 68,214) for the treatment of HAE was submitted to the Division of Gastrointestinal and Coagulation Drug Products April 8, 2004 and subsequently transferred to the Division of Pulmonary and Allergy Products (DPAP). The New Drug Application (NDA 22-150) was submitted to DPAP on October 26, 2007. The application was deemed not approvable under section 505(d) of the Act and 21 CFR 314.125(b) on April 23, 2008 with the action letter outlining a series of deficiencies. Item 6 (a) (9) of the letter states:

6. Submit revised drug substance specifications to include the following:

a. The following impurities in the drug substance and the drug product are not considered to be adequately qualified for safety.

9. (b) (4)

Conduct an adequate qualification study (a 3-month repeat-dose toxicity study in an appropriate species) that provides an appropriate margin of safety (generally 10-fold) at a NOAEL dose or modify your proposed acceptance criterion to less than (b) (4)

In this submission (SDN66), Jerini re-submitted the following studies in support of their proposed 14-day SC rat repeat dose toxicology (b) (4) qualification study: acute intravenous toxicity in rats (JE049-0155), Bacterial Reverse Mutation Test (JE049-0154), and *In vitro* mammalian chromosome aberration test in cultured human lymphocyte (JE049-156). These studies were previously reviewed under the NDA 22-150 as a chemistry consult (DFS document dated March 14, 2008). Based on this detailed review, it was concluded that a 3-month SC repeat dose toxicity study was needed to qualify the (b) (4) impurity.

Also included in this submission was an amended final study report (JE049-0167: induction of micronuclei in the bone marrow of treated mice with (b) (4) and a proposed draft Protocol v5 for their proposed 14-day subcutaneous administration toxicity study in the rat (sponsor no. 1001955). The study report and the proposed protocol are reviewed herein.

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2.6.6 TOXICOLOGY

2.6.6.4 Genetic toxicology

Study title: Induction of micronuclei in the bone marrow of treated mice

Key findings:

- Intravenous doses up to 60 mg/kg in mice did not induce the formation of MN-PCEs.

Study no.: JE049-0167

Volume #, and page #: Electronic
Conducting laboratory and location: (b) (4)
Date of study initiation: March 19, 2008
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: (b) (4) batch no 1005323, and 78.4% pure

Methods

Strains/species/cell line: Male Crl:CD-1 (ICR) mice (n= 6 males/dose group)

Doses used in definitive study: 0 (vehicle), 20, 60 and 200 mg/kg administered intravenously in dose volumes of 10 mL/kg with an infusion rate of 1.0 mL/min for 2 consecutive days

Basis of dose selection: An IV dose-range finding study was conducted in male and female mice up to 300 mg/kg/day (n= 1/sex) in a dose volume of 10 mL/kg and infused at 2.0 mL/min. Both the male and female convulsed and died immediately after dosing. An additional study was conducted dosing males and females (n=2/sex) with 200 mg/kg IV for 2 days and observed two day post-dosing. The sponsor stated that there were no gender differences in clinical signs of toxicity and therefore selected male mice for the micronucleus experiment. Male animals showed clinical signs of lethargy, pallor or extremities and decreased activity. One female showed decreased activity. The 200 mg/kg IV dose was tolerated and therefore, picked as the maximum dose for the micronucleus study.

Negative controls: Vehicle (0.9% NaCl)

Positive controls: 40 mg/kg Cyclophosphamide (CPA)

Incubation and sampling times: Male mice (n=6/group) were intravenously dosed with controls or treatment and observed immediately post-dose, at least 4 h post-dose and prior to the second administration for clinical signs of toxicity. Individual body weights were recorded. Test article and vehicle treated mice were sampled in groups, 24 h post the second administration. The CPA

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treated mice were sampled 24 h post-administration. Mice were sacrificed by an overdose of sodium pentobarbitone given by intraperitoneal injection and then cervical dislocation. Using a syringe and needle bone marrows were flushed from marrow cavity of femurs and then pelleted. The pellets were then prepared and fixed on slides and stained with Giemsa. Polychromatic and normochromatic erythrocytes were determined until at least a total of 1000 cells had been analyzed. Micronucleated polychromatic erythrocytes (MN-PCEs) were counted and ratios were determined and compared for the controls and the (b) (4) treated groups.

Results

Study validity: The sponsor considered the assay valid if the incidence and distribution of the MN-PCEs in the VC were consistent with historical control data; at least 5 animals out of each group were available for analysis, and the positive control group showed a statistically significant increased in frequency of MN-PCEs. The sponsor considered the clastogenic or aneugenic if all of the following are true: a statistically significant increase in frequency of MN-PCEs occurred at one or more dose levels, the incidence and distribution of MN-PCEs in individual animals exceeded historical control data, a dose-response trend in the proportion of MN-PCE was observed in 2 or more dose levels. If these criteria are not met then the test article is considered negative.

Study outcome: Four out of seven (4/7) mice convulsed and died prior to cell harvest in the 200 mg/kg, IV treated group. No cells were collected from any of the study animals in the HD group. The MTD was apparently exceeded in the 200 mg/kg IV group based upon observed mortalities. No clinical signs of toxicity were observed in the 20 and 60 mg/kg/day treated mice. The MN-PCE levels in the 20 and 60 mg/kg/day IV groups were within historical control levels and were similar to those seen in concurrent VC group. The dose of 60 mg/kg IV was within 1/3 of the MTD and considered to be acceptable as the high dose given the loss of the 200 mg/kg IV group. The cyclophosphamide positive control produced expected increases in the frequency of micronuclei. Based on the negative and positive controls providing the expected results and the (b) (4) treated animals showing no induction of micronuclei formation up to 60 mg/kg, IV, the study was considered valid and (b) (4) was negative for inducing micronuclei.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

(b) (4) is formed via hydrolysis over time. The sponsor proposed a specification of (b) (4) (b) (4) for (b) (4) in their drug product. This specification is beyond the threshold of (b) (4) (b) (4) for peptides as recommended during the TIDES Conference of 2005 (see NDA 22-150 Chemistry Consult). For peptide impurities or degradants that are (b) (4) the impurity or degradant needs to be fully identified, characterized and qualified for safety. In the Not Approvable action letter (April 23, 2008), the sponsor was notified that a 3-month SC repeat dose toxicology study was needed to qualify this degradant. Alternatively, they should reduce the level of (b) (4)

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The sponsor submitted the final study report of an in vivo mouse micronucleus study conducted with (b) (4) acute intravenous toxicity in rats (JE049-0155), Bacterial Reverse Mutation Test (JE049-0154), *In vitro* mammalian chromosome aberration test in cultured human lymphocyte (JE049-156), and a proposed draft study protocol for a 14-day subcutaneous rat study as a proposal to qualify the degradants. (b) (4) The acute intravenous toxicity in rats (JE049-0155), Bacterial Reverse Mutation Test (JE049-0154), and *In vitro* mammalian chromosome aberration test in cultured human lymphocyte (JE049-156) studies were previously reviewed under the NDA 22-150 as a chemistry consult (DFS document dated March 14, 2008). The *in vivo* mouse micronucleus assay was reviewed in this current submission and determined to be negative. The following is a review of the sponsor's proposed 14-day SC rat study.

Toxicology draft study protocol:

1. (b) (4) **14-day subcutaneous (injection) administration toxicity study in rat**

Methods: The 14-day study will be performed under GLP compliant conditions at a (b) (4) (b) (4) Rat Crl:WI (Han) rats (between 10 and 12 weeks old) will be assigned to one of 2 groups (control= sterile isotonic saline 0.9% NaCl, and 0.3 mg/kg/day) to receive once daily SC injections for 14 days.

Group	Main Study	Dose (mg/kg)	Dosing Frequency
Control	10M + 10F	0	Once daily
High	10M + 10F	0.3	Once daily

Rats will be administered 2 mL/kg of body weight of control or icatibant acetate. Seven injection sites will be used in rotation (first administration into left shoulder [IJ1], second administration into right shoulder [IJ2], third administration into right mid-side [IJ3], fourth administration into right hip [IJ4], fifth administration into rump [IJ5], sixth administration into left hip [IJ6], and seventh administration into left mid-side [IJ7]). The formulation will be prepared daily and filtered for sterility.

Observations will include: clinical signs (twice daily on dosing days with a detailed examination once weekly), local tolerance (once daily weeks 1 and 2), body weights (at randomization, twice weekly throughout the dosing period), food consumption (once weekly throughout the dosing period), hematology (a complete battery), coagulation, clinical chemistry (a complete battery), gross examination, organ weights, bone marrow smears, histopathology (all tissues from control and high dose animals; a complete histopathology battery excluding lacrimal glands).

Internal comments: The 14-day SC rat toxicology study is not adequately designed to support the safety qualification of (b) (4) As previously recommended in the Not Approvable action letter dated April 23, 2008, a 3-month SC repeat dose toxicity study in an appropriate species should be conducted to qualify the degradant. As HAE is considered a chronic intermittent indication, a study duration of at least 3-months is necessary to qualify this degradant. Additionally, the maximum human daily dose of the parent drug is 90 mg/day, or 1.8

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mg/kg. Considering the proposed (b) (4) specification for (b) (4) the maximum daily exposure to the degradant may be (b) (4). This clinical level would not be adequately supported by the proposed 0.3 mg/kg/day dose in the 14-day SC rat study (potential safety margin of 8.3 on a mg/kg basis with the assumption of 50 kg human body weight). The study should provide an appropriate margin of safety (~10-fold) at a NOAEL dose. Although dosing is at the sponsor's discretion, administration of only one dose strength may not allow for the identification of a NOAEL. Generally, three doses are used that allow for a characterization of toxicity as well as identification of a NOAEL.

Alternatively, the proposed acceptance criterion should be modified to less than (b) (4)

External comments (to sponsor):

Your proposed 14-day SC rat toxicology study is not adequately designed to support the safety qualification of (b) (4) As previously recommended in the Not Approvable action letter to your NDA 22-150 dated April 23, 2008, a 3-month SC repeat dose toxicity study in an appropriate species should be conducted to qualify this degradant. As hereditary angioedema is considered a chronic intermittent indication, a study duration of at least 3-months is necessary to qualify this degradant. Additionally, the maximum human daily dose of the parent drug is 90 mg/day, or 1.8 mg/kg based on 50 kg human body weight. Considering the proposed (b) (4) specification for (b) (4) the maximum daily exposure to the degradant may be (b) (4) (b) (4). This clinical level would not be adequately supported by the proposed 0.3 mg/kg/day dose in the 14-day SC rat study (potential safety margin of 8.3 on a mg/kg basis). The study should provide an appropriate margin of safety (~10-fold) at a NOAEL dose. Although dosing is at your discretion, administration of only one dose strength may not allow for the identification of a NOAEL. Generally, three doses are used that allow for a characterization of toxicity as well as identification of a NOAEL. An alternative to conducting the 3-month rat study is to modify your proposed acceptance criterion to less than (b) (4)

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12.6 Appendix 6: Carcinogenicity Protocol Assessment IND 68,214 (Serial No. 80), September 30, 2010

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

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	68,214
Supporting document/s:	#092 (SX)
Sponsor's letter date:	September 30, 2010
CDER stamp date:	September 30, 2010
Product:	Firazyr® (Icatibant); Bradykinin Type 2 (B_2) Receptor Antagonist (synthetic decapeptide with 5 non proteinogenic amino acids)
Indication:	Treatment of acute attacks of hereditary angioedema
Sponsor:	Shire Human Genetic Therapies 700 Main Street Cambridge, MA 02139
Review Division:	Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Timothy W. Robison, Ph.D., D.A.B.T.
Supervisor/Team Leader:	Molly Topper, Ph.D.
Division Director:	Badrul Chowdhury, M.D., Ph.D.
Project Manager:	Carol Hill

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

In the present submission, the sponsor provided a report for a second 13-week dose range finding toxicology study with mice as well as a dose selection proposal and draft protocol for a new 2-year carcinogenicity study with mice.

The ECAC originally considered dose selections and designs for the 2-year carcinogenicity studies with mice and rats on June 10, 2008 (see attached minutes in the Appendix). The ECAC recommended doses of 5, 15, and 50 mg/kg/day for the mouse. However, the sponsor initiated the 2-year carcinogenicity study with mice with doses of 0, 10, 30, and 100 mg/kg/day, respectively, which were not in agreement with doses recommended by the ECAC. The study was halted on Day 12 of treatment after severe local reactions were observed in animals receiving 30 and 100 mg/kg/day. Animals were subsequently terminated.

1.2 Brief Discussion of Nonclinical Findings

In a second 13-week subcutaneous toxicology study (JE049-0170), mice received icatibant at doses of 0, 5, 15, and 50 mg/kg twice per week. One male animal (#33) given 50 mg/kg/dose was sacrificed for welfare reasons on day 53 due to findings of epidermal ulceration on the neck at subcutaneous injection sites. These findings were directly related to the subcutaneous administration of the test article. Sores/lesions were observed at injection sites in a dose-related manner for mice at 15 and 50 mg/kg/dose. There was some imbalance between the clinical signs and gross pathologic/histopathologic findings in which the incidence of findings at injection sites for the 15 mg/kg/dose group was lower with the gross pathologic/histopathologic examinations. Target tissues/organs of toxicity were the injection sites, skin + subcutis, liver, axillary and inguinal lymph nodes, stomach, urinary bladder, vagina, and Harderian glands. Findings for injection sites #1, 2, and/or 3 were observed for males and females in the mid and/or high dose groups and consisted of dose-related increased incidences of scab formation, epidermal hyperplasia, epidermal ulceration, dermal inflammatory cell infiltration, dermal fibrosis, panniculus muscle degeneration, folliculitis, and/or dermal mineralization. Findings of epidermal ulceration were generally confined to males and females in the high dose group with the exception of one male in the mid dose group. Other histopathological findings did not appear to be dose limiting with respect to identification of a MTD. The MTD was identified at 15 mg/kg/dose based upon the moribund sacrifice of one male in the 50 mg/kg/dose group due to findings of epithelial ulceration on the neck associated with subcutaneous administration of the test article as well as findings at injection sites for the 50 mg/kg/dose group that included high incidences of scab formation, epidermal hyperplasia, epidermal ulceration, dermal inflammatory cell infiltration, dermal fibrosis, panniculus muscle degeneration, folliculitis, and/or dermal mineralization. The ulceration is a particular concern.

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1.3 Recommendations

Based upon the ECAC recommendations and conclusions from the meeting on November 2, 2010, the sponsor should revise doses for the 2-year carcinogenicity study with mice to 2, 5, and 15 mg/kg/dose administered twice per week.

2 Drug Information

2.1 Drug

Trade name: Firazyr

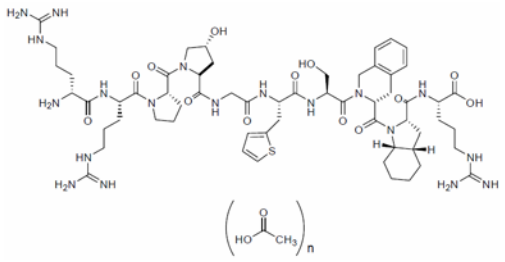
Generic Name: Icatibant

Chemical Name: D-Arginyl-L-arginyl-L-prolyl-L-[(4*R*)-4-hydroxyprolyl]-glycyl-L[3-(2-thienyl)alanyl]-L-seryl-D-(1,2,3,4-tetrahydroisoquinolin-3-ylcarbonyl)-L[(3a*S*,7a*S*)-octahydroindol-2-ylcarbonyl]-Larginine, acetate salt

Abbreviated Chemical Name: H-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-OH, acetate salt

Molecular Formula/Molecular Weight: $C_{59}H_{89}N_{19}O_{13} \cdot C_2H_4O_2$/ MW = 1304.6 g/mole

Structure:



Pharmacologic class: Bradykinin Type 2 (B_2) Receptor Antagonist

2.2 Relevant INDs, NDAs, and DMFs

None

2.4 Proposed Clinical Population and Dosing Regimen

Icatibant acetate is being developed for the treatment of patients with hereditary angioedema (HAE) who are 18 years old and older. Icatibant will be administered by the subcutaneous route. The anticipated clinical dosing of icatibant in patients having an HAE attack is 30 mg/day up to 3 times daily SC. Therefore, the maximum recommended human dose (MRHD) of icatibant is 90 mg/day, which is associated with AUC levels of 14.27 μg·hr/mL (extrapolated AUC levels observed in elderly females treated with 30 mg/day SC dosing).

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2.8 Regulatory Background

The ECAC originally considered dose selections and designs for the 2-year carcinogenicity studies with mice and rats on June 10, 2008 (see attached minutes in the Appendix). The ECAC recommended doses of 5, 15, and 50 mg/kg/day for the mouse.

However, the sponsor initiated the 2-year carcinogenicity study with mice with doses of 0, 10, 30, and 100 mg/kg/day, respectively, which were not in agreement with doses recommended by the ECAC. The study was halted on Day 12 of treatment after severe local reactions were observed in animals receiving 30 and 100 mg/kg/day. The mice in Groups 3 and 4 (30 and 100 mg/kg/day) developed sores and lesions at the site of injection within 7 days of dosing. As a result of these observations, dosing was suspended for welfare reasons and all animals were maintained off dose while investigations were conducted. The sponsor claimed that these local reactions were not consistent with the findings in the high dose group (100 mg/kg/day) in the 13-week dose-range finding study (JE049-0159); however, mice were only dosed with 100 mg/kg/day for 4 weeks due to findings of hemorrhage, ulceration, and necrosis at multiple injection sites in males and females.

A series of investigative studies to determine possible causes for the severe dose-site reaction seen in the early stages of the 104 week mouse carcinogenicity study was performed. The investigative studies were designed to assess the impact of several different variables of the icatibant preparations on the formation of injection site reactions in mice. Specifically, the experiments addressed; the presence of an identification transponder, pH, and osmolarity of the injection solutions and compared formulations used in the previous 13 week dose range finding and 104 week carcinogenicity studies. Based on the results of the investigative studies, there did not appear to be a distinct difference in skin reactions to icatibant acetate when an identification transponder was present or when different batches of icatibant acetate which varied in pH and osmolarity were administered subcutaneously at 100mg/kg/day over 14 days. Additionally, when the batch and formulation that was used in the early stages of the 104 week carcinogenicity study were retested in the investigative study, skin reactions were observed soon after the initiation of dosing. Based on the results of these investigative studies it is possible to conclude that the lack of skin reactions to icatibant in the previous 13 week dose range finding study (JE049-0159) was not attributable to the formulation characteristics of the test article, Icatibant in regards to pH and osmolarity, nor to the presence of an identification transponder used in these studies.

Two factors were identified that could have been responsible for the unexpected results in the mouse carcinogenicity study (JE049-0161). The first factor was that a majority of the mice from the 13-week study (JE049-0159) were non naïve towards icatibant at the start of the dosing period. The second factor was that the carcinogenicity study was mistakenly initiated with the doses proposed in the protocol submitted to the ECAC (0, 10, 30, and 100 mg/kg/day) rather than the FDA recommended doses noted in the June

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11, 2008 ECAC report (0, 5, 15, 50 mg/kg/day), resulting in a 2-fold increase in the intended dose.

Upon agreement with the FDA (FAX dated November 24, 2008), the 104 week study was suspended and a new dose range finding study (JE049-0170) was initiated.

The Division provided comments (see Review and FAX dated May 11, 2009 and May 20, 2009, respectively) on the design of the 13-week subcutaneous dose ranging toxicology study (JE049-0170) that was provided in the present submission.

3 Studies Submitted

3.1 Studies Reviewed

Icatibant: 13-Week Subcutaneous Administration Toxicity Study in the Mouse (JE049-0170)

3.3 Previous Reviews Referenced

1. June 10, 2008 ECAC Meeting Minutes for IND 68,214
2. PharmTox Review of IND 68,214 dated June 12, 2008 – Review of SPAs for mouse and rat carcinogenicity studies
3. PharmTox Review of IND 68,214 dated May 11, 2009- Review of protocol for the 13-week dose range finding toxicology study with mice (JE049-0170)

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6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: **Icatibant: 13-Week Subcutaneous Administration Toxicity Study in the Mouse**

Study no.:	Sponsor Reference Number JE049-0170
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	June 29, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Icatibant acetate, batch number 1015228 (Purity: 81.4% (net assay) or 96.2% when corrected for water, acetic acid, and solvent contents)

Key Study Findings

● In a 13-week subcutaneous toxicology study, mice received icatibant at doses of 0, 5, 15, and 50 mg/kg twice per week.

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● One male animal (#33) given 50 mg/kg/dose was sacrificed for welfare reasons on day 53 due to findings of epidermal ulceration on the neck at subcutaneous injection sites. These findings were directly related to the subcutaneous administration of the test article.

● A female animal (#72) given 50 mg/kg/dose was sacrificed on day 72 due to the presence of a large skin mass that was determined to be an osteosarcoma. The relationship of this finding to treatment was questionable.

● Clinical observations of mice found that 10 of 10 males and 9 of 10 females in the 50 mg/kg/dose group had sores/lesions associated with subcutaneous injections. Two of 10 males and 4 of 10 females in the 15 mg/kg/dose group had sores/lesions. Postdose observations recorded hunched appearance with piloerection and semi-closed eyes for the majority of mice in the 50 mg/kg/dose group between 1 and 2 hr postdose. These findings started during the first week of dosing. There was some imbalance between the clinical signs and gross pathologic/histopathologic findings in which the incidence of findings at injection sites for the 15 mg/kg/dose group was lower with the gross pathologic/histopathologic examinations.

● Absolute body weights and body weight gains were unaffected.

● Target tissues/organs of toxicity were the injection sites, skin + subcutis, liver, axillary and inguinal lymph nodes, stomach, urinary bladder, vagina, and Harderian glands.

● Findings for injection sites #1, 2, and/or 3 were observed for males and females in the mid and/or high dose groups and consisted of dose-related increased incidences of scab formation, epidermal hyperplasia, epidermal ulceration, dermal inflammatory cell infiltration, dermal fibrosis, panniculus muscle degeneration, folliculitis, and/or dermal mineralization. Findings of epidermal ulceration were confined to males and females in the high dose group with the exception of one male in the mid dose group. These findings of ulceration are considered dose limiting.

● Findings for the liver consisted of a reduction in glycogen vacuolation for males and females in the mid and high dose groups, increased sinusoidal cells for two males in the high dose group, increased incidences of inflammatory cell foci for males in the high dose group and all female treatment groups, and clear cell foci for one male in the high dose group. Increased sinusoidal cells, inflammatory cell foci, and clear cell foci may potentially be pre-neoplastic lesions. These findings do not appear to be dose limiting.

● For the axillary and inguinal lymph nodes, increased cellularity was observed for all male treatment groups and females in the mid and high dose group. Incidences for male treatment groups did not display a dose-response relationship. These findings do not appear to be dose limiting.

● Findings for the stomach consisted of nonglandular epithelial hyperplasia for one male and three females in the high dose group and nonglandular mucosal inflammation for

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one female in the high dose group. The stomach was not examined for females in the low and mid dose group although the finding of nonglandular epithelial hyperplasia was not regarded as dose limiting.

● Urinary bladder distension was observed for 1 low dose male and 3 of 10 high dose males; however, examinations of the low and mid dose groups were limited to gross lesions. Gross pathological examinations found distension for 2 of 10 low dose males, 1 of 10 mid dose males, and 3 of 10 males and 1 of 10 females in the high dose group. The relationship of this finding to treatment was questionable based upon the lack of a dose-response. It was unclear if the observed finding might be a pharmacological effect of icatibant.

● For the vagina, anoestrous morphology of the vaginal epithelium was observed for females in the 15 or 50 mg/kg/dose groups. This finding was characterized by the presence of numerous goblet cells within the superficial layers of the vaginal epithelium. These findings do not appear to be dose limiting.

● Findings for the Harderian glands consisted of increased incidences of necrosis with interstitial inflammation for males and females in the high dose group although the concurrent control background incidence of this finding was high and a relationship to treatment was questionable.

● The MTD was identified at 15 mg/kg/dose based upon the moribund sacrifice of one male in the 50 mg/kg/dose group due to findings of epithelial ulceration on the neck associated with subcutaneous administration of the test article as well as findings at injection sites for the 50 mg/kg/dose group that included high incidences of scab formation, epidermal hyperplasia, epidermal ulceration, dermal inflammatory cell infiltration, dermal fibrosis, panniculus muscle degeneration, folliculitis, and/or dermal mineralization. The ulceration is a particular concern.

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Methods

Doses:	0, 5, 15, and 50 mg/kg/dose, Administered twice per week
Frequency of dosing:	Administered twice per week
Route of administration:	Subcutaneous; Three separate injection sites, identified as IJ1 (scruff), IJ2 (left hip) and IJ3 (right hip) were used in rotation starting with IJ1.
Dose volume:	1 mL/kg
Formulation/Vehicle:	0.9% Physiological Saline
Species/Strain:	Hsd:ICR (CD-1®) mice were obtained from (b) (4)
Number/Sex/Group:	10 mice/sex/group
Age:	10 weeks old at the start of treatment
Weight:	Body weights ranges were 31.0 to 46.6 g for males and 23.9 to 35.3 g for females
Satellite groups:	For toxicokinetic evaluation, there were 6 mice/sex/group in the control group and 18 mice/sex/group in each treatment group
Unique study design:	Dose level selection was based on previous repeat dose studies via the subcutaneous route. A previous 104 week carcinogenicity study conducted in mice with daily administration was halted on Day 12 of treatment after severe local reactions were observed in animals receiving 30 and 100 mg/kg/day. Dose levels based on a prior 13-week dose-range finding study are now accepted as potentially flawed in design.
Deviation from study protocol:	Deviations were minimal and had no impact on the study.

Table 1 Study Design of 13-week toxicology study with mice

Study design

Four groups of mice of the Hsd:ICR (CD-1®) strain were dosed as follows:

Group Number	Description	Dose level+ (mg/kg/dose)	Number of animals in group			
			Main study		Satellite study#	
			Male	Female	Male	Female
1	Control	0	10	10	6	6
2	Low	5	10	10	18	18
3	Intermediate	15	10	10	18	18
4	High	50	10	10	18	18

+ dose levels are expressed in terms of the free base
# for toxicokinetic investigations only; no other experimental observation data from these animals has been reported

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Observations and Results

Mortality: All animals were observed at the beginning and the end of the working day to ensure the animals were in good health. Any animal which showed marked signs of ill health was isolated.

There were two mice in the 50 mg/kg/dose group that were sacrificed during the dosing period. The sacrifice of at least one of these mice was clearly due to findings induced by treatment.

One male animal (#33) given 50 mg/kg/dose was sacrificed for welfare reasons on day 53 due to findings of epidermal ulceration on the neck at subcutaneous injection sites. A number of macroscopic and microscopic findings were present at the injection sites in this animal that included moderate epidermal ulceration, minimal epithelial hyperplasia, moderate dermal inflammatory cell infiltration, slight panniculus muscle degeneration, and minimal dermal fibrosis. These findings were directly related to the administration of the test article.

A female animal (#72) given 50 mg/kg/dose was sacrificed on day 72 due to the presence of a skin mass (28x20x15 mm on the left hip involving left femur, left sciatic nerve, and left popliteal LN). The histopathological diagnosis was osteosarcoma. This finding was confined to a solitary animal and no proliferative lesions were identified in the bone or musculoskeletal system of other mice in this study. No proliferative bone changes were identified in previous studies in mice where icatibant was administered at higher doses over the same time period ((b) (4) Study No. 1966/040) although the reliability of this study is in question.

Three satellite animals (Group 4M: 2/18; Group 4F: 1/18) died on Day 1 after dosing (before the toxicokinetic bleed) and were immediately replaced. The replacements were not dosed on Day 1. The numbers replaced were 129 (Group 4M), 140 (Group 4M) and 199 (Group 4F).

Clinical Signs: All animals were observed daily for signs of ill health or overt toxicity. In addition, each animal was given a detailed physical examination at weekly intervals. On both dosing days during week 1, toxicity animals were observed 1 to 2 hr postdose. On the non-dosing days within the week, the animals were observed during the morning of each day. Toxicity animals were observed once weekly from week 2 onwards. They were observed 1 to 2 hr after the second dose in each week. The injection sites of all toxicity animals were observed once daily during week 1 and once weekly thereafter. Inflammatory skin reactions were graded using a standardized scale based on OECD guidelines for testing of chemicals 404.

Clinical observations of mice found that 10 of 10 males and 9 of 10 females in the 50 mg/kg/dose group had sores/lesions (back, neck, injection sites #1 and/or #3) associated with subcutaneous injection sites. There were additional findings of hair loss

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or thinning fur on the dorsal side. One male in the 50 mg/kg/dose group, observed with ulcerated neck in week 8, was subsequently sacrificed (see Mortality section above).

Two of 10 males and 4 of 10 females in the 15 mg/kg/dose group had sores/lesions associated with subcutaneous injections. In addition, there were observations of thinning fur on the dorsal side for these males and females.

There was some imbalance between the clinical signs and gross pathologic/histopathologic findings in which the incidence of findings at injection sites for the 15 mg/kg/dose group was lower with the gross pathologic/histopathologic examinations.

Table 2 Clinical observations (number of days with observed finding/number of animals)

Clinical observations	Males				Females			
	0	5	15	50	0	5	15	50
Sores/Lesions, Injection Site #1	0/0	0/0	0/0	21/4	0/0	0/0	1/1	23/7
Sores/Lesions, Back, Injection Site #1	0/0	0/0	0/0	11/6	0/0	0/0	0/0	6/3
Sores/Lesions, Back	0/0	0/0	0/0	15/3	0/0	0/0	2/1	6/2
Sores/Lesions, Neck	0/0	0/0	4/2	10/2	0/0	0/0	7/3	11/3
Sores/Lesions, Neck, Injection Site #1	0/0	0/0	0/0	4/2	0/0	0/0	0/0	5/4
Sores/Lesions, Back, Injection Sites #1 and #3	0/0	0/0	0/0	2/1	0/0	0/0	0/0	0/0
Ulcerated neck	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0
Thinning fur, dorsal	0/0	0/0	7/1	55/7	0/0	0/0	19/2	8/1
Thinning fur, dorsal, Injection Site #1	0/0	0/0	6/1	0/0	0/0	0/0	0/0	31/7
Hair loss, dorsal	0/0	0/0	0/0	13/3	0/0	0/0	0/0	9/1

Examination of injection sites found scabbing for 2 of 10 males and 3 of 10 females in the 15 mg/kg/dose group and 10 of 10 males and 9 of 10 females in the 50 mg/kg/dose group. Patch hair (evidence of normal repair response following depilation) was observed for 1 of 10 males and 5 of 10 females in the 15 mg/kg/dose group and 10 of 10 males and 9 of 10 females in the 50 mg/kg/dose group. Scabbing and patchy hair growth were observed during the first week of dosing. Erythema was observed for 2 of 10 females in the 15 mg/kg/dose group and 2 of 10 males and 1 of 10 females in the 50 mg/kg/dose group.

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Table 3 Injection site observations (number of weeks[1] with observed finding/number of animals)

Injection site observations	Males				Females			
	0	5	15	50	0	5	15	50
Scabbing	0/0	0/0	4/2	51/10	0/0	0/0	16/3	37/9
Patchy hair growth[2]	0/0	0/0	4/1	58/10	0/0	0/0	19/5	44/9
Erythema	0/0	0/0	0/0	4/2	0/0	0/0	3/2	1/1

1. Estimate – Injection sites were observed daily during the first week of treatment and weekly thereafter
2. Evidence of normal repair response following depilation

Postdose observations recorded hunched appearance with piloerection and semi-closed eyes for the majority of mice in the 50 mg/kg/dose group between 1 and 2 hr postdose. These findings started during the first week of dosing. Red tail was recorded between 1 and 2 hr after dosing for 4 males and 9 females in Week 12 and for 2 males and 5 females in Week 13.

Red tail was recorded for 10 of 10 females in the 15 mg/kg/dose group during week 13.

Table 4 Group incidence post-dosing observations

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Test Article	Control	Icatibant		
Group	1	2	3	4
Level (mg/kg/dose)	0	5	15	50

Group and Sex	Clinical sign	Number of animals in group showing sign in Week (Day): 1 (1)	1 (4)	2 (11)	3 (18)	4 (25)	5 (29)	5 (32)
4M	**Number examined:**	10	10	10	10	10	10	10
	Hunched	4	8	9	5	1	4	4
	Piloerection	4	6	7	4		10	4
	Semi-closed eyes	4	8	6	1	2	3	4
4F	**Number examined:**	10	10	10	10	10	10	10
	Hunched		3	9	6		10	6
	Piloerection		2	6	1		10	6
	Semi-closed eyes	5	3	3	3	5	2	6

Group and Sex	Clinical sign	Number of animals in group showing sign in Week (Day): 12 (81)	13 (88)
3F	**Number examined:**	10	10
	Red tail		10
4M	**Number examined:**	9#	9
	Red tail	4	2
	Hunched	3	6
	Piloerection	3	
	Semi-closed eyes	3	4
4F	**Number examined:**	9$	9
	Red tail	9	5
	Hunched	9	7
	Piloerection	5	
	Semi-closed eyes	5	2

(n) = day number
Post -dose observations performed 1 to 2 hours after dose
Any other group/week – no observed

# animal number 33, Group 4M, sporadic Week 8
$ animal number 72, Group 4F, sporadic Week 11

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Body Weights: Individual body weights were recorded on day -7, before treatment on the first day of dosing, at weekly intervals, and before necropsy.

Absolute body weights were slightly increased for male treatment groups although there was no dose-response relationship. Body weight gains were decreased for males in the low and mid dose groups; however, body weight gain was unaffected for males in the high dose group suggesting that decreases for the low and mid dose groups were not related to treatment. Changes of absolute body weight or body weight gain would appear to have no influence on identification of an MTD.

Absolute body weights were unaffected for female treatment groups. Body weight gains were increased for females in the mid and high dose groups.

Table 5 Absolute body weights and body weight gains

Males				
Parameter	Control	5 mg/kg	15 mg/kg	50 mg/kg
BW, Day 1	35.0	37.0	37.2	37.1
BW, Week 13	40.6	42.4	41.4	43.0
%Control (Abs)	100.0	104.4	102.0	105.9
Δ	5.6	5.4	4.2	5.9
%Initial BW	16.0	14.6	11.3	15.9
%Control (BWG)	100.0	91.2	70.6	99.4
Females				
Parameter	Control	5 mg/kg	15 mg/kg	50 mg/kg
BW, Day 1	29.6	29.4	30.2	28.3
BW, Week 13	34.1	34.0	35.9	33.9
%Control (Abs)	100.0	99.7	105.3	99.4
Δ	4.5	4.6	5.7	5.6
%Initial BW	15.2	15.6	18.9	19.8
%Control (BWG)	100.0	102.9	124.2	130.2

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Figure 1 Group mean body weights - males

Test Article	Control	Icatibant		
Group	1	2	3	4
Level (mg/kg/dose)	0	5	15	50

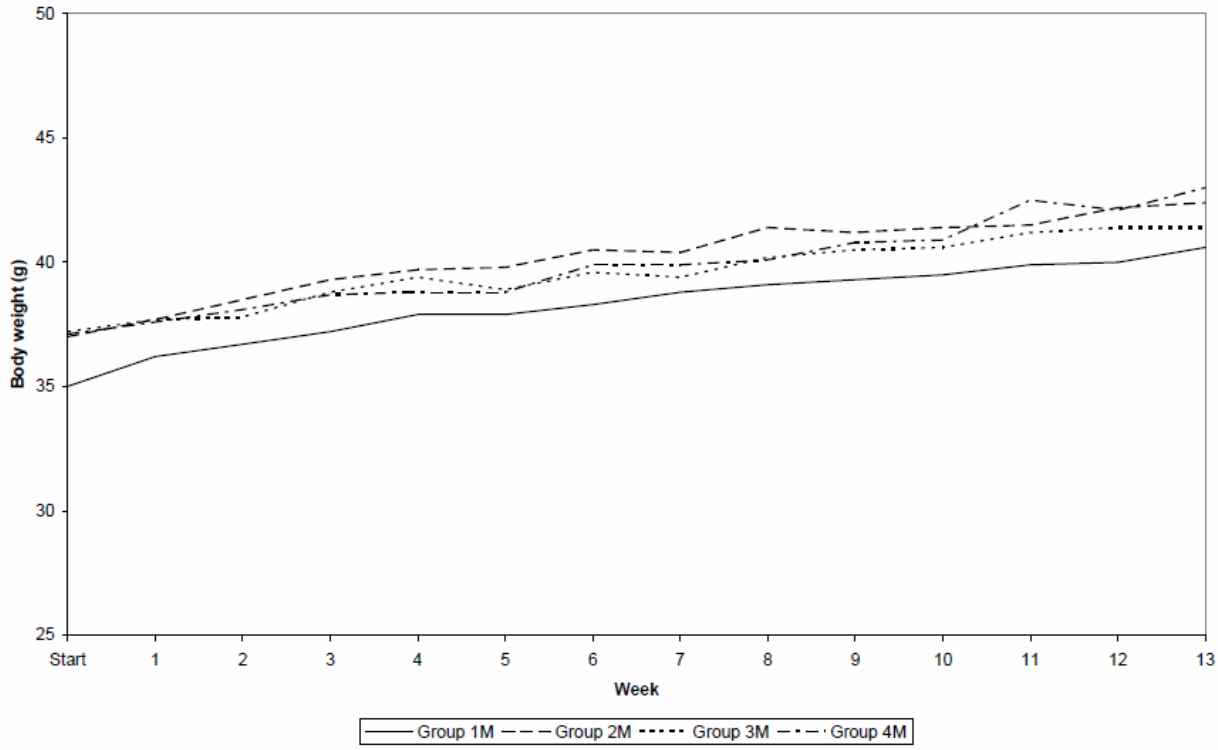
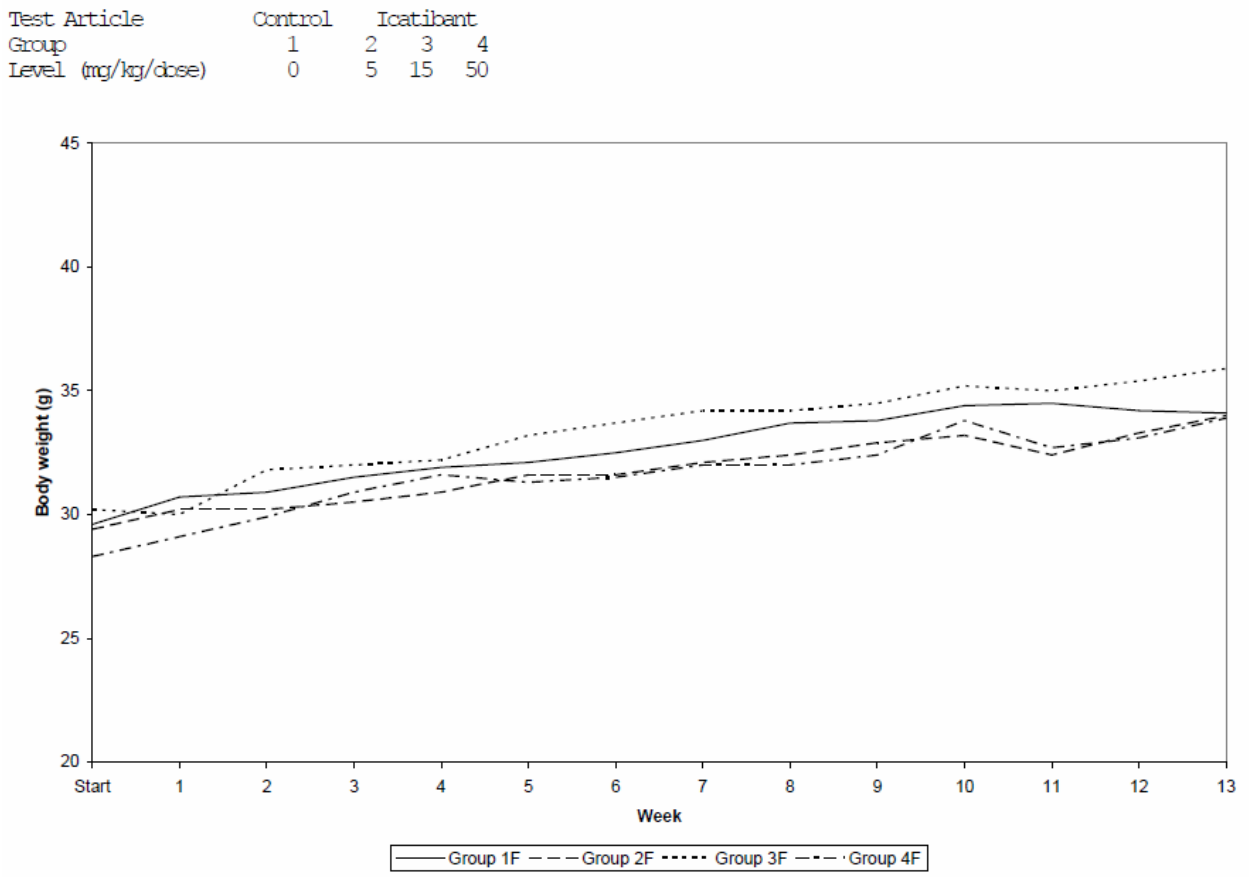


Figure 2 Group mean body weights - females



Feed Consumption: The amount of food consumed by each animal was determined weekly (from Week 1). Consumption was calculated as g/animal/week.

Food consumption was increased for male and female treatment groups over the 13-week treatment period. Statistical significance was achieved for males in the high dose group.

Table 6 Food consumption (g/animal/week), Weeks 1 to 13

Dose mg/kg/dose	Males	Females
0	41.2	39.1
5	43.9 (106.6%)	41.9 (107.1%)
15	46.4 (112.6%)	42.4 (108.4%)
50	48.8* (118.5%)	45.4 (116.1%)

* Statistical significance was achieved, p≤0.05

Ophthalmoscopy: Not performed.

ECG: Not performed.

Hematology: Blood samples were collected during week 13 for measurement of a complete panel of hematology parameters from the first 5 mice/sex/group.

Changes of hematology parameters were generally small and/or lacked dose-response relationships. Platelet counts were increased for females in the high dose group. White blood cell counts were increased for all female treatment groups that were attributed to elevations of neutrophil and lymphocytes counts. However, neutrophil counts were decreased for males in the mid and high dose groups. Slight increases of reticulocyte counts were evident for all male and female treatment groups.

Table 7 Hematology parameters at week 13

Parameter	Males				Females			
	0	5	15	50	0	5	15	50
Platelets 10^9/L	NC	NC	NC	NC	1429	1383	1489	1630* (114%)
Platelet crit (PCT) %	NC	NC	NC	NC	1.13	1.09	1.19	1.31 (116%)
White blood cells 10^9/L	NC	NC	NC	NC	3.7	4.3 (116%)	6.2 (168%)	5.3 (143%)
Neutrophils 10^9/L	1.0	0.9	0.7 (70%)	0.7 (70%)	0.5	0.5	0.9 (180%)	0.9 (180%)
Neutrophils %	15	15	14	11 (73%)	15	12	15	17 (113%)
Lymphocytes 10^9/L	NC	NC	NC	NC	2.9	3.6 (124%)	4.8 (166%)	4.1 (141%)
Reticulocytes %	2.4	2.5	2.8 (117%)	3.0* (125%)	2.6	3.4 (131%)	3.0 (115%)	3.0 (115%)
Reticulocytes 10^9/L	225.7	225.9	254.1 (113%)	260.0 (115%)	235.7	302.2 (128%)	268.1 (114%)	267.2 (113%)

NC = No change
*Statistical significance achieved, $p \leq 0.05$

Clinical Chemistry: Blood samples were collected during week 13 for measurement of a complete panel of clinical chemistry parameters from the last 5 mice/sex/group.

Changes of clinical chemistry parameters were generally small and appear to have minimal toxicologic significance. Inorganic phosphate concentrations were increased for all male treatment groups. The glucose concentration was significantly increased for males in the high dose group. Total bilirubin concentrations were increased for all male treatment groups although a dose-response relationship was not observed. HCRE concentrations were decreased for males and females in the high dose group.

Table 8 Clinical chemistry parameters at week 13

Parameter	Males				Females			
	0	5	15	50	0	5	15	50
Inorganic Phosphate mmol/L	1.5	1.7 (113%)	1.8 (120%)	2.0* (133%)	NC	NC	NC	NC
Glucose mmol/L	9.3	10.7	9.6	11.6* (125%)	NC	NC	NC	NC
Total Bilirubin	2.0	2.7	2.6	2.6	2.1	2.1	1.7	1.4

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µmol/L		(135%	(130%)	(130%)				
HCRE µmol/L	12	10	11	8 (67%)	13	10	11	9 (69%)

Urinalysis: Not performed.

Gross Pathology: All toxicity animals including decedents were subjected to necropsy. A full macroscopic examination was performed under the general supervision of a pathologist and all lesions were recorded. Bone marrow smears were prepared at necropsy but not examined.

Gross pathological findings were evident for injection sites, skin + subcutis, axillary and inguinal lymph nodes, liver, urinary bladder, and spleen.

Injection site findings consisted of sores for males in the high dose group and females in the mid and high dose groups and lesions, red focus, redness, and red area for males in the high dose group. Redness was also observed for one female in the mid dose group.

In the skin + subcutis, there was a finding of a mass for one female in the high dose group that was determined by histopathological examination to be an osteosarcoma. Other findings included sore, red focus, fur loss, and red area although dose-response relationships were generally not evident.

Large axillary and/or inguinal lymph nodes were observed for males in the high dose group and all female treatment groups. These findings correlated with histopathological findings of increased cellularity. Increased incidences of redness of the axillary LN were observed for males and females in the mid dose group and females in the mid and high dose groups although dose-response relationships were not evident.

Findings in the liver consisted of paleness for all male treatment groups, caudate lobe lesion for 1 male in the mid dose group, and large for one male in the high dose group and two females in the mid dose group. Dose-response relationships were generally not evident for these findings in the liver. There were no corresponding histopathological findings.

Increased incidences of urinary bladder distention were observed for all male treatment groups and one female in the high dose group although incidences for male treatment groups did not display a dose-response relationship. This finding correlated with histopathological findings of urinary bladder distention. Dark contents were observed for one male in the high dose group.

Increased incidences of large spleen were observed for females in the mid and high dose groups although a dose-response relationship was not evident.

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Table 9 Gross pathological findings

Organ/Tissue	Males				Females			
	0	5	15	50	0	5	15	50
Injection Site #1 (Scruff)	10	10	10	10	10	10	10	10
-sore	0	0	0	5	0	0	1	4
-lesion	0	0	0	1	0	0	0	0
-red focus	0	0	0	1	0	0	0	0
Injection Site #3 (Right Hip)	10	10	10	10	10	10	10	10
-red	0	0	0	2	0	0	1	0
-red area	0	0	0	1	0	0	0	0
Skin + Subcutis	10	10	10	10	10	10	10	10
-mass #1	0	0	0	0	0	0	0	1
-sore	0	1	0	0	0	0	0	0
-red focus	0	0	1	0	0	0	0	0
-fur loss	0	0	0	0	0	0	2	0
-red area	0	0	0	1	0	0	0	1
Axillary LN	10	10	10	10	10	10	10	10
-large	0	0	0	1	0	0	1	2
-red	0	0	1	0	1	0	3	1
Inguinal LN	10	10	10	10	10	10	10	10
-large	0	0	0	0	0	1	1	3
Liver	10	10	10	10	10	10	10	10
-pale	0	1	1	1	0	0	1	1
-caudate lobe lesion	0	0	1	0	0	0	0	0
-large	0	0	0	1	0	0	2	0
Urinary bladder	10	10	10	10	10	10	10	10
-distension	0	2	1	3	0	0	0	1
-dark contents	0	0	0	1	0	0	0	0
Spleen	10	10	10	10	10	10	10	10
-large	0	0	0	0	0	0	4	2

Organ Weights: Organ weights were measured for the brain, heart, kidneys, liver, prostate, spleen, thymus, and testes + epididymides.

Observed changes of organ weight changes were small (<10%) and any relationships to treatment were questionable.

Histopathology: Fixed tissues were delivered to [redacted] (b) (4) that performed the histopathological examinations of tissues. Tissues were embedded in paraffin wax BP (block stage), sectioned at a nominal 5 µm, and stained with hematoxylin and eosin. Paired tissues/organs were processed to allow samples of both left and right tissue/organ to be examined. All tissues were examined for the control and high dose groups. For the low and mid dose groups, histopathological examinations were limited to the liver, spleen, kidneys, injection sites, axillary and inguinal lymph nodes, ovaries, uterus, vagina, and gross lesions. Microscopic examination of the tissues was performed by the nominated study pathologist on behalf of the sponsor.

Adequate Battery: A complete battery of organs and tissues were examined for the control and high dose groups. In general, the low and mid dose groups were not examined with the exception of the liver, spleen, kidneys, injection sites, axillary and

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inguinal lymph nodes, ovaries, uterus, vagina, and gross lesions that appeared to be acceptable.

Peer Review: A peer review of the histopathological examination performed by the pathologist was not performed.

Histological Findings: Target tissues/organs of toxicity were the injection sites, skin + subcutis, liver, axillary and inguinal lymph nodes, stomach, urinary bladder, vagina, and Harderian glands.

Findings for injection sites #1, 2, and/or 3 were observed for males and females in the mid and/or high dose groups and consisted of dose-related increased incidences of scab formation, epidermal hyperplasia, epidermal ulceration, dermal inflammatory cell infiltration, dermal fibrosis, panniculus muscle degeneration, folliculitis, and/or dermal mineralization. Findings of epidermal ulceration were generally confined to males and females in the high dose group with the exception of one male in the mid dose group.

Findings for the liver consisted of a reduction in glycogen vacuolation for males and females in the mid and high dose groups, increased sinusoidal cells for two males in the high dose group, increased incidences of inflammatory cell foci for males in the high dose group and all female treatment groups, and clear cell foci for one male in the high dose group. Increased sinusoidal cells, inflammatory cell foci, and clear cell foci may potentially be pre-neoplastic lesions.

For the axillary and inguinal lymph nodes, increased cellularity was observed for all male treatment groups and females in the mid and high dose group. Incidences for male treatment groups did not display a dose-response relationship.

Findings for the stomach consisted of nonglandular epithelial hyperplasia for one male and three females in the high dose group and nonglandular mucosal inflammation for one female in the high dose group. The stomach was not examined for females in the low and mid dose group although the finding of nonglandular epithelial hyperplasia was not regarded as dose limiting.

Urinary bladder distension was observed for 1 low dose male and 3 of 10 high dose males; however, examinations of the low and mid dose groups were limited to gross lesions. Gross pathological examinations found distension for 2 of 10 low dose males, 1 of 10 mid dose males, and 3 of 10 males and 1 of 10 females in the high dose group. The relationship of this finding to treatment was questionable based upon the lack of a dose-response. It was unclear if the observed finding might be a pharmacological effect of icatibant. Meini et al. (European Journal of Pharmacology 388, 177-182, 2000 and 615: 10-16, 2009) reported that icatibant (1–10 nmol/kg IV), a selective bradykinin B_2 receptor antagonist dose-dependently reduced the bradykinin (100 nmol/kg IV)-induced urinary bladder contraction. Icatibant recognizes with similar potencies, the bradykinin B_2 receptors expressed in the detrusor muscle of both humans and rats. Icatibant

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reduces both bladder hyperreflexia and plasma protein extravasation in models of chemically-induced cystitis.

For the vagina, anoestrous morphology of the vaginal epithelium was observed for females in the 15 or 50 mg/kg/dose groups. This finding was characterized by the presence of numerous goblet cells within the superficial layers of the vaginal epithelium.

Findings for the Harderian glands consisted of increased incidences of necrosis with interstitial inflammation for males and females in the high dose group although the concurrent control background incidence of this finding was high and a relationship to treatment was questionable.

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Table 10 Histopathological findings

Organ/Tissue	Males				Females			
	0	5	15	50	0	5	15	50
Injection Site #1 (Scruff)	10	10	10	10	10	10	10	10
-scab formation, minimal-moderate	0	0	0	3	0	0	2	3
-epidermal hyperplasia, minimal-moderate	0	0	3	7	0	0	5	10
-epidermal ulceration, minimal-moderate	0	0	0	2	0	0	0	2
-dermal inflammatory cell infiltration, minimal-moderate	0	0	1	3	0	0	2	7
-dermal fibrosis, minimal-moderate	0	0	2	7	0	0	5	9
-panniculus muscle degeneration, minimal-slight	0	0	1	4	0	0	0	3
Injection Site #2 (Left Hip)	10	10	10	10	10	10	10	10
-scab formation, slight	0	0	0	0	0	0	0	1
-epidermal hyperplasia, minimal-slight	1	0	0	0	1	0	0	3
-epidermal ulceration, slight	0	0	0	0	0	0	0	1
-dermal fibrosis, minimal	0	0	0	1	0	0	2	5
-dermal inflammatory cell infiltration, minimal-slight	0	0	0	0	0	0	0	4
-panniculus muscle degeneration, minimal-moderate	0	0	2	4	0	0	2	5
-folliculitis, minimal	0	0	0	0	1	0	0	2
-dermal mineralization, minimal	0	0	0	1	0	0	0	0
Injection Site #3 (Right Hip)	10	10	10	10	10	10	10	10
-scab formation, minimal	0	0	1	0	0	0	1	0
-epidermal hyperplasia, minimal	0	0	0	1	0	0	1	1
-epidermal ulceration, minimal	0	0	1	0	0	0	0	0
-dermal inflammatory cell infiltration, minimal-slight	0	0	1	2	0	2	1	2
-dermal fibrosis, minimal-slight	0	0	0	2	0	0	0	4
-panniculus muscle degeneration, minimal-moderate	0	0	1	4	0	1	1	1
Skin with Subcutis	10	1	0	10	10	0	2	10
-scab formation, minimal-slight	0	1	-	0	0	-	0	1

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Organ/Tissue	Males				Females			
	0	5	15	50	0	5	15	50
-epidermal hyperplasia, minimal	0	1	-	0	0	-	2	1
-epidermal ulceration, moderate	0	0	-	0	0	-	0	0
-dermal inflammatory cell infiltration, minimal-slight	1	0	-	0	0	-	1	1
-osteosarcoma	0	0	-	0	0	-	0	1
Liver	10	10	10	10	10	10	10	10
-glycogen vacuolation, minimal-slight	10	8	3	1	10	7	2	1
-increased sinusoidal cells, minimal-slight	1	1	0	2	0	0	0	0
-inflammatory cell foci, minimal-slight	1	1	0	2	1	3	4	2
-clear cell foci	0	0	0	1	0	0	0	0
Axillary LN	10	10	10	10	10	10	10	10
-increased cellularity, minimal-slight	0	3	1	2	0	0	2	5
Inguinal LN	10	10	10	10	10	10	10	10
-increased cellularity, minimal-slight	0	1	1	1	0	0	2	5
Stomach	10	0	1	10	10	0	0	10
-nonglandular epithelial hyperplasia, minimal-slight	0	-	0	1	0	-	-	3
-nonglandular mucosal inflammation, minimal	0	-	0	0	0	-	-	1
Urinary bladder	10	2	1	10	10	0	0	10
-distension	0	1	0	3	0	-	-	0
Vagina					10	10	10	10
-anoestrus					0	0	3	2
Harderian gland	10	0	0	10	10	2	0	10
-necrosis with interstitial inflammation , minimal-marked	6	-	-	9	6	1	-	8

Toxicokinetics: On day 1 and during week 13, blood samples for measurement of plasma drug concentrations were collected from toxicokinetic animals at 0.5, 1, 2, 4, 8, and 24 hr postdose. Blood samples were collected from controls animals only at 2 and 24 hr postdose. Samples were transported frozen on dry ice (b) (4) for plasma analysis and toxicokinetic evaluation. All toxicokinetic animals were killed and discarded following the week 13 blood sampling. Concentrations of icatibant, M1, and M2 in mouse plasma samples were measured by LC-MS/MS over the calibration range of 25-5000 ng/mL for icatibant and 2.5-500 ng/mL for M1 and M2.

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Icatibant was the predominant form with lesser amounts of M1 and M2 (Icatibant > M1 > M2). C_{max} and AUC values for icatibant, M1, and M2 on day 1 and during week 13 increased with elevating doses in a generally dose proportional manner. Exposure values for icatibant, M1, and M2 during week 13 were generally less values on day 1, which correlated with increased clearance (Cl/F) values during week 13 as compared to day 1. There were no consistent sex-related differences in exposure to icatibant, M1, or M2. Half-lives of icatibant, M1, and M2 (day 1 and during week 13 combined) were 0.31 to 0.77 hr, 0.4 to 3.07 hr, and 0.54 to 3.07 hr, respectively. Volume of distribution (Vz) values during week 13 were greater than values on day 1, with the most marked differences for M1 and M2.

Table 11 Toxicokinetic parameters for icatibant on day 1

i) Males

Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h*ng/mL)	$AUC_{0-\infty}$ (h*ng/mL)	$AUC_{0-\infty}$ Ext. %	Cl/F (ml/min/kg)	Vz (L/kg)
5	9140	0.50	0.62	11400	13300	14	6.26	0.330
15	18700	1.0	0.56	28500	28900	1.3	8.66	0.420
50	49900	1.0	0.62	115000	116000	0.046	7.21	0.380

ii) Females

Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h*ng/mL)	$AUC_{0-\infty}$ (h*ng/mL)	$AUC_{0-\infty}$ Ext. %	Cl/F (ml/min/kg)	Vz (L/kg)
5	10700	0.50	0.31	8570	8760	2.2	9.52	0.250
15	21500	1.0	0.37	28500	28600	0.20	8.74	0.280
50	38200	1.0	0.77	81700	81800	0.12	10.2	0.680

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Table 12 Toxicokinetic parameters for M1 on day 1

i) Males

Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h*ng/mL)	$AUC_{0-\infty}$ (h*ng/mL)	$AUC_{0-\infty}$ Ext. %	Cl/F (ml/min/kg)	Vz (L/kg)
5	813	0.5	0.4	1100	1110	0.3	75.3	2.61
15	1550	1	0.73	2860	2870	0.1	87.2	5.52
50	9410	2	0.81	23500	23600	0.4	35.4	2.50

ii) Females

Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h*ng/mL)	$AUC_{0-\infty}$ (h*ng/mL)	$AUC_{0-\infty}$ Ext. %	Cl/F (ml/min/kg)	Vz (L/kg)
5	669	0.5	0.4	689	691	0.2	121	4.21
15	1610	0.5	0.66	3040	3100	1.9	80.7	4.62
50	2870	2	0.95	7940	7980	0.6	104	8.58

Table 13 Toxicokinetic parameters for M2 on day 1

i) Males

Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h*ng/mL)	$AUC_{0-\infty}$ (h*ng/mL)	$AUC_{0-\infty}$ Ext. %	Cl/F (ml/min/kg)	Vz (L/kg)
5	1610*	0.50	0.94	1360	1360	0.28	61.2	4.98
15	828	1.0	3.5	2320	2350	0.93	107	32.1
50	4670	2.0	3.6	9770	9810	0.40	84.9	26.2

**One animal showed high levels of M2 at the 0.5h time point, elevating the mean C_{max} for the group*

ii) Females

Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h*ng/mL)	$AUC_{0-\infty}$ (h*ng/mL)	$AUC_{0-\infty}$ Ext. %	Cl/F (ml/min/kg)	Vz (L/kg)
5	432	0.50	1.1	696	701	0.66	119	11.1
15	1060	1.0	3.0	2480	2490	0.34	100	26.4
50	3170	2.0	3.3	7690	7720	0.29	108	30.9

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Table 14 Toxicokinetic parameters for icatibant on day 91

i) Males

Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h*ng/mL)	$AUC_{0-\infty}$ (h*ng/mL)	$AUC_{0-\infty}$ Ext. %	Cl/F (ml/min/kg)	Vz (L/kg)
5	3590	0.5	0.54	3250	3640	10.9	22.9	1.060
15	14000	1	0.37	16300	16300	0.1	15.3	0.496
50	37200	1	0.83	77200	77400	0.2	10.8	0.778

ii) Females

Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h*ng/mL)	$AUC_{0-\infty}$ (h*ng/mL)	$AUC_{0-\infty}$ Ext. %	Cl/F (ml/min/kg)	Vz (L/kg)
5	6150	0.5	0.43	5800	6210	6.5	13.4	0.505
15	17000	0.5	0.43	22700	22700	0.3	11.0	0.408
50	42100	1	0.65	68400	68400	0.0	12.2	0.682

Table 15 Toxicokinetic parameters for M1 on day 91

i) Males

Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h*ng/mL)	$AUC_{0-\infty}$ (h*ng/mL)	$AUC_{0-\infty}$ Ext. %	Cl/F (ml/min/kg)	Vz (L/kg)
5	169	0.5	1.61	239	453	47.1	184	25.6
15	894	1	0.57	1060	1070	1.4	233	11.5
50	2540	1	2.07	6360	6360	0.0	131	23.5

ii) Females

Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h*ng/mL)	$AUC_{0-\infty}$ (h*ng/mL)	$AUC_{0-\infty}$ Ext. %	Cl/F (ml/min/kg)	Vz (L/kg)
5	317	1	0.54	491	496	1.2	168	7.86
15	832	1	0.75	1620	1620	0.1	154	9.95
50	3130	1	3.07	5920	5930	0.1	141	37.4

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Table 16 Toxicokinetic parameters for M2 on day 91

i) Males

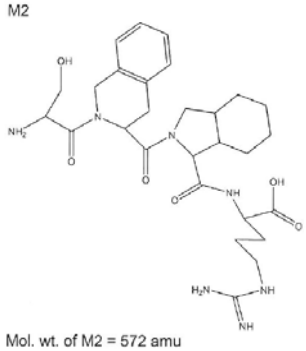
Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h*ng/mL)	$AUC_{0-\infty}$ (h*ng/mL)	$AUC_{0-\infty}$ Ext. %	Cl/F (ml/min/kg)	Vz (L/kg)
5	117	1	0.95	254	255	0.4	327	27.0
15	696	1	6.44	996	1020	2.1	246	137
50	1680	1	3.01	5150	5170	0.4	161	42.0

ii) Females

Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h*ng/mL)	$AUC_{0-\infty}$ (h*ng/mL)	$AUC_{0-\infty}$ Ext. %	Cl/F (ml/min/kg)	Vz (L/kg)
5	229	1	0.71	382	395	3.4	211	13.0
15	580	2	3.79	1510	1510	0.3	165	54.1
50	2430	1	5.58	5520	5580	1.0	149	72.2

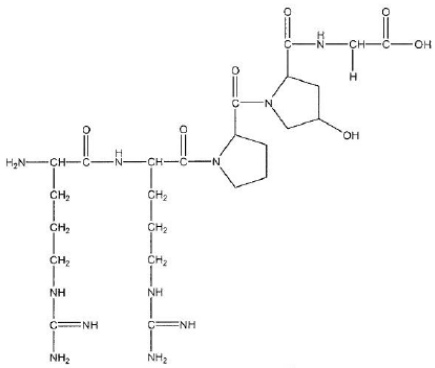
Figure 3 Structures of M1 and M2

M2



Mol. wt. of M2 = 572 amu

(b) (4)



Mol. wt. of M1 = 597.68 amu

Dosing Formulation Analysis: Formulations for use in weeks 1 and 13 were analyzed to determine achieved concentration. The target range for preparation of the formulations was 90 to 110% of nominal.

Concentrations were acceptable with the exception of Group 2 at 85% of the nominal concentration during week 13.

Table 17 Dosing formulation analysis

Achieved concentration analysis

	Results as % nominal		
Week	Group 2	Group 3	Group 4
1	96, 96	105, 104	102, 102
13	85, 85, 85	93, 93	92, 90

8 Carcinogenicity

Draft Protocol for a 104 Week Subcutaneous Administration Carcinogenicity Study in the Mouse (Version 1)

The objective of the study is to determine the effects of the test article, icatibant, on the incidence and morphology of tumors following subcutaneous administration to the mouse, twice weekly for 104 weeks. The toxicokinetic profile of the test article will also be assessed. For the carcinogenicity assessment, there will be 51 mice/sex/group. For the toxicokinetic assessment, there will be 3 mice/sex/group in the control group and 18 mice/sex/group in the treatment groups.

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Table 18 Design of the 104-week carcinogenicity study with mice

Group number	Group description	Dose level* (mg/kg/day)	Animal numbers			
			Carcinogenicity		Toxicokinetics	
			Male n=51	Female n=51	Male n=3 or 18	Female n=3 or 18
1	Control	0	1-51	205-255	409-411	466-468
2	Low	TBA	52-102	256-306	412-429	469-486
3	Intermediate	TBA	103-153	307-357	430-447	487-504
4	High	TBA	154-204	358-408	448-465	505-522

* Dose administered twice weekly
Dose levels are expressed in terms of the free base. A correction factor of X will be applied when using Sponsor lot number Y.

Group number	Group description	Dose level* (mg/kg/day)	Animal numbers: Toxicokinetic - Spare	
			Male n=3	Female n=3
1	Control	0	523-525	535-537
2	Low	TBA	526-528	538-540
3	Intermediate	TBA	529-531	541-543
4	High	TBA	532-534	544-546

* Dose administered twice weekly
Dose levels are expressed in terms of the free base. A correction factor of X will be applied when using Sponsor lot number Y.
Spare: Animals will be dosed but not sampled, unless used as replacements for TK animals terminated early

Dose selection for the 2-year carcinogenicity study with mice was based upon a 13-week dose range finding toxicology study. Icatibant acetate (vehicle, 5, 15 or 50 mg/kg/dose) was administered by the subcutaneous route, twice weekly, for a period of 13 consecutive weeks ((b) (4) study 8200994, Sponsor Study JE049-0170). There were non-adverse treatment-related findings in the liver, vagina, injection sites, and drainage lymph nodes. In addition, treated animals consumed more food compared to controls. Based on AUC_{0-t} values, exposure data demonstrated there was no accumulation of icatibant, M1 or M2; the dose response was approximately proportional and there was no evidence of a sex difference in exposure. Based on data generated from the 13-week study, dose levels for this two year carcinogenicity study will be 5, 15 and 50 mg/kg/dose, twice weekly, at a dose volume of 1 mL/kg.

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Table 19 Administration of the test article in the 104-week carcinogenicity study

1.7 Test article administration	
Route	Three injection sites will be used in rotation (first administration into scruff [IJ1], second administration into left hip [IJ2] and third administration into right hip [IJ3]).
Justification	Possible human therapeutic route
Frequency of dosing	Twice weekly for at least 104 weeks, excluding the day of necropsy
Dose volume	1 mL/kg. Individual dose volumes will be based on individual body weight

For the carcinogenicity and toxicokinetic studies, Hsd:ICR (CD-1®) mice will be obtained from [redacted] (b) (4) At start of dosing, mice will be approximately 10 weeks old and anticipated to be sexually mature. Males will be singly housed and females will be house in groups of up to three. Mice will have *ad libitum* access to SQC Rat and Mouse Maintenance Diet No 1 (expanded).

Clinical examinations will be conducted on all animals once weekly. They will be removed from the cage and physical examinations will be performed that include palpation for tissue masses. Animals will be observed postdose as detailed in the table below.

Table 20 Postdose observations in the carcinogenicity study

5.3 Post dosing observations		
Animal cohort	**Frequency of observation**	**Detail**
Carcinogenicity	Week 1: once daily 1-4 hours post dose; 10 animals/sex/group Week 2-end of dosing period: 10 animals/sex/group - once weekly 1-4 hours post-dose for 16 weeks, then once every 4 weeks thereafter.	Observations related to time of dosing Character and timing of reactions to treatment will be recorded The timing and frequency of observations may be modified at the discretion of the Study Director

Injection sites will be observed during the study as shown in the table below.

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Table 21 Injection site observations

5.4 Dose site observations		
Animal cohort	**Frequency of observation**	**Detail**
Carcinogenicity	Week 1: once daily 1-4 hours post dose; 10 animals/sex/group Week 2-end of dosing period: 10 animals/sex/group - once weekly 1-4 hours post-dose for 16 weeks, then once every 4 weeks thereafter.	Inflammatory skin reactions will be graded using a standardised scale based on OECD guideline for testing of chemicals 404
Toxicokinetic	Not required	Not required

Body weights and food consumption will be measured once weekly from day 1 to week 16 and once every 4 weeks thereafter.

Ophthalmic examinations will be conducted as detailed in the table below.

Table 22 Ophthalmic examinations

5.7 Ophthalmic examinations		
Animal cohort	**Frequency of observation**	**Detail**
Carcinogenicity	Pre-treatment: Group 1, 2, 3, 4; all animals Week 26, 52, 78, 104 : Group 1 and 4; 20 animals/sex/group Week 26, 52, 78, 104: If deemed necessary, Groups 3 and then 2; 20 animals/sex/group	Mydriatic agent administered prior to indirect ophthalmoscopy examination
Toxicokinetic	Not required	Not required

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The sponsor plans to collect blood samples from male mice at necropsy and from male decedents, if possible, for testosterone measurements.

Blood samples for measurement of plasma drug concentrations will be collected on day 1 and during week 26 as shown in the table below.

Table 23 Blood sampling for toxicokinetic measurements

6.2	Toxicokinetics
6.2.1	Sampling schedule: Day 1 and Week 26

Group/sex (Description)	Sample time (hours post dose)					
	0.5	1	2	4	8	24
	Animal numbers					
1M (Control)			409-411			
2M (Low)	412-414	415-417	418-420	421-423	424-426	427-429
3M (Intermediate)	430-432	433-435	436-438	439-441	442-444	445-447
4M (High)	448-450	451-453	454-456	457-459	460-462	463-465
1F (Control)			466-468			
2F (Low)	469-471	472-474	475-477	478-480	481-483	484-486
3F (Intermediate)	487-489	490-492	493-495	496-498	499-501	502-504
4F (High)	505-507	508-510	511-513	514-516	517-519	520-522
Additional information:						
Animal numbers may change if deaths occur						

Mice will be submitted to necropsy examinations and organs and tissues will be collected and prepared for histopathological examination as shown in the table below.

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Table 24 Necropsy examinations and preparation of organs and tissues for histopathological examinations

7.2 Carcinigenicity animals	
7.2.1 Necropsy	
Animal fate	Groups 1, 2, 3, 4, including decedents: Necropsied
Macroscopic examination	Groups 1, 2, 3, 4, including decedents
Organs weighed (W)	Not required
Tissues preserved (P)	Groups 1, 2, 3, 4, including decedents
Additional information	Histology and microscopic pathology will be carried out by external Principal Investigators; see Section 7.1.
7.2.2 Histology: Principal Investigator	
Tissue trimming, processing and embedding (E)	Groups 1, 2, 3, 4: all tissues Decedents: all tissues Testes and epididymides from early decedents be processed at Covance as follows: These tissues will be fixed in Bouin's fluid following Covance Histology standard operating procedures for cutting down, processing and embedding. This will ensure the tissue architecture is maintained for microscopic examination.
Microtomy and staining (E)	Groups 1, 2, 3, 4: all tissues Decedents: all tissues
Additional information	None
7.2.3 Pathology: Principal Investigator	
Microscopic evaluation (E)	Groups 1, 2, 3, 4: all tissues Decedents: all tissues

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Table 25 Tissue list for the carcinogenicity study

7.4 Tissue list							
Tissue / organ				**Tissue / organ**			
Bone marrow smear (femur)				Salivary glands – mandibular (x1), sublingual (x1), parotid (x1)	P		E
Eyes (x2)	P[1]		E				
Optic nerves (x2)	P		E	Mandibular lymph nodes (x1)	P		E
Harderian glands (x2)	P[2]			Thymus (x1)	P		E
Skin and subcut tissue (x1)	P		E	Lungs (x2)	P		E
Mammary (x1)	P		E	Heart (x1)	P		E
Muscle (quadriceps) (x1)	P		E	Aorta (x1)	P		E
Femur with bone marrow and stifle joint (x1)	P		E	Trachea (x1)	P		E
				Oesophagus (x1)	P		E
Sternum with bone marrow (x1)	P		E	Tongue (x1)	P		E
Sciatic nerves (x2)	P		E	Thyroids (x2) + parathyroids (x2)	P		E
Liver (x 2)	P		E	Larynx (x1)	P		E
Spleen (x1)	P		E	Pituitary (x1)	P		E
Pancreas (x1)	P		E	Brain (x3)	P		E
Mesenteric lymph nodes (x1)	P		E	Spinal cord cervical (x1)	P		E
Stomach (x1)	P		E	Spinal cord thoracic (x1)	P		E
Duodenum (x1)	P		E	Spinal cord lumbar (x1)	P		E
Jejunum (x1)	P		E	Lacrimal glands (x2)	P[2]		
Ileum (x1)	P		E	Zymbal glands (x2)	P		
Caecum (x1)	P		E	Bronchial lymph nodes (x1)			
Colon (x1)	P		E	Trachea bifurcation (x1)	P		
Rectum (x1)	P		E	Head (not processed)	P		
Gall bladder (x1)	P		E	Nasal cavity (x2)	P		
Adrenals (x2)	P		E	Nasal cavity (x2)	P		
Kidney (x2)	P		E	Nasopharynx (x1)	P		
Testes and epididymides (x2)	P		E	Nares (x1)	P		
Ovaries (x2)	P		E	Popliteal lymph nodes (x2)	P		E
Seminal vesicles (x2)	P		E	Ureters (x2)	P		E
Urinary bladder (x1)	P		E	Peyer's patch (x1)	P		E
Prostate (x1)	P		E	Oviducts (x2)	P		E
Uterus including cervix (x3)	P		E	Dosing sites[3] (variable)	P		E
Vagina (x1)	P		E	Animal identification	P		
Brown fat	P		E	Gross lesions (variable)	P		E
				Tissue masses (variable)	P		E
Legend:							
P = Tissues preserved; W = Organs weighed; E = Tissues processed and examined microscopically							
(xN) = number of sections for histopathology							
Footnotes:							
1: Tissue taken into Davidson's fixative							
2: Preserved with the head (*in situ*) unless histopathological processing is required							
3: All dose sites preserved 3 dose sites examined (IJ1, IJ2 and IJ3)							
Additional information:							
Fixative will be neutral buffered 10% formalin unless specified otherwise							
Left and right organs will be weighed together							
Lungs to include mainstem bronchi and bronchioles							
Bone tissue designated for histopathological examination will be decalcified using Kristenson's fluid							

Evaluation: The dose of 50 mg/kg in the 13-week dose range finding toxicology study was identified as a lethal dose. Further, epithelial ulceration was identified at injection sites was identified was several animals in the 50 mg/kg group. The sponsor should revise doses to 2, 5, and 15 mg/kg/dose administered twice per week per ECAC recommendations and conclusions on November 2, 2010.

The ophthalmic examination is generally not required for a carcinogenicity study and might be removed from the study protocol.

Other aspects of the study design appear to be acceptable. Ablation of testosterone levels was observed in the 26-week toxicology study with rats.

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11 Integrated Summary and Safety Evaluation

In the present submission, the sponsor provided a report for a second 13-week dose range finding toxicology study with mice (JE049-0170) as well as a dose selection proposal and draft protocol for a new 2-year carcinogenicity study.

The ECAC originally considered dose selections and designs for the 2-year carcinogenicity studies with mice and rats on June 10, 2008 (see attached minutes in the Appendix). The ECAC recommended doses of 5, 15, and 50 mg/kg/day for the mouse. However, the sponsor initiated the 2-year carcinogenicity study with mice with doses of 0, 10, 30, and 100 mg/kg/day, respectively, which were not in agreement with doses recommended by the ECAC. The study was halted on Day 12 of treatment after severe local reactions were observed in animals receiving 30 and 100 mg/kg/day. A series of investigative studies to determine possible causes for the severe dose-site reaction seen in the early stages of the 104 week mouse carcinogenicity study was performed. Two factors were identified that could have been responsible for the unexpected results in the mouse carcinogenicity study (JE049-0161). The first factor was that a majority of the mice in the first 13-week dose range finding toxicology study (JE049-0159) were non naïve towards icatibant at the start of the dosing period. The second factor was that the carcinogenicity study was mistakenly initiated with the doses proposed in the protocol submitted to the SPA Carcinogenicity Committee (0, 10, 30, and 100 mg/kg/day) rather than the FDA recommended doses noted in the June 11, 2008 ECAC report (0, 5, 15, 50 mg/kg/day), resulting in a 2-fold increase in the intended dose. Upon agreement with the FDA (FAX dated November 24, 2008), the 104 week study was suspended, and a new 13-week dose range finding study (JE049-0170) was initiated.

The Division provided comments (see Review and FAX dated May 11, 2009 and May 20, 2009, respectively) on the design of the 13-week subcutaneous dose ranging toxicology study that was provided in the present submission.

In a 13-week subcutaneous toxicology study (JE049-0170), mice received icatibant at doses of 0, 5, 15, and 50 mg/kg twice per week. One male animal (#33) given 50 mg/kg/dose was sacrificed for welfare reasons on day 53 due to findings of epidermal ulceration on the neck at subcutaneous injection sites. These findings were directly related to the subcutaneous administration of the test article. Clinical observations of mice found that 10 of 10 males and 9 of 10 females in the 50 mg/kg/dose group had sores/lesions associated with subcutaneous injections. Two of 10 males and 4 of 10 females in the 15 mg/kg/dose group had sores/lesions. Postdose observations recorded hunched appearance with piloerection and semi-closed eyes for the majority of mice in the 50 mg/kg/dose group between 1 and 2 hr postdose. These findings started during the first week of dosing. There was some imbalance between the clinical signs and gross pathologic/histopathologic findings in which the incidence of findings at injection sites for the 15 mg/kg/dose group was lower with the gross pathologic/histopathologic examinations. Absolute body weights and body weight gains were unaffected. Target tissues/organs of toxicity were the injection sites, skin + subcutis, liver, axillary and inguinal lymph nodes, stomach, urinary bladder, vagina, and Harderian glands. Findings

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for injection sites #1, 2, and/or 3 were observed for males and females in the mid and/or high dose groups and consisted of dose-related increased incidences of scab formation, epidermal hyperplasia, epidermal ulceration, dermal inflammatory cell infiltration, dermal fibrosis, panniculus muscle degeneration, folliculitis, and/or dermal mineralization. Findings of epidermal ulceration were generally confined to males and females in the high dose group with the exception of one male in the mid dose group. These findings of ulceration are considered dose limiting. Histopathological findings in other organs and tissues did not appear to be dose limiting with respect to identification of a MTD. The MTD was identified at 15 mg/kg/dose based upon the moribund sacrifice of one male in the 50 mg/kg/dose group due to findings of epithelial ulceration on the neck associated with subcutaneous administration of the test article as well as findings at injection sites for the 50 mg/kg/dose group that included high incidences of scab formation, epidermal hyperplasia, epidermal ulceration, dermal inflammatory cell infiltration, dermal fibrosis, panniculus muscle degeneration, folliculitis, and/or dermal mineralization. The ulceration is a particular concern.

Exposure margins between the MTD of 15 mg/kg/dose in mice and clinical doses of icatibant at 30 and 90 mg/day are shown in the table below. There is adequate coverage for icatibant; however, M2 is not cover (AUC ratio is <1). The proposed low dose at 5 mg/kg/dose in mice provides exposure margins of 1.6 and 0.53 for icatibant exposures achieved with clinical doses of 30 and 90 mg/day, respectively; however, exposure margins for M2 are significantly less than 1.

Table 26 Exposure margins for clinical doses of 30 and 90 mg/day

Analyte	13-week mouse study (JE049-0170) MTD = 15 mg/kg/dose	Clinical dose of 30 mg	Exposure margins	Clinical dose of 90 mg/day	Exposure margins
	AUC_{0-t}, ng*hr/mL (M+F on day 91)	AUC ng*hr/mL		AUC ng*hr/mL	
Icatibant	19500	4107	4.7	12300	1.6
M1	1340	?		?	
M2	1253	3023	0.4	9100	0.14

Reference ID: 2977968

The sponsor provided a draft protocol for the 2-year carcinogenicity study with mice. Dose should be revised to 2, 5, and 15 mg/kg/dose administered twice weekly per November 2, 2010 ECAC Recommendations and Conclusions. The sponsor proposed to conduct ophthalmic examinations during the 2-year study although this would appear unnecessary and could potentially subject the animals to manipulation and stress that could result in mortality. Other aspects of the study were acceptable.

3 SPECIFIC QUESTIONS FOR DISCUSSION

3.1 Non-Clinical

► Question 1

A 13 week dose-range finding study in the mouse was performed using a twice weekly dosing regimen at dose levels of 0, 5, 15 and 50 mg/kg. Based on the findings from Study JE049-0170, the maximum dose suggested for subsequent carcinogenicity studies is twice weekly dosing at 50 mg/kg/dose.

Does the Executive Carcinogenicity Assessment Committee (ECAC) agree that the proposed dosing regimen of 0, 5, 15 and 50 mg/kg administered twice weekly, based on results of study JE049-0170, is acceptable for the proposed 104 week carcinogenicity study in the mouse?

Response: The MTD in study JE049-0170 was identified at 15 mg/kg/dose based upon the moribund sacrifice of one male in the 50 mg/kg/dose group due to findings of epithelial ulceration on the neck associated with subcutaneous administration of the test article as well as findings at injection sites for the 50 mg/kg/dose group that included high incidences of scab formation, epidermal hyperplasia, epidermal ulceration, dermal inflammatory cell infiltration, dermal fibrosis, panniculus muscle degeneration, folliculitis, and/or dermal mineralization. The ulceration is a particular concern.

Doses for the proposed 2-year carcinogenicity study with mice should be revised to 2, 5, and 15 mg/kg/dose administered twice per week per November 2, 2010 ECAC recommendations and conclusions.

► Question 2

Does the Executive Carcinogenicity Assessment Committee (ECAC) agree that the proposed study design (e.g. duration, sample size, animal model) for a 104 week mouse carcinogenicity study is acceptable?

Response: The general study design with respect to duration, numbers of animals per group, animal model, and histopathological examinations of tissues appear to be acceptable. Ophthalmic examinations during the 2-year study appear unnecessary and

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could potentially subject the animals to manipulation and stress that could result in mortality.

12 Appendix/Attachments

1. ECAC Meeting Minutes dated June 10, 2008

Reference ID: 2977968

12.7 Appendix 7: ECAC Minutes, June 11, 2008

Food and Drug Administration
Center for Drug Evaluation and Research
Office of New Drugs

FACSIMILE TRANSMITTAL SHEET

DATE: June 11, 2008

To: Glen Park	**From:** Adele Seifried
Company: Jerini AG	OND IO
Fax number: (212) 681-2105	**Fax number:** 301-796-9855
Phone number: (212) 681-2100	**Phone number:** 301-796-0535

Subject: Response to Carcinogenicity Special Protocol Assessment Request - Final CAC Report - IND 68,214

Total no. of pages including cover: 6

Comments:

Document to be mailed: ☐ YES
☑ NO

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Executive CAC
Date of Meeting: June 10, 2008

Committee: David Jacobson Kram, OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Lois Freed, Ph.D., DNP, Alternate Member
Timothy McGovern, Ph.D., DPAP, Team Leader
Molly Shea, Ph.D., DPAP, Presenting Reviewer

Author of Minutes: Molly Shea, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

The Committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogenicity bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND # 68, 214
Drug Name: Icatibant Acetate (Firazyr)
Sponsor: Jerini AG

Background: Icatibant acetate is a synthetic decapeptide being developed for the treatment of patients with hereditary angioedema (HAE) who are 18 years old and older. In Type I (quantitative deficiency) and Type II (qualitative deficiency) HAE a deficiency of C1-esterase inhibitor (C1-INH) results in an increased release of bradykinin (BK). BK binds to the bradykinin type-2 receptor (B_2) to trigger activation of many cascades leading to vasodilatation, increased vascular permeability and smooth muscle cell contraction, resulting in the increased vascular permeability and acute inflammation observed in HAE patients. The anticipated clinical dosing of icatibant in patients having an HAE attack is 30 mg/day up to 3 times daily SC. Therefore, the maximum recommended human dose (MRHD) of icatibant is 90 mg/day, which is associated with AUC levels of 14.27 mcg*h/mL (extrapolated from AUC levels observed in elderly females treated with 30 mg/day SC dosing).

Absorption, distribution, metabolism and elimination (ADME) studies were conducted in the mouse and rat. However, these studies were limited for the mouse. In both the mouse and the rat, icatibant is metabolized to the active metabolites M1 (b) (4) and M2 (b) (4) by hydrolysis of icatibant's peptide backbone, thought to be induced by peptidases. M1 is formed at >90%, ~50% and 70% in human, mouse and rat, respectively. M2 is formed in rat ~9% and in human ~75%. After repeat SC dosing in rats, icatibant is rapidly distributed and metabolized with the M2 metabolite appearing from 0.2 to 3 hours post-dose. Neither icatibant nor M2 accumulated after repeat dosing in male and female rats up to 13-weeks dosing as was observed in the NDA. The biological half-life was more than 5 hours after a 26 µg/kg SC dose in rats and

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terminal half-life was over 6 days in rats after 10 µg/kg, SC. There were no differences observed in rats in the systemic exposures of males and females. Serum protein binding of icatibant was low with 44 and 49% in human and rat, respectively. No serum protein data are available for the mouse.

Neither icatibant nor the M1 or M2 metabolites is metabolized by human hepatocytes or microsomes. Additionally, icatibant is not an inducer of CYP450 isozymes. Based on excretion studies, the primary route of elimination after SC administration of icatibant is via the kidneys in mice (~97.1%) and rats (~61%) with small amounts excreted in the feces (8.0% to 33%).

Jerini completed a full genetic toxicity battery assessment for icatibant. Icatibant did not induce genetic toxicity in the presence or absence of metabolic activation in the bacterial reverse mutation assays or *in vitro* mammalian chromosomal aberration studies or micronuclei formation in *in vivo* assays under the conditions tested.

The sponsor sought concurrence for dose selection and regimen for two 104-week carcinogenicity studies in mice and rats.

Mouse Carcinogenicity Study or Mouse Dose Selection

The sponsor proposed a standard 2-year carcinogenicity study in CD-1 mice at SC doses of 0 (vehicle control), 10, 30 and 100 mg/kg/day. Three injections sites are to be used with daily rotation. The sponsor considered icatibant doses up to 100 mg/kg/day in mice as the maximum tolerated dose based on local tolerance and stated that this dose provides an exposure which is at least ≈7 times that reached in the clinic at the MRHD of 90 mg/day.

Based on a detailed review of the dose-range finding 13-week SC mouse study, the dose-limiting toxicity was injection site reaction with hemorrhage, ulceration and necrosis observed at multiple injection sites in males and females that increased in incidence and severity at the 100 mg/kg/day dose. Of note, mice were dosed with 100 mg/kg/day for only 4 weeks (from Weeks 9 to Weeks 13) and severe local reactions were observed for this truncated dosing interval compared to the 13-week dosing up to 50 mg/kg/day. Therefore, the MTD was exceeded at 100 mg/kg/day SC doses based on the injection site toxicity. The MTD was determined to be 50 mg/kg/day based on tolerable levels of injection site reaction.

Rat Carcinogenicity Study or Rat Dose Selection

The sponsor proposed two alternative dosing regimens for the 2-year rat carcinogenicity study at doses that were based on the MTD, pharmacodynamic action of icatibant and on previously conducted repeat dose toxicity studies in the dog which used similar alternative dosing regimens. These regimens alternated seven injection sites and were presented as follows:

Option A: 0 (0.9% physiological saline-VC), 3 mg/kg t.i.d (twice weekly), 10 mg/kg t.i.d (twice weekly) and 10 mg/kg/day

Option B: 0 (0.9% physiological saline-VC), 10 mg/kg t.i.d. (twice weekly), 3.3 mg/kg/day and 10 mg/kg/day

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The sponsor proposed a HD of 10 mg/kg/day of icatibant in each of the alternative dosing regimens. The two dosing regimens incorporated daily and intermittent dosing but differed in whether the dose response relationship of any eventual findings would be investigated based on daily or intermittent dosing. The sponsor stated that 10 mg/kg t.i.d to be administered twice weekly allows for a greater daily dose while allowing for sufficient rotation of injection sites.

Based on a detailed review of the dose-range finding 13-week SC Wistar rat study, the dose-limiting toxicities were reduced body weight gain or body weight loss in all treated male groups and high dose females and injection site reactions in males and females. The MTD was 10 mg/kg/day in females based on no change in group mean body weight gains, no injection site reactions and reduced incidence of effects observed in the thymus, adrenals and female reproductive organs at this dose. For males, the MTD was not identified based on male body weight gain reduction exceeding 10% in all icatibant treated animals and injection site reaction at doses > 10 mg/kg/day.

Executive CAC Recommendations and Conclusions:

Mouse:

The Committee recommended daily doses of 0 (VC), 5, 15, and 50 mg/kg/day, by SC injection and injection site rotation in males and females, based on an MTD criterion (severe local toxicity).

Rat:

The Committee concluded that the data were insufficient to make dose recommendations for male rats as all tested doses exceeded the MTD based on significant reductions in body weight gain in comparison to control animals. The proposed alternative dosing regimens (t.i.d dosing, 2 times per week) are not supported by the 13-week repeat dose rat study which administered drug on a daily basis. Due to the absence of TK data in the draft study report for the dose-ranging study, rat to human exposure comparisons can not be calculated.

The Committee recommended daily doses of 0 (VC), 1, 3, and 10 mg/kg/day for female rats, by SC injection with site rotation, based on MTD (severe local toxicity).

If a dosing regimen other than daily dosing is desired, the range-finding study should be conducted using that regimen.

General comments:

The sponsor should contact the Division prior to terminating any groups or making any dose adjustments.
The proposed hematological and urine measurements should be omitted.
TK evaluations are not needed beyond 6 months.

David Jacobson Kram, Ph.D.

Reference ID: 2977968

Chair, Executive CAC

cc:\
/Division File, DPAP
/TMcGovern, DPAP
/MShea, DPAP
/CHill, RPM, DPAP
/ASeifried, OND IO

Reference ID: 2977968

12.8 Appendix 8: ECAC Minutes, November 3, 2010

Food and Drug Administration
Center for Drug Evaluation and Research
Office of New Drugs

FACSIMILE TRANSMITTAL SHEET

DATE: November 3, 2010

To: Thomas Class	**From:** Adele Seifried
Company: Shire Human Genetic Therapies	OND IO
Fax number: (978) 869-1542	**Fax number**: 301-796-9855
Phone number: (781) 482-9130	**Phone number:** 301-796-0535

Subject: Response to Carcinogenicity Special Protocol Assessment Request - Final CAC Report - IND 68,214

Total no. of pages including cover: 4

Comments:

Document to be mailed: ☐ YES ☑ NO

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Executive CAC
Date of Meeting: November 2, 2010

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Adam Wasserman, Ph.D., DAAP, Alternate Member
Molly Topper, Ph.D., DPARP, Supervisor
Tim Robison, Ph.D., DPARP, Presenting Reviewer

Author of Draft: Tim Robison, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

The committee did not address the sponsor's proposed statistical evaluation for the carcinogen bioassay, as this does not affect the sponsor's ability to initiate the bioassay. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following the CDER/CBER Guidance for Industry, Providing Regulatory Submission in Electronic Format- Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (June 2008) and the associated Study Data Specifications document.

IND # 68,214
Drug Name: Firazyr® (Icatibant)
Sponsor: Shire Human Genetic Therapies

Mouse Carcinogenicity Study Protocol and Dose Selection

Background: In a second 13-week subcutaneous toxicology study (JE049-0170), CD-1 mice received icatibant at doses of 0, 5, 15, and 50 mg/kg in 0.9% physiological saline twice per week with rotation among three sites. One male animal given 50 mg/kg/dose was sacrificed for welfare reasons on day 53 due to findings of epidermal ulceration on the neck at subcutaneous injection sites. These findings were directly related to the subcutaneous administration of the test article. Sores/lesions were observed at injection sites in a dose-related manner for mice at 15 and 50 mg/kg/dose. Target tissues/organs of toxicity were the injection sites, skin + subcutis, liver, axillary and inguinal lymph nodes, stomach, urinary bladder, vagina, and Harderian glands. Findings for injection sites #1, 2, and/or 3 were observed for males and females in the mid and/or high dose groups and consisted of dose-related increased incidences of scab formation, epidermal hyperplasia, epidermal ulceration, dermal inflammatory cell infiltration, dermal fibrosis, panniculus muscle degeneration, folliculitis, and/or dermal mineralization. Findings of epidermal ulceration were generally confined to males and females in the high dose group with the exception of one male in the mid dose group. Other histopathological findings did not appear to be dose limiting with respect to identification of a MTD. The MTD was identified at 15 mg/kg/dose based upon the moribund sacrifice of one male in the 50 mg/kg/dose group due to findings of epithelial ulceration on the neck associated with subcutaneous administration of the

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test article as well as findings at injection sites for the 50 mg/kg/dose group that included high incidences of scab formation, epidermal hyperplasia, epidermal ulceration, dermal inflammatory cell infiltration, dermal fibrosis, panniculus muscle degeneration, folliculitis, and/or dermal mineralization. The ulceration is a particular concern.

Executive CAC Recommendations and Conclusions:

- The Committee recommended doses of 0, 2, 5, and 15 mg/kg/dose by subcutaneous injection twice per week, based on MTD (epithelial ulceration associated with subcutaneous injections).

- The sponsor should contact the Review Division if skin lesions require dose reductions during the study.

- The committee noted that the ophthalmic examinations in the 2-year carcinogenicity study with mice are not required.

If the sponsor plans histological evaluation of tissues from only control and high dose treatment groups, they will also need to conduct histopathologic examination of other dose groups under any of the following circumstances:

(a) for any macroscopic findings in the low and mid dose groups for a given tissue, they will need to look at that tissue for all of the dose groups
(b) for statistically significant or otherwise remarkable findings in the high dose group, the sponsor will need to look at the affected tissues in all of the dose groups.
(c) for an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc.; see McConnell et al, JNCI 76:283, 1986) they should look at all relevant tissues for that dose level and the next lower dose level,
(d) for an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DPARP
/MTopper, DPARP
/TRobison, DPARP
/CHill, DPARP
/ASeifried, OND IO

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/s/

HANS M ROSENFELDT
07/23/2011

TIMOTHY W ROBISON
07/23/2011
I concur

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D.
OND IO
NDA: 22-150
Date NDA received: 26 October 2007
Drug: icatibant acetate
Sponsor: Jerini U.S. Inc.
Indication: Treatment of hereditary angioedema in patients 18 years old and older
Reviewing Division: Division of Pulmonary and Allergy Products

Comments:
I have reviewed the pharmacology/toxicology review and the supervisory pharmacologist memo for this NDA. The reviewer and the supervisor found the nonclinical information submitted to be adequate to support the use of the product for up to 13 weeks duration. The NDA did not contain chronic toxicity data or carcinogenicity data. Hereditary angioedema patients could require repeated treatment over their lifetime. Therefore, even though treatment with icatibant acetate may only occur intermittently, the total duration of exposure could be prolonged. The division recommended to the sponsor in a preNDA meeting on January 24, 2007 that such prolonged intermittent use should be supported by chronic toxicity studies (6 month rodent and 9 month nonrodent) and carcinogenicity studies in mouse and rat. It appears that the chronic toxicity studies are currently underway and the carcinogenicity studies may be initiated soon.

I concur that the information submitted would be adequate to support dosing in humans for up to 13 weeks total duration and that the NDA is, therefore, approvable from a pharm/tox perspective for this duration. I also concur that the chronic toxicity data should be submitted to support labeling of the product for use beyond 13 weeks. The division has indicated that they may be willing to permit submission of the carcinogenicity studies post-approval based on the severity of the indication and the results of the studies reviewed to date. This is a reasonable position.

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/s/

Paul Brown
4/3/2008 01:19:54 PM
PHARMACOLOGIST

Supervisory Pharmacologist Review

NDA: 22-150 – Firazyr (icatibant acetate for injection, 30 mg)
FROM: Timothy J. McGovern, Ph. D., Supervisory Pharmacologist
DATE: March 18, 2008

I concur with the recommendation by Dr. Molly Shea, the pharmacology/toxicology reviewer, that the pharmacology and toxicology of icatibant acetate has been adequately studied and that the drug product is approvable from a nonclinical standpoint (see NDA review dated March 18, 2008) for the treatment of hereditary angioedema (HAE). Of note, the available toxicology data supports a clinical dosing regimen up to 13 weeks.

Nonclinical studies were either previously reviewed under INDs 68,124 (subcutaneous dosing for HAE), (b) (4) IND 68,124 was originally assigned to the Division of Gastrointestinal and Coagulation Products but was reassigned to DPAP in August 2005. A pre-NDA meeting was held between Jerini on January 24, 2007 and Jerini was informed that the Division considers the potential dosing of HAE patients with no more than eight 30 mg subcutaneous injections a month over the lifetime of the HAE patient as chronic intermittent dosing. Therefore, nonclinical chronic repeat dose toxicity studies via the subcutaneous route of administration in the rat (6 month) and the dog (9 month) and 2 carcinogenicity studies (1 mouse and 1 rat) to address the carcinogenic potential of icatibant would be needed.

Pharmacology: Icatibant is a synthetic decapeptide with a structure similar to endogenous bradykinin (BK). It is a potent, selective BK type 2 (B_2) receptor antagonist that binds to B_2 with similar affinity as BK. Icatibant binding at the B_2 receptor inhibits BK induced processes and is hypothesized to contribute to the decrease of vascular permeability and acute inflammation observed in HAE patients. The pharmacology of icatibant was characterized in *in vitro* and *in vivo* models in which the selectivity for the B_2 receptor was demonstrated using a rabbit aorta model. Other pharmacology further characterized the binding affinity and activity of icatibant and two of its major metabolites, M1 (b) (4) and M2 (b) (4) on BK receptors demonstrating that icatibant is a competitive inhibitor of the B_2 receptor. Jerini also investigated the mechanism of observed injection site irritation and demonstrated that icatibant activates the release of histamine, LTC4 and PGD2 from mast cells and induces mobilization of calcium nerve fibers, which may contribute to local injection site irritation.

Safety pharmacology: The safety pharmacology of icatibant has been adequately addressed. Studies were conducted to assess icatibant related effects on cardiovascular, respiratory, central nervous and gastrointestinal systems. Icatibant and its metabolites M1 and M2 did not inhibit cardiac potassium currents up to 300 µM concentrations. Icatibant decreased coronary blood flow in isolated guinea pig heart, aggravated the duration of post-ischemic reperfusion arrhythmias in isolated working hearts in Wistar rats and impaired cardiodynamics. In repeat dose toxicology studies in dogs up to 13-weeks duration, there were no statistically significant changes in EKG parameters or changes in

blood pressures in icatibant treated males and females compared to the vehicle treated animals after 4 weeks dosing. In a 13-week study, there was a slight but statistically significant decrease in heart rate (21-29%) and a decrease in diastolic BP in males (-39%) at 6 h post-dose at a dose of 100 mg/kg. There were no significant differences in other measured parameters. No changes in respiratory function or GI transit were observed after intravenous dosing but icatibant did decrease gastric acid volume and acidity. Icatibant decreased urine output, sodium and potassium excretion and, to a lesser extent, GFR, renal plasma flow and osmolar clearance in beagle dogs at 0.3 mg/kg, IV. Icatibant also significantly inhibited BK-induced hypotension in anesthetized rats and produced decreased motor activity, assumption of prone position, reduced respiration, ptosis, piloerection and mydriasis at 1-10 mg/kg IV. These effects on central nervous system (CNS), cardiovascular (CVS) and renal systems are considered the result of the partial agonistic property of icatibant on B-receptors and may present toxicological concerns.

General toxicology: Subcutaneous toxicity studies up to 13 weeks were conducted in rats and dogs. In rats, 13-week treatment at doses up to 10 mg/kg produced no significant systemic toxicity; local injection site toxicity was observed. In contrast, a 14-day study at doses up to 50 mg/kg produced clinical signs of toxicity (injection site irritation), decreases in prostate and testes weights, and an increase in thymic weights in the HD group were observed in females. Histopathological findings were observed in the epididymides (oligospermia and aspermia) and testes (hypospermia) in all icatibant treated groups. No NOAEL could be defined due to male reproductive effects of icatibant at all doses. A follow-up 14-day study using the same doses in slightly older male rats also assessed the reversibility of the male reproductive organ effects. High dose males demonstrated decreased testicular and prostate weights. Histopathological findings included injection site irritation in the HD group, oligospermia and cellular debris in the lumen of the epididymis and hypospermia in the testes. The NOAEL for male reproductive organ effects in this study was considered 1 mg/kg. Interestingly, the identified NOAEL for this effect increased from 1 to 10 mg/kg as duration of treatment increased from 14-days to 13-weeks. Possible explanations for this observation include adaptive responses in the test species or the presence of drug batch impurities in the earlier batches used for the 14-day studies which used a different manufacturing process.

One 13-week SC toxicity study was conducted that dosed dogs up to 100 mg/kg/day. Clinical signs included erythema of the face, injection site irritation and several incidences of gastrointestinal disturbances (diarrhea and/or vomiting) at all doses. Due to abnormal vocalization in the HD group at day 12, the dose was decreased to 2 x 30 mg/kg/day. The target organs of toxicity included the male and female reproductive organ (decreased organ weight, immature), adrenal (increased weight), liver (increased weight), and thymus (decreased weights). Microscopic changes in the male (testes, epididymides, and prostate) and female (mammary gland, ovaries, uterus and vagina) reproductive organs, at the injection site (mainly HD groups but sporadic in LD and MD groups), in the adrenal gland (vacuolated cortical cell in HD males and females), liver (vacuolated hepatocyte in 1/4 males) and thymus (all doses) were observed. No NOAEL can be determined for this study. Exposure levels (AUC) in dogs ranged from 35 µg*h/mL at the low dose to 230 µg*h/mL at the high dose.

A second 13-week study was conducted with either single daily doses of 3 or 10 mg/kg/day of icatibant or 3.3 mg/kg TID administered twice weekly. Similar findings were noted in animals dose daily as in the previous 13-week study but no effects were observed in the male reproductive organs with twice weekly dosing; females treated with SC administration of icatibant twice weekly still showed delayed or impaired maturation of the reproductive organs. Observed clinical signs included increased erythema and edema at multiple sites that increased with increased dose. After the 13-week recovery period, 1/2 males showed the testes and prostate as immature where 1/2 males had recovered and there were no findings in female reproductive organs suggesting these adverse effects were reversible. Hormonal analysis showed that icatibant induced a decrease of testosterone, LH and FSH in male dogs, which secondarily induced sexual immaturity. As in the previous study, no NOAEL was determined in this study. However, twice weekly administration of icatibant resulted in less severe toxicity in males than with daily dosing.

Overall, the toxicology program supports the proposed clinical use of the drug product up to a duration of 13 weeks. Although a NOAEL was not identified in the 13-week dog studies, the achieved exposures to icatibant exceed those anticipated in the clinical setting and the toxicity profile has been adequately characterized. Given the indication of HAE, the lack of a safety margin based on toxicity studies is currently considered to be acceptable.

During the pre-NDA meeting, Jerini was advised that chronic toxicology studies would be necessary to support the anticipated use of this product. The Division was willing to consider accepting these studies as a Phase 4 commitment given the regulatory history of this application and the indication. However, the Division expected that the studies would be initiated promptly. In the Filing Letter for this NDA dated January 2008, the sponsor was advised that the icatibant labeling will reflect a clinical dosing regimen that is supported by the nonclinical toxicology studies in both dose and duration; currently the sponsor has nonclinical support for subcutaneous clinical dosing for up to 13-weeks duration. Jerini has indicated that their chronic toxicology studies are underway.

<u>Reproductive toxicity:</u> The reproductive toxicology program conducted with icatibant included a male mouse fertility study, rat and rabbit teratology studies, a rat fertility and early embryofetal development study that was incorporated into a 13-week toxicology study, and a rat pre- and post-natal development study in rats. The data support a Pregnancy Category of “C”. Treatment for 5 weeks did not affect fertility in male mice at doses up to 80.8 mg/kg, SC. Segment II studies in rats and rabbits produced no evidence of teratogenicity at SC doses up to 25 mg/kg and 10 mg/kg, respectively. However, a dose of 10 mg/kg was toxic to the embryo, reducing the litter sizes as a result of increased intrauterine embryofetal death rate. Icatibant treatment resulted in dose-related decreases for total implantations and total number of live fetuses as well as dose-related increases for percent of pre-implantation loss when compared to the control values.

The sponsor also evaluated the effects on fertility and early embryonic development in a 13-week repeat dose toxicity study in rats at doses up to 10 mg/kg. HD treated females demonstrated an increase in pre-implantation loss. Untreated females paired with HD treated males showed no increase in pre-implantation loss compared to untreated females paired with vehicle control treated males, indicating that icatibant treatment of females and not males resulted in the pre-implantation loss. Icatibant had no effects on male sperm count, motility, or morphology. No toxicity was observed in regard to male seminology, mating or fertility indices.

A peri- and post-natal development study in rats evaluated doses up to 10 mg/kg/day. Icatibant treatment delayed parturition at all doses, resulting in F_0 deaths at 3 and 10 mg/kg/day. Clinical signs of toxicity (pallor of eyes and extremities, round back, piloerection and reddish discharge in vagina) were observed in 1 MD and in multiple HD F_0 females. Based on the observed maternal toxicities including clinical signs, prolonged gestation periods for all icatibant dosage groups, F_0 spontaneous deaths and early sacrifice of icatibant treated dams, litter deaths, and necropsy findings in the MD and the HD groups, the maternal toxicity NOAEL could not be defined. The F_1 generation showed an increase in the number of pup deaths and cannabalism in the MD and HD. The HD F_1 generation also showed a slight decrease in air righting and hair growth. After SC administration, icatibant and the M2 metabolite is found in rat milk.

Genotoxicity: The sponsor has conducted multiple genetic toxicology studies for icatibant and other unnatural amino acids. Preliminary studies evaluated under IND (b) (4) included an Ames test with only 2 microbial strains and an *in vivo* micronucleus test conducted in mice. Under IND (b) (4), five genetic toxicology studies were conducted: 4 Ames assays (2 with icatibant and 2 with the unnatural amino acids (b) (4)) and an *in vitro* cytogenetic test of bone marrow cells from Chinese hamsters. Three new genetic toxicology studies were submitted in this NDA and included a bacterial reverse mutation assay, an *in vitro* mammalian chromosome aberration test, and an *in vivo* bone marrow micronucleus assay in Sprague-Dawley rats. The bacterial reverse mutation assay and the *in vivo* micronucleus study in rats were studies used to bridge the change in manufacturer for the drug substance. Icatibant was negative in all studies.

Carcinogenicity: No carcinogenicity studies are available at this time. At the pre-NDA meeting of January 2007, the sponsor was informed that two carcinogenicity studies will be required for the proposed indication. However, given the regulatory history of the application and the indication, the Division is willing to consider accepting the studies as a Phase 4 commitment; though it was expected Jerini would initiate the studies in a timely manner. Jerini has indicated that the studies will be initiated in April to July of 2008.

Labeling: The review team decided not to conduct a review of the product label in this review cycle.

In conclusion, the sponsor has completed an adequate nonclinical program to support clinical use up to 13 weeks. Chronic toxicity studies are underway and carcinogenicity studies are planned to be initiated in 2008. The anticipated clinical exposures have been covered in the conducted 13-week toxicology studies in rats and dogs and the primary toxicities appear to be reproductive organ effects and local injection site irritation. Of note, the latter finding has been observed in the clinic. Although NOAEL doses were not identified in supporting dog studies, the toxicity profile has been characterized and the findings are considered acceptable given the severity of the indication.

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/s/

Timothy McGovern
3/18/2008 05:48:51 PM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-150**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**10/22/07**
PRODUCT:	**Firazyr (Icatibant)**
INTENDED CLINICAL POPULATION:	**Patients with hereditary angioedema 18 years of age or older**
SPONSOR:	**Jerini US, Inc.**
DOCUMENTS REVIEWED:	**Electronic document**
REVIEW DIVISION:	**Division of Pulmonary and Allergy Products**
PHARM/TOX REVIEWER:	**Molly E. Shea, Ph.D.**
PHARM/TOX SUPERVISOR:	**Timothy J. McGovern, Ph.D.**
DIVISION DIRECTOR:	**Badrul Chowdhury, M.D, Ph.D.**
PROJECT MANAGER:	**Carol Hill**

Date of review submission to Division File System (DFS): March 18, 2008

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The nonclinical discipline recommends an approval of the application (NDA 22-150). The sponsor has submitted adequate nonclinical data to support the safety of Firazyr (Icatibant) for up to 13-weeks of clinical administration. Clinical administration beyond 13-weeks is not supported by the currently available nonclinical data. In the Division's Filing Letter dated January 8, 2008, the sponsor was informed that the icatibant labeling will reflect a clinical dosing regimen that is supported by the nonclinical toxicology studies in both dose and duration.

In the future, Jerini plans to complete two repeat dose subcutaneous chronic toxicity studies (one in rats and one in dogs) and two carcinogenicity studies (one in mice and one in rats) to support their proposed chronic intermittent indication. Additionally, Jerini will conduct two carcinogenicity studies (1 mouse and 1 rat) with icatibant. The toxicity profile of icatibant has been characterized adequately up to 13-weeks with the completion of repeat-dose subcutaneous studies up to 13-weeks. Each of the excipients included in the subcutaneous injection formulation are at or below levels found in other currently approved products that are administered via the same route of administration. However, impurities have been identified in the substance and the drug product that require qualification studies. Comments to the sponsor will be forthcoming from the ONDQA reviewer.

B. Recommendation for nonclinical studies

Chronic toxicity studies in two species (at least one non-rodent will be required to support chronic clinical use of the drug product. In addition, carcinogenicity studies will be needed to address the carcinogenic potential of icatibant under the anticipated chronic intermittent usage to treat hereditary angioedema. The following comment should be forwarded to the sponsor based on the Division's filing letter of January 2008:

The completion of nonclinical chronic repeat dose toxicity studies (6-month in rat and 9- month in dog) via the subcutaneous route of administration is required to support chronic intermittent clinical dosing. The icatibant labeling will reflect a clinical dosing regimen that is supported by the nonclinical toxicology studies in both dose and duration. At this time you have nonclinical support for subcutaneous clinical dosing for up to 13-weeks duration. We acknowledge your submission stating that chronic toxicity studies have been initiated as of June 2007 (dog study) and July 2007 (rat study) and the carcinogenicity studies will be initiated April 8, 2008 and July 7, 2008 for mice and rats, respectively.

C. Recommendations on labeling

The review team has concluded that the recommended action for this NDA is Not Approvable. Therefore, a detailed review of the proposed labeling will not be performed at this time.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The toxicity profile of icatibant acetate has been well characterized up to 13-weeks administration via the subcutaneous route of administration. Exposures of the parent compound in animals have achieved 1.4- to 3.6-fold multiples of the anticipated human exposure at the recommended maximum clinical dose of 90 mg. The primary toxicities observed in animal studies are the male (immature testes, epididymides, and prostate) and female reproductive organs (immature ovaries and uterus) and the injection site irritation (with possible histamine release). In males, testosterone levels were reversibly reduced between 90 to 95% when icatibant was administered daily. However, when icatibant was administered twice weekly, the effects on the male reproductive organs were significantly blunted and testosterone levels recovered between icatibant doses. For females, twice weekly dosing did not reduce the icatibant-induced effects on reproductive organs. Additional toxicities in animals were observed in the thymus (lymphoid depletion), adrenal (cortical cellular vacuolation) and liver (vacuolated hepatocyte). In rats treated with a newly manufactured batch of icatibant (different manufacturer) for 13-weeks via SC administration resulted in no toxicities (NOAEL of 10 mg/kg, associated with icatibant exposure that is 1.4-fold greater than maximum clinical exposure at 90 mg). In studies of a shorter duration (14-weeks) and at higher icatibant doses using an icatibant batch manufactured at a different site than that used in the 13-week study, the primary target organs of toxicity were also the male and female reproductive organs, the injection site and thymus. In dogs treated with icatibant daily via SC administration for 13-weeks, the primary target organs of toxicity were the male and female reproductive organs, injection sites, adrenal gland, liver and thymus. Although a NOAEL was not identified in 13-week studies with daily dosing, the dosing regimen provided adequate exposure coverage compared to that anticipated in the clinic.

During icatibant's drug development, Jerini has evaluated icatibant's effects on the CNS, CVS, respiratory, renal and GI systems. Icatibant had no effect on cardiac potassium currents using *in vitro* test systems, on respiratory function or on GI transit but did decrease gastric acid volume and acidity. Partial agonistic properties of icatibant were observed on CNS (decreased motor activity, reduced respiration, ptosis, piloerection and mydriasis), CVS (decreased coronary blood flow and aggravated duration of post-ischemic

reperfusion arrhythmias in isolated heart models) and renal systems (decreased urine output, sodium and potassium excretion renal plasma flow and osmolar clearance). In a 13-week dog study dosed up to 100 mg/kg/day, dogs showed a decrease in heart rate (-25%) and a decrease in diastolic BP (-39%) 6 h post-dose on Week 1 of dosing at 100 mg/kg/day. No effects on QT interval or other safety pharmacology findings were observed.

A complete genetic toxicology battery for icatibant was evaluated. Under the conditions tested, icatibant was not mutagenic. Carcinogenicity studies have not been conducted. Jerini plans to initiate 1 mouse and 1 rat carcinogenicity study using icatibant April 2008.

The reproductive and development toxicity battery has been completed for icatibant. The sponsor conducted a 5-week fertility study in male mice, a fertility and early embryonic development to implantation study in rats, 2 definitive teratogenic studies (1 in rats and 1 in rabbits), and a pre- and post-natal development in pregnant rats. Icatibant had no effect on male fertility in mice up to 80.8 mg/kg SC. In rats treated up to 10 mg/kg/day SC, Icatibant had no effects on male sperm count, motility, or morphology. An increase in pre-implantation loss in females was observed at 10 mg/kg/day. Icatibant was not teratogenic in rats (up to 25 mg/kg/day, SC) or rabbits (up to 10 mg/kg/day, SC). However, at 10 mg/kg/day, icatibant was embryo toxic in rabbits reducing the litter sizes as a result of increased intrauterine embryofetal death rate. Icatibant treatment resulted in dose-related decreases for total implantations and total number of live fetuses as well as dose-related increases for percent of pre-implantation loss when compared to the control values. In the pre- and post-natal development study conducted in rats (up to 10 mg/kg/day, SC), icatibant did not effect fertility or have any significant effect on pup development. However, icatibant did prolong gestation, resulting in F_0 spontaneous deaths and litter deaths. The F_1 generation showed an increase in the number of pup deaths and cannabalism in the MD and HD compared to the VC groups. After SC administration, icatibant and the M2 metabolite is found in rat milk. Based on these results, icatibant should be classified as Pregnancy Category C.

B. Pharmacologic activity

In Type I (quantitative deficiency) and Type II (qualitative deficiency) hereditary angioedema (HAE) a deficiency of C1-esterase inhibitor (C1-INH) results in an increased release of bradykinin (BK). BK binds to the bradykinin type-2 receptor (B2) to trigger activation of many cascades leading to cellular proliferation, excitation of neurons, vasodilatation, increased vascular permeability and smooth muscle cell contraction. These events are believed to contribute to the increased vascular permeability and acute inflammation observed in HAE patients.

Icatibant is a synthetic decapeptide with similar structure as endogenous bradykinin. It is a potent, selective bradykinin-2 (B_2) receptor antagonist that binds to the B_2-receptor with similar affinity as bradykinin. Icatibant binding at the B_2 receptor inhibits bradykinin induced processes and is hypothesized to contribute to the decrease of vascular permeability and acute inflammation observed in HAE patients.

C. Nonclinical safety issues relevant to clinical use

The primary toxicities in animal studies treated with icatibant were injection site irritation and immature male (testes, epididymides and prostates) and female (ovaries and uterus) reproductive organs. As observed in reproductive studies, icatibant treatment may induce pre-implantation loss (embryo-toxic), increase spontaneous abortions and prolong gestation length. Icatibant should not be administered to pregnant women. Icatibant has been shown to be excreted in milk of lactating rats. The sponsor has identified injection site irritation in clinical studies.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

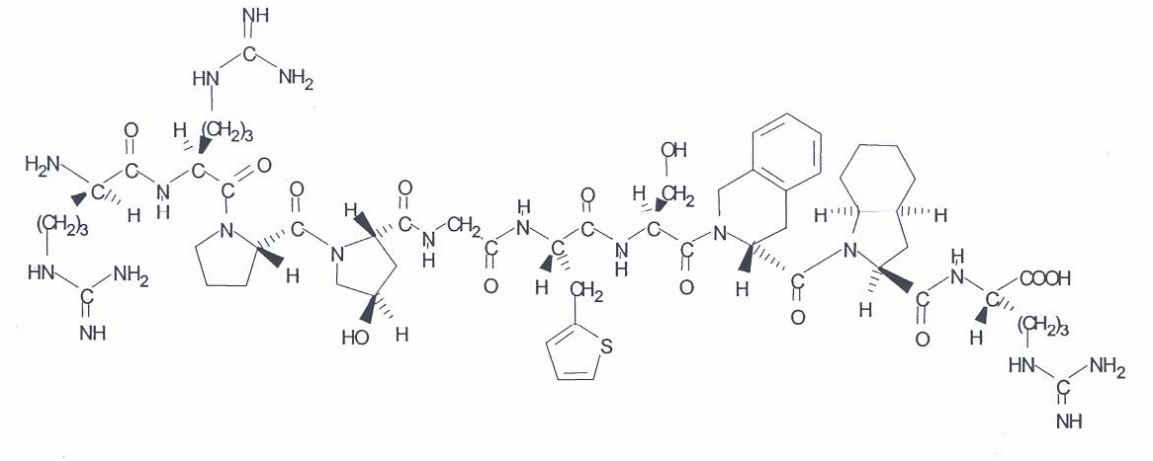
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-150
Review number: 1 Original review
Sequence number/date/type of submission: 000
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Jerini US Inc.
Manufacturer for drug substance: (b) (4)

Reviewer name: Molly E. Shea, Ph.D.
Division name: Division of Pulmonary and Allergy Products (DPAP)
Review completion date: March 18, 2008

Drug:

Trade name: Firazyr
Generic name: Icatibant acetate
Code name: JE 049/HOE 140/S 89 0140
Chemical name: D-arginyl-L-arginyl-L-prolyl-L[(4R)-4-hydroxyprolyl]-glycyl-L-[3-(2-thienyl)alanyl]-L-seryl-D-(1,2,3,4-tetrahydroisoquinolin-3-yl-carbonyl)-L-3aS,-7aS)-octahydroindol-2-yl-carbonyl]-L-arginine acetate
CAS registry number: NA
Amino acid sequence: H-D-Arg-L-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-OH· nCH_3COOH
Molecular formula/molecular weight: $C_{59}H_{89}N_{19}O_{13} \cdot C_2H_4O_2$/1304.6
Structure:



Relevant INDs/NDAs/DMFs: IND 68,214 (Subcutaneous for HAE); (b) (4) DMF (b) (4) DMF (b) (4)

Drug class: Bradykinin type 2 (B_2) receptor antagonist

Intended clinical population: Patients 18 years of age or older with acute hereditary angioedema (HAE).

The proposed labeling recommends repeated injections, if required, at intervals not less than 6 hours and not to exceed 3 injections in 24 hours for a maximum daily dose of 90 mg/day.

Clinical formulation: Icatibant 30 mg solution for injection is delivered as a 10 mg/mL sterile, isotonic, acetate-buffered solution. The following table provides the formulation of icatibant for subcutaneous injection.

Ingredient	Quantity per mL	Function
Icatibant	10.0 mg	Active ingredient
Sodium hydroxide, NF/Ph.Eur.	(b) (4)	
Glacial acetic acid, USP/Ph. Eur.		
Sodium chloride, USP/Ph. Eur.		
Water for injection, USP/Ph. Eur.		

Route of administration: Subcutaneous injection (SC)

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

Studies reviewed within this submission:

Pharmacology

JE049-0003	In vitro pharmacology: Human B_1 and B_2 Receptors. Study of icatibant
JE049-0004	In vitro pharmacology: Human B_1 and B_2 Receptors. Study of M1, M2 and Icatibant
JE049-0201	Study of icatibant (HOE 140) in various receptor binding and enzyme assays
JE049-0218	In vitro pharmacology: high throughput profile. Study of icatibant, M2 and M1
JE049-0217	In vitro pharmacology: LH-RH receptor binding assay. Study of icatibant, M2 and M1
JE049-0215	Characterization of the effects of icatibant on human mast cells
JE049-0216	Icatibant dose- and calcium-dependently induces calcitonin gene-related peptide release from isolated skin in wild-type, B2 -/- and KITW/KITW-v but less in TRPV1 -/- mice

JE049-0013	Project summary for: Analysis of species specificity and potential agonistic activity of icatibant using MAPK activation and calcium mobilization assays
JE049-0202	Effect of HOE 140 on cardiac potassium channels expressed in Xenopus oocytes
JE049-0203	Electrophysiological examination of the effects of icatibant and its metabolites M1 and M2 on the HERG-mediated potassium current
JE049-0208	Icatibant acetate safety pharmacology study on respiratory function after intravenous administration in the conscious rat (whole body plethysmography)
JE049-0209	Icatibant acetate evaluation of effects on gastrointestinal transit after intravenous administration in the rat
JE049-0210	Icatibant acetate evaluation of effects on gastric acid secretion after intravenous administration in the rat

Pharmacokinetics

JE049-0318	Icatibant metabolites M1, M2: Metabolic stability in human liver microsomes
JE049-0312	HOE 140- (oic-6,7-^{3}H): Whole-body autoradiographic studies in male rats after a single subcutaneous dose of 1 mg/kg body weight
JE049-0309	HOE 140- (oic-6,7-^{3}H): Radiokinetics study in male dogs after single subcutaneous administration of approx. 0.2 mg/kg body weight
JE049-0306	HOE 140- (oic-6,7-^{3}H): Radiokinetics study in male rats after single subcutaneous administration of approx. 1 mg/kg body weight
JE049-0311	HOE 140- (prolyl-3,4-^{3}H): Metabolism studies in male rats after subcutaneous administration of approx. 1 mg/kg body weight
JE049-0310	HOE 140-^{3}H: Determination of protein binding *in vitro* in human, monkey, dog and rat serum
JE049-0321	HOE 140- ^{3}H: Metabolism studies in male mice after a single subcutaneous dose of 1 mg/kg body weight
JE049-0323	Metabolism studies in rat following subcutaneous administration of 12 µg/kg ^{3}H-HOE 140
JE049-0326	HOE 140- ^{3}H: Excretion route, kinetics and organ distribution in male mice after a single subcutaneous dose of about 1 mg/kg body weight
JE049-0327	HOE 140- ^{3}H: Excretion route, kinetics and organ distribution in male rats after a single subcutaneous dose of about 1 mg/kg body weight
JE049-0330	Icatibant: Cytochrome P450 inhibition in human liver microsomes
JE049-0331	Icatibant: Measurement of cytochrome P450 induction on enzyme activity level in freshly isolated, pre-plated human hepatocytes
JE049-0317	Icatibant acetate: Metabolic stability in liver microsomes and S9 of Dog and Human

JE049-0329	HOE 140- ^{3}H: Radiokinetic study of excretion with the milk in lactating rats after a single subcutaneous dose of about 1 mg/kg body weight
	Repeat Dose Toxicology
JE049-0127	HOE 140: 14-day subcutaneous toxicity study in rats
JE049-0128	HOE 140: 14-day subcutaneous toxicity study in male rats with an 8-week recovery period
JE049-0108	Combined 13-week toxicity and effects on fertility and early embryonic development to implantation in rats by subcutaneous administration
JE049-0115	Two-phase dose range-finding study by subcutaneous administration of icatibant in Beagle dogs
JE049-0016	4-week toxicity study by subcutaneous route in Beagle dogs with twice weekly T.I.D. dosing and an 8-week treatment free period
JE049-0117	13-week toxicity study by subcutaneous route in Beagle dogs
JE049-0118	13-week toxicity study by subcutaneous route in sexually immature Beagle dogs with daily or twice weekly T.I.D. dosing followed by a 14-week treatment from period
	Genetic Toxicology
JE049-0132	Bacterial reverse mutation test
JE049-0133	*In vitro* mammalian chromosome aberration test in cultured human lymphocytes
JE049-0135	Bone marrow micronucleus test by intravenous route in rats
	Reproductive Toxicology
JE049-0108	Combined 13-week toxicity and effects on fertility and early embryonic development to implantation in rats by subcutaneous administration
JE049-0141	Study for effects on pre- and post-natal development in rats by subcutaneous administration
	Local Tolerance
JE049-0142	Local tolerance after a single administration by subcutaneous route in rabbits
JE049-0143	Single-dose local tolerance study in rabbits (intra-arterial/intravenous/paravenous)
	Special Toxicology
JE049-0151	H-Oic-OH: Single-dose subcutaneous study in mice
JE049-0152	H-D-Tic-OH: Single-dose subcutaneous toxicity study in mice
JE049-0159	*In vitro* hemolysis test of HOE 140
JE049-0166	Assessment of antibody responses towards icatibant in hyperimmunization studies
JE049-0155	Acute intravenous toxicity in rats

JE049-0154 Bacterial reverse mutation test
JE049-0156 *In vitro* mammalian chromosome aberration test in cultured human lymphocytes

Studies not reviewed within this submission: Pharmacology, pharmacokinetic and toxicology studies (IV, IN, IH and SC routes of administration) have been previously reviewed under INDs (b) (4) 68,214. Additionally, the sponsor submitted to their NDA new toxicology studies that have not been reviewed but used different routes of administration than the subcutaneous route proposed for icatibant. The following table outlines the studies previously reviewed under INDs (b) (4) 68,214. Following this table is a list of studies that have not been reviewed previously and are not reviewed here as they do not contribute to the support of icatibant's safety for the subcutaneous injection route of administration.

Previously Reviewed Nonclinical Studies

Study No.	Study Title	IND No.
Pharmacology studies		
JE049-0001	• Antagonistic effect of HOE 140 in BK-stimulated cultured endothelial cells • HOE 140 and D-Arg-[Hyp^{2}, Thi^{5,8}, D-Phe]-BK in isolated organs • Effects of HOE 140 and D-Arg-[Hyp^{2}, Thi^{5,8}, D-Phe]-BK on changes of coronary flow in isolated guinea pig hearts • Effect of HOE 140 on bradykinin induced endothelium dependent relaxations in isolated bovine coronary arteries • Inhibition of BK-induced hypotension in anesthetized rats by HOE 140 • Effect of HOE 140 on BK-induced fall in blood pressure in rats after nasal application • Antiinflammatory effect in the carrageenan-induced paw edema in rats • Inhibitory effect on S 89 0140 on burn edema in rat paws • Effect of HOE 140 on carrageenan paw edema in the rat and delayed-type hypersensitivity (DTH) in the rat and mouse • Analgesic activity in the modified Randall-Selitto test (yeast-induced motor impairment in rats) • Analgesic effect in mice: acetic acid writhing test • Bronchodilatory activity in anesthetized guinea pigs • IgE-mediated allergic reaction: passive cutaneous anaphylaxis (PCA) in rats • The effects of HOE 140 (S 89 0140) on the release of histamine from rat peritoneal mast cells • Inhibition of endotoxin-induced shock • Lack of effect of the bradykinin-antagonist HOE 140 on the endotoxin-induced drop of blood pressure in anesthetized rats • Effects of IV administration of HOE 140 on blood pressure and heart rate in conscious dogs • Effects of HOE 140 on postischemic reperfusion arrhythmias in isolated working rat hearts • Influence of intravenous infusion of the bradykinin-antagonist S 89 0140 on the development of acute renal vascular hypertension in anesthetized rats	(b) (4)

Study No.	Study Title	IND No.
	• Effects of S 89 0140 on plasma rennin activity in comparison with D-Arg-[Hyp^{2}, Thi^{5,8}, D-Phe]-BK in rats • Behavioural observation in mice • Spontaneous motor activity in mice (6 hours) • Inhibition of electroschock-induced tonic convulsions • Protection from Pentetrazol-induced convulsions in mice by S 89 0140 • Effects of S 89 0140 on prevention of tetrabenazine-induced ptosis • Effects of S 89 0140 on D,L-5-hydroxytryptophan-potentiation in mice • Effects of S 89 0140 on potentiation of hexobarbital sleep in mice • Effects of S 89 0140 on inhibition on apomorphine-induced climbing in mice • Effects of sodium nitrite-induced hypoxia in mice • Inhibitory avoidance (step-through avoidance) in mice scopolamine-induced amnesia (subcutaneous treatment) • Blood glucose in normal rats after intravenous treatment with S 89 0140	
JE049-0002	Effects of HOE 140 on bradykinin receptors and on bradykinin-induced responses, in guinea pig tracheal epithelial cells and in human synovial cells	(b) (4)
JE049-0005	Novel bradykinin analogues act as dual antagonists on the guinea pig ileum	
JE049-0007	Effect of HOE 140 after intraduodenal application	
JE049-0008	Effect of HOE 140 after nasal application	
JE049-0010	Inhibition of nociceptive responses to intradermal injections of bradykinin by HOE 140	
JE049-0211	Inhibitory effect of HOE 140 on histamine-stimulated gastric acid secretion in stomach lumen perfused rats	
JE049-0212	Salidiuretic effect of HOE 140 after subcutaneous administration in rats	
JE049-0204	Intravenous bolus dose of the bradykinin antagonist HOE 140 lead to a short depressor response on rats in contrast to high subcutaneous and intranasal dose	
JE049-0213	Effect of HOE 140 on urinary excretion of sodium and potassium, and on renal clearances in Beagle dogs	
JE049-0214	Effect of HOE 140 on histamine release from human skin mast cells	
JE049-0206	Effects of the bradykinin antagonist HOE 140 in a model of myocardial infarction in the anesthetized dog	
JE049-0207	The effects of HOE 140 on ischemia-induced arrythmias in anesthetized rats with and without preconditioning	
JE049-0205	The hemodynamic and electrocardiographic effects on HOE 140 in conscious dogs under conditions of exercise	
JE049-0009	Effect of HOE 140 on bradykinin-induced bronchoconstriction in anesthetized guinea pigs: comparison of aerosol with intravenous administration	
JE049-0012	Effects of S 89 0140, a bradykinin antagonist, and endothelin on carrageenin-induced footpad edema and vascular permeability in urate crystal-induced inflammation rats	
JE049-0006	HOE 140 is a competitive bradykinin antagonist in anesthetized rats	
Pharmacokinetics		
JE049-0305	HOE 140-^{3}H: Studies on excretion and distribution after a single	

Study No.	Study Title	IND No.
	subcutaneous administration of 12 µg/kg body weight to rats	
JE049-0319	*In vitro* metabolism of HOE 140	(b) (4)
JE049-0303	Pilot study on toxicokinetics in mice after subcutaneous and intravenous administration	
JE049-0304	Exploratory toxicokinetics in rats after subcutaneous and intravenous administration	
JE049-0307	Pilot study of toxicokinetics in dogs after subcutaneous administration	
Single Dose Toxicity		
JE049-0101	HOE 140: Single dose subcutaneous toxicity study in mice	
JE049-0102	HOE 140: Single dose intravenous toxicity study in mice	
JE049-0103	HOE 140: Single dose subcutaneous toxicity study in rats	
JE049-0104	HOE 140: Single dose intravenous toxicity study in mice	
JE049-0105	Single-dose intravenous infusion toxicity study of HOE 140 in rats	IND 68,214
JE049-0106	Single-dose intravenous toxicity study of HOE 140 in dogs	IND 68,214
JE049-0107	Single-dose intravenous infusion toxicity study of HOE 140 in dogs	IND 68,214
Repeat-Dose Toxicity		
JE049-0109	Repeated-dose (1 month) intravenous toxicity study of HOE 140 in rats	(b) (4)
JE049-0110	HOE 140 (S 890140): Comparative repeated-dose (1-month) toxicity study in rats (nasal./intravenous/subcutaneous)	
JE049-0111	Subacute (28-day) repeated dose inhalation toxicity study with HOE 140 in rats	
JE049-0112	Repeated-dose (1-month) nasal toxicity study of HOE 140 (S890140) in rats	
JE049-0121	Repeated-dose (1-month) intravenous toxicity study of HOE 140 (S890140) in dogs	
JE049-0123	28-Day inhalation toxicity study with HOE 140 (S890140) in the dog	
JE049-0124	Repeated-dose (1-month) nasal toxicity study of HOE 140 (S 890140) nasal spray in Beagle dogs	
Genetic Toxicology		
JE049-0126	S 89 0140 (HOE 140): Study of the mutagenic potential in strains of *Salmonella typhimurium* (Ames test)	
JE049-0130	S 89 0140: Study of the mutagenic potential in strains of *Salmonella typhimurium* (Ames test) and *Eschericia coli*	
JE049-0131	S 89 0140: Study of the mutagenic potential in strains of *Salmonella typhimurium* (Ames test) and *Eschericia coli*	
JE049-0134	S 89 0140 (HOE 140): Micronucleus test in male and female NMRI mice after intravenous administration	
JE049-0136	Evaluation of S 89 0140 (HOE 140) in the *In vivo* cytogenetic test in bone marrow cells of the Chinese hamster-chromosome analysis	
Reproductive and Developmental Toxicity		
JE049-0137	Effects of 5-week subcutaneous administration of HOE 140 on fertility of male mice	
JE049-0138	Effects of 5-week intravenous administration of HOE 140 on fertility of male mice	
JE049-0139	HOE 140: Subcutaneous embryotoxicity study in Wistar rats (effect no morphological and postnatal development including fertility) Segment II	
JE049-0140	HOE 140 (S 89 0140): Subcutaneous embryotoxicity study in Himalayan rabbits (Segment II)	
Local Tolerance		
JE049-0144	Single-dose intra-articular local tolerance study of HOE 140 (S 89 0140) injection solution in rabbits	
JE049-0145	HOE (S 89 0140): Primary skin irritation study on shorn intact and abraded rabbit skin (Patch test)	

Study No.	Study Title	IND No.
Special Toxicology Studies		
JE049-0146	HOE 140 (S 89 0140): Mucosal tolerance in the rabbit eye	(b) (4)
JE049-0147	Antigenicity study of HOE 140 in Guinea pigs. Homologous 4-hour passive cutaneous anaphylaxis test	
JE049-0148	HOE 140, drug substance (S 89 0140): testing for sensitizing properties in the Pirbright-White guinea pig in the maximization test	
JE049-0149	Bradykinin antagonist HOE 140: S.C. injection vs SC infusion in male rats: effect on pituitary-testicular axis	

The following studies have not been reviewed. These studies do not contribute to the safety of icatibant or do not support the subcutaneous route of administration proposed for this NDA.

Methods of Analysis

JE049-0301	Radioimmunoassay method of icatibant
JE049-0302	HOE 140: Determination in human plasma by high performance liquid chromatography and method comparison vs radioimmunoassay
JE049-0141	Method validation in rat plasma and rat milk
JE049-0332	Icatibant and metabolite M2: Determination of freeze thaw stability in rat plasma
JE049-0333	Icatibant and metabolite M2: Determination of short term stability in rat plasma
JE049-0334	Icatibant and metabolite M2: Determination of long term stability in rat plasma
JE049-0335	Icatibant and metabolite M2: Determination of post preparative stability in rat plasma
JE049-0341	Icatibant and metabolite M2: Determination of freeze and thaw stability in rat milk
JE049-0342	Icatibant and metabolite M2: Determination of short term stability in rat milk
JE049-0343	Icatibant and metabolite M2: Determination of long term stability in rat milk
JE049-0344	Icatibant and metabolite M2: Determination of post preparative stability in rat milk
JE049-0122	Icatibant and metabolite M2: Bioanalytical method validation for and analysis of dog plasma samples
JE049-0337	Icatibant and metabolite M2: Determination of freeze and thaw stability in Beagle plasma
JE049-0338	Icatibant and metabolite M2: Determination of short term stability in Beagle plasma
JE049-0339	Icatibant and metabolite M2: Determination of long term stability in Beagle plasma
JE049-0340	Icatibant and metabolite M2: Determination of post preparative stability in Beagle plasma

JE049-0336	Icatibant, metabolite M2 and internal standard (S 91 1486): Determination of stock solution stability
	Pharmacology
JE049-0011	Effect of HOE 140 on antigen-induced airway hyperresponsiveness in guinea pigs
	Pharmacokinetics
JE049-0320	*In vitro* metabolism of HOE 140 in the 9000g lung fractions of man and rat
	Repeat Dose Toxicity
JE049-0113	HOE 140 (S 890140): Repeated-dose (6 months) nasal toxicity study in rats
JE049-0114	HOE 140 (S 890140): Repeated-dose (6 months) nasal toxicity study in rats
JE049-0119	HOE 140 (S 890140): Pilot toxicity study (non-GLP). Testing for toxicity by repeated intravenous administration to dogs
JE049-0120	2-week toxicity study of icatibant by intravenous route in Beagle dogs
JE049-0122	4-week toxicity study by intravenous route (1-hour infusion) in Beagle dogs
JE049-0125	Repeated-dose (6-month) nasal toxicity study of HOE 140 (S 890140) in dogs

INTRODUCTION AND DRUG HISTORY

On October 22, 2007 Jerini US, Inc (Jerini) submitted this New Drug Application (NDA) for Firazyr (Icatibant), a new molecular entity, for the treatment of hereditary angioedema (HAE) in adults (patients ≥18 years old). Icatibant is formulated as a pre-filled syringe for a single 30 mg subcutaneous injection. No more than 3 injections of icatibant within a 24-hour period are recommended. Currently, there are no approved treatments for HAE.

The original Investigational New Drug application (IND 68,214) for the treatment of HAE was submitted to the Division of Gastrointestinal and Coagulation Products April 8, 2004. On August 16, 2005, the IND 68,214 for icatibant acetate was transferred from the Division of Gastrointestinal and Coagulation Products to the Division of Pulmonary and Allergy Products (DPAP). A pre-NDA meeting was held between Jerini and DPAP on January 24, 2007 to discuss the registration process and any potential deficiencies in the drug development program. Jerini was informed that the Division considers the potential dosing of HAE patients with no more than eight 30 mg subcutaneous injections a month over the lifetime of the HAE patient as chronic intermittent dosing. To support the clinical chronic intermittent dosing schedule, Jerini was notified that nonclinical chronic repeat dose toxicity studies via the subcutaneous route of administration in the rat (6 month) and the dog (9 month) and 2 carcinogenicity studies (1 mouse and 1 rat) to address the carcinogenic potential of icatibant would be needed. Following the pre-NDA meeting, Jerini (May 1, 2007) requested comments on their proposed nonclinical study protocol designs from the Division for a 26-week subcutaneous repeat dose rat study, a 39-week subcutaneous repeat dose dog study, two 13-week subcutaneous pre-carcinogenicity screening study in rats and mice and two 104 week subcutaneous carcinogenicity study in rats and mice. Comments were relayed to Jerini on these proposed studies June 5, 2007.

For this NDA, Jerini submitted pharmacology, pharmacokinetics and toxicology studies conducted for the development of icatibant for the intranasal/inhalation, intravenous and subcutaneous routes of administration. Pharmacology, pharmacokinetic and toxicology studies have been previously reviewed under INDs (b) (4) 68,214 (subcutaneous administration of HAE). IND (b) (4) includes reviews of studies that were reviewed in IND (b) (4) Jerini has a Letter of Authorization allowing cross reference of IND (b) (4). Reference is made to the nonclinical reviews of INDs (b) (4) 68,214 (subcutaneous administration of HAE) for the detailed review of the nonclinical studies. The nonclinical studies that support the development of icatibant for the subcutaneous route of administration are reviewed herein.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Icatibant is a synthetic decapeptide with similar structure as endogenous bradykinin (BK). It is a potent, selective BK type 2 (B_2) receptor antagonist that binds to B_2 with similar affinity as BK. Icatibant binding at the B_2 receptor inhibits BK induced processes and is hypothesized to contribute to the decrease of vascular permeability and acute inflammation observed in HAE patients. The pharmacology of icatibant has been well characterized using various *in vitro* and *in vivo* models, which were previously reviewed under INDs (b) (4) and 68,214. Under these reviews, the selectivity for the B_2 receptor demonstrated using a rabbit aorta model, which contains only BK type 1 (B_1) receptors. Incubation of rabbit aorta with BK resulted in contraction of the aorta. After incubation with BK and icatibant, no inhibition of rabbit aorta contraction was observed, demonstrating that icatibant did not competitively antagonized the B_1 receptor. However, icatibant was shown to inhibit BK-induced effects in cultured endothelial cells ($IC_{50}= 10^{-8}$ and 10^{-9} mol/L for BK-induced release of endothelium derived relaxing factors and release of intracellular calcium, respectively) and isolated guinea-pig hearts ($IC_{50}= 5.4 \times 10^{-9}$ mol/L for BK-induced contraction).

In addition to these previously reviewed studies, the sponsor submitted additional pharmacology studies that further characterized the binding affinity and activity of icatibant and two of its major metabolites, M1 (b) (4) and M2 (b) (4) on BK receptors. These studies demonstrated that using *in vitro* receptor binding studies and measuring the displacement of ^{3}H-BK in guinea pig ileum, guinea pig trachea epithelium and human synovial cells, icatibant was a competitive inhibitor of the B_2 receptor with an IC_{50} within the 1-4 nM range. In Chinese Hamster ovary (CHO) cells expressing recombinant B_1 or B_2 receptors, icatibant had an IC_{50} of 6.0 µM and 4.3 nM, respectively. Slight binding of additional receptors was observed with icatibant in a receptor screening study. However, this binding did not interfere with the receptors enzymatic activity with the exception of ACE and NK2 enzymatic activity.

Additionally, Jerini investigated the mechanism of observed injection site irritation by exposing icatibant to human mast cells, nociceptive nerve fibers and measuring MAPK and calcium levels using *in vitro* techniques. These data showed that icatibant does activate the release of histamine, LTC4 and PGD2 from mast cells and induces mobilization of calcium nerve fibers, which may contribute to local injection site irritation.

Several new safety pharmacology studies were submitted to the NDA. These studies included 2 cardiovascular studies evaluating icatibant and metabolites M1 and M2 effects on cardiac potassium currents, one respiratory function safety study conducted in rats and 2 gastrointestinal (GI) studies conducted in rats. Icatibant did not inhibit cardiac potassium currents up to 300 µM concentrations under the conditions tested. After intravenous (IV) administration of icatibant to Wistar rats up to 10 mg/kg, no changes in

respiratory function were observed compared to the vehicle control group. In the GI studies, single IV doses of icatibant up to 10 mg/kg in Wistar rats had no effect on GI transit but did decrease gastric acid volume and acidity.

Additional safety pharmacology studies were reviewed under IND (b) (4) Reference is made to Dr. Young Choi's review of IND (b) (4) for the detailed review of these studies. Based on Dr. Choi's review, icatibant decreased coronary blood flow [IC_{50}= 3.1 x 10^{-8} mol/L (0.5 mL/min) resulting in a % inhibition ranging from 22% to 67%] in isolated guinea pig heart, aggravated the duration of post-ischemic reperfusion arrhythmias in isolated working hearts in Wistar rats and impaired cardiodynamics. At a rate of 0.5 ng/kg/min, intracoronary infusion of icatibant in an anesthetized myocardial infarction dog model increased mortality rate 2-fold (58%) over saline ischemia (28%) and produced delayed deaths. Also, icatibant at 0.3 mg/kg, IV decreased urine output, sodium and potassium excretion (~40% for all 3 parameters), to a lesser extent for GFR, renal plasma flow and osmolar clearance in beagle dogs. Icatibant also significantly inhibited BK-induced hypotension in anesthetized rats and produced decreased motor activity, assumption of prone position, reduced respiration, ptosis, piloerection and mydriasis at 1-10 mg/kg IV. These effects on central nervous system (CNS), cardiovascular (CVS) and renal systems are considered the result of the partial agonistic property of icatibant on B-receptors and may present toxicological concerns.

In the 4-week subcutaneous (JE049-0016) and 13-week subcutaneous (JE049-0117) repeat dose toxicology studies conducted in the Beagle dog, electrocardiographic (EKG) examinations were conducted in the presence of icatibant. In the 4-week study, dogs were dose up to 10 mg/kg twice weekly (administered as 3.3 mg/kg TID twice weekly). There were no statistically significant changes in EKG parameters or changes in blood pressures in icatibant treated males and females compared to the vehicle treated animals. In the 13-week study, dogs were administered daily doses of icatibant up to 100 mg/kg/day. There was a slight but statistically significant decrease in heart rate in HD males (-29%) and HD females (-21%) 6 hours post dose during Week 1 of dosing compared to the vehicle control groups. A decrease in diastolic BP was noted in HD males (-39%) 6 h post-dose on Week 1 of dosing compared to the vehicle control group. At Week 13 of dosing, there were no statistically significant differences in heart rate, PQ- QRS- or QT-intervals evaluated in the icatibant treated males and females compared to the vehicle control animals. There were no statistically significant changes in QT corrected for heart rate using either Bazett or Fridericia formulae in the icatibant treated animals compared to the vehicle treated animals. However, a statistically significant decrease in systolic BP (-19%) was observed in HD males 1 h post-dose in Week 12 and (-22%) was observed in HD females before dosing in Week 13, respectively.

During icatibant's drug development, Jerini has evaluated icatibant's effects on the CNS, CVS, respiratory, renal and GI systems. Taking these data as whole, the safety pharmacology of icatibant has been adequately addressed.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: In Type I (quantitative deficiency) and Type II (qualitative deficiency) hereditary angioedema (HAE) a deficiency of C1 esterase inhibitor (C1-INH) results in an increased release of bradykinin (BK). BK binds to the BK type-2 receptor (B_2) to trigger activation of many intracellular processes. Included are cascades, two of which are coupled by the $G_{\alpha q/11}$ and $G_{\alpha i}$ proteins, leading to calcium mobilization and MAPK activation, respectively. Calcium mobilization and MAPK activation induces many cellular events including cellular proliferation, excitation of neurons, vasodilatation, increased vascular permeability and smooth muscle cell contraction. These events are believed to contribute to the increased vascular permeability and acute inflammation observed in HAE patients.

Icatibant is a synthetic decapeptide with similar structure as endogenous BK. It is a potent, selective B_2 receptor antagonist that binds to B_2 with similar affinity as BK. Icatibant binding at the B_2 receptor inhibits BK induced processes and is hypothesized to contribute to the decrease of vascular permeability and acute inflammation observed in HAE patients. *In vitro* studies have shown that icatibant binds to both B_1 and B_2 receptors (Ki values of 2 nM and 1.2 µM, respectively) and inhibits BK with IC_{50} of 6 µM and 4.3 nM, respectively. These data show that icatibant is selective for the B_2 receptor subtype over the B_1 receptor (Study no. JE049-0003).

The sponsor submitted 5 pharmacology studies that used isolated receptors (*in vitro* and using high-throughput screening), cell cultures and tissue models to evaluate the binding affinity and activity of icatibant and two of its major metabolites, M1 [redacted] (b) (4) and M2 [redacted] (b) (4) on BK receptors. Briefly, these studies showed that icatibant bound preferentially to the B_2 receptor subtype over the B_1 receptor which resulted in significant inhibition of native BK binding. Icatibant metabolites M1 and M2 also bound to B2 receptors but with less inhibition than the parent drug and M2 having greater inhibition of BK than the M1 metabolite. Neither icatibant nor M1 or M2 bound to the LH-RH receptor. Slight binding of additional receptors was observed with icatibant in a receptor screening study. However, this binding did not interfere with the receptors enzymatic activity with the exception of ACE and NK2 enzymatic activity. These data are summarized in the following table.

Study no/Title	Study Design	Study Outcomes
JE049-0003 *In vitro* pharmacology: Human B_1 and B_2 receptors-study of icatibant (non-GLP, Dec 20003)	*In vitro* competitive binding assay using human recombinant B_1 and B_2 receptors isolated from Chinese hamster ovary cells. B_1 and B_2 receptors were incubated with radiolabeled ligand and unlabeled icatibant (batch no. E084) to determine the IC_{50} and K_i of icatibant at each receptor.	B_1 : IC_{50}= 6.0E-06 K_i= 1.2E-06 B_2 : IC_{50}= 4.3E-09 K_i= 2.0E-09
JE049-0004 *In vitro* pharmacology: Bradykinin receptors-	*In vitro* competitive binding assay using human recombinant B_1 and B_2 isolated from Chinese hamster ovary cells and	Human B_1 M1= -8 % inhibition M2= 20% inhibition

Study no/Title	Study Design	Study Outcomes
Study of M1, M2 and Icatibant (non-GLP, Nov 2003)	guinea pig ileum isolated B_2 receptor B_1 and B_2 receptors (human and guinea pig) were incubated with radiolabeled bradykinin ligand and unlabeled icatibant (batch no. E084), M1 and M2 metabolites to determine the % inhibition of control specific binding	Icatibant= 83% inhibition Human B_2 M1= 10 % inhibition M2= 45 % inhibition Icatibant= 100% inhibition Guinea pig B_2 M1= 48 % inhibition M2= 58% inhibition Icatibant= 100% inhibition
JE049-0217 *In vitro* pharmacology: LH-RH receptor binding assay-Study of icatibant, M2 and M1 (non-GLP, Nov 2006)	LH-RH receptor was isolated from rat pituitary gland and incubated with radiolabeled LH-RH receptor ligand and unlabeled icatibant (batch no. E084), M2 and M1 metabolites to determine the % inhibition of control specific binding	There was no binding of icatibant, M1 or M2 to the LH-RH receptor.
JE049-0201 Study of icatibant (HOE 140) in various receptor binding and enzyme assays (non-GLP, Dec 2001)	30 different receptor types were incubated with their radiolabeled ligand and with unlabeled icatibant to determine if icatibant could bind with any affinity to these receptors. 7 different enzymes were assayed in the presence of radiolabeled substrate and icatibant to determine whether icatibant had any effect on their activities Icatibant was assayed in these experiments up to 10 µM	Receptor binding: < 10% inhibition of 25/30 receptors Slight inhibition in 5 receptors: M4 (16%), opiate (19%), EP1 (13%), VIP1 (56%) and VIP2 (41%)] Enzymatic assays: Icatibant resulted in an IC_{50} = 2.3 µM in the ACE assay. No other enzymes were affected by icatibant.
JE049-0218 *In vitro* pharmacology: high throughput profile-study of icatibant, M2 and M1 (non-GLP, Dec 2006)	Using a high throughput screening method consisting of competitive binding assays ~76 different receptor types were assayed to test for binding of icatibant or either the M2 or M1 metabolites. The percent inhibition of the radiolabeled ligand by icatibant, M2 or M1 was determined.	B_2 bound icatibant (98% inhibition), M2 (62% inhibition) and M1 (2% inhibition) M3-receptor bound icatibant (52% inhibition) but neither M1 nor M2 affected this receptor NK2 bound icatibant (96% inhibition) but neither M1 nor M2 bound this receptor IC_{50} for NK2 receptor= 4.2E-07 M V1a bound icatibant (66% inhibition), M2 (10% inhibition) but not M1.

2.6.2.3 Secondary pharmacodynamics

In the nonclinical toxicology studies (see Toxicology Section of this review) injection site irritation is observed after subcutaneous injection of icatibant. This has also been observed after subcutaneous injection in humans. Jerini investigated the mechanism of this injection site irritation by exposing human mast cells and nociceptive nerve fibers to icatibant, and measuring MAPK and calcium levels using *in vitro* experimental methods. These data showed that icatibant does activate the release of histamine, LTC4 and PGD2

from mast cells. Additionally, icatibant mobilizes calcium release from nerve fibers. Each of these actions may contribute to icatibant irritation after subcutaneous injection. These studies are summarized below.

Study no/Title	Study Methods	Study Outcomes
JE049-0215 Characterization of the effects of icatibant on human mast cells (non-GLP, Nov 2006)	Mast cells harvested from human skin were exposed to various concentrations of icatibant and to determine the concentration at which histamine, tryptase, leukotriene-C4 (LTC4) and prostaglandin (PGD2) may be released. Concentrations of icatibant (E084) tested in each assay ranged from 1 x 10^{-3} to 1 x 10^{-10} M.	• Human mast cells are activated by icatibant • More than 40% of preformed histamine is released at 10^{-4}M icatibant concentration • Icatibant at 10^{-3} and 10^{-4} M increased the release of tryptase by ~5% compared to no treatment • LTC4 and PGD2 was released from mast cells at icatibant concentrations from 10^{-3} to 10^{-6} M.
JE049-0013 Analysis of species specificity and potential agonistic activity of icatibant using MAPK activation and calcium mobilization (non-GLP, Dec 2006)	Agonistic and antagonistic properties of B_2 receptor from human (HF15 and HEK-293 cells), rat (Rat2, PC-12, RFL-6, RBL-2 and A7r5 cells) and dog (MDCK and CF52.Tr cells) cells were determined by measuring MAPK activation and calcium mobilization	• Nanomolar concentrations of icatibant exerted partial agonistic activity on MAPK activation using the rat and dog B_2R but not the human B_2R. • At icatibant concentrations < 50 µM, no partial agonistic activity on calcium mobilization was detected in rat, dog and human cells.
JE049-0216 Icatibant dose- and calcium-dependently induces calcitonin gene-related peptide release from isolated skin in wild-type, B2 -/- and KITW/KITW-v but less in TRPV1 -/- mice (non-GLP, Feb 2006)	Neuropeptide release was measured after icatibant-stimulation (1, 3.1 and 10 mM) in isolated mouse skin (5 mice/sex/strain) from several different mouse strains. C57BL/6 =Controlcontrol B2 -/- = bradykinin 2 receptor knockout model TRPV -/- = capsaicin receptor knockout mouse KITW/KITW-v = Mast cell deficient mice	• Icatibant induces a dose-dependent and partially extracellular calcium dependent release of iCGRP from nociceptive nerve fibers in skin • 10 mM icatibant regularly induced a sustained release of iCGRP • Acute action of icatibant (3.1 and 10 mM) mobilizes internal calcium stores

2.6.2.4 Safety pharmacology

Cardiovascular effects: Two *in vitro* experiments were conducted to evaluate the effects of icatibant and metabolites M1 and M2 on the inward rectifying potassium currents expressed in either Xenopus oocytes or Chinese hamster ovary cells. In the first study (study no. JE049-0202, non-GLP), Xenopus oocytes expressing cardiac potassium channels were incubated in the presence of icatibant at 10, 30 and 100 µM or vehicle control (physiological buffer solution) alone and potassium currents were measured using patch clamp. There was no statistically significant change in potassium currents in the icatibant treated cells compared to the vehicle control currents.

In a second study (study no. JE049-0203, non-GLP), the effects of icatibant, M1 (b) (4) and M2 (b) (4) on whole cell potassium currents were tested in Chinese hamster ovary cells that expressed the HERG potassium channel. Icatibant and the two metabolites were tested at 10, 100 and 300 µM concentrations (n= 3 assays/concentration). Compared to the vehicle (physiological saline and DMSO) treated cells, icatibant and the two metabolites did not inhibit potassium currents. At 10, 100 and 300 µM, icatibant induced a current change of 1, 3 and 6%; M1 at 3, 5 and 5%; and M2 at 0, 0 and 2%, respective to concentration. The negative (vehicle; n=3) and positive controls (100 nM Haloperidol; n=3) resulted in no inhibition of potassium currents and significant inhibition of potassium currents (90% inhibition), respectively.

Taken these two studies together, icatibant and metabolites M1 and M2 did not inhibit potassium channel currents under these assay conditions.

Pulmonary effects: The effects of single intravenous doses of icatibant on respiratory parameters using whole body plethysmography were investigated in conscious rats (study no. JE049-0208, GLP conditions). Single IV doses of icatibant (batch no. E084) were administered at 0 (vehicle control-physiological saline), 1, 3 and 10 mg/kg to male Wistar rats (mean BW range 309-359 g; n=8/dose). To serve as the positive control, theophylline (16 mg/kg) was administered intravenously under the same experimental conditions. The respiratory parameters assessed were inspiratory time, expiratory time, peak inspiratory flow, peak expiratory flow, tidal volume, respiratory rate, relaxation time, pause and enhanced flow. Icatibant had no statistically significant effects on inspiratory time, expiratory time, peak inspiratory flow, peak expiratory flow, tidal volume, respiratory rate, relaxation time, pause or enhanced flow compared to the vehicle control group. The theophylline treated rats produced the expect changes in respiratory parameters demonstrating the validity of the assay.

Gastrointestinal effects: Two studies investigated icatibant's effects on the gastrointestinal (GI) system. The first study, (JE049-209, GLP) intravenously administered icatibant to male Wistar rats (mean BW range 140-174 g; n=8/group) and the GI transit time was evaluated. Rats were administered single doses of 0 (vehicle-physiological saline), 1, 3 or 10 mg/kg icatibant intravenously 10 minutes prior to charcoal suspension gavage. Atropine (1 mg/kg, IV) was administered as a positive control. The distance covered by charcoal in the small intestine in the icatibant treated

rats was compared to the vehicle treated rats. At all doses tested, icatibant had no statistically significant effect on the distance covered in the small intestine by charcoal compared to the vehicle control group (+6%, +8% and -5% for 1, 3 and 10 mg/kg IV, respectively, compared to VC). Atropine reduced charcoal transit by 27% compared to the vehicle control group.

In the second study (JE049-0210, GLP) the effects of icatibant on gastric acid secretion were investigated in rats. Male Wistar rats (mean BW range 216-234 g; n=8/group) were intravenously administered 0 (vehicle-physiological saline), 1, 3 and 10 mg/kg of icatibant and the gastric fluid volume, pH free chlorhydric acidity and total acidity were measured. To serve as a positive control, atropine sulfate (2 mg/kg, IV) was administered. At 1 and 3 mg/kg, IV, of icatibant, no effect on the gastric fluid volume, pH, free chlorhydric acidity and total acidity was observed compared to the vehicle control animals. The HD group (10 mg/kg) reduced gastric fluid volume (-48%), free chlorhydric acidity (-42%) and total acidity (-26%) compared to the vehicle control group. The positive control, atropine, reduced gastric fluid volume (-83%), free chlorhydric acidity (-32%) and total acidity (-26%) compared to the vehicle control group.

2.6.2.5 Pharmacodynamic drug interactions

No new data were submitted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

No new data were submitted.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Jerini conducted two 14-day toxicity studies (each in rats), a 10-day dose-ranging toxicity study in dogs, a 4-week toxicity study in dogs and three 13-week toxicity studies (1 in rats and 2 in dogs) using the subcutaneous route of administration. These studies showed that after subcutaneous administration, icatibant is rapidly distributed and metabolized with the M2 metabolite [redacted] (b) (4) appearing from 0.2 to 3 hours post-dose in rats and dogs. In rats and dogs, increases in icatibant doses from LD to MD doses (0.1 to 3 mg/kg in rats and 10 to 30 mg/kg/day in dogs) produced approximately dose proportionate increases in systemic exposures. Increases in icatibant doses from MDs to HDs resulted in super-proportionate increases in systemic exposures in rats (increased ~128-fold instead of the expected ~50-fold) and approximately dose proportionate increases in dogs. Using an alternate dosing schedule in dogs (1 or 3.3 mg/kg TID of icatibant twice weekly), AUC levels of icatibant and M2 increased in an approximate dose proportionate increase. The t_{max} was achieved from 0.5 to 3.0 h post-dose in rats and dogs. Neither icatibant nor M2 accumulated after repeat dosing in males and females up

to 13-weeks dosing. There were no gender differences observed in rats or dogs in the systemic exposures of males and females.

Distribution studies showed that icatibant (or its metabolites) is distributed into the kidneys, liver, urinary bladder, lungs and spleen 1 hour and it still detectable in the liver, kidneys, spleen, bone marrow, adrenals and injection sites at 24-hours post SC dose in rats. Serum protein binding of icatibant was low with 44 and 49% in man and rat, respectively, and 39% in monkey and dogs. Icatibant is excreted in the milk of rats after IV administration. M1 [redacted] (b) (4) and M2 [redacted] (b) (4) are the primary metabolites of icatibant and are formed by hydrolysis of the peptide backbone, thought to be induced by peptidases. Neither icatibant nor the M1 or M2 metabolites are metabolized by human hepatocytes or microsomes. Additionally, icatibant is not an inducer of CYP450 isozymes. Based on excretion studies, the primary route of elimination after SC administration of icatibant is via the kidneys in mice (~97.1%), rats (~61%) and dogs (~63%) with small amounts excreted in the feces (8.0% to 33%).

As reported in the nonclinical review of IND [redacted] (b) (4) (reviewed by Dr. Choi), the major metabolite in man is M1 (>90%). M1 is formed in rat (~70%), dog (~60%) and mouse (~50%). M2 is formed in rat (~9% of observed parent levels), dog (~3%) and human (~75%). The biological half-life was more than 5 hours after 26 µg/kg SC in rats and terminal half-life was over 6 days in rats after 10 mcg/kg, SC. The comparative pharmacokinetics of icatibant after intravenous administration in man, rat, dog and mouse were summarized by Dr. Choi in the table below:

Parameter	Man	Rat	Dog	Mouse
Dose (mg/kg)	0.2	1.0	0.1	1.0
AUC (ng/mL*h)	1518	2290	120-125	1130
C_{max} (ng/mL)	970	1269-3600	63-70	1310
T_{max} (h)	1.0*	0.5**	1**	0.5**
$T_{1/2}$ (h)	4.6	-	-	-

*- After 1 h infusion
**-First sampling point
- data are not available.

After single dose SC and IV administration of icatibant to healthy subjects, clinical pharmacokinetic analysis was conducted. As observed in the following table, after 0.4 mg/kg SC and IV administration, icatibant was rapidly absorbed with a T_{max} achieved for icatibant at ~0.5 to 0.6 h post-dose. The M2 metabolite T_{max} was observed at ~ 2 h post-dose after SC administration and ~ 1.5 h post-dose after IV administration, each T_{max} being significantly longer in humans than in mouse, rat or dog. After a 0.4 mg/kg SC dose in man, AUC_{0-t} (ng*h/mL) values for icatibant were significantly lower than those observed in rats and dogs from 13-week toxicology studies. The M2 metabolite exposure was ~ 25% lower than exposure to the parent compound in humans. This is in contrast to data in rats and dogs where M2 accounted for only ~9% and ~3%, respectively, of the parent compound exposure.

Parameter	Icatibant Dose (mg/kg)					
	PK of Icatibant			PK of M_2 Metabolite		
	0.4 SC (20 mg/mL)	0.4 SC (10 mg/mL)	0.4 IV (1 mg/mL)	0.4 SC (20 mg/mL)	0.4 SC (10 mg/mL)	0.4 IV (1 mg/mL)
C_{max} (ng/mL)	1133	1387	2876	354	373	463
T_{max} (h)	0.561	0.595	0.500	2.24	2.182	1.52
AUC_{0-t} (ng*h/mL)	2492	2984	3034	2057	1974	2079

Extrapolating from these data, the proposed clinical dose of 30 mg icatibant SC (0.6 mg/kg for 50 kg human) is speculated to achieve an approximate systemic exposure of 4107 ng*h/mL and 3023 ng*h/mL for icatibant and the M2 metabolite, respectively. At the maximum recommended dose of 90 mg/day, the respective exposures project to 12.3 µg*h/ml and 9.1 µg*h/mL.

2.6.4.2 Methods of Analysis

For evaluation of plasma levels of icatibant and the M2 metabolite (the formation of the M1 metabolite was not evaluated in these studies) from rat and dog plasma samples were prepared from whole blood samples. These plasma samples were evaluated using liquid chromatography-MS/MS analytic methods. In the rat, the lower limit of quantitation (LLOQ) ranged from 2 to 30 ng/mL plasma for icatibant and was 10 ng/mL plasma for the M2 metabolite. In the dog, the LLOQ for icatibant ranged from 1.3 to 26.54 ng/mL plasma and for M2 ranged from 0.431 to 65.14 ng/mL plasma. Using the data obtained from LC-MS/MS the TK parameters C_{max}, t_{max} and AUC could be determined.

2.6.4.3 Absorption

Jerini conducted two 14-day toxicology studies and one 13-week toxicology study in Sprague-Dawley rats using the subcutaneous route of administration. In the Beagle dogs, the sponsor conducted a dose-ranging study up to 10 days dosing, a 4 week toxicology study and two 13-week toxicology studies. In each of these studies, blood samples were collected and concentrations of icatibant or icatibant and the M2 metabolite were determined.

In a 14-day subcutaneous rat study (JE049-0127) rats were dosed with 0 (vehicle-water for injection), 1, 10 or 50 mg/kg/day of icatibant, SC. Blood samples were taken on Day 1 and Day 14 from the TK animals (3/sex/group) to determine icatibant levels. Blood samples were taken at 0.5, 1, 2, 3, 7 and 24 h post-dose on each day from the retrobulbal venous plexus. Plasma samples were prepared from whole blood and analyzed using LC-MS/MS analytical methods. TK parameters determined included: C_{max}, t_{max} and AUC. The lower limit of quantitation (LLOQ) for icatibant was 10 ng/mL plasma.

Sampling period	TK Parameter	Icatibant Dose (mg/kg/day)		
		1	10	50

		M	F	M	F	M	F
Day 1	C_{max} (µg/mL)	1.35	1.11	7.49	7.51	20.6	16.9
	T_{max} (h)	0.5	0.5	1.0	1.0	0.5	0.5
	AUC_{0-t} (µg*h/mL)	1.36	1.0	17.4	15.4	85	94.1
Day 14	C_{max} (µg/mL)	1.36	1.15	9.48	10.1	31.6	37.7
	T_{max} (h)	0.5	0.5	1.0	0.5	1.0	0.5
	AUC_{0-t} (µg*h/mL)	1.47	0.98	22.1	16.9	93.7	80

There were no significant gender differences in the TK parameters evaluated in rats on Days 1 and 14 of study. The systemic exposure levels increased in an approximately dose proportionate manner from the LD to the MD. However, systemic exposure increased less than dose proportional from the MD to the HD with an approximate increase of 2.5-fold instead of the expected 5-fold on Day 1. However, at Day 14, the systemic exposure levels were approximately dose proportional. The t_{max} was achieved from 0.5 to 1.0 h post-dose for both males and females. After repeated SC administration for 14-days, there was no accumulation of icatibant in either male or female rats.

In a second 14-day subcutaneous rat study (JE049-0128), male rats (females were not included in this study) were dosed with 0 (vehicle-water for injection), 0.1, 1 or 50 mg/kg/day of icatibant, SC. Blood samples were taken on Day 1 and Day 14 from the TK animals (3/sex/time point) to determine icatibant levels. Blood samples were taken at 0.5, 1, 2, 3, 7 and 24 h post-dose on each day from the retrobulbal venous plexus. Plasma samples were prepared from whole blood and analyzed using LC-MS/MS analytical methods. TK parameters determined included: C_{max}, t_{max} and AUC. The lower limit of quantitation (LLOQ) for icatibant was 2 ng/mL plasma.

Sampling period	**TK Parameter**	**Icatibant Dose (mg/kg/day)**		
		0.1	**1**	**50**
Day 1	C_{max} (µg/mL)	0.073	0.738	11.258
	T_{max} (h)	0.5	0.5	3.0
	AUC_{0-24}(µg*h/mL)	0.04	0.956	122.423
Day 14	C_{max} (µg/mL)	0.078	1.355	26.786
	T_{max} (h)	0.5	0.5	3.0
	AUC_{0-24}(µg*h/mL)	0.044	1.0	177.5

On Day 1 of dosing, the C_{max} and AUC increased in an approximately dose-proportionate manner from LD to MD. From MD to HD, the C_{max} increased but in a less than dose-proportional manner (increased by 15-fold instead of ~50-fold). The AUC increased from the MD to the HD by ~128-fold instead of ~50-fold, making it a super-proportional increase to dose. After repeated dosing for 14 days, the C_{max} increased in an approximately dose proportional manner from the LD to the MD. From the MD to the HD, the C_{max} increased in a sub-proportional manner by ~20-fold instead of the ~ 50-fold. The AUC increased in an approximately dose-proportional manner from the LD to the MD. However, from the MD to the HD the AUC increased in supra-proportional manner by ~177-fold. Based on these data, after 14-day repeat dosing, icatibant does not accumulate in males. The T_{max} ranges from 0.5 h to 3.0 h on Day 1 of dosing and from 0.5 h to 1.0 h on Day 14 of dosing.

In study no. JE049-0108, male and female rats were subcutaneously administered 0 (vehicle-0.9% NaCl), 1, 3 and 10 mg/kg/day for 13 weeks. Satellite males and females (6 rats/sex/group) had blood samples collected on Day 1 and during Week 13 at 0.5, 1, 2 and 4 h post-dosing. Plasma was prepared from samples and assessed for icatibant and the metabolite M_2 [redacted] (b) (4) levels using LC-MS/MS analytical methods. TK parameters determined included: t_{max} and AUC. The lower limit of quantitation (LLOQ) for icatibant was 30 ng/mL plasma and for M_2 metabolite was 10 ng/mL plasma, respectively.

	Parameter	Icatibant Dose (mg/kg/day)											
		TK of Icatibant						TK of M_2 Metabolite					
		1		3		10		1		3		10	
		M	F	M	F	M	F	M	F	M	F	M	F
Day 1	C_{max} (µg/mL)	0.96	0.81	2.1	2.4	5.4	6.1	0.14	0.06	0.20	0.17	0.47	0.25
	T_{max} (h)	0.5	0.5	0.5	1	1	1	1	1	1	1	2	2
	$AUC_{0\ 5\text{-}4\ h}$ (µg*h/mL)	0.82	0.66	2.5	3.7	12.4	12.6	0.13	0.07	0.42	0.29	1.12	0.64
Wk 13	C_{max} (µg/mL)	1.0	1.1	2.3	2.7	6.1	6.8	0.09	0.06	0.18	0.13	0.39	0.18
	T_{max} (h)	0.5	0.5	0.5	1	2	1	1	1	2	1	2	2
	$AUC_{0\ 5\text{-}4\ h}$ (µg*h/mL)	1.7	1.2	5.6	5.0	15.9	17.2	0.15	0.08	0.48	0.31	0.88	0.59

NC- not calculated because individual values were invalid (QC rejected).

On Day 1 and Week 13 of dosing, icatibant C_{max} and AUC levels increased in an approximate dose proportional manner in both males and females. There were no gender differences and icatibant did not accumulate after 13 weeks of daily SC dosing. The M2 metabolite C_{max} increased with increased icatibant dose on Day 1 and Week 13 in a sub-proportional at all doses. AUC levels were proportional from LD to MD and sub-proportional from MD to HD. The metabolite formed between 1 and 2 hours post-icatibant dose. The metabolite formed between 1 and 2 hours post-icatibant dose, demonstrating rapid metabolism of the parent molecule.

In a dose-ranging study (JE049-0115), a single Beagle dog/sex was dosed 100 mg/kg/day for 10 days. Blood samples were taken on Day 1 and Day 10 from each animal from Phase 2 to determine icatibant levels and the metabolite M_2 [redacted] (b) (4) levels. On Day 1 and Day 10 samples were taken at: predose, 0.5, 1, 2, 4, 8 and 24 h post-dose. Plasma samples were prepared from whole blood and analyzed using LC-MS/MS analytical methods. TK parameters determined included: C_{max}, t_{max} and AUC. The lower limit of quantitation (LLOQ) for icatibant was 11.4-29.07 ng/mL plasma and for M2 metabolite was 18.59-65.14 ng/mL plasma, respectively. These data showed that dogs were exposed to icatibant and that the M2 metabolite was present in dogs.

Day	Parameter	Icatibant 100 mg/kg/day			
		TK for Icatibant		TK for M2 metabolite	
		M	F	M	F

Day 1	C_{max} (µg/mL)	52.2	54.2	2.01	0.73
	T_{max} (h)	1	2	24	24
	AUC_{0-24} (µg*h/mL)	458	507	19	10
Day 10	C_{max} (µg/mL)	77.4	57.8	3.38	1.13
	T_{max} (h)	1	1	0	0
	AUC_{0-24} (µg*h/mL)	415	522	6	13

Blood samples were taken from 1 dog/sex at the indicated time points at one dose (100 mg/kg/day). Based on the available data, icatibant C_{max} and AUC levels were similar between the male and the female at Day 1 and at day 10 of the study. There was no accumulation of icatibant over the 10 day dosing period in either the male or the female. The M2 metabolite was ~3-fold higher in males than in females for C_{max} on day 1 and day 10 of the study. The AUC of the M2 metabolite was approximately the same in males and females on Day 1 and Day 10.

In a 4-week toxicity study (JE049-0116), Beagle dogs were subcutaneously administered 0 (vehicle-0.9% NaCl), 1 or 3.3 mg/kg TID of icatibant twice weekly. These doses equaled to 3 and 10 mg/kg icatibant twice weekly. Blood samples were taken on Day 22 from all animals to determine icatibant levels and the metabolite M2 (b) (4) levels. Samples were taken at: predose, 1, 3, 6, 7, 12, 13 and 24 h post-dose. Plasma samples were prepared from whole blood and analyzed using LC-MS/MS analytical methods. TK parameters determined included: t_{max} and AUC. The lower limit of quantitation (LLOQ) for icatibant was 2.27 and 8.49 ng/mL plasma and 0.431 and 1.40 ng/mL plasma for M2 metabolite, respectively.

	TK Parameter	**Icatibant Dose (mg/kg/day)**			
		1 TID		**3.3 TID**	
		M	**F**	**M**	**F**
TK for Icatibant	AUC_{Tot}(µg*h/mL)	18.2	10.9	43.4	44.5
TK for M2	AUC_{Tot} (µg*h/mL)	0.575	NC	0.735	0.851

NC- not calculated because individual values were invalid (QC rejected).

On Day 22, AUC levels of icatibant increased with increased dosage in both males and females in an approximately dose proportionate manner. The M_2 metabolite also increased with increased dose but in a sub-proportionate manner for males (no data were available for females at the low dose). The t_{max} was reached between 1 and 2 hours post-dose in both males and females. No gender differences were observed in systemic exposure levels on Day 22 of the study.

Study no. JE049-0117, dosed Beagle dogs with 0 (vehicle- 0.9% NaCl), 10, 30 and 100 mg/kg/day of icatibant for 13 weeks. Blood samples were taken on Day 1 and in week 13 from all animals to determine icatibant levels and the metabolite M_2 (b) (4) levels. On Day 1 and during Week 13 samples were taken at: predose, 0.5, 1, 2, 4, 8 and 24 h post-dose. Plasma samples were prepared from whole blood and analyzed using LC-MS/MS analytical methods. TK parameters determined included: C_{max}, t_{max} and AUC. The lower limit of quantitation (LLOQ) for icatibant was 6.94-26.54 ng/mL plasma and for M2 metabolite was 4.14-9.36 ng/mL plasma, respectively.

	Parameter	Icatibant Dose (mg/kg/day)											
		TK of Icatibant						TK of M2 Metabolite					
		10		30		100		10		30		100	
		M	F	M	F	M	F	M	F	M	F	M	F
Day 1	C_{max} (µg/mL)	10.9	11.2	47.7	41.4	NC	74.4	0.40	0.32	1.16	1.01	NC	1.38
	T_{max} (h)	1.0	1.0	1.5	1.75	NC	2.0	1.75	2.00	3.00	2.50	NC	4.0
	AUC_{0-t} (µg*h/mL)	24.6	22.9	190	164	NC	383	1.14	0.90	6.14	5.26	NC	7.65
Wk 13	C_{max} (µg/mL)	17.6	17.7	34.4	41.6	82.3	71.0	0.39	0.31	NC	NC	0.63	0.77
	T_{max} (h)	0.75	0.75	1.75	1.50	0.88	0.75	2.0	2.0	NC	NC	2.0	2.0
	AUC_{0-t} (µg*h/mL)	38.2	31.6	80.9	83	246	213	1.15	0.77	NC	NC	3.10	3.46

NC- not calculated because individual values were invalid (QC rejected).

On Day 1 and Week 13 of dosing, the C_{max} and AUCs of icatibant were similar between males and females, suggesting that there were no significant gender differences. This was also observed for the M2 metabolite. On Day 1 of dosing, the C_{max} increased in an approximately dose-proportional manner from the LD to the MD. However, from the MD to the HD, the C_{max} of icatibant was sub-proportional with an increase by ~1.8-fold instead of the expected ~ 3-fold increase. The AUCs for icatibant increased in a supra-proportional manner from LD to MD (8-10 fold increase) on Day 1. At week 13, the C_{max} and AUC increased in a dose proportional manner form the LD to the MD but C_{max} was sub-proportional from the MD to the HD (~1.7-fold increase instead of ~ 3-fold increase). AUC increased approximately dose-proportionately. However, the dosing schedule was modified throughout the study period in the HD group.

The M2 metabolite formed within ~ 2-4 hours post-icatibant administration. The M2 metabolite C_{max} and AUCs increased from the LD to the MD in an approximately dose related manner. From the MD to the HD, M2 only slightly increased in C_{max} and AUC levels for those levels that were quantifiable. This increase was sub-proportionate to dose. However, the dosing schedule was modified throughout the study period in the HD group.

In a second 13-week subcutaneous repeat dose dog study (JE049-0118), sexually immature Beagle dogs were treated with icatibant daily at 3 or 10 mg/kg or with 3.3 mg/kg TID administered twice weekly. Venous blood samples were taken on Day 1 and in week 13 from all animals to determine icatibant levels and the metabolite M_2 (b) (4) [redacted] levels. On Day 1 and during Week 13 samples were taken at: predose, 0.5, 1, 2, 4, 8 and 24 h post-dose for the VC, 3 and 10 mg/kg/day Groups 1, 2 and 3, respectively. For Group 4, samples were taken at pre-dose, 1, 3, 6, 7, 12, 13 and 24 h post-dosing. Plasma samples were prepared from whole blood and analyzed using LC-MS/MS analytical methods. TK parameters determined included: C_{max}, t_{max} and AUC. The lower limit of quantitation (LLOQ) for icatibant was 1.3-3.2 ng/mL plasma and for M2 metabolite was 0.37-1.05 ng/mL plasma, respectively.

	Parameter	Icatibant Dose (mg/kg/day)

		TK of Icatibant						TK of M2 Metabolite					
		3		10		3.3 TID		3		10		3.3 TID	
		M	F	M	F	M	F	M	F	M	F	M	F
Day 1	C_{max} (µg/mL)	5.1	4.4	14.6	16.1	-	-	0.16	0.14	0.28	NC	-	-
	T_{max} (h)	0.7	1.1	0.9	1.0	-	-	3.6	2.0	3.3	NC	-	-
	AUC_{0-t} (µg*h/mL)	13.1	11.3	44.2	41.4	-	-	0.99	0.78	1.98	NC	-	-
	AUC_{tot} (µg*h/mL)	-	-	-	-	47.1	50.8	-	-	-	-	NC	1.52
Wk 13	C_{max} (µg/mL)	5.1	4.8	21.8	20.6	-	-	0.19	0.24	0.23	0.32	-	-
	T_{max} (h)	0.6	0.8	0.8	0.9	-	-	3.3	3.2	4.0	3.2	-	-
	AUC_{0-t} (µg*h/mL)	13.9	13.6	55.2	58.2	-	-	1.4	1.5	2.3	2.7	-	-
	AUC_{tot} (µg*h/mL)	-	-	-	-	NC	NC	-	-	-	-	1.9	NC

NC- not calculated because individual values were invalid (QC rejected).
- Values not determined.

Exposure levels of icatibant increased with increased daily dose (3 to 10 mg/kg/day) in both males and females in an approximately dose-proportional manner. There was no accumulation after repeated daily SC administration of icatibant in males or females when comparing exposure levels at Day 1 to Week 13. The t_{max} for icatibant was achieved rapidly (0.6- 1 h post-dose) after SC administration in males and females. There were no PK gender differences observed after daily administration of icatibant to males and females. After twice weekly administration of icatibant, plasma concentrations and systemic exposure levels were below the level of detection in both males and females.

The M_2 metabolite of icatibant increased in systemic exposure with increased icatibant dose in supra-proportional manner. There was no accumulation of M_2 after repeat daily SC administration of icatibant and there were no significant PK gender differences in M_2. Systemic exposure to M_2 after twice daily administration of icatibant was not detectable.

2.6.4.4 Distribution

Two whole body autoradiographic studies were completed in male Wistar rats. Each study administered a single subcutaneous dose of radiolabeled icatibant. The first study (study no. JE049-0311) dosed rats with 1 mg/kg of icatibant radiolabeled at prolyl-3,4-^{3}H and the second study (study no. JE049-0312) dosed rats with 1 mg/kg of icatibant radiolabeled at oic-6,7-^{3}H at 1 mg/kg. The distribution of the test compound was examined at 5 min and 1, 4 and 24 h post-dose. The quantitative level of radioactivity distributed in tissues was not provided. However, an overall distribution summary was reported.

Study no JE049-0311 showed that from 1 h post-dose, radioactivity occurred in the kidneys, liver, lungs and spleen. Radioactivity was present in the pancreas, testes, epididymides, wall of both the stomach and intestines, walls of large vessels, wall of the eye, adrenals and in tissues chiefly or exclusively connective structures and bone marrow.

Radioactivity of relatively high concentration was found in the epiphysial cartilage and radioactivity of lower concentration was also found in the articular cartilage of the tubular bones. The highest concentrations were contained in the kidneys, urinary tract and liver. In the brain, radioactivity slightly accumulated in the anterior hypophysis and in the wall of the great cerebral vein. In the vascular system, the radioactivity concentrations in the vessel wall were higher than in the blood itself, where they remained low and at all times below those in the large organs. At 24 h post-dose, detectable radioactivity was still to be found in several organs, the liver, kidneys, bone marrow, adrenals and lymph nodes being among these.

Study no. JE049-0312 the radioactivity absorbed into the body from the site of administration was negligible after 5 mins and could only be observed in the kidneys. From 1 h post-dose, radioactivity occurred in the kidneys, liver, and in the urinary tracts and urine. The lymph nodes and epiphyseal cartilage exhibited similar or slightly lower concentrations. The concentrations in these organs and tissues were higher than those in the blood. The lungs, spleen and skin showed similar concentrations as the blood, and the testes and epididymides slightly lower concentrations. The values in the skeletal and cardiac muscle were lower than the above mentioned organs. No radioactivity was detectible in the CNS and adipose tissue. In the intestines, the radioactivity was chiefly found in the anterior segments and more in the contents than in the wall. Four hours after dosing, the concentrations were lower but the highest concentrations remained in the liver, lymph nodes and the kidneys. At the end of the 24 h period, radioactivity had further decreased but was still detectable in the live, lymph nodes, spleen, kidneys, contents of intestines and bone marrow and at the site of administration. The liver had the highest concentration.

In study no. JE049-0310 (non-GLP), the binding of icatibant to the serum proteins of man, monkey, dog and rat was determined. Icatibant (concentrations ranging from 0.01 to 10 µg/mL) was incubated at room temperature for 30 minutes with serum proteins and then ultrafiltration was used to determine the percentage of protein binding, The protein binding in man and rat was 44 and 49%, respectively, which was ~6 and 11% higher than the protein binding in monkey and dog (~38%).

Under GLP conditions (study no. JE049-0329), 1 mg/kg of radiolabeled icatibant (propyl-3,4-^{3}H) was intravenously administered into 4 lactating female Wistar rats. The concentrations of radioactivity in blood and milk were determined up to 48 h post-dose. As early as 0.5 h post-dose, radioactivity was detected in the milk of rats (0.32 µg equivalents/g). Blood levels at 0.5 h were 1.11 µg equivalents/g. In the milk, radioactivity concentrations increased with time during the first 7 hours post-dose with maximal levels at 1.06 µg equivalents/g. The elimination radioactivity from blood was rapid at the beginning with a $t_{1/2}$= 0.5 h. In the milk, the $t_{1/2}$= 5.3 h. As from 24 h post-dose, there was no further decrease of levels in milk. At the last time point (48 h), the mean concentration was 0.10 µg equivalents/g in blood and 0.34 µg equivalents/g in milk. The portion of radioactivity excreted with the milk was estimated to be approximately 2% of the original dose. In conclusion, icatibant is excreted in the milk of rats after IV administration.

PK parameter	1.0 mg/kg IV Icatibant	
	Blood	Milk
C_{max} (µg equivalent/g)	1.109	1.061
T_{max} (h)	0.75	7.0
$T_{1/2}$ (h)	0.5	5.3

2.6.4.5 Metabolism

Jerini conducted 4 *in vitro* human and dog hepatocyte and microsome studies examining the potential effects of icatibant on cytochrome P450 enzymes (CYP450). In the first study, study no. JE049-0330 (GLP), the inhibitory effect of icatibant (batch no. E084) on catalytic activity of cytochrome P450 isoforms was tested. Nine major human hepatic CYP isoenzymes (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4) from pooled human liver microsomes were incubated with icatibant at 2, 10 and 50 µg/mL for 30 mins. The positive controls used for each isoenzyme showed clear inhibition (>30%). Icatibant did not exert any inhibitory effect on the activity of the 9 CYP isoenzymes tested up to 50 µg/mL under the experimental conditions tested. The following table summarizes the inhibition of CYP marker reactions by icatibant (mean of triplicates).

CYP	Marker Reaction	% Inhibition by Icatibant		
		2 µg/mL	10 µg/mL	50 µg/mL
1A2	7-ethoxyresorufin O-deethylation	-17.3 ± 8.6	-46 ± 5.2	-43.1 ± 4.3
2A6	Coumarin 7-hydroxylation	-9.6 ± 10.1	-2.6 ± 8.5	-10.3 ± 11.1
2B6	S-mephenytoin N-demethylation	1.2 ± 9.7	8.4 ± 16.9	-11.6 ± 16.0
2C8	Paclitaxel 6a-hydroxylation	-3.1 ± 15.0	4.2 ± 12.7	-0.3 ± 9.8
2C9	Diclofenac 4'-hydroxylation	11.6 ± 11.5	5.9 ± 10.4	14.1 ± 14.2
2C19	S-mephenytoin 4'-hydroxylation	-1.7 ± 10.8	12.5 ± 8.3	10.3 ± 12.6
2D6	Bufuralol –hydroxylation	3.4 ± 14.0	-13.1 ± 13.0	0.7 ± 10.8
2E1	Chlorzoxazone 6-hydroxylation	-7.6 ± 4.3	-5.5 ± 6.3	-9.0 ± 9.2
3A4	Testosterone 6 b-hydroxylation	-23.2 ± 12.5	-9.7 ± 12.6	-13.9 ± 7.4

The sponsor stated that inhibition rates ±20% were considered mainly due to statistical variation.

In the second study (study no. JE049-0317), the metabolic stability of icatibant acetate in liver microsomes and S9 of dog and humans was investigated. Icatibant (75 µg/mL) was incubated for 60 or 120 minutes at 37°C with dog liver microsomes or human liver microsomes. Additionally, icatibant (75 µg/mL) was incubated with dog or human S9 fraction for 2 hrs at 37°C with samples taken at 15, 30, 45, 60 and 120 minutes to test for metabolism. Icatibant incubated up to 2 hours with liver microsomes of dog or humans or with S9 fractions from dog or humans, did not metabolize icatibant significantly. The positive controls were rapidly metabolized in the presence of dog or human microsomes and dog or human S9 fractions. These data taken together, suggest that icatibant is metabolized independently from the CYP450 enzymes.

The metabolic stability of icatibant metabolites, M1 and M2, were evaluated in human hepatocytes in study JE049-0318 (non-GLP). M1 (6.09 µg/mL) and M2 (6.83 µg/mL) were incubated with 0.5 microsomal protein for 60 min at 37°C. Samples of M1 and M2 were taken at 0, 15, 30, 45 and 60 minutes and analyzed for metabolism. Under these study conditions, neither M1 nor M2 were metabolized by human microsomal proteins.

In study no. JE049-0331 (GLP), icatibant was incubated with freshly prepared human hepatocytes to test for icatibant's potential to induce CYP450 1A2 and 3A4 enzyme activity. Icatibant at 2, 10 and 50 µg/mL concentrations was incubated with hepatocytes from 3 different donors for 1A2 and 7 different donors for 3A4. A known inducer for each 1A2 (omeprazole) and 3A4 (rifampicine) was incubated with respective donors to serve as a positive control. Icatibant did not induce enzymatic activity for either 1A2 or 3A4 in freshly prepared human hepatocytes.

2.6.4.6 Excretion

The sponsor conducted 6 excretion studies administering radiolabeled icatibant subcutaneously and collecting urine and feces in mice, rats and dogs. Male mice were administered 1 mg/kg of radiolabeled icatibant (prolyl-3,4-^{3}H) formulated in 0.9% NaCl in 3 GLP studies (study no. JE049-0321, JE049-0326, and JE049-0327). A mean total of ~ 91.7% of radioactivity administered was excreted via urine and only 8.0% was excreted into feces. Renal excretion was complete by 24 h post-dose. The renal elimination was bi-phasic with a $t_{1/2}$ at 4.5h and a second $t_{1/2}$ at 15.2 h. Excretion in the feces was ~8.8% of the injected dose. Radioactivity in mice was distributed to the liver, kidneys, and urinary bladder with the highest concentrations. The recovery rate was ~97.1% of injected dose. The chromatographic analysis of urine showed ~64.3% of unchanged icatibant and 36% of M1 metabolite in the urine from 0-8 h urine collection. At 8-24 h urine collection, the proportion of M1 increased slight to 48.6%.

Two excretion studies were conducted in rats. In study no. JE049-0323 (non-GLP), 3 male Wistar rats were subcutaneously administered a single 12 µg/kg radiolabeled icatibant. Approximately 61% of radioactivity was collected in urine and less than 3% was detected in feces. After chromatographic analysis, it was determined that less than 0.5% of parent product was recovered from urine. One metabolite (M1) was recovered at 57% in urine.

In the second rat study (study no. JE049-0306, GLP), 6 male Wistar rats were administered a single 1 mg/kg subcutaneous dose of radiolabeled icatibant (Oic-6,7-^{3}H). Five min post-administration, measurable concentrations of radioactivity (mean 0.048 µg equivalents/gram) were already present in animal blood. The maximum concentrations were reached after 1 h in all animals and were between 0.425 and 0.499 µg equivalents/g. The elimination of the radioactivity from blood was a 2-phase process. A rapid first phase occurred with individually different half-lives of between 0.38 and 0.89 h and a terminal phase with half-lives between 5.5 and 20.6 h. The greater part of the excretion occurred via the kidneys (mean 68.5%). In a dried sample, the corresponding portion was 67.5%. The elimination via feces was appreciably less with a mean values being 30.1%. The majority of radioactivity excreted via this route was eliminated 8-24 h post-administration. The mean balance of radioactivity recovered was 100.6 % (samples measured directly) and 99.6% (dried samples).

PK parameter	1 mg/kg SC Icatibant

C_{max} (µg equivalent/g)	0.4601
T_{max} (h)	1
$T_{1/2}$ Phase 1 (h)	0.63
Time range Phase 1 (h)	1-4
$T_{1/2}$ Phase 2 (h)	13.93
Time range phase 2 (h)	8-48

In study no JE049-0309 (GLP), 2 male Beagles were administered a single 0.2 mg/kg subcutaneous dose of radiolabeled icatibant (labels on Oic-6,7-^{3}H). Five min post-administration, measurable concentrations of radioactivity were already present in blood and plasma. These amounted to mean 0.03 or 0.06 µg equivalents/g in blood and plasma, respectively. The maximum concentration was reached after 0.75 h and was 0.20 and 0.33 µg equivalents/g in blood and plasma, respectively. The elimination of the radioactivity from blood was a monophasic with a half-life of 1.6 h. The last measurable concentrations in the blood were detected 8 h post-dose. The mean radioactivity excreted via the kidneys was 63% of the total radioactivity administered. This renal excretion mainly occurred within 24 h post-dose. The elimination via feces was appreciably less with a mean values being 33.4%. The majority of radioactivity excreted this route was eliminated 24-48 h post-administration. The mean balance of radioactivity recovered was 97 % (samples measured directly) and 96.4% (dried samples).

PK parameter	**0.2 mg/kg SC Icatibant**	
	Blood	**Plasma**
C_{max} (µg equivalent/g)	0.20	0.33
T_{max} (h)	0.75	0.75
$T_{1/2}$ Phase 1 (h)	1.6	1.4
Time range Phase 1 (h)	1-8	0.5-8
$T_{1/2}$ Phase 2 (h)	-	91.5
Time range phase 2 (h)	-	24-72

Based on excretion studies conducted in mice, rats and dogs, the primary route of elimination of total radioactivity of radiolabeled icatibant administered subcutaneously is via the kidneys with a small amount of radioactivity excreted into the feces. After subcutaneous administration, radiolabeled icatibant is excreted rapidly with the majority recovered in the urine within 24 hours.

2.6.4.7 Pharmacokinetic drug interactions

No new data were submitted.

2.6.4.8 Other Pharmacokinetic Studies

No new data were submitted.

2.6.4.9 Discussion and Conclusions

- Icatibant is rapidly metabolized to M1 and M2: the major metabolite in man is M1 (>90%). Icatibant metabolism is through hydrolysis of the peptide backbone

believed to result from peptidase activity. M1 is formed in rat (~70%), dog (~60%) and mouse (~50%). The M2 metabolite [redacted] (b) (4) appears from 0.2 to 3 hours post-dose in rats and dogs. Neither icatibant nor the M1 or M2 metabolites are metabolized by human hepatocytes or microsomes. Additionally, icatibant is not an inducer of CYP450 isozymes.

- Neither icatibant, nor M2 accumulated after repeat dosing in males and females up to 13-weeks dosing. There were no gender differences observed in rats or dogs in the systemic exposures of males and females.
- The t_{max} was achieved from 0.5 to 3.0 h post-dose in rats and dogs.
- In rats and dogs, increases in icatibant doses from LD to MD doses (0.1 to 3 mg/kg in rats and 10 to 30 mg/kg/day in dogs) produced approximately doses proportionate increases in systemic exposures. Increases in icatibant doses from MDs to HDs resulted in super-proportionate increases in systemic exposures in both rats (increased ~128-fold instead of the expected ~50-fold) and approximately dose proportionate in dogs. Using an alternate dosing schedule in dogs (1 or 3.3 mg/kg TID of icatibant twice weekly), AUC levels of icatibant and M2 increased in an approximate dose proportionate increase.
- Icatibant (and/or its metabolites) is distributed into the kidneys, liver, urinary bladder, lungs and spleen 1 hour and it still detectable in the liver, kidneys, spleen, bone marrow, adrenals and injection sites at 24-hours post SC dose in rats.
- Serum protein binding of icatibant was low with 44 and 49% in man and rat, respectively, and 39% in monkey and dogs. Icatibant is excreted in the milk of rats after IV administration.
- Based on excretion studies, the primary route of elimination after SC administration of icatibant is via the kidneys in mice (~97.1%), rats (~61%) and dogs (~63%) with lesser amounts excreted in the feces (8.0% to 33%).

2.6.4.10 Tables and figures to include comparative TK summary

Not applicable because no new data were submitted.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Not applicable because no new data were submitted.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: Under IND [redacted] (b) (4) acute toxicology studies conducted in the mouse, rat and dog were reviewed (Dr. Choi's review). The highest non-lethal IV dose was 30.7 mg/kg and the minimum lethal dose (MLD) was 48.3 mg/kg for mice. For rats, the highest non-lethal IV dose was 12.3 mg/kg and the MLD was 19.2 mg/kg. After IV dosing, rapid respiration, twitching and convulsions were observed prior to death. In an acute IV toxicity study, male dogs (n=2/group) were administered 0.4, 2 and 10 mg/kg icatibant. No mortality was observed in this study. However, numerous clinical signs,

many suggestive of massive histamine release, were observed in the MD and HD groups. In a separate study, male dogs (n= 3/group) were administered single IV doses (infused over 5 h period) of 15, 30 and 60 mg/kg. An increase in serum CPK levels were above historical background (153 mU/mL) was noted in 1 MD animal (406 mU/mL) and two HD dogs (843 and389 mU/mL, respectively. The NOAEL in this study was 15 mg/kg. In acute SC toxicity studies, the highest non-lethal dose was 614 mg/kg and the MLD was 767 mg/kg for mice. In rats, no mortality was observed after a single SC injection of 1227 mg/kg icatibant. After SC injection, injection sites showed bluish discoloration that progressed to necrosis.

During icatibant's development, intranasal (IN), inhalation (IH), IV and SC routes of administration have been explored. Under INDs (b) (4), the main repeat dose toxicology studies investigated the intranasal (IN), inhalation (IH) and IV routes of administration with a few studies conducted using the SC route. Due to low bioavailability, no toxicity was observed in most IN toxicity studies. However, reduced testicular weights and spermiogenesis in the testes were observed in a single 1-month IN toxicity study carried out in the rat at 125, 1250 or 12500 µg/kg. The NOAEL was considered 125 µg/kg based on these testicular findings.

In a comparative IV/SC/IN 1-month toxicity study, male rats were either intravenously (10 mg/kg/day), subcutaneously (10 mg/kg/day) or intranasally (2.5 mg/animal/day) administered icatibant. Renal tubular atrophy was observed in 7/10 rats and renal tubular necrosis in 4/10 rats treated via the IV route. Impairment in sperm maturation was observed in 8/10 rats and oligospermia in the epididymides in 7/10 rats after IV administration. Tubular atrophy (4/10) and tubular necrosis (1/10) were also observed in SC treated rats. Despite similar exposure levels, oligospermia in the epididymides in 1/10 animals was the only male reproduction effect observed in SC treated animals. No control groups were included in this study. TK analysis showed the exposure ($AUC_{0\text{-}4h}$) to be similar between IV (20.33 µg/mL) and SC (17.3 µg*h/mL) treated animals. No adverse effects were reported in the intranasal group.

In a 1-month toxicity study, rats were intravenously administered 0.1, 1 and 10 mg/kg/day of icatibant. Renal toxicity was observed in 10/10 males and 5/10 females with areas of epithelial necrosis, areas of repair and foci of tubular atrophy at the 10 mg/kg/day dose. Irreversible tubular atrophy was present in 5/5 males and 1/5 females after a 28-day recovery period. The NOAEL was 1 mg/kg/day. In a 1-month study, dogs were intravenously administered 0.1, 0.25 and 0.8 mg/kg/day. All HD males 3/3 had swelling of flews that was attributed to histamine release. The major part of the glandular portions in the basal mucosa was missing from the stomach of 1 HD male that was reported as a relatively rare change. The NOAEL was 0.25 mg/kg/day.

Based on the previous reviews (b) (4) of the 1-month repeat dose toxicity studies in rats and dogs, the target organs toxicity in the rats were the male reproductive organs (decreased spermatogenesis and decreased weights of testes, epididymis, prostate, and/or seminal vesicles), kidneys (uneven color, increased weight, epithelial necrosis of proximal tubules, foci of tubular atrophy, increased blood urea, uric acid, creatinine, hemoglobin and epithelial cells in urine), stomach (taut and enlarged)

and liver (increased wt). In the dogs, the target organs were swelling of flews (histamine release reaction) and the stomach (chronic gastritis).

In support of the proposed SC route of administration for this NDA, the sponsor conducted two 14-day SC toxicity studies in the rat, one 13-week SC toxicity study in the rat (submitted as a combined 13-week toxicity and fertility and early embryonic development study), a SC dose-ranging study in the dog, a 4-week SC toxicity study in the dog (twice weekly dosing), and two 13-week SC toxicity studies in the dog (1 with daily SC dosing and 1 with daily and TID administered twice weekly SC dosing). In the first 14-week SC toxicity study, male and female rats were administered 0 (vehicle), 1, 10 or 50 mg/kg/day of icatibant. Only 5 rats/sex/group were dosed, which is considered an inadequately powered rat study to identify potential drug induced toxicities. Clinical signs of toxicity were limited to injection site irritation at the high-dose (50 mg/kg/day). Decreases in prostate weights in the MD (-33%) and HD (-39), and testes weights relative to brain weights in MD (-29%) and HD (-30%) were observed in males and increases in thymic weights in the HD group (30-38%) were observed in females. Histopathological findings included: epididymides (oligospermia and aspermia) and testes (hypospermia) were observed in all icatibant treated groups and injection site irritation was observed in the HD group. No NOAEL could be defined by the reviewer due to male reproductive effects of icatibant at all doses.

A second 14-day SC rat study (this study was followed by an 8-week recovery period), was conducted only in males (n=10 rats/group) to explore the reproductive organ effects in older animals. Rats were administered 0 (vehicle), 0.1, 1 or 50 mg/kg/day of icatibant SC and 1 group of untreated juvenile male rats were maintained as a second control group. A slight decrease in creatinine levels (-18%) in the HD group compared to the VC group was observed. Males in the HD had decreased testicular and prostate weights compared to the VC group. The sponsor did not evaluate a full histopathological battery of tissues in this study, with the rationale that no systemic toxicity other than reproductive organ effects was observed in the first 14-day SC study in males or females. Therefore, the sponsor selected only the male reproductive organs for microscopic examination. Histopathological findings included injection site irritation in the HD group (observed macroscopically and microscopically), oligospermia and cellular debris in the lumen of the epididymis and hypospermia in the testes. No NOAEL could be determined by the reviewer based on the incomplete histopathological battery in this study. For the male reproductive organ findings alone, the NOAEL is considered 1 mg/kg by the reviewer; this NOAEL was associated with an AUC of 22 µg*h/Ml.

In the 13-week SC toxicity study, male and female rats were administered 0 (vehicle), 1, 3 and 10 mg/kg/day of icatibant. In addition to extending the exposure period of rats to icatibant, this study was used to bridge the change in manufacturer of the drug substance. There were slight decreases in thyroid absolute weights (-6 to -18%) and weights relative to BWs (-3 to -16%) in all icatibant treated males but this was not associated with any macroscopic or microscopic findings. There were no microscopic findings in this study with the exception of injection site irritation. The NOAEL was 10 mg/kg/day based on the absence of systemic toxicity; this NOAEL was associated with an $AUC_{0.5\text{-}4\ h}$ of 16.55

µg*h/mL. This study was used to bridge the change in manufacturer for the drug substance. Using this new batch of the drug substance, a NOAEL was established in rats compared to no NOAEL identified in the 14-day toxicity studies conducted with the old drug substance manufacturer. Possible explanations include dosing in the 13-week study was only up to 10 mg/kg while the 14-day studies dosed up to 50 mg/kg, adaptive responses at lower doses from 14-days to 13 weeks, and the possible presence of impurities in the earlier batches.

A 13-week SC toxicity study was conducted in dogs that dosed animals once daily with 0 (vehicle), 10, 30 and 100 mg/kg/day. The top dose was selected based on a dose-range finding study conducted in dogs that observed minimal toxicity at 100 mg/kg/day. In the 13-week study, clinical signs of toxicity included erythema of the face, injection site irritation and several incidences of gastrointestinal disturbances (diarrhea and/or vomiting) at all doses. Due to abnormal vocalization in the HD group at day 12, the dose was decreased to 2 x 30 mg/kg/day. Decreases in leukocyte counts in HD males and increases in platelet counts in HD females were observed. Triglycerides and ALP were increased in HD males and decreased CK, creatinine, LDH and ASAT levels in HD males were observed compared to the vehicle control animals. Male and female reproductive organ weights (absolute and relative to BWs) were decreased, adrenal weights relative to BWs were increased in males and females, liver weights relative to BW were increased in MD and HD males, and thymic weights (absolute and relative to BWs) were decreased in both males and females compared to respective vehicle control treated animals. Macroscopic findings were observed at injection sites (hematomas and edema), the male (testes, epididymides and prostate) and female (ovaries and uterus) reproductive organs (organs all immature at all doses), thymus, liver and adrenal gland. Microscopic changes in the male (testes, epididymides, and prostate) and female (mammary gland, ovaries, uterus and vagina) reproductive organs, at the injection site (mainly HD groups but sporadic in LD and MD groups), in the adrenal gland (vacuolated cortical cell in HD males and females), liver (vacuolated hepatocyte in 1/4 males) and thymus (all doses) were observed. Based on the arrest of sexual maturity in all icatibant treated males and females, lymphoid depletion in the thymus and on injection site irritation, no NOAEL can be determined for this study. Exposure levels (AUC) in dogs ranged from 35 µg*h/mL at the low dose to 230 µg*h/mL at the high dose.

Based on the findings observed in dogs after daily administration of icatibant, the sponsor proposed an alternative dosing schedule to minimize the histamine release reactions (erythema of face) and to reduce systemic toxicity. In the 4-week SC toxicity study, Beagle dog were administered 0 (vehicle), 1 and 3.3 mg/kg TID as twice weekly dosing. This resulted in 3 and 10 mg/kg of icatibant administered twice weekly. Erythema was observed in low incidence in males and females at each dose. Gross and microscopic findings were limited to injection site irritation (hematomas) which did not follow a dose response. Testosterone levels were significantly decreased in males treated with icatibant at 1 or 3.3 mg/kg TID twice weekly but these levels rebounded between dosing intervals. A NOAEL of 3.3 T.I.D. (10 mg/kg) administered twice weekly (associated with an AUC of 44 µg*h/mL) was determined by the reviewer based on no microscopic drug-related findings.

The sponsor conducted a second 13-week SC toxicity study in dogs, dosing dogs either daily with 0 (vehicle), 3 or 10 mg/kg/day of icatibant or administered icatibant at 3.3 mg/kg TID twice weekly (10 mg/kg twice weekly). Observed clinical signs included increased erythema and edema at multiple sites that increased with increased dose. With cessation of treatment erythema abated. After daily administration of icatibant at 3 and 10 mg/kg/day, the thymus (lymphoid depletion) and the male (immature testes, epididymides and prostate) and female (immature ovaries, oviducts, uterus and vagina) reproductive tissues showed toxicity as reflected by reduction in these organ weights relative to body weights, macroscopic findings of reduced size and microscopic findings of reduced size. After the 13-week recovery period, 1/2 males showed the testes and prostate as immature where 1/2 males had recovered and there were no findings in female reproductive organs suggesting these adverse effects were reversible. When males were treated twice weekly with icatibant, no affects were observed in the male reproductive organs but females treated with SC administration of icatibant twice weekly showed delayed or impaired maturation of the reproductive organs. Lymphoid depletion in the thymus was observed in both males and females after twice weekly dosing. Hormonal analysis showed that icatibant induced a decrease of testosterone, LF and FSH in male dogs, which secondarily induced sexual immaturity. No NOAEL was determined in this study based on the adverse effects on male and female reproductive organs and lymphoid depletion observed in the thymus. However, twice weekly administration of icatibant resulted in less severe toxicity in males than with daily dosing.

At 13-weeks daily SC dosing of rats showed no toxicity after administration of a newly manufactured batch of icatibant from a different site manufacturing site. However, after IV and SC administration of icatibant for 1-month or 14-days, respectively, the target organs of toxicity were the male reproductive organs, kidneys, stomach and the injection sites (using old icatibant manufacturer). Dogs administered icatibant SC daily for 13 weeks showed erythema of the face (histamine reaction), male and female reproductive organ immaturity, thymus (lymphoid depletion), adrenal (cortical cellular vacuolation), liver (vacuolated hepatocyte) and injection site irritation. After 1-month IV administration, erythema and chronic gastritis were observed in dogs. When altering the dosing schedule in dogs from daily administration to twice weekly dosing, the effects on the male reproductive organs were reduced. However, females showed immature reproductive organs.

Genetic toxicology: Throughout the development of icatibant, the sponsor has conducted multiple genetic toxicology studies for icatibant and unnatural amino acids. The preliminary studies were evaluated under IND (b) (4). An Ames test (tested only 2 microbial strains) and an *in vivo* micronucleus test conducted in mice were conducted. The results of the Ames test with TA98 and TA100 up to 4000 µg/plate were negative with and without metabolic activation and the results of the micronucleus test were also negative. Under IND (b) (4), Dr. Choi reviewed five genetic toxicology studies: 4 Ames assays (2 with icatibant and 2 with the unnatural amino acids (b) (4) and an *in vitro* cytogenetic test of bone marrow cells from Chinese hamsters. The results of all Ames tests with *Salmonella typhimurium* strains and an

Escherichia coli strain, WP2uvrA, were negative with and without metabolic activation up to 5,000 µg/mL for all compounds. Icatibant did not produce significant increases in chromosomal aberrations of bone marrow cells in the *in vitro* cytogenetic test.

The sponsor submitted three new genetic toxicology studies in this NDA. These studies were: a bacterial reverse mutation assay (a complete bacterial strain battery), an *in vitro* mammalian chromosome aberration test, and an *in vivo* bone marrow micronucleus assay in Sprague-Dawley rats using the icatibant. The bacterial reverse mutation assay and the *in vivo* micronucleus study in rats were studies used to bridge the change in manufacturer for the drug substance. Icatibant did not induced genetic toxicity in these three studies under the conditions assayed.

Carcinogenicity: The sponsor has not submitted studies to evaluate icatibant's carcinogenic potential. At the pre-NDA meeting held on January 24, 2007 between the Division of Pulmonary and Allergy Products and Jerini, Jerini was informed of the need for 2 carcinogenicity studies to address the carcinogenic potential of icatibant. In an information request sent to Jerini following the Mid-Cycle Review meeting on January 23, 2008. Jerini was asked to provide a status update of their carcinogenicity studies. In Jerini's January 28, 2008 response to the request for information, Jerini indicated that the carcinogenicity studies would be initiated April 8, 2008 and July 7, 2008 for mice and rats, respectively.

Reproductive toxicology: Under IND (b) (4) a 5-week fertility study in male mice was conducted. Mice were dosed with 0 (vehicle-physiological saline) 16.2 and 80.8 mg/kg/day SC for 5 weeks then mated with untreated F mice for 1 week. There were no effects on copulation rate in icatibant treated animals compared to the vehicle control group. However, the conception rate was reduced in the 80.8 mg/kg/day group compared to the vehicle control group by 14%. The results showed no significant differences between control and treated groups for general health, body weight gain, mating ability, organ weight, number of spermatozoa or motility in left or right epididymis or female reproductive parameters. Therefore, 5 weeks SC treatment of male mice did not affect male fertility. The male fertility study in mice was carried out, but this study is known to be one of the most insensitive tests for spermatogenesis or testicular activity. Also, it has been known that fertility of rodents is not affected unless testicular activity or sperm counts are reduced by 90-95%. Additionally, this study did not follow current Segment 1 study guidelines.

Also under IND (b) (4), two teratogenic studies were reviewed (1 in rats and 1 in rabbits), which were preceded by dose-ranging studies. In the dose-ranging study conducted in rats, 3 pregnant rats/group were treated with 25, 50 and 100 mg/kg/day (no vehicle control was used) of icatibant SC from Days 7 through 18 of pregnancy. On Day 21 of pregnancy, pregnant rats were sacrificed and their uteri examined. At 25 mg/kg/day slight swelling at the injection site in 2/3 dams (Day 3) was observed. No additional toxicities were observed in dams or in their concepti. The third 25 mg/kg/day female died after the 10th injection on Day 16 of pregnancy. This female had blue discoloration of skin, panting, reduced activity, abdominal position and then death. The cause of death

was unclear. Dosing for the MD group (50 mg/kg/day) stopped after the 7th injection due to local intolerance but the dams were kept until Day 21 of pregnancy. From the 4th injection, swelling, hardening and scabbing occurred at the injection sties. Food consumption and body weight of dams or concepti were not affected. Pregnant rats in the HD group (100 mg/kg/day) had to be killed after the 4th injection on Day 10 of pregnancy due to local intolerance: marked swelling and reddening. One dam also had scabbing and another was observed with a "weeping skin wound". Concepti from this group were considered unremarkable with no teratogenicity observed.

In the definitive study (reviewed under IND (b) (4) by Dr. Choi), the effects of icatibant SC administration on morphological and post-natal development including fertility in Wistar rats was examined. Female rats (30 pregnant rats/group) were treated with 0 (vehicle control- 0.9% NaCl), 0.25, 2.5 and 25 mg/kg/group of icatibant SC from Days 7 through 18 of pregnancy. Twenty females/group were sacrificed and delivered by Cesarean-section on Day 21 of pregnancy and the fetuses were examined for morphological changes (skeletal examination on 50% and body cross section on 50%). The remaining 10 pregnant females/group were allowed to litter and raise their young and the fetuses were examined for viability, physical development and functional behavioral changes. One M and 1 F (10 pairs) from each litter of F_1 generation were raised to maturity for mating and their fertility was examined. After the birth of F_2 generation, the parent and pups were sacrificed. SC injection of icatibant did not produce any significant effects on dams for the following: general physical condition, food consumption, body weight gain, organ weight change (liver, spleen and kidney), pregnancy rate, duration of pregnancy in F_0 or F_1 generation, live or dead pups or sex distribution. There were no significant major external of internal anomalies reported in pups for F_1 or F_2 generations. However, 1 or 2 fetuses in the 0.25 and 2.5 mg/kg group had multiple minor skeletal malformations or variations. Post-natal development of treated fetuses and fertility of F_1 generation were comparable to the control group. Besides a kinky tail, especially 1 fetus in the 25 mg/kg/group, no major terata were produced in the study although 1 dam in the 25 mg/kg/day group had empty implantation sites in the uterus, and fetuses in the 25 mg/kg/day group had slightly greater numbers with less ossification of skeletons and variations of liver and kidneys than the control group (4 fetuses had lobus dexter of liver and 3 fetuses had distended pelvises in the kidney, while 1 fetus in the control group had the same findings). Therefore, icatibant was not teratogenic in rats under the conditions studied.

In a preliminary dose-ranging in rabbits (under IND (b) (4)), 2 pregnant rabbits/group were treated daily with SC injections of 10, 25 or 50 mg/kg/day of icatibant from Days 6 through 18 (no vehicle control was used). Rabbits were sacrificed on Day 29 of pregnancy and their uteri were examined. The reviewer stated that impairment of pregnancy was observed in all groups, although no impairment of body weights was noted. One dam in the 10 mg/kg group had only empty implantation sites in the uterus, indicating a clinically unnoticed abortion, but the second dam had 8 normally developed live fetuses. All dams in the 25 and 50 mg/kg/ groups had empty implantation sites in the uterus. Based on this study, doses of 0.1, 1 and 10 mg/kg/day were selected.

In the definitive rabbits study, Himalayan rabbits (15 pregnant females/group) were treated with 0 (vehicle control- 0.9% NaCl), 0.1, 1.0 and 10 mg/kg/day of icatibant, SC once daily from Days 7 through 18 of pregnancy. On Day 29 of pregnancy, surviving dams were sacrificed and fetuses delivered by C section. Fetuses were kept in an incubator for 24 h to determine survival rate and then examined morphologically for developmental disorders. Due to the occurrence of abortions, premature births, increased light-grey depression on the surface of kidneys in the icatibant treated groups, this study was repeated using 0.1 and 1.0 mg/kg icatibant SC and a 0.9% Na Cl group. Rabbits used for this section of the study were New Zealand White rabbits.

In the main study, 4 unexpected abortions were produced in 2 dams each in all 3 treated groups and a premature birth in 0.1 mg/kg group, while none of these occurred in the control group. No other toxic effects were produced in the icatibant treated dams. At autopsy, light-grey depression was found on the surface of kidneys in 5-7 dams each in all 3 icatibant treated groups but only 1 dam in the control group. To determine if these effects were drug induced, the study was repeated but with 2 lower doses only and the vehicle group. The abortions or premature births were not reproduced in this study, and incidence of light grey colored kidneys in the treated groups was not increased, but it was also reported in 1 dam each in treated groups only. The depression on the kidney was due to chronic interstitial nephritis, and it was attributed to the strain of rabbits by the sponsor.

Morphological examination of fetuses showed no increase in incidence of major malformations in the treated groups. In fact, the control fetuses had greater multiple malformations or variation of skeletons including limbs. Minor anomalies or variations of liver or kidneys were reported in some fetuses, but they were reported as within the normal ranges. Among the dead fetuses examined (6 in control, 4 in 1 mg/kg and 15 in the 10 mg/kg groups), 10 mg/kg had significantly more stunted (8/15) or retarded fetuses (7/15) over the control group (2/6 and 1/6, respectively). Icatibant was not teratogenic under the test conditions. All 3 doses of icatibant did not have significant toxic effects on the dams. However, 10 mg/kg was toxic to the embryo reducing the litter sizes as a result of increased intrauterine embryofetal death rate. Icatibant treatment resulted in dose-related decreases for total implantations and total number of live fetuses as well as dose-related increases for percent of pre-implantation loss when compared to the control values.

Under the conditions tested in the rats and rabbits, icatibant did not induce major terata. However, icatibant has a potential to produce abortions/premature births, intrauterine death or resorptions.

Included in this NDA submission, were 2 reproductive toxicology studies to complete the reproductive toxicology battery. The sponsor combined the 13-week repeat dose toxicity study and the effects on fertility and early embryonic development to implantation study in rats. Male Sprague-Dawley rats were given 0 (vehicle control- 0.9% NaCl), 1, 3 and 10 mg/kg/day of icatibant SC doses for 10 weeks prior to mating, during and after mating. Female rats were given 0 (vehicle control- 0.9% NaCl), 1, 3 and 10 mg/kg/day of icatibant SC doses daily for 2 weeks prior to mating, during mating and until 7 days post-coitum. HD treated females (10 mg/kg/day) had an increase in pre-implantation loss

compared to vehicle treated females. Untreated females paired with HD treated males showed no increase in pre-implantation loss compared to untreated females paired with vehicle control treated males, indicating that icatibant treatment of females and not males resulted in the pre-implantation loss. Icatibant had no effects on male sperm count, motility, or morphology. Based on no toxicity observed in male seminology and no effects on mating or fertility indices, the NOAEL for male fertility was 10 mg/kg/day. Based on an increase of pre-implantation loss observed in HD treated females, the NOAEL for female fertility was 3 mg/kg/day. These conclusions were in agreement with the sponsor's conclusions. This fertility and early embryonic study was conducted using current standard guidelines and fulfills the requirement for completion of a Segment 1 study.

In the second reproductive toxicity study submitted in this NDA, the effects of daily SC administration of icatibant on pre- and post-natal development in rats were evaluated. Sprague-Dawley rats were administered 0 (vehicle), 1, 3 and 10 mg/kg/day to mated females from Day 6 post-coitum until Day 21 post-partum. The effects of icatibant on the F_0 dams (maternal toxicity) and F_1 generation (systemic and developmental toxicity) were evaluated. Icatibant treatment delayed parturition at 1, 3 and 10 mg/kg/day, resulting in F_0 deaths (found dead or early sacrifice) in the 3 and 10 mg/kg/day groups. Clinical signs of toxicity (pallor of eyes and extremities, round back, piloerection and reddish discharge in vagina) were observed in 1 MD and in multiple HD F_0 females. After SC administration, icatibant and the M2 metabolite is found in rat milk. Based on the observed maternal toxicities including clinical signs, prolonged gestation periods for all icatibant dosage groups, F_0 spontaneous deaths and early sacrifice of icatibant treated dams, litter deaths, and necropsy findings in the MD and the HD groups, the maternal toxicity NOAEL could not be defined. The F_1 generation showed an increase in the number of pup deaths and cannabalism in the MD and HD compared to the VC groups. Therefore, the NOAEL for systemic toxicity in the F_1 generation was determined to be 1 mg/kg/day. The F_1 generation showed a slight decrease in air righting and hair growth compared to the VC control group. Therefore, the NOAEL for development of the F_1 generation was considered 3 mg/kg/day.

The sponsor has adequately completed the reproductive toxicity battery for icatibant. The relevant data indicate that the Pregnancy category for this drug should be C based on the the presence of increased pre-implantation loss, increased spontaneous abortions and increased pup deaths.

Special toxicology: Several special toxicology studies were previously reviewed under IND (b) (4) by Dr. Choi. Due to changes observed in male reproductive organs in IV and SC repeat dose rat toxicology studies, the sponsor conducted a 28-day repeat dose SC study to evaluate the effects of icatibant treatment on the pituitary-testicular axis. Male rats dosed with 1.25 mg/kg/day by SC injections or infusions for 28-days showed no significant effects on male reproductive organs or hormone levels during the dosing phase. The absence of male reproductive organs was likely due to the low doses of icatibant used. Interestingly, an unexpected activation significantly increased release and synthesis of testosterone in the serum and testes, after the 8-week recovery period was

observed. Also noted after the recovery period were increased organ weights of testes, prostate, seminal vesicles and adrenal glands, especially after SC infusion. The increased testosterone in serum and testes and increased weights of testes, prostate and seminal vesicles after the recovery period suggested that these organs may have been suppressed during the 4-weeks of treatment period at a sub-threshold level and most likely due to low systemic drug exposure.

Dr. Choi reviewed a local tolerance study in rabbits that were injected intra-articularly with single 10 or 60 µg/mL doses of icatibant in saline into the right knee joints of rabbits. Icatibant was well tolerated. In two local irritation studies (primary skin irritation study of rabbit skin and mucosal tolerance study in rabbit eye) icatibant was considered a non-irritant at 0.5 mg/mL icatibant solution. Antigenic potential of icatibant was studied in guinea pigs by homologous 4-hour PCA reactions and maximization tests. In the antigenicity study, when guinea pigs were sensitized with icatibant alone in intranasal or SC route, no anti-icatibant antibodies were produced. Thus, PCA test were negative. But, when guinea pigs were sensitized subcutaneously with icatibant plus Freund's adjuvant, antibody formation was observed when challenged with icatibant-BSA conjugated in one of 5 sera samples, with a titer of 23-25. Therefore, icatibant was a sensitizer under the conditions tested. In another study, a guinea pig maximization test, 8/10 icatibant treated guinea pigs plus Freund's adjuvant had slight erythema, while the control group showed no reaction. Therefore, icatibant was a sensitizer when administered with Freund's adjuvant in 2 studies.

Submitted to this NDA were 2 local toxicity studies conducted in rabbits using single dose SC administration in one study and intrarterial/intravenous/paravenous administrations in the second study. Icatibant administered via the SC route up to 40 mg in acetate buffer, pH 5.5 was comparable to the vehicle control for local irritation. In the second study, administration of icatibant up to 0.5 mg intra-arterially and intravenously and 0.1 mg paravenously resulted in little injection site irritation.

Two single-dose studies administering two unnatural, synthetic peptides that are both found in the icatibant drug substance, either [redacted] (b) (4) at 1000 mg/kg, SC were conducted by the sponsor. Neither study was adequately designed with the absence of control groups. No animals died after [redacted] (b) (4) administration. However, after [redacted] (b) (4) (2/3) females died within 24-h post-dose.

An *in vitro* hemolysis test of icatibant showed that 0.1% of icatibant did not induce hemolysis of human whole blood after incubation for 30 min where incubation with the positive control (distilled water for injection) did induce hemolysis.

In an assessment of antibody responses in rat, dog and monkey, the sponsor concluded that hyperimmunization attempts, even with icatibant-protein conjugates in combination with adjuvant, did not elicit a sustained antibody response against icatibant. Based on the lack of information provided by the sponsor (icatibant lot and purity and detailed methods of study), no concrete conclusions can be drawn from this study report.

However, based on the summary data provided by the sponsor, icatibant-thyroglobulin conjugate did not elicit a strong anti-icatibant antibody response.

For safety qualification of the (b) (4) impurity, the sponsor conducted 1 acute IV toxicity study, a bacterial reverse mutation study and a chromosome aberration study. (b) (4) was negative in the Ames test but positive in the chromosomal aberration test for inducing genotoxicity. Although positive for genotoxicity in the chromosome aberration test, (b) (4) is a member of the peptide drug class which is considered to have low genotoxic risk. The acute IV toxicity study dosed rats up to 100 mg/kg. No toxicity was observed in rats. However, the duration of this study is not considered acceptable to qualify this impurity for safety. Therefore, (b) (4) has not been adequately qualified for safety. A chemistry consult was completed to address issues related to drug substance and product impurities and comments to the sponsor will be addressed by the ONDQA reviewer, Dr. Eugenia Nashed.

2.6.6.2 Single-dose toxicity

No new data were submitted.

2.6.6.3 Repeat-dose toxicity

Study title: HOE 140: 14-day subcutaneous toxicity study in rats

Key study findings:

- Only 5 rats/sex/group were dosed in the main study. This study is considered inadequately powered to identify potential toxicities induced by icatibant.
- Clinical signs of toxicity were limited to injection site irritation.
- ↓ prostate wts in MD (-33%) and HD (-39) males; ↓ testes wts relative to brain weights in MD (-29%) and HD (-30%) groups, and ↑ female thymic wts in the HD group (30-38%)
- Gross findings included small male reproductive organs (testes, epididymides and prostate) and injection site irritation.
- Histopathological findings included: epididymides (oligospermia and aspermia), testes (hypospermia) were observed in all icatibant treat groups and injection site irritation was observed in the HD group.
- No NOAEL could be defined by the reviewer due to male reproductive effects of icatibant at all doses. Local effects were observed at the injection site at the HD. The sponsor called the NOAEL 10 mg/kg.

Study no.: JE049-0127
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)

Date of study initiation: February 3, 2005
GLP compliance: Yes

QA report: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, lot # D081, and 96.2% pure

Methods

Doses: 0 (vehicle), 1, 10 or 50 mg/kg/day
Species/strain: Sprague Dawley rats
Number/sex/group or time point (main study): 5/sex/group
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in water for injection, administered in 1.0 mL/kg and infusion rate was not applicable.
Satellite groups used for toxicokinetics or recovery: 3 rats/sex/group were used for TK analysis.
Age: 6-7 weeks old
Weight: males: 166-197 g and females: 136-168 g
Sampling times: See below
Unique study design or methodology (if any): None.

Observations, Times and Results

Mortality: All animals were checked for mortality at least twice a day.
There were no deaths prior to scheduled sacrifice throughout this study.

Clinical signs: All animals were observed daily for clinical signs of toxicity. The type of findings observed and the incidence of each finding from study Days 1 to 14 are summarized below. Injection site irritation with alopecia, scabbing and scales was observed in HD males and females with the presence of reddened skin.

Observation	**Icatibant Dose (mg/kg/day)**							
	0		**1**		**10**		**50**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Injection site alopecia								
No observations	-	-	-	-	-	-	9	22
Number of animals	-	-	-	-	-	-	1/5	4/5
Days from-to	-	-	-	-	-	-	7-15	8-15
Injection site scabbed								
No observations	-	-	-	-	-	-	23	33
Number of animals	-	-	-	-	-	-	4/5	5/5
Days from-to	-	-	-	-	-	-	7-15	6-15
Injection site wh scales								
No observations	-	-	-	-	-	-	4	20
Number of animals	-	-	-	-	-	-	1/5	3/5
Days from-to	-	-	-	-	-	-	12-15	8-15
Skin reddened								
No observations	-	-	-	-	-	-	7	6
Number of animals	-	-	-	-	-	-	2/5	2/5
Days from-to	-	-	-	-	-	-	9-12	8-10

Body weights: Animals were weighed once during pretest and on Days 1, 3, 5, 8 and 14 of study.

There were no statistically significant differences in group mean body weights or group mean body weight gains in males or females treated with icatibant compared to vehicle treated control animals.

Food consumption: Food consumption was determined once during pretest and on Days 1, 3, 5, 8 and 14.

There were no treatment related affects on food consumption in the icatibant treated groups compared to the vehicle treated group.

Ophthalmoscopy: Ophthalmic examinations were not performed.

EKG: EKG examinations were not performed.

Hematology: Blood samples were taken from a retrobulbar venous plexus of fasted animals. A complete hematological battery was assessed.

There were no statistically significant changes in the hematology parameters for males or females treated with icatibant compared to the vehicle control treated animals.

Clinical chemistry: Blood samples were taken from a retrobulbar venous plexus of fasted animals. A complete clinical chemistry battery was assessed.

There were no statistically significant changes in clinical chemistry parameters for males or females treated with icatibant compared to the vehicle control treated animals.

Urinalysis: Urine was collected from all animals overnight on ice in metabolism cages prior to necropsy. A complete urinalysis was conducted.

There were no statistically significant changes in urine parameters in the icatibant treated animals compared to the vehicle treated animals.

Gross pathology: A complete necropsy was conducted on all study animals.

Macroscopic findings were observed at injection sites in males and females and the male reproductive organs (testes, epididymides and prostate). Observations in the reproductive organs were most likely related to drug due to the absence of these observations in the vehicle control group. However, the effects on the male reproductive organs did not follow a dose-response. Injection site irritation was observed primarily in the HD group.

Observation	**Icatibant Dose (mg/kg/day)**							
	0		**1**		**10**		**50**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Testis		NA		NA		NA		NA
Abnormal content, material, ill-defined	-		-		1/5		-	
Small	-		1/5		1/5		1/5	
Epididymis		NA		NA		NA		NA
Small	-		1/5		1/5		-	
Prostate		NA		NA		NA		NA
Small	-		-		1/5		-	
Injection site								
Focus/area, crateriform, red	-	-	-	-	-	-	-	1/5

Observation	Icatibant Dose (mg/kg/day)							
	0		1		10		50	
	M	F	M	F	M	F	M	F
Focus/area raised, firm/hard, dark, red	-	-	-	-	-	-	1/5	-
Focus/area, raised, firm/hard, red, focal	-	-	-	-	-	-	1/5	1/5
Focus/area, raised, red, multifocal	-	-	-	-	-	-	3/5	-
Focus/area, raised soft, red, multifocal	-	-	-	-	-	-	-	2/5

- No macroscopic finding observed
NA- Not applicable

Organ weights: The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary, prostate with seminal vesicles, spleen, testes, thymus and thyroid gland were weighed. The absolute organ weights and relative organ weights to body weights and to brain weights were determined.

Decreases in male reproductive organ weights (prostate and testes) and an increase in female thymic weights were observed in icatibant treated rats compared to the vehicle control treated animals. The decrease in prostate weight was observed in the MD and HD for absolute weight and prostate weight relative to brain weight. A slight decrease in prostate weight was observed in the LD when assessing prostate weight relative to body weight. An increase in thymus weights were observed in females alone mainly in the HD group.

Organ weight change relative to controls

Organ	Sex	Icatibant dose (mg/kg/day)		
		1	10	50
Prostate wt	M	-	-31%	-39%
	F	NA	NA	NA
Prostate wt rel BW	M	-17%	-33%	-39%
	F	NA	NA	NA
Prostate wt rel to BR	M	-	-35%	-42%
	F	NA	NA	NA
Testes wt	M	-	-	-
	F	NA	NA	NA
Testes rel to BW	M	-	-	-
	F	NA	NA	NA
Testes rel to BR	M	-	-29%	-30%
	F	NA	NA	NA
Thymus wt	M	-	-	-
	F	-	-	+37%
Thymus wt rel to BW	M	-	-	-
	F	-	+15%	+30%
Thymus wt rel to BR	M	-	-	-
	F	-	-	+38%

- No statistically significant difference compared to controls
NA- Not applicable

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

All study animals had tissues prepared for histopathological examination. However, tissues from the control and the 50 mg/kg/day group animals were examined. The testis,

epididymis, prostate, seminal vesicles and injection site from all other animals were examined. Also, macroscopic findings from the main study animals were evaluated. A complete histological battery was examined with the exception of the cervix. However, the vagina, ovaries, fallopian tubes and uterus were examined.

Observation	**Icatibant Dose (mg/kg/day)**							
	0		**1**		**10**		**50**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Epididymis	N=5	NA	N=5	NA	N=5	NA	N=5	NA
Cellular debris: lumen	2 (2.0)		2 (2.5)		4 (2.3)		5 (2.8)	
Oligospermia	-		1 (2.0)		2 (3.5)		4 (3.3)	
Aspermia	-		1 (5.0)		1 (5.0)		-	
Testis	N=5	NA	N=5	NA	N=5	NA	N=5	NA
Hypospermia	-		1 (5.0)		3 (2.3)		3 (1.7)	
Giant cell: multinucleated	-		-		2 (2.0)		3 (1.7)	
Injection site	N=5	N=5	N=5	N=5	N=5	N=4	N=5	N=5
Acanthosis	-	-	-	-	-	-	5 (2.2)	5 (2.0)
Necrosis	-	1 (1.0)	-	-	-	-	3 (2.0)	2 (2.5)
Inflammation:exudative	-	-	-	-	-	-	2 (2.5)	4 (2.5)
Hemorrhage	-	-	1 (2.0)	1 (1.0)	-		4 (2.5)	-

- No macroscopic finding observed
NA- Not applicable
(#)- mean severity: grade 1= minimal; grade 2= slight; grade 3= moderate; grade 4= marked; grade 5= massive

After repeated 14-day daily SC dosing of rats, the target organs of toxicity were the epididymides, testes and the injection site. Oligospermia and aspermia were observed in epididymides at all icatibant doses and increased in incidence and severity with increased icatibant dose. Hypospermia was observed at all icatibant doses in the testes with increased incidence and severity. Other than injection site irritation, there were no additional histopathology findings in females. No NOAEL could be determined in this study due to male reproductive affects observed at all doses.

Toxicokinetics: Blood samples were taken on Day 1 and Day 14 from the TK animals (3/sex/group) at 0.5, 1, 2, 3, 7 and 24 h post-dose. Plasma concentrations were determined. The TK data are summarized in the following table.

Sampling period	**TK Parameter**	**Icatibant Dose (mg/kg/day)**					
		1		**10**		**50**	
		M	**F**	**M**	**F**	**M**	**F**
Day 1	C_{max} (mcg/mL)	1.35	1.11	7.49	7.51	20.6	16.9
	T_{max} (h)	0.5	0.5	1.0	1.0	0.5	0.5
	AUC_{0-t} (mcg*h/mL)	1.36	1.0	17.4	15.4	85	94.1
Day 14	C_{max} (mcg/mL)	1.36	1.15	9.48	10.1	31.6	37.7
	T_{max} (h)	0.5	0.5	1.0	0.5	1.0	0.5
	AUC_{0-t} (mcg*h/mL)	1.47	0.98	22.1	16.9	93.7	80

There were no significant gender differences in the TK parameters evaluated in rats on Days 1 and 14 of study. The systemic exposure levels increased in an approximately dose proportionate manner for all doses on Day 1 and Day 14. The t_{max} was achieved from 0.5

to 1.0 h post-dose for both males and females. After repeated SC administration for 14-days, there was no accumulation of icatibant in either male or female rats.

Study title: HOE 140: 14-day subcutaneous toxicity study in male rats with an 8-week recovery period

Key study findings:

- This study focused on male reproductive organ toxicity
- ↓ creatinine levels (-18%) in the HD group
- ↓ testis and prostate wts in the HD groups
- Histopathological findings included injection site irritation in the HD group (observed macroscopically and microscopically), oligospermia and cellular debris in the lumen of the epididymis and hypospermia in the testes. After the 8-week recovery period, the epididymal findings were mostly reversible with 1/10 animals having cellular debris and oligospermia remaining and hypospremia in the testes remained in HD males with similar incidence and slightly higher severity than the VC group.
- The sponsor did not evaluate a full histopathological battery of tissues in this study.
- No NOAEL could be determined by the reviewer based on the incomplete Histopathological battery. However, for male reproductive organ findings alone the NOAEL is considered 1 mg/kg by the reviewer; this dose is associated with an AUC of 1 µg*h/mL. The sponsor considered the NOAEL to be 1 mg/kg/day.

Study no.: JE049-0128
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: September 16, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, lot # D081, and 96.5% pure

Methods

Doses: 0 (untreated, juvenile), 0 (vehicle), 0.1, 1 or 50 mg/kg/day
Species/strain: Sprague Dawley rats
Number/sex/group or time point (main study): 10/group
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in water for injection, administered in 1.0 mL/kg and infusion rate was not applicable.
Satellite groups used for toxicokinetics or recovery: 3 rats for the vehicle control group and 9 rats/icatibant dose group were used for TK analysis and 10 additional male rats/group in all dosage groups with the exception of the untreated controls to be observed as recovery animals.
Age: 10 weeks old

Weight: males: 150-208 g
Sampling times: See below
Unique study design or methodology (if any): This study examined the effects of repeated subcutaneous administration of icatibant to 10 week old (sexually mature) rats and examined its effects on male reproductive organs. In the previous 14-day study (JE049-0127) conducted on sexually immature rats (6-7 weeks old), males showed hypospermatogenesis and oligospermia which were associated with reduced accessory sex gland weights. These findings were observed at all doses examined (1-50 mg/kg) and were attributed by the sponsor to a delay in sexual maturation that the sponsor considered as species-specific. The sponsor addressed whether these same findings would be observed in sexually mature male rats in this current study. Untreated juvenile rats were maintained as a control group for histopathological examination only.

Observations, Times and Results

Mortality: All animals from the main study groups were checked for mortality at least twice a day.
There were no deaths prior to scheduled sacrifice throughout this study.

Clinical signs: All animals were observed daily for clinical signs of toxicity. The type of findings observed and the incidence of each finding from study Days 1 to 14 are summarized below. There were no clinical signs of toxicity observed in the vehicle control group or the LD or MD icatibant groups. Skin lesions were observed in the HD icatibant treatment group from study Day 3 of treatment throughout the treatment phase and continued to be observed for 23 days into the recovery period in the HD recovery animals. No additional clinical signs of toxicity were observed in any of the treatment groups.

Observation	**Icatibant Dose (mg/kg/day)**			
	0	**0.1**	**1**	**50**
Skin lesion Number of animals (Days from-to)	- -	- -	- -	5/20 (Day 3) 15/20 (Day 4) 16/20 (Day 7) 17/20 (Day 9) 19/20 (Days 10-14) 10/10 (Recov Days 1-12) 9/10 (Recov. Day 13) 4/10 (Recov Day 14) 2/10 (Recov. Days 15-23)

Body weights: Animals were weighed weekly including pretest and recovery.
There were no statistically significant differences in group mean body weights or group mean body weight gains in males treated with icatibant compared to vehicle treated control animals.

Food consumption: Food consumption was determined weekly.

There were no treatment related affects on food consumption in the icatibant treated groups compared to the vehicle treated group.

Ophthalmoscopy: Ophthalmic examinations were not performed.

EKG: EKG examinations were not performed.

Hematology: Blood samples were taken from a retrobulbar venous plexus of fasted animals. In the untreated control group, animals were sacrificed Day 2 of study and blood was collected for hematological assessment. For the vehicle control group and the icatibant treated groups, animals were sacrificed the day after the last dose was administered or at the end of the recovery period. A complete hematological battery was assessed.

There were no statistically significant changes in the hematology parameters for males treated with icatibant compared to the vehicle control treated animals.

Clinical chemistry: Blood samples were taken from a retrobulbar venous plexus of fasted animals. In the untreated control group, animals were sacrificed Day 2 of study and blood was collected for clinical chemistry assessment. For the vehicle control group and the icatibant treated groups, animals were sacrificed the day after the last dose was administered or at the end of the recovery period. A complete clinical chemistry battery was assessed.

There were no statistically significant changes in clinical chemistry parameters for males treated with icatibant compared to the vehicle control treated animals with the exception of a slight decrease in creatinine levels in the HD compared to the vehicle treated animals. This difference was not observed in the recovery animals.

Δ% relative to vehicle treated controls

Parameter	**Icatibant (mg/kg/day)**		
	0.1	**1.0**	**50**
Creatinine (mcmol/L)	-	-	-18%
Recovery Creatine (mcmol/L)	-	-	-

- No statistically significant difference.

Urinalysis: Urine was collected from all animals overnight on ice in metabolism cages prior to necropsy. A complete urinalysis was conducted.

There were no statistically significant changes in urine parameters in the icatibant treated animals compared to the vehicle treated animals.

Gross pathology: A complete necropsy was conducted on all study animals.

Macroscopic findings were observed at injection sites and the male (testes, epididymides and seminal vesicles) reproductive organs. The findings in the male reproductive organs were not observed immediately after 14-days of repeated subcutaneous dosing but were observed in 1/10 males after the 8-week recovery period. The macroscopic findings of small male reproductive organs were observed in a single HD male no. 96.

Observation	Icatibant (mg/kg/day)			
	0	0.1	1.0	50
Injection site, N=10				
Erosion/ulceration	-	-	-	1
Focus/area red	-	-	2	7
Scab/crust, crateriform, firm/hard, red	-	-	-	1
Scab/crust, red	-	-	-	1
Scab/crust	2	-	2	8
Recovery Injection site, N=10				
Erosion/ulceration	-	-	-	-
Focus/area red	-	-	-	-
Scab/crust, crateriform, firm/hard, red	-	-	-	-
Scab/crust, red	-	-	-	-
Scab/crust	-	-	-	-
Testis N=10				
Small	-	-	-	-
Recovery Testis N=10				
Small	-	-	-	1
Epididymis N=10				
Small	-	-	-	-
Recovery Epididymis N=10				
Small	-	-	-	1
Seminal vesicle N=10				
small	-	-	-	-
Recovery Seminal vesicle N=10				
small	-	-	-	1

- No finding.

Organ weights: The adrenals, brain, epididymides, heart, kidneys, liver, pituitary, prostate with seminal vesicles, spleen, testes, thymus and thyroid gland were weighed. The absolute organ weights and relative organ weights to body weights and to brain weights were determined.

Minor decreases in male reproductive organ weights (prostate and testes) were observed in icatibant treated rats at the highest dose compared to the vehicle control treated animals. The decrease in prostate weight was observed in the HD for absolute weight, prostate weights relative to body weights and prostate weight relative to brain weight. After the 8-week recovery period there was no statistically significant difference in prostate gland weights compared to the control group. A slight decrease in testes weights relative to body weights and to brain weights were observed in the HD treated groups after the dosing phase of the study and the 8-week recovery period.

Organ weight change relative to controls

Organ	Icatibant (mg/kg/day)		
	0.1	1.0	50
Prostate Gland			
Absolute wt change	-	-	-21%
Relative to body wt	-	-	-19%
Relative to brain wt	-	-	-21%
Recovery Prostate Gland			
Absolute wt change	-	-	-

Organ	Icatibant (mg/kg/day)		
	0.1	1.0	50
Relative to body wt	-	-	-
Relative to brain wt	-	-	-
Testis			
Absolute wt change	-	-	-
Relative to body wt	-	-	-8%
Relative to brain wt	-	-	-10%
Recovery Testis			
Absolute wt change	-	-	-
Relative to body wt	-	-	-13%
Relative to brain wt	-	-	-12%

- No statistically significant difference.

Histopathology: Adequate Battery: yes (), no (X)—explain
Peer review: yes (X), no ( )

All study animals had tissues prepared for histopathological examination. However, limited tissues from the untreated juvenile and the vehicle treated control groups and the HD group were examined. The tissues microscopically examined included the testes, epididymis, prostate, seminal vesicle, kidney, injection sites (for VC and treated HD groups) and macroscopic findings. The slides for the specified tissues examined in this study were peer reviewed by Dr. B. Durchfeld-Meyer. The following table shows the icatibant related findings observed in the male reproductive organs and at the injection sites compared to the untreated juvenile male rats (reproductive organ comparison only) and the vehicle control (VC) males (comparison of both injection sites and reproductive organs).

Observation	Icatibant (mg/kg/day)				
	Juvenile untreated control 0	VC 0	0.1	1.0	50
Epididymis,	N=10	N=10	N=10	N=10	N=10
Cellular debris: lumen					
Grade 1	3	2	-	-	5
Grade 2	7	-	-	-	5
Oligospermia					
Grade 1	-	1	-	-	5
Grade 2	-	-	-	-	5
Grade 3	-	-	-	-	-
Grade 4	2	-	-	-	-
Grade 5	8	-	-	-	-
Recovery Epididymis,	NA	N=10	N=10	N=10	N=10
Cellular debris: lumen					
Grade 1		-	-	-	1
Grade 2		-	-	-	-
Oligospermia					
Grade 5		-	-	-	1

Observation	Icatibant (mg/kg/day)				
	Juvenile untreated control 0	**VC 0**	**0.1**	**1.0**	**50**
Testis, Hypospermia	N=10	N=10	N=10	N=10	N=10
Grade 1	-	-	-	-	1
Grade 2	2	1	-	-	2
Giant cell: multinucleated, grade 2	-	-	-	-	1
Recovery Testis, Hypospermia	NA	N=10	N=10	N=10	N=10
Grade 1		-	-	-	-
Grade 2		-	-	-	1
Grade 3		-	-	-	-
Grade 4		-	-	-	-
Grade 5		-	-	-	1
Injection site back	NA	N=10	N=10	N=10	N=10
Necrosis of epidermis					
Grade 2		-	-	1	2
Grade 3		-	-	-	2
Inflammation					
Grade 1		-	-	-	1
Grade 2		-	-	-	2
Grade 3		-	-	-	2
Edema					
Grade 1		1	-	-	-
Grade 2		-	-	-	1
Recovery Injection site back	NA	N=10	N=10	N=10	N=10
Necrosis of epidermis		-	-	-	-
Inflammation		-	-	-	-
Edema		-	-	-	-
Injection site neck	NA	N=10	N=10	N=10	N=10
Necrosis of epidermis					
Grade 1		1	-	-	-
Grade 2		1	1	2	1
Grade 3		1	1	1	7
Grade 4		-	-	-	1
Necrosis: pannicular muscle					
Grade 2		-	1	-	-
Grade 3		-	1	1	1
Acanthosis					
Grade 1		-	-	1	3
Grade 2		-	-	2	6
Grade 3		-	-	-	1
Inflammation					
Grade 1		-	1	-	-
Grade 2		3	-	1	4
Grade 3		1	-	-	2
Edema					
Grade 2		1	3	2	3
Grade 3		-	-	-	2
Infiltration: mixed inflam cell					
Grade 1		3	2	-	-

Observation	Icatibant (mg/kg/day)				
	Juvenile untreated control 0	**VC 0**	**0.1**	**1.0**	**50**
Grade 2		-	1	3	3
Hemorrhage					
Grade 2		-	-	-	2
Grade 3		-	-	-	2
Recovery Injection site neck	NA	N=10	N=10	N=10	N=10
Necrosis of epidermis		-	-	-	-
Necrosis: pannicular muscle		-	-	-	-
Acanthosis		-	-	-	-
Inflammation		-	-	-	-
Edema		-	-	-	-
Infiltration: mixed inflame cell		-	-	-	-
Hemorrhage		-	-	-	-

- No finding.
NA- Not applicable.
(#)- mean severity: grade 1= minimal; grade 2= slight; grade 3= moderate; grade 4= marked; grade 5= massive

The untreated juvenile controls showed that the secretory content of the accessory sex glands and cellular debris and low numbers of sperm in the epididymis are characteristic of this immature rat population. Comparing the VC treated rats with the icatibant treated rats showed an increase in incidence and severity of oligospermia and cellular debris in the lumen of the epididymis in the HD group. The HD males showed an increase in incidence and severity hypospermia in the testes compared to the VC group animals. After the 8-week recovery period, the epididymal findings were mostly reversible with 1/10 animals having cellular debris and oligospermia remaining. Hypospremia in the testes remained in HD males after the recovery period with similar incidence and slightly higher severity than the VC group. Injection site irritation at the two sites of administration, the back and the neck, resulted in severe irritation at the HD of icatibant. Injection site irritation resolved after the 8-week recovery period.

The macroscopic findings of small male reproductive organs were observed in a single HD male no. 96. Oligospermia was observed in the epididymis at Grade 5 and hypospermatogenesis in the testes was observed with a Grade 5 severity as well. There were no microscopic findings in the seminal vesicle in this animal.

Although the sponsor did not evaluate a full histopathological battery of tissues in this study, the previous study (JE049-0127) that was conducted in males and females at 1, 10 and 50 mg/kg/day showed no additional histopathological findings other than those observed in the male reproductive organs and the local injection site. However, this previous study was inadequately powered (n= 5/sex/group), potentially not missing drug-induced toxicities. The findings observed in the male reproductive organs in this study were limited to the HD group. Taking the data from both the 14-day study conducted in males and females and the 14-day study conducted in males alone, the combination of these 2 studies do not fulfill a complete histopathology assessment due to inadequate animal numbers in the first study and inadequate histopathology assessment in the second study.

The sponsor considered the NOAEL to be 1 mg/kg/day. Based on the study results with increased incidence and severity of epididymal and testes findings in the HD

and injection site irritation, the reviewer agrees with the sponsor with a NOAEL of 1 mg/kg/day for the male reproductive findings and the injection site. However, no overall NOAEL can be determined for this study due to the incomplete histopathological battery.

Toxicokinetics: Blood samples were taken on Day 1 and Day 14 from the TK animals (3/sex/time point) at 0.5, 1, 2, 3, 7 and 24 h post-dose. Icatibant plasma concentrations were determined. The TK data are summarized in the following table.

Sampling period	TK Parameter	Icatibant Dose (mg/kg/day)		
		0.1	**1**	**50**
Day 1	C_{max} (µg/mL)	0.073	0.738	11.258
	T_{max} (h)	0.5	0.5	3.0
	AUC_{0-24}(µg*h/mL)	0.04	0.956	122.423
Day 14	C_{max} (µg/mL)	0.078	1.355	26.786
	T_{max} (h)	0.5	0.5	3.0
	AUC_{0-24}(µg*h/mL)	0.044	1.0	177.5

On Day 1 of dosing, the C_{max} and AUC increased in an approximately dose-proportionate manner from LD to MD. From MD to HD, the C_{max} increased but did not increase in a dose-proportional manner (increased by 15-fold instead of ~50-fold). The AUC increased from the MD to the HD by ~128-fold instead of ~50-fold, making it a super-proportional increase to dose. After repeated dosing for 14 days, the C_{max} increased in an approximately dose proportional manner from the LD to the MD. From the MD to the HD, the C_{max} increased in a sub-proportional manner by ~20-fold instead of the ~ 50-fold. The AUC increased in an ~ dose-proportional manner from the LD to the MD. However, from the MD to the HD the AUC increased in a supra-proportional manner by ~177-fold. Based on these data, after 14-day repeat dosing, icatibant does not accumulate in males. The T_{max} ranges from 0.5 h to 3.0 h on Day 1 of dosing and from 0.5 h to 1.0 h on Day 14 of dosing.

Study title: Combined study of 13-week toxicity and effects on fertility and early embryonic development to implantation in rats by subcutaneous administration

Key study findings:

- Slight decreases in thyroid absolute weights (-6 to -18%) and weights relative to BWs (-3 to -16%) were observed in males but this was not associated with macroscopic or microscopic findings.
- There were no microscopic findings in this study with the exception of injection site irritation.
- The NOAEL for systemic toxicity was 10 mg/kg/day; this dose is associated with an AUC of 16.55 µg*h/mL. However, no NOAEL for local effects was identified due to injection site irritation and necrosis at all doses; these findings are considered clinically monitorable.

Study no.: JE049-0108
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)

Date of study initiation: February 17, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batches 0565984 (98.1% pure) and 0565985 (98.1%)

Methods

Doses: 0 (vehicle), 1, 3 and 10 mg/kg/day
Species/strain: Sprague Dawley rats
Number/sex/group or time point (main study): 10/sex/group
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in isotonic saline solution (0.9% NaCl), administered in 1.0 mL/kg and infusion rate was not applicable.
Satellite groups used for toxicokinetics or recovery: 6 rats/sex/group for TK analyses
Age: 5-6 weeks old
Weight: males: 470 g and females: 260 g
Sampling times: See below
Unique study design or methodology (if any): The sponsor combined the 13-week repeat dose toxicity study and the effects on fertility and early embryonic development to implantation study in rats. Under this section, the 13-week repeat dose section of the study report is reviewed. See the reproductive toxicology section for the review of the effects on fertility and early embryonic development to implantation study in rats. Male and female rats were administered 0 (VC), 1, 3 or 10 mg/kg/day of icatibant for 13 weeks and a standard toxicology assessment was conducted as described below.

Observations, Times and Results

Mortality: All animals were checked for morbidity or mortality twice a day.

One LD male (no.H20778) was found dead Week 2 after anesthesia administration on Day 14. No other animals died prior to scheduled sacrifice. This death was deemed accidental due to anesthesia.

Clinical signs: All animals were observed at least once a day for clinical signs of toxicity.

There were no drug-related clinical signs of toxicity observed throughout the study period.

Body weights: Body weights were recorded for each animal at least once during the pre-treatment period, on the first day of treatment and then twice a week until the end of the study.

There were no statistically significant differences in group mean body weights or group mean body weight gains in males or females treated with icatibant compared to vehicle treated control animals.

Food consumption: Food consumption was recorded for each animal once a week through sacrifice.

There were no treatment related affects on food consumption in the icatibant treated groups compared to the vehicle treated group.

Ophthalmoscopy: Ophthalmoscopy was not conducted in this study.

EKG: EKG was not measured in this study.

Hematology: Blood samples were taken from the orbital sinus from all animals. Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period and at the end of the 13-week treatment period. A complete hematological battery was assessed with the exception of measurement of hematocrit.

There were no statistically significant differences in the hematological parameters assessed in the icatibant treated animals compared to the vehicle treated animals.

Clinical chemistry: Blood samples were taken from the orbital sinus from all animals. Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period and at the end of the 13-week treatment period. A complete clinical chemistry battery was assessed.

There were no statistically significant differences in the hematological parameters assessed in the icatibant treated animals compared to the vehicle treated animals.

Urinalysis: Urine was collected from all animals individually placed in metabolism cages for an overnight period of 14 hours once before the treatment period and at the end of the 13-week treatment period. A complete urinalysis was conducted.

There were no statistically significant changes in urine parameters in the icatibant treated animals compared to the vehicle treated animals.

Gross pathology: A complete necropsy was conducted on all study animals found dead or that were sacrificed at the end of the 13-week study period.

There were no drug-related macroscopic changes in the icatibant treated animals compared to the VC animals.

Organ weights: The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus and thyroids with parathyroids were weighed from all animals. Absolute organ weights and organ weights relative to body weights were determined. No organ weights relative to brain weights were provided.

Slight decreases in male thyroid weights (absolute and relative to body weight) were observed. Female thyroid weights did not change compared to the vehicle control females. No other changes in organ weights were observed.

Organ weight change relative to controls

Organ	Sex	Icatibant dose (mg/kg/day)		
		1	3	10
Thyroid wt	M	-6.3%	-12.2%	-18%
	F	-	-	-
Thyroid rel BW	M	-2.6%	-12.2%	-16%

Organ	Sex	Icatibant dose (mg/kg/day)		
		1	3	10
	F	-	-	-

- No statistically significant difference compared to controls

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

Histopathological evaluation was conducted on control and HD animals sacrificed at the end of the treatment period and on any animals that died prior to the scheduled sacrifice. All macroscopic lesions of all animals in the LD and MD were also examined microscopically. The injection sites, prostate, seminal vesicles, right epididymis, right testes, ovaries (with oviducts), mammary gland, vagina and uterus were examined in the LD and MD groups. At least 10% of the histological slides from control (group 1) and HD (Group 3) in each sex group and all slides from identified target organs were peer reviewed. A complete histological battery was microscopically examined with the exception of the lachrymal gland, pharynx and cervix (however, the uterus, ovaries and fallopian tubes were examined). All other tissues were examined. The following table summarizes the icatibant induced toxicities observed after repeated SC administration for 13 weeks.

Observation	Icatibant Dose (mg/kg/day)							
	0		1		3		10	
	M	F	M	F	M	F	M	F
Injection sites	N=10	N=10	N=10	N=10	N=10	N=10	N=10	N=10
Acute inflam, level 2	-	-	-	-	-	-	1 (2)	-
Area necros, level 1-last site; muscle; multifl	-	-	-	-	-	-	2 (2)	-
Area necros; level 1-next to last site; muscle; multifocal	-	-	1 (1)	-	3 (2)	-	1 (3)	-
Area necros; level 2; muscle; focal	-	-	-	-	1 (1)	-	1 (3)	-
Area necros; level 2; muscle; multifocl	-	-	-	-	1 (1)	-	1 (3)	1 (2)
Ulceration; level 2	-	-	-	-	-	-	1 (3)	1 (1)
Inflam; level 1; subcutaneous; diffuse	2 (1)	1 (1)	1 (1)	(2)	3 (1)	3 (1.2)	4 (2)	6 (1.5)
Inflam; level 2; subcutaneous; diffuse	1 (1)	1 (1)	3 (1.5)	1 (1)	5 (2)	1 (1)	6 (2.0)	2 (1)
Inflam; level 2-next to last site, subcut diff	1 (1)	-	3 (1.3)	-	5 (1.8)	-	6 (1.8)	-

- No macroscopic finding observed
(#)- mean severity: grade 1= minimal; grade 2= slight; grade 3= moderate; grade 4= marked; grade 5= massive

After 13-weeks of daily SC administration of icatibant to male and female rats, the target organ of toxicity was local injection site. Injection site irritation was observed in both males and females with increased incidence and severity with increased icatibant doses. Some inflammation was also noted in the vehicle control group. Based on the absence of any systemic toxicity at all doses the systemic NOAEL was considered 10 mg/kg/day.

Toxicokinetics: Satellite males and females had blood samples collected on Day 1 and during Week 13 at 0.5, 1, 2 and 4 h post-dosing. Plasma was prepared from samples and assessed for icatibant and the metabolite M_2. TK data are summarized in the following table.

	Parameter	Icatibant Dose (mg/kg/day)											
		TK of Icatibant						TK of M_2 Metabolite					
		1		3		10		1		3		10	
		M	F	M	F	M	F	M	F	M	F	M	F
Day 1	C_{max} (mcg/mL)	0.96	0.81	2.1	2.4	5.4	6.1	0.14	0.06	0.20	0.17	0.47	0.25
	T_{max} (h)	0.5	0.5	0.5	1	1	1	1	1	1	1	2	2
	$AUC_{0\ 5\text{-}4\ h}$ (mcg*h/mL)	0.82	0.66	2.5	3.7	12.4	12.6	0.13	0.07	0.42	0.29	1.12	0.64
Wk 13	C_{max} (mcg/mL)	1.0	1.1	2.3	2.7	6.1	6.8	0.09	0.06	0.18	0.13	0.39	0.18
	T_{max} (h)	0.5	0.5	0.5	1	2	1	1	1	2	1	2	2
	$AUC_{0\ 5\text{-}4\ h}$ (mcg*h/mL)	1.7	1.2	5.6	5.0	15.9	17.2	0.15	0.08	0.48	0.31	0.88	0.59

NC- not calculated because individual values were invalid (QC rejected).

On Day 1 and Week 13 of dosing, icatibant C_{max} and AUC levels increased in an approximate dose proportional manner in both males and females. There were no gender differences and icatibant did not accumulate after 13 weeks of daily SC dosing. The M2 metabolite C_{max} increased with increased icatibant dose on Day 1 and Week 13 in a sub-proportional at all doses. AUC levels were proportional from LD to MD and sub-proportional from MD to HD. The metabolite formed between 1 and 2 hours post-icatibant dose.

Other: Epididymal sperm was sampled prior to sacrifice and assessed for motility, morphology and sperm count. The left testis of each male was weighed and then sperm heads were counted.

There were no icatibant related effects on epididymal sperm count, motility or morphology or on testicular sperm head count or daily sperm production compared to the vehicle treated males.

Study title: Two-phase dose range-finding study by subcutaneous administration of icatibant in Beagle dogs

Key study findings:

- No animals died in either the Phase 1 or the Phase 2 study.
- The maximum non-lethal dose was 150 mg/kg (highest dose tested) in males and females, at which females showed a decrease in body weight and food consumption.
- Injection site irritation (scabbing, hematomas and thickening at the injection site) was observed in males and females dosed with ≥75 mg/kg/day.
- The sponsor determined that 100 mg/kg/day was the maximum dose to be used for the 13-week repeat dose toxicology study.

Study no.: JE049-0115
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: December 15, 2004 (Phase 1) and January 11, 2005 (Phase 2)

GLP compliance: No
QA report: yes () no (X)
Drug, lot #, and % purity: Icatibant, batch # E084, and 95.9% pure

Methods

Doses: Dogs received icatibant subcutaneous injections as follows:
Phase 1: Days 1 to 3: 10 mg/kg/day
Days 4 to 6: 25 mg/kg/day
Days 7 to 9: 75 mg/kg/day
Days 10 to 12: 100 mg/kg/day
Days 13 to 15: 150 mg/kg/day
Phase 2: 100 mg/kg/day for 10 days
Species/strain: Beagle dogs
Number/sex/group or time point (main study): 1 dog/sex was administered doses of icatibant following the previously described schedule as Phase 1 of the study. Phase 2 of the study used a different male and female dog (1/sex) to receive the test item by daily subcutaneous injection for 10 days at 100 mg/kg/day.
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in isotonic saline solution (0.9% NaCl), administered in 0.4 mL/kg and infusion rate was not applicable.
Satellite groups used for toxicokinetics or recovery: None.
Age: 7 and 9 months old
Weight: males: 7.4 and 6.6 kg and females: 7.3 and 6.8 kg
Sampling times: See below
Unique study design or methodology (if any): This study escalates doses of icatibant in Beagle dogs to evaluate the potential systemic toxicity and local tolerance to icatibant after 3 days dosing (Phase 1) and 10 days dosing (Phase 2). The aim is to establish a maximum tolerated dose (MTD) in dogs and to use these data to select doses for the 13-week toxicity study. There were no treatment free days between successive dose-levels.

Observations, Times and Results

Mortality: Each animal was checked for morbidity or mortality twice a day.
There were no premature deaths in either Phase 1 or Phase 2 of this study.

Clinical signs: Each animal was observed at least twice a day for clinical signs of toxicity. The primary clinical sign observed in either Phase 1 or Phase 2 of this study was injection site irritation with thickening at the injection site and erythema. In the Phase 1 study, injection site irritation was observed to increase with increased icatibant dose.

Phase 1: Injection site reactions were observed in the male and female as described in the table.

Injection Site	Male G50501	Female G50551
Thickening		
Site no. 6	-	Days 8-12, slight (75-100 mg/kg)
Site no. 7	Days 8 and 9, moderate (75	From day 8, moderate (75 mg/kg)

	mg/kg)	
Site no. 8	Day 9, moderate (75 mg/kg) Days 10 and 12, moderate (100 mg/kg)	Day 9, slight (75 mg/kg) From Day 10, moderate (100 mg/kg)
Site no. 9	Days 10 to 12, moderate (100 mg/kg)	Days 10 to 13, moderate (100 mg/kg)
Site no. 10	Days 10 to 11, moderate (100 mg/kg)	Days 10 to 13, moderate (100 mg/kg)
Site no. 13	From day 14, moderate (150 mg/kg)	Day 14, moderate From Day 15, marked (150 mg/kg)
Site no. 14	From Day 15, moderate (150 mg/kg)	From day 15, moderate (150 mg/kg)
Erythema		
Site no. 13	-	From Day 14, moderate to severe, before dosing (150 mg/kg)

Phase 2: Injection site reactions were observed in the male and female as described in the table. Each animal was dosed with 100 mg/kg/day

Injection Site	Male G50502	Female G50552
Thickening		
Site no. 1	-	Day 1, marked From day 3, moderate
Site no. 2	From Day 3, moderate	From Day 3, slight
Site no. 6	-	From Day 10, moderate
Site no. 7	-	From Day 10, moderate
Erythema		
Site no. 2	Day 3, very slight before dosing	-

Body weights: For Phase 1, body weight was recorded for each animal once before dosing, on the first day of treatment, on the day of each change of dose-levels, on Day 12 and prior to necropsy. For Phase 2, body weight was recorded for each animals twice before the beginning of treatment and then on Days 1, 4, 7 and 10.

Phase 1: The mean body weight in the male was unaffected by icatibant treatment. The females in Phase 1 lost 0.3 kg between Days 12 and 16 of dosing at 150 mg/kg/day.

Phase 2 (100 mg/kg/day SC for 10 days: The mean body weight in the male was not affected by icatibant treated over the 10 days of daily dosing. The female lost 0.4 kg between Days 1 and Day 4 of dosing.

Food consumption: Food consumption was recorded for each animal by noting the difference between the quantity given and the remaining food left the next morning. Food intake per animal and per day was calculated for 12 days (Phase 1) or 14 days (Phase 2) before the beginning of the treatment period and then throughout the study. Food consumption was expressed as a percentage of the quantity given.

Phase 1: Food consumption in the male was not affected by icatibant treatment but the female treated with 150 mg/kg/day reduced intake by 50%.

Phase 2 (100 mg/kg/day SC for 10 days): Food consumption in the male was not affected by icatibant treatment. The females reduced food intake by ~50% on days 1, 8 and 10 of dosing.

Ophthalmoscopy: Ophthalmic examination was not conducted in this study.

EKG: EKG examinations were not performed in this study.

Hematology: Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period, and on Day 11 (Phase 2 animals). A complete hematological battery was assessed for Phase 2 animals.

There were no changes in the hematological parameters in the icatibant treated animals when comparing the pretreatment period to the Day 11. Due to the low number of animals used in this study (1/sex at a single dose) and the absence of a control group, no conclusions can be drawn about the affects of icatibant on hematology.

Clinical chemistry: Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period, and on Day 11 (Phase 2 animals). A complete clinical chemistry battery was assessed for Phase 2 animals.

There were no changes in clinical chemistry parameters in the icatibant treated animals when comparing the pretreatment period to the Day 11. Due to the low number of animals used in this study (1/sex at a single dose) and the absence of a control group, no conclusions can be drawn about the affects of icatibant on clinical chemistry.

Urinalysis: Urine was collected once before the treatment period, and on Day 11 (Phase 2 animals). A complete urinalysis was conducted for Phase 2 animals.

There were no changes in urine parameters in the icatibant treated animals when comparing the pretreatment period to the Day 11 urine. Due to the low number of animals used in this study (1/sex at a single dose) and the absence of a control group, no conclusions can be drawn about the affects of icatibant on urinalysis.

Gross pathology: Phase 1 animals were sacrificed on Day 16 of study and Phase 2 animals were sacrificed on Day 11 of their study period. Each dog was anesthetized by intravenous injection of thiopental sodium and sacrificed by exsanguination. A complete necropsy was conducted on all study animals from Phase 1 and Phase 2.

Macroscopic findings were observed at injection sites (hematomas and thickening at injection sites) in both the Phase 1 and Phase 2 studies. In the Phase 2 study, 1 male showed adhesions in the lung. No controls were used in this study.

Phase 1 animals

Finding	Male N=1	Female N=1
Injection site 10 Hematoma subcutaneous tissue	1	-
Injection site 11 Hematoma subcutaneous tissue	1	1
Injection site 12 Hematoma subcutaneous tissue	1	1
Injection site 13 Thickened subcutaneous tissue	1	1
Injection site 14 Thickened subcutaneous tissue	1	1

Phase 2 animals

Finding	Male N=1	Female N=1
Injection site 1 Hematoma subcutaneous tissue	1	-
Injection site 1 Thickened subcutaneous tissue	-	1
Injection site 2 Thickened subcutaneous tissue	1	1
Injection site 4 Hematoma subcutaneous tissue	-	1
Injection site 5 Thickened subcutaneous tissue	1	-
Injection site 6 Thickened subcutaneous tissue	-	1
Injection site 7 Hematoma subcutaneous tissue	1	-
Injection site 7 Thickened subcutaneous tissue	-	1
Injection site 8 Hematoma subcutaneous tissue	1	-
Injection site 9 Thickened subcutaneous tissue	-	1
Injection site 10 Hematoma subcutaneous tissue	1	-
Lung, adhesions	1	-

Organ weights: No organs were weighed from Phase 1 animals. The adrenals, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thymus and thyroids with parathyroids were weighed. The brain and the prostate were no weighed in this study. Due to the low number of study animals (1/sex) at the single dose (100 mg/kg/day) and the absence of a control group, no conclusions can be made about organ weight in this study.

Histopathology: Adequate Battery: yes (), no (X)—explain
Peer review: yes (), no (X)

No animals from the Phase 1 study were evaluated microscopically. Only limited tissues were evaluated in this study from Phase 2 animals. The kidneys, injection sites showing macroscopic abnormalities and injection sites No. 8, 9 and 10 were microscopically examined. In the absence of any control animals in this study, the findings can not be determined to be icatibant related or considered background. Injection site irritation was observed in the male (injection sites 1, 2, 5, 7, 8, 9 and 10) and tubular epithelial degeneration/necrosis and proteinaceous casts were observed in the kidneys in males. Additionally, monocyte cellular aggregation in the lungs was observed in the male. Injection site irritation was observed in the icatibant treated females (injection sites 1, 2, 4, 6, 7, 8, 9 and 10) and interstitial fibrosis, mineralization, tubular basophilia, tubular epithelial degeneration/necrosis, proteinaceous cast and interstitial monocyte cellular aggregation was observed in the kidney.

Toxicokinetics: Blood samples were taken on Day 1 and Day 10 from each animal from Phase 2 to determine icatibant levels and the metabolite M_2 (b) (4) levels. TK data are summarized in the following table.

Day	Parameter	Icatibant 100 mg/kg/day			
		TK for Icatibant		TK for M2 metabolite	
		M	F	M	F
Day 1	C_{max} (mcg/mL)	52.2	54.2	2.01	0.73
	T_{max} (h)	1	2	24	24
	AUC_{0-24}(mcg*h/mL)	458	507	19	10
Day 10	C_{max} (mcg/mL)	77.4	57.8	3.38	1.13
	T_{max} (h)	1	1	0	0
	AUC_{0-24}(mcg*h/mL)	415	522	6	13

Blood samples were taken from 1 dog/sex at the indicated time points at one dose (100 mg/kg/day). Based on the available data, icatibant C_{max} and AUC levels were similar between the male and the female at Day 1 and at day 10 of the study. There was no accumulation of icatibant over the 10 day dosing period in either the male or the female. The M2 metabolite was ~3-fold higher in males than in females for C_{max} on day 1 and day 10 of the study. The AUC of the M2 metabolite was approximately the same in males and females on Day 1 and Day 10.

Study title: 4-week toxicity study by subcutaneous route in Beagle dogs with twice weekly T.I.D. Dosing and an 8-week treatment free period.

Key study findings:

- Erythema was observed in low incidence in males and females at each dose.
- Gross and microscopic findings were limited to injection site irritation (hematomas) which did not result in a dose response and were reversible.
- Testosterone levels were decreased in males treated with icatibant at 1 or 3.3 mg/kg TID twice weekly on the days of icatibant dosing. However, testosterone levels rebound in males during dosing intervals.
- A NOAEL of 3.3 T.I.D. administered twice weekly was defined in males (corresponding AUC_{0-24} 43.4 mcg*h/mL) and females (corresponding AUC_{0-24} h 44.5 mcg*h/mL), which is in agreement with the sponsor's study conclusion.

Study no.: JE049-0116
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: November 23, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batch # E083, and 82.7% pure

Methods

Doses: 0 (vehicle), 3 and 10 mg/kg (administered as 1 and 3.3 mg/kg T.I.D. twice weekly)
Species/strain: Beagle dogs
Number/sex/group or time point (main study): 3/sex/group
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in isotonic saline solution (0.9% NaCl), administered in 0.4 mL/kg and infusion rate was not applicable.
Satellite groups used for toxicokinetics or recovery: 2/sex/group for recovery
Age: 12 months old (sexually mature)
Weight: males: 6.5-9.1 kg and females: 5.3-7.6 kg
Sampling times: See below
Unique study design or methodology (if any): Beagle dogs were dosed twice weekly with 0, 3 or 10 mg/kg/day using the SC route of administration. For each of the 2 days/week of dosing, animals were dosed T.I.D with 0, 1 (low-dose) or 3.3 mg/kg TID (high-dose) to reach the respective 0, 3 or 10 mg/kg/day icatibant dose. All dogs (n=5/sex/group) were dosed for 4 weeks and then 3/sex/group were sacrificed for analyses. Two dogs/sex/group were observed for a further 8 weeks as the recovery group animals. In this study, sexually mature dogs were used to determine the effects of icatibant treatment on the reproductive organs. Other repeat dose toxicology studies using sexually immature dogs showed delayed reproductive organ maturity in both females and males.

Observations, Times and Results

Mortality: All animals were checked for morbidity or mortality twice a day.
There were no deaths prior to scheduled sacrifice throughout this study.

Clinical signs: All animals were observed at least once a day for clinical signs of toxicity. The type of findings observed and the incidence of each finding from study Days 1 to 27 are summarized below. Erythema was observed as potential drug-related clinical sign of toxicity. In a single HD male and female generalized erythema was observed on Day 18. In the LD females, erythema of the right and left ears was observed on Day 18 and in one HD females was observed earlier on Day 15. Erythema was well-defined or moderate to severe in these animals.

Observation	**Icatibant Dose (mg/kg)**					
	0		**1 TID**		**3.3 TID**	
	M	**F**	**M**	**F**	**M**	**F**
Erythema:						
Right ear	-	-	-	1 (Day 18)	-	1 (Day 15)
Left ear	-	-	-	1 (Day 18)	-	1 (Day 15)
Generalized	-	-	-	-	1 (Day 18)	1 (Day 18)

Body weights: Body weights were recorded for each animal at least twice during the pre-treatment period, on the first day of treatment and then once a week until the end of the study.

There were no statistically significant differences in group mean body weights or group mean body weight gains in males or females treated with icatibant compared to vehicle treated control animals.

Food consumption: Food consumption was recorded for each animal by noting the difference between the quantity given and the remaining food left the next morning. This was estimated daily for at least 5 days before the beginning of treatment and throughout the study period. Food consumption was expressed as a percentage of the quantity given.

There were no treatment related effects on food consumption in the icatibant treated groups compared to the vehicle treated group.

Ophthalmoscopy: Ophthalmoscopy was not conducted in this study.

EKG: Telemetry was carried out for all animals. EKG examinations were performed on Days 1, 15 and 25. Recording began at least 45 minutes before the first (morning) administration and ended at least 20 hours after the first administration. These recordings corresponded with the T_{max} of icatibant, which was achieved at ~ 1 to 2 hours post-dose. This examination was performed using leads I, II, III and Cardiovit AT-6 Schiller. Heart rate, PQ- and QT-intervals and QRS-complex duration were determined, and QT was corrected using Bazett and Fridericia formulae. Blood pressure was also evaluated on Day 18 of dosing.

There were no statistically significant differences in EKG parameters in the icatibant treated animals compared to the vehicle treated animals. There was no statistically significant difference in BP in the icatibant treated males and females compared to the vehicle treated males and females at Day 18 of dosing.

Hematology: Blood samples were taken from a jugular vein. Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period and Day 24 at the treatment period. A complete hematological battery was assessed with the exception of measurement of hematocrit.

There were no statistically significant differences in the hematological parameters assessed in the icatibant treated animals compared to the vehicle treated animals.

Clinical chemistry: Blood samples were taken from a jugular vein. Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period and Day 24 at the treatment period. A complete clinical chemistry battery was assessed.

There were no statistically significant differences in the hematological parameters assessed in the icatibant treated animals compared to the vehicle treated animals.

Urinalysis: Urine was collected from all animals from individual diuresis cages once before the treatment period and Day 24 at the treatment period.

There were no statistically significant changes in urine parameters in the icatibant treated animals compared to the vehicle treated animals.

Gross pathology: A complete necropsy was conducted on all study animals.

Macroscopic findings observed were limited to slight injection site irritation (hematomas), which were not observed after the 8-week recovery period.

Observation	Icatibant (mg/kg)					
	0		1 TID		3.3 TID	
	M	F	M	F	M	F
Injection site 23, N=3 Hematoma subcutaneous tissue, diffuse	-	1	-	-	1	-
Rec Injection site 23, N=2 Hematoma subcutaneous tissue, diffuse	-	-	-	-	-	-
Injection site 24, N=3 Hematoma subcutaneous tissue, diffuse	-	-	1	1	-	1
Rec Injection site 24, N=2 Hematoma subcutaneous tissue, diffuse	-	-	-	-	-	-

- No macroscopic finding observed

Organ weights: The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus and thyroids with parathyroids were weighed from all animals. Absolute organ weights and organ weights relative to body weights were determined. No organ weights relative to brain weights were provided.

Decreases in prostate weights relative to body weights (-32.6%) were observed in HD males treated with icatibant compared to the vehicle treated males. However, this decrease was not statistically significant due to high variability between the control group (0.0685 ± 0.045) and the HD group (0.04647 ± 0.012). HD males showed an increase in group mean thymic weights relative to body weights (+79.5%) compared to the vehicle control group. This was not observed in females treated with icatibant. During the treatment period, there were no other statistically significant organ weight changes compared to vehicle controls. However, after the 8-week recovery period, slight decreases in absolute thymic weights in MD (-58%) and HD (-64%) females and a decrease in thymic weights relative to body weights in MD (-53%) and HD (-61%) females were observed in the previously icatibant exposed animals compared to the vehicle control treated animals. There were no microscopic correlates to these findings and they are of unclear toxicological significance.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

Histopathological evaluation was conducted on all study animals in both the treatment group and the recovery group. At least 20% of the histological slides from control (group 1) and HD (Group 3) in each sex group and all slides from identified target organs were peer reviewed. A complete histological battery was microscopically examined with the exception of the lachrymal gland, pharynx and seminal vesicles. All other tissues were examined. The following table summarizes the potential icatibant related histopathology findings after 4 weeks treatment compared to the VC group.

Observation	Icatibant (mg/kg)					
	0		1 TID		3.3 TID	
	M	F	M	F	M	F
Injection site 21	N=3	N=3	N=3	N=3	N=3	N=3

Observation	Icatibant (mg/kg)					
	0		1 TID		3.3 TID	
	M	F	M	F	M	F
Hemorrhage	-	-	-	-	-	1 (1)
Inflammation	-	-	-	-	-	1 (1)
Rec Injection site 21	N=2	N=2	N=2	N=2	N=2	N=2
Hemorrhage	-	-	-	-	-	-
Inflammation	-	-	-	-	-	-

- No macroscopic finding observed

(#)- mean severity: grade 1= minimal; grade 2= slight; grade 3= moderate; grade 4= marked; grade 5= massive

After repeated administration of icatibant and vehicle, there were sporadic incidences of injection site irritation in dogs from the VC and the icatibant treated groups. At injection site 21, the HD female group showed a low incidence and severity of local irritation. There were no additional histopathological findings in this study. Local injection sites are easily monitorable. Therefore, the reviewer determined the NOAEL to be the HD (3.3 mg/kg TID) administered twice weekly.

Toxicokinetics: Blood samples were taken on Day 22 from all animals to determine icatibant levels and the metabolite M_2 (b) (4) levels. TK data are summarized in the following table.

	TK Parameter	Icatibant Dose (mg/kg/day)			
		1 TID		3.3 TID	
		M	F	M	F
TK for Icatibant	AUC_{Tot}(mcg*h/mL)	18.2	10.9	43.4	44.5
TK for M_2	AUC_{Tot} (mcg*h/mL)	0.575	NC	0.735	0.851

NC- not calculated because individual values were invalid (QC rejected).

On Day 22, AUC levels of icatibant increased with increased dosage in both males and females in an approximately dose proportionate manner. The M_2 metabolite also increased with increased dose but in a sub-proportionate manner for males (no data were available for females at the low dose). The t_{max} was reached between 1 and 2 hours post-dose in both males and females. No gender differences were observed in systemic exposure levels on Day 22 of the study.

Other: Hormonal assays: Blood was sampled to determine hormone levels. Using radioimmunoassay, luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone (PROG), estradiol (ESTR) and testosterone (TESTO) levels were measured at predose and then before and 24 hours after the first dose on Days 15 and 25. Vaginal smears were taken from each female to determine estrus cycle immediately before blood sampling for hormonal assays.

There were no statistically significant effects of icatibant treatment on LH, estradiol and progesterone levels in females compared to vehicle control animals. FSH levels were highly variable in females and males throughout the study. LH levels in icatibant treated males were also highly variable. Therefore, no conclusive effect of icatibant treatment could be determined. On the days of icatibant treatment significant decreases to almost ablation of testosterone levels were observed in males compared to the vehicle control levels. However, prior to dosing on Day 15, there were no significant

differences in testosterone levels compared to the VC group. On Day 25 prior to dosing, testosterone levels had rebound to levels beyond the VC levels.

Parameter	Week	Icatibant (mg/kg)	
		1 TID	3.3 TID
		Males	Males
Testo	Day 15 (before 1st injection)	-	-
	Day 15 24h post-dose	-96%	-97%
	Day 25 (before 1st injection)	+166%	+154
	Day 25 24h post-dose	-96%	-97%

- No statistically significant difference compared to vehicle controls.
NA- Not applicable.

Vaginal smears: There were not differences in vaginal smears in the females treated with icatibant and those treated with the vehicle control.

Study title: 13-week toxicity study by subcutaneous route in beagle dogs

Key study findings:

- Clinical signs of toxicity included erythema of the face, injection site irritation and several incidences of gastrointestinal disturbances (diarrhea and/or vomiting).
- ↓ leukocyte counts in HD males and ↑ platelet counts in HD females
- ↑ triglycerides and ALP in HD males and ↓ CK, creatinine, LDH and ASAT levels in HD males compared to the vehicle control animals.
- ↓ reproductive organ wts (absolute and relative to BWs) in males and females, ↑ adrenal wts relative to BWs in males and females, ↑ liver wts relative to BW in MD and HD males, ↓ thymic wts (absolute and relative to BWs) in both males and females compared to respective vehicle control treated animals.
- Macroscopic findings were observed at injection sites (hematomas and edema), the male (testes, epididymides and prostate) and female (ovaries and uterus) reproductive organs, thymus, liver and adrenal gland.
- The target organs of toxicity included: male (testes, epididymides, and prostate) and female (mammary gland, ovaries, uterus and vagina) reproductive organs, the injection site, adrenal gland, liver and thymus.
- Based on the arrest of sexual maturity in all icatibant treated males and females, lymphoid depletion in the thymus and on injection site irritation, no NOAEL can be determined for this study. This is in agreement with the sponsor's conclusions. Systemic exposures to icatibant from 35 - 230 µg*h/mL and for M2 from 0.96 - 3.28 µg*h/mL ranged from the low to high doses.

Study no.: JE049-0117
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: February 21, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batch # E083, and 82.7% pure

Methods

Doses: 0 (vehicle), 10, 30 and 100* mg/kg/day
Species/strain: Beagle dogs
Number/sex/group or time point (main study): 4/sex/group
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in isotonic saline solution (0.9% NaCl), administered in 0.4 mL/kg and infusion rate was not applicable.
Satellite groups used for toxicokinetics or recovery: None.
Age: 6.6 to 7.3 months old
Weight: males: 6.5-8.5 kg and females: 6.4-7.4 kg
Sampling times: See below
Unique study design or methodology (if any): Beagle dogs were dosed once daily with 0, 10, 30 or 100 mg/kg/day using the SC route of administration. *The sponsor observed the HD animals (100 mg/kg/day) to not tolerate the drug and decided to split the dose-level starting Day 12 to 2 SC doses of 50 mg/kg to equal 100 mg/kg/day dosing. The sponsor miscalculated the dose on Days 17 to 18 resulting in a higher daily dosing (200 mg/kg/day) and again Days 19-29 (120 mg/kg/day). On Day 30 these animals were dosed with 60 mg/kg/day and allowed 1 day as a washout period. From Day 32 to the end of the study animals were dosed with 60 mg/kg/day as two SC doses of 30 mg/kg.

Observations, Times and Results

Mortality: All animals were checked for morbidity or mortality at least twice a day. There were no deaths prior to scheduled sacrifice throughout this study.

Clinical signs: All animals were observed at least once a day for clinical signs of toxicity. The type of findings observed and the incidence of each finding from study Days 1 to 93 are summarized below. Injection site irritation and edema (increase size) of face and injection sites were observed. Injection site erythema was observed 3-5 weeks, 6-10 weeks and 10 weeks for the low-dose, mid-dose and high-dose, respectively. Edema was observed starting Day 1 of study until 2 weeks post study initiation. Vomiting was observed the first 3 days of dosing. The sponsor reported abnormal vocalization in HD animals, hypoactivity in females on days 1 and 2, loss of balance and tremors in a single female Day 1 at Day 18, resulting in a reduction of dose-levels. The sponsor was notified by the CRO of abnormal vocalization and itching in HD females post-dose. Strong reactions to icatibant administration was observed on day 11 of dosing with abnormal vocalization, 1 male and 3 females rolled on the ground and 3 females licked themselves immediately after injection at the injection site. The doses were subsequently reduced. It was concluded by the CRO that SC injection of icatibant at 250 mg/mL and at 140 mg/mL was not tolerated in males or females.

Observation	**Icatibant Dose (mg/kg/day)**							
	0		**10**		**30**		**100/2x30**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Thickening at injection site: Site 1	0	0	0	0	0	0	1	0

Observation	Icatibant Dose (mg/kg/day)							
	0		10		30		100/2x30	
	M	F	M	F	M	F	M	F
Site 2	0	0	0	0	0	1	2	2
Site 12	0	0	0	0	0	0	1	3
Site 7	0	0	0	0	0	0	0	1
Site 17	0	0	0	0	0	1	0	1
Site 18	0	0	0	0	0	0	0	3
Site 21	0	0	0	0	0	0	0	1
Erythema at injection site:								
Site 2	0	0	0	0	0	0	1	0
Site 13	0	0	0	0	0	0	1	0
Site 18	0	0	0	0	0	0	1	1
Site 21	0	0	0	0	0	0	1	0
Erythema:								
Tongue	0	0	0	0	0	0	1	0
Right ear	1	2	3	3	3	0	0	0
Left ear	1	2	3	3	3	0	0	0
Neck	0	0	1	0	0	0	0	0
Generalized	0	0	4	4	4	4	4	4
Head	0	0	0	0	0	1	0	0
Abnormal vocalization	0	1	0	1	0	0	3	3
Feces								
Soft	0	1	0	1	0	3	2	3
liquid	0	0	0	1	0	0	3	3
Vomit	0	0	1	0	4	0	1	0
Vomit/whitish	0	0	0	1	1	1	4	4
Increase in size:								
Head	0	0	2	2	3	2	0	2
Muzzle	0	0	1	0	2	0	0	0
Right ear	0	0	3	4	4	4	4	4
Left ear	0	0	3	4	4	4	4	4
Right lower eyelid	0	0	1	0	3	0	0	0
Left lower eyelid	0	0	1	0	3	0	0	0
Right upper eyelid	0	0	1	0	3	0	0	0
Left upper eyelid	0	0	1	0	3	1	0	0
Around the right eye	0	0	2	4	4	4	4	4
Around the left eye	0	0	2	4	4	4	4	4
Injection site 1	0	0	0	0	0	1	3	4
Injection site 2	0	0	0	2	0	1	4	4
Injection site 7	0	0	0	2	0	3	4	4
Injection site 11	0	0	0	0	0	0	4	3
Injection site 12	0	0	0	0	3	3	3	4
Injection site 13	0	0	0	0	1	0	1	4
Injection site 14	0	0	0	0	0	0	1	1
Injection site 17	0	0	0	0	1	2	2	4
Injection site 18	0	0	0	0	0	0	1	3
Injection site 3	0	0	0	0	0	0	0	2
Injection site 4	0	0	0	0	0	0	0	1
Injection site 6	0	0	0	0	0	0	0	1
Injection site 10	0	0	0	0	0	0	0	2
Injection site 16	0	0	0	0	0	0	0	1
Injection site 20	0	0	0	0	0	0	0	1
Injection site 21	0	0	0	0	0	0	0	2

Body weights: Body weights were recorded for each animal at least twice during the pre-treatment period, on the first day of treatment and then once a week until the end of the study.

There were no statistically significant differences in group mean body weights or group mean body weight gains in males or females treated with icatibant compared to vehicle treated control animals.

Food consumption: Food consumption was recorded for each animal by noting the difference between the quantity given and the remaining food left the next morning. This was estimated daily for at least 7 days before the beginning of treatment and throughout the study period. Food consumption was expressed as a percentage of the quantity given.

There were no treatment related effects on food consumption in the icatibant treated groups compared to the vehicle treated group.

Ophthalmoscopy: All animals were examined prior to treatment and on 1 occasion at the end of the treatment period. Pupillary light and blink reflexes were recorded. Pupils were dilated with tropicamide and appendages, optic media and fundus were examined by indirect ophthalmoscopy. Slit-lamp was used to evaluate any abnormalities.

There were no treatment related observations in icatibant treated animals compared to the vehicle treated animals.

EKG: EKG examinations were performed on all animals before the treatment period, at the end of Week 1 at predose, 1 and 4 hours post-dose, and during Weeks 12 or 13 at predose and 1 and 4 hours post-dose. This examination was performed using leads I, II, III and Cardiovit AT-6 Schiller. Heart rate, PQ- and QT-intervals and QRS-complex duration were determined, and QT was corrected using Bazett and Fridericia formulae. Blood pressure was also evaluated.

There were no statistically significant differences in heart rate, PQ- QRS- or QT-intervals in males or females treated with icatibant compared to the vehicle control treated animals at Week 1 of dosing prior to dosing and 1 hours after dosing. However, a slight but statistically significant decrease in heart rate was observed in HD males (-29%) and HD females (-21%) 6 hours post dose during Week 1 of dosing compared to the vehicle control groups. A decrease in diastolic BP was noted in HD males (-39%) 6 h post-dose on Week 1 of dosing compared to the vehicle control group.

At Week 13 of dosing, there were no statistically significant differences in heart rate, PQ- QRS- or QT-intervals evaluated in the icatibant treated males and females compared to the vehicle control animals. There were no statistically significant changes in QT corrected for heart rate using either Bazett or Fridericia formulae in the icatibant treated animals compared to the vehicle treated animals. However, a statistically significant decrease in systolic BP (-19%) was observed in HD males 1 h post-dose in Week 12 and (-22%) was observed in HD females before dosing in Week 13, respectively.

Hematology: Blood samples were taken from a jugular vein. Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before

the treatment period, during Week 5 and then at Week 13 of the treatment period. A complete hematological battery was assessed.

A slight decrease in leukocyte number was observed in HD males at Weeks 5 and 13 compared to vehicle control animals. There was no exacerbation of this finding from Week 5 to 13 and this was not observed in females. HD females had a slight increase in platelet counts Weeks 5 and 13 of dosing compared to the vehicle control animals. There was no exacerbation of this finding in females and this finding was not observed in males.

Parameter	**Week**	**Sex**	**Icatibant Dose (mg/kg/day)**		
			10	**30**	**100/2x30**
Leukocytes (g/L)	5	M	-	-	- 41%
	13	M	-	-	-42%
	5	F	-	-	-
	13	F	-	-	-
Plat (g/L)	5	M	-	-	-
	13	M	-	-	-
	5	F	-	-	+40%
	13	F	-	-	+31%

Clinical chemistry: Blood samples were taken from a jugular vein. Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period, during Week 5 and then at Week 13 of the treatment period. A complete clinical chemistry battery was assessed.

Changes in clinical chemistry parameters in the icatibant treated animals compared to the vehicle control treated animals were limited to males. Most changes in clinical chemistry parameters were observed in HD males with occasional changes in the LD or MD male groups.

Parameter	**Week**	**Sex**	**Icatibant Dose (mg/kg/day)**		
			10	**30**	**100/2x30**
Triglycerides (mmol/L)	5	M	-	-	+111%
	13	M	+67%	+97%	+161%
	5	F	-	-	-
	13	F			
ALP (IU/L)	5	M	-	-	+190%
	13	M	-	-	+143%
	5	F	-	-	-
	13	F			
ASAT (IU/L)	5	M	-	-29%	-32%
	13	M	-	-	-68%
	5	F	-	-	-
	13	F	-	-	-
LDH (IU/L)	5	M	-	-	- 40%
	13	M	-	-	-39%
	5	F	-	-	-
	13	F	-	-	-
Creatinine (umol/L)	5	M	-	-	-
	13	M	-	-	-25%
	5	F	-	-	-

Parameter	Week	Sex	Icatibant Dose (mg/kg/day)		
			10	30	100/2x30
	13	F	-	-	-
CK (IU/L)	5	M	-	-	-
	13	M	-36%	-26%	-48%
	5	F	-	-	-
	13	F	-	-	-

Urinalysis: Urine was collected from all animals once before the treatment period, during Week 5 and then at Week 13 of the treatment period. A complete urinalysis was conducted.

There were no statistically significant changes in urine parameters in the icatibant treated animals compared to the vehicle treated animals.

Gross pathology: A complete necropsy was conducted on all study animals.

Macroscopic findings were observed at injection sites (hematomas and edema), the male (testes, epididymides and prostate) and female (ovaries and uterus) reproductive organs (reduced size), thymus (reduced size), liver (enlarged) and adrenal gland (enlarged).

Observation	Icatibant Dose (mg/kg/day)							
	0		10		30		100/2x30	
	M	F	M	F	M	F	M	F
Adrenal Glands enlarged	-	-	-	-	-	-	1	-
Epididymides reduced in size	-	NA	4	NA	4	NA	4	NA
Injection site 1								
Hematoma subcutaneous tissue	-	-	-	-	-	-	1	-
Hematoma subcutaneous tissue, diffuse	-	-	-	-	-	-	2	1
Injection site 10								
Hematoma subcutaneous tissue	-	-	-	-	-	-	1	-
Hematoma subcutaneous tissue, diffuse	-	-	-	-	-	-	1	2
Injection site 13								
Hematoma subcutaneous tissue	-	-	-	-	-	-	1	-
Hematoma subcutaneous tissue, diffuse	1	1	-	-	2	1	2	4
Injection site 15								
Hematoma subcutaneous tissue, diffuse	-	-	-	-	-	-	1	-
Injection site 16								
Hematoma, diffuse	2	-	-	2	3	-	3	2
Injection site 18								
Hematoma subcutaneous tissue, diffuse	-	-	-	-	-	1	1	1
Injection site 19								
Diffuse	-	-	-	-	-	-	1	-
Edema	-	-	-	-	2	-	1	1
Hematoma diffuse	2	1	1	1	2	2	2	2
Injection site 21								
Hematoma	-	-	-	-	-	-	1	-
Thickened	-	-	-	-	-	-	1	-
Injection site 2								
Edema	-	-	-	-	-	2	-	2
Hematoma, diffuse	-	1	-	-	-	2	-	3
Injection site 3								
Hematoma, diffuse	-	-	-	-	-	-	1	-

Observation	Icatibant Dose (mg/kg/day)							
	0		10		30		100/2x30	
	M	F	M	F	M	F	M	F
Injection site 4								
Hematoma, diffuse	-	-	-	-	-	-	2	-
Injection site 5								
Hematoma, diffuse	-	-	-	-	-	1	1	-
Injection site 7								
Thickened tissue	-	-	-	-	-	-	1	-
Edema	-	-	-	-	-	-	-	1
Hematoma	-	-	-	-	-	-	-	1
Hematoma diffuse	-	-	-	-	-	1	-	2
Injection site 12								
Hematoma, diffuse	-	-	-	-	-	-	-	1
Liver								
Enlarged	-	-	-	-	-	-	1	-
Prostate reduced in size	-	NA	4	NA	4	NA	4	NA
Testes reduced in size	-	NA	4	NA	4	NA	4	NA
Thymus reduced in size	-	-	1	1	2	2	2	2
Ovaries, reduced in size	NA	-	NA	3	NA	2	NA	2
Uterus, reduced in size	NA	-	NA	4	NA	4	NA	4

- No macroscopic finding observed
NA- Not applicable

Organ weights: The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus and thyroids with parathyroids were weighed. Absolute organ weights and organ weights relative to body weights were determined. No organ weights relative to brain weights were provided.

Decreases in reproductive organ weights (absolute and relative to body weights) were observed in males and females compared to the vehicle control treated animals. Adrenal weights relative to body weights were increased in males and females compared to the vehicle control treated animals. Liver weights relative to body weights were increased in MD and HD males compared to the vehicle control treated animals. This was not observed in females. Thymic weights (absolute and relative to body weights) were decreased in both males and females compared to respective vehicle control treated animals. No organ weights relative to brain weights were provided.

Organ weight change relative to controls

Organ	Sex	Icatibant dose (mg/kg/day)		
		10	30	100/2x 30
Adrenals wt	M	-	-	-
	F	-	-	+35%
Adrenals rel to BW	M	-	-	+46%
	F	+14%	+24%	+36%
epididymides wt	M	-59%	-65%	-60.5%
	F	NA	NA	NA
epididymides rel BW	M	-57%	-64%	-58%
	F	NA	NA	NA
Liver wt	M	-	-	+46.5%
	F	-	-	-
Liver rel BW	M	-	+34%	+60%

Organ	Sex	Icatibant dose (mg/kg/day)		
		10	30	100/2x 30
	F	-	-	-
Prostate wt	M	-75%	-78%	-73%
	F	NA	NA	NA
Prostate rel BW	M	-73%	-76%	-70%
	F	NA	NA	NA
Testes wt	M	-81%	-79%	-80%
	F	NA	NA	NA
Testes rel BW	M	-81%	-79%	-78%
	F	NA	NA	NA
Thymus wt	M	-25%	-42%	-43%
	F	-	-	-
Thymus rel BW	M	-22%	-41%	-39%
	F	-9.0%	-34%	-22%
Ovary wt	M	NA	NA	NA
	F	-76%	-77%	-72%
Ovary rel BW	M	NA	NA	NA
	F	-74%	-77%	-74%

- No statistically significant difference compared to controls
NA- Not applicable

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

All study animals had tissues prepared for histopathological examination. All macroscopic lesions from all animals were microscopically examined, All tissues from Groups 1 to 4 were microscopically evaluated and the least recent and most recent injection sites from all animals were examined from Groups 1 to 4. At least 10% of the histological slides from control (group 1) and HD (Group 4) in each sex group and all slides from identified target organs were peer reviewed by Dr. James Armstrong. A complete histological battery was microscopically examined with the exception of the lachrymal gland, pharynx and seminal vesicles. All other tissues were examined.

Observation	Icatibant Dose (mg/kg/day)							
	0		10		30		100/2x30	
	M	F	M	F	M	F	M	F
Injection site 1 n=4								
Subcut hemorrhage	-	-	-	-	-	-	2 (3.5)	1 (2.0)
Subcut inflam cells	-	-	-	-	-	-	3 (1.7)	-
Subcut collagen degener	-	-	-	-	-	-	1 (3.0)	-
Subcut hemosider	-	-	-	-	-	-	1 (2.0)	-
Subcut fibrosis	-	-	-	-	-	-	3 (2.0)	1 (2.0)
Injection site 10 n=4								
Subcut hemorrhage	-	-	-	-	-	-	2 (3.5)	2 (3.0)
Subcut collagen degener	-	-	-	-	-	-	2 (3.0)	2 (3.0)
Subcut inflam cells	-	-	-	-	-	-	3 (3.0)	2 (2.5)
Subcut fibrosis	-	-	-	-	-	-	2 (2.0)	1 (2.0)
Subcut hemosider	-	-	-	-	-	-	2 (2.0)	-
Deg./nec. Myop. Sub. m	-	-	-	-	-	-	3 (2.0)	1 (3.0)
Injection site 11 n=4								
Subcut inflam cells	-	-	-	-	-	-	1 (3.0)	-

Observation	Icatibant Dose (mg/kg/day)							
	0		10		30		100/2x30	
	M	F	M	F	M	F	M	F
Subcut hemorrhage	-	-	-	-	-	-	1 (1.0)	-
Subcut hemosider	-	-	-	-	-	-	1 (3.0)	-
Subcut fibrosis	-	-	-	-	-	-	1 (3.0)	-
Deg./nec. Myop. Sub. m	-	-	-	-	-	-	1 (1.0)	-
Injection site 13 n=4								
Subcut hemorrhage	1(2.0)	1(3.0)	-	-	2 (1.5)	1 (3.0)	3 (3.7)	4 (2.8)
Subcut inflam cells	1(1.0)	-	-	-	2 (3.0)	1 (3.0)	3 (2.7)	4 (3.0)
Subcut collagen degener	-	-	-	-	2 (2.5)	-	3 (2.7)	3 (3.0)
Subcut edema	-	-	-	-	-	-	1 (2.0)	-
Subcut fibrosis	-	-	-	-	2 (1.5)	1 (2.0)	2 (1.5)	2 (2.0)
Deg./nec. Myop. Sub. M	-	1 (1.0)	-	-	2 (3.0)	1 (3.0)	3 (3.0)	4 (3.8)
Subcut hemosider	-	-	-	-	-	-	-	1 (1.0)
Injection site 15 n=4								
Subcut inflam cells	-	-	-	-	1 (2.0)	-	1 (2.0)	-
Subcut collagen degener	-	-	-	-	1 (2.0)	-	1 (2.0)	-
Subcut fibrosis	-	-	-	-	1 (2.0)	-	1 (2.0)	-
Deg./nec. Myop. Sub. m	-	-	-	-	1 (2.0)	-	1 (2.0)	-
Injection site 16 n=4								
Subcut hemorrhage	2(2.0)	-	-	-	1 (2.0)	-	3 (3.0)	2 (3.0)
Subcut inflam cells	-	-	-	-	3 (2.3)	-	3 (3.3)	2 (3.0)
Subcut collagen degener	-	-	-	-	2 (2.5)	-	3 (3.0)	2 (2.5)
Subcut edema	-	-	-	-	1 (3.0)	-	3 (2.7)	-
Subcut hemosider	-	-	-	-	-	-	1 (2.0)	-
Deg./nec. Myop. Sub. m	-	-	-	-	3 (3.0)	-	3 (2.7)	2 (2.5)
Injection site 17 n=4								
Subcut inflam cells	-	-	-	-	-	-	1 (3.0)	-
Subcut hemosider	-	-	-	-	-	-	1 (2.0)	1 (2.0)
Subcut fibrosis	-	-	-	-	-	-	1 (3.0)	1 (1.0)
Injection site 18 n=4								
Subcut hemorrhage	-	-	-	-	-	-	1 (4.0)	1 (3.0)
Subcut inflam cells	-	-	-	-	-	1 (2.0)	1 (2.0)	2 (2.5)
Subcut fibrosis	-	-	-	-	-	1 (2.0)	1 (2.0)	2 (2.0)
Deg./nec. Myop. Sub. M	-	-	-	-	-	1 (1.0)	2 (2.0)	2 (1.5)
Injection site 19 n=4								
Subcut collagen degener	2(1.5)	-	1 (3.0)	-	4 (3.0)	2 (3.0)	4 (3.8)	2 (3.5)
Subcut edema	-	-	-	-	2 (3.0)	2 (2.0)	4 (2.8)	2 (3.5)
Subcut hemosider	-	-	-	-	-	-	1 (1.0)	1 (2.0)
Subcut inflam cells	-	2 (1.0)	-	1 (2.0)	-	2 (3.0)	-	3 (2.7)
Subcut hemorrhage	-	-	-	1 (2.0)	-	2 (2.0)	-	2 (4.0)
Subcut fibrosis	-	-	-	1 (2.0)	-	2 (1.0)	-	4 (1.3)
Deg/nec. Myop.sub m.	-	1 (1.0)	-	1 (2.0)	-	2 (3.0)	-	3 (3.0)
Injection site 20 n=4								
Subcut hemorrhage	-	-	-	-	-	-	1 (1.0)	-
Subcut inflam cells	-	-	-	-	1 (2.0)	-	1 (3.0)	1 (2.0)
Subcut collagen degener	-	-	-	-	-	-	1 (3.0)	-
Subcut edema	-	-	-	-	-	-	1 (2.0)	-
Deg./nec. Myop. Sub. M	-	-	-	-	1 (3.0)	-	1 (2.0)	-
Subcut hemosider	-	-	-	-	-	-	-	1 (2.0)
Injection site 21 n=4								
Subcut inflam cells	-	-	-	-	-	-	4 (2.3)	1 (4.0)
Subcut collagen degener	-	-	-	-	-	-	2 (3.0)	1 (2.0)
Subcut edema	-	-	-	-	-	-	2 (3.0)	-

Observation	Icatibant Dose (mg/kg/day)							
	0		10		30		100/2x30	
	M	F	M	F	M	F	M	F
Subcut hemosider	-	-	-	-	-	-	3 (1.3)	2 (2.0)
Subcut fibrosis	-	-	-	-	-	-	3 (1.7)	2 (2.0)
Deg./nec. Myop. Sub. m	-	-	-	-	-	-	4 (2.5)	1 (3.0)
Injection site 2 n=4								
Subcut inflam cells	-	4 (1.5)	-	2 (2.0)	2 (1.0)	3 (3.3)	3 (1.7)	4 (3.3)
Subcut hemosider	-	-	-	-	-	-	2 (3.0)	1 (2.0
Subcut collagen degener	-	1 (2.0)	-	1 (2.0)	-	3 (3.0)	2 (2.0)	4 (3.3)
Subcut mineralization	-	-	-	-	-	-	2 (1.5)	-
Deg./nec. Myop. Sub. M	-	2 (1.0)	1 (1.0)	1 (1.0)	-	3 (3.3)	1 (1.0)	2 (2.5)
Subcut hemorrhage	-	2 (2.0)	-	1 (1.0)	-	3 (2.3)	-	3 (3.0)
Subcut edema	-	-	-	-	-	3 (2.7)	-	3 (3.7)
Injection site 3 n=4								
Subcut fibrosis	-	-	-	-	-	-	1 (2.0)	-
Subcut mineralization	-	-	-	-	-	-	1 (1.0)	-
Injection site 4 n=4								
Subcut hemorrhage	-	-	-	-	-	-	2 (2.5)	-
Subcut inflam cells	-	-	-	-	-	-	3 (1.7)	-
Subcut collagen degener	-	-	-	-	-	-	1 (3.0)	-
Subcut hemosider	-	-	-	-	-	-	2 (2.0)	-
Deg./nec. Myop. Sub. m	-	-	-	-	-	-	3 (2.7)	-
Injection site 5 n=4								
Subcut hemorrhage	-	-	-	-	-	-	1 (3.0)	-
Subcut inflam cells	-	-	-	-	-	-	2 (2.0)	-
Subcut collagen degener	-	-	-	-	-	-	1 (3.0)	-
Subcut edema	-	-	-	-	-	-	1 (3.0)	-
Subcut fibrosis	-	-	-	-	-	1 (1.0)	1 (2.0)	-
Subcut hemosider	-	-	-	-	-	-	1 (2.0)	-
Deg./nec. Myop. Sub. m	-	-	-	-	-	-	2 (1.5)	-
Injection site 6 n=4								
Subcut inflam cells	-	-	-	-	-	-	1 (2.0)	-
Subcut hemosider	-	-	-	-	-	-	1 (2.0)	-
Subcut fibrosis	-	-	-	-	-	-	1 (3.0)	-
Deg./nec. Myop. Sub. m	-	-	-	-	-	-	1 (1.0)	-
Injection site 7 n=4								
Subcut inflam cells	-	-	-	-	-	1 (4.0)	3 (1.3)	3 (3.7)
Subcut fibrosis	-	-	-	-	-	-	3 (2.3)	1 (2.0)
Subcut hemosider	-	-	-	-	-	-	3 (1.7)	-
Subcut mineralization	-	-	-	-	-	-	1 (2.0)	-
Deg./nec. Myop. Sub. M	-	-	-	-	-	1 (1.0)	1 (3.0)	3 (3.7)
Subcut hemorrhage	-	-	-	-	-	1 (2.0)	-	4 (2.3)
Subcut collagen degener	-	-	-	-	-	1 (3.0)	-	3 (3.3)
Subcut edema	-	-	-	-	-	-	-	2 (3.0)
Injection site 9 n=4								
Subcut inflam cells	-	-	-	-	-	-	1 (1.0)	-
Subcut hemorrhage	-	-	-	-	-	-	1 (2.0)	-
Subcut hemosider	-	-	-	-	-	-	2 (2.0)	-
Subcut fibrosis	-	-	-	-	-	-	2 (2.5)	-
Deg./nec. Myop. Sub. m	-	-	-	-	-	-	2 (2.0)	-
Injection site 12 n=4								
Subcut hemorrhage	-	-	-	-	1 (2.0)	-	-	1 (2.0)
Subcut inflam cells	-	-	-	-	1 (3.0)	-	1 (2.0)	1 (2.0)
Subcut collagen degener	-	-	-	-	1 (3.0)	-	-	-

Observation	Icatibant Dose (mg/kg/day)							
	0		10		30		100/2x30	
	M	F	M	F	M	F	M	F
Subcut edema	-	-	-	-	1 (2.0)	-	-	-
Subcut hemosider	-	-	-	-	-	-	1 (2.0)	-
Subcut fibrosis	-	-	-	-	-	-	1 (2.0)	1 (2.0)
Deg./nec. Myop. Sub. m	-	-	-	-	1 (3.0)	-	1 (1.0)	-
Liver n=4								
Vacuolated hepatocy	-	-	-	-	-	-	1 (3.0)	-
Thymus n=4								
Lymphoid depletion	-	3 (1.7)	4 (2.5)	4 (2.5)	4 (2.5)	4 (3.5)	2 (4.0)	4 (4.5)
Adrenal Glands n=4								
Cort. Cell. Hypertrophy	-	-	-	-	-	-	1 (3.0)	-
Vacuol. Cortical cell	-	-	-	-	-	-	3 (2.7)	4 (2.8)
Prostate n=4								
Immature	-	NA	4 (NA)	NA	4(NA)	NA	4(NA)	NA
Testes n=4								
Immature	-	NA	4(NA)	NA	4(NA)	NA	4(NA)	NA
Epididymides n=4								
Immature	-	NA	4(NA)	NA	4(NA)	NA	4(NA)	NA
Mammary gland area								
No mammary development	NA	-	NA	4(NA)	NA	4(NA)	NA	4(NA)
Ovaries n=4								
Immature	NA	-	NA	4(NA)	NA	4(NA)	NA	4(NA)
Uterus n=4								
Immature	NA	-	NA	4(NA)	NA	4(NA)	NA	4(NA)
Vagina n=4								
Immature	NA	-	NA	4(NA)	NA	4(NA)	NA	4(NA)

- No macroscopic finding observed

NA- Not applicable

(#)- mean severity: grade 1= minimal; grade 2= slight; grade 3= moderate; grade 4= marked; grade 5= massive

After daily SC administration of icatibant in males and female dogs, the primary target organs of toxicity were the reproductive organs (males- testes, epididymides and prostate and females- ovaries, uterus, vagina and mammary glands), adrenal glands, injection sites, thymus and liver. Male and female reproductive organs were immature in all animals at all doses of icatibant. Adrenal gland findings were observed only at the HD. Injection site effects were noted at multiple sites and were shown most frequently and with most severity at the HD. Lymphoid depletion was observed in the thymus and increased in incidence and severity with increased icatibant dose. Liver changes were low in incidence and only observed at the HD of icatibant.

Based on the immature reproductive organ findings in the male and female dogs and lymphoid depletion in the thymus at all doses of icatibant, no NOAEL could be defined for this study.

Toxicokinetics: Blood samples were taken on Day 1 and in week 13 from all animals to determine icatibant levels and the metabolite M_2 (b) (4) levels. TK data are summarized in the following table.

	Parameter	Icatibant Dose (mg/kg/day)

		TK of Icatibant						TK of M2 Metabolite					
		10		30		100		10		30		100	
		M	F	M	F	M	F	M	F	M	F	M	F
Day 1	C_{max} (mcg/mL)	10.9	11.24	47.7	41.4	NC	74.4	0.40	0.32	1.16	1.01	NC	1.38
	T_{max} (h)	1.0	1.0	1.5	1.75	NC	2.0	1.75	2.00	3.00	2.50	NC	4.0
	AUC_{0-t} (mcg*h/mL)	24.6	22.9	190	164	NC	383	1.14	0.90	6.14	5.26	NC	7.65
Wk 13	C_{max} (mcg/mL)	17.6	17.7	34.4	41.6	82.3	71.0	0.39	0.31	NC	NC	0.63	0.77
	T_{max} (h)	0.75	0.75	1.75	1.50	0.88	0.75	2.0	2.0	NC	NC	2.0	2.0
	AUC_{0-t} (mcg*h/mL)	38.2	31.6	80.9	83	246	213	1.15	0.77	NC	NC	3.10	3.46

NC- not calculated because individual values were invalid (QC rejected).

On Day 1 and Week 13 of dosing, the C_{max} and AUCs of icatibant were similar between males and females, suggesting that there were no significant gender differences. This was also observed for the M2 metabolite. On Day 1 of dosing, the C_{max} increased in an approximately dose-proportional manner from the LD to the MD. However, from the MD to the HD, the C_{max} of icatibant was sub-proportional with an increase by ~1.8-fold instead of the expected ~ 3-fold increase. The AUCs for icatibant increased in a supra-proportional manner from LD to MD (8-10 fold increase) on Day 1. At week 13, the C_{max} and AUC increased in a dose proportional manner form the LD to the MD but C_{max} was sub-proportional from the MD to the HD (~1.7-fold increase instead of ~ 3-fold increase). AUC increased approximately dose-proportionately. However, the dosing schedule was modified throughout the study period in the HD group.

The M2 metabolite formed within ~ 2-4 hours post-icatibant administration. The M2 metabolite C_{max} and AUCs increased from the LD to the MD in an approximately dose related manner. From the MD to the HD, M2 only slightly increased in C_{max} and AUC levels for those levels that were quantifiable. This increase was sub-proportionate to dose.

Study title: 13-week toxicity study by subcutaneous route in sexually immature beagle dogs with daily or twice weekly T.I.D. dosing followed by a 14-week treatment-free period

Key study findings:

- Clinical signs included ↑ erythema and edema at multiple sites with ↑ dose of icatibant, which were reversible after cessation of treatment.
- The target organs of toxicity in the dog after daily SC treatment with icatibant are thymus and the male (testes, epididymides and prostate) and female (ovaries, oviducts, uterus and vagina) reproductive tissues as reflected by reduction in these organ wts relative to body weights, macroscopic findings of reduced size and microscopic findings of reduced size. After the 13-week recovery period, 1 of 2 males showed the testes and prostate as immature where 1 male had recovered and there were no findings in female reproductive organs suggesting these adverse effects were reversible.

- When males were treated twice weekly with icatibant, no effects were observed in the male reproductive organs when icatibant was administered SC twice weekly but females treated with SC administration of icatibant twice weekly showed delayed or impaired maturation of the reproductive organs and lymphoid depletion in the thymus was observed in males and females.
- Hormonal analysis showed that icatibant induced a decrease of testosterone, LH and FSH in male dogs, which secondarily induced sexual immaturity.
- No NOAEL was determined in this study based on the adverse effects on male and female reproductive organs and thymic lymphoid depletion. However, twice weekly administration of icatibant resulted in less severe toxicity in males than with daily dosing. Systemic exposures to icatibant ranged from 13.7 to 57.2 µg*h/mL for daily administration (no AUC was determined for TID dosing) and M2 ranged from 1.45 to 2.5 µg*h/mL in this study.

Study no.: JE049-0118
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: October 27, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batch # E083, and 82.7% pure

Methods

Doses: 0 (vehicle-Group 1), 3 (Group 2), 10 (Group 3) mg/kg daily and 10 mg/kg (Group 4) administered twice weekly at 3.3 mg/kg TID
Species/strain: Beagle dogs
Number/sex/group or time point (main study): 3/sex/group
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in isotonic saline solution (0.9% NaCl), administered in 0.4 mL/kg and infusion rate was not applicable.
Satellite groups used for toxicokinetics or recovery: An additional 2 dogs/sex/group were used as the recovery group
Age: 6.5 months old
Weight: males: 7.0-9.3 kg and females: 6.4-7.9 kg
Sampling times: See below
Unique study design or methodology (if any): For the VC, 3 and 10 mg/kg/day groups (Groups 1, 2 and 3, respectively), 5 dogs/sex/group were administered either the vehicle or icatibant via the subcutaneous route once daily. Group 4 was administered 10 mg/kg of icatibant as 3 SC injections which were delivered twice weekly. At the end of the treatment period (total of 13-weeks), animals were sacrificed, except for the last 2 surviving dogs of each sex and group, which were kept for a 14-week recovery period.

Observations, Times and Results

Mortality: All animals were checked for morbidity or mortality at least twice a day.
There were no deaths prior to scheduled sacrifice throughout this study.

Clinical signs: All animals were observed at least once a day for clinical signs of toxicity. The type of findings observed and the incidence of each finding during the treatment and the recovery periods are summarized below. Erythema and edema (increased size at multiple sites) increased in incidence with increase dose of icatibant. Gastrointestinal disturbances (vomiting and soft feces) were observed in the icatibant treated dogs and were not observed in the vehicle control treated animals. After cessation of treatment, all signs were reversible.

Observation	**Icatibant Dose (mg/kg/day)**							
	0		**3**		**10**		**3.3 TID**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Erythema: N=5								
Right ear	0	1	5	3	5	5	5	5
Left ear	0	1	4	3	5	4	4	5
Generalized	0	0	2	1	2	2	2	4
Recov Erythema: N=2								
Right ear	0	0	1	1	0	0	1	0
Left ear	0	0	1	0	0	0	1	0
Generalized	0	0	0	0	1	0	0	0
Feces, Soft N=5	0	0	0	1	3	0	0	0
Recov Feces, Soft N=2	0	0	0	0	0	0	0	0
Vomit/whitish N=5	0	0	0	0	1	0	0	0
Vomit/yellowish	0	0	0	0	1	0	0	0
Recov Vomit/whitish N=2	0	0	0	0	0	0	0	0
Vomit/yellowish	0	0	0	0	0	0	0	0
Increase in size: N=5								
Head	0	0	3	0	3	3	3	4
Right ear	0	0	5	5	5	4	4	4
Left ear	0	0	1	5	5	4	4	4
Around right eye	0	0	1	0	0	0	0	0
Right forelimb	0	0	0	0	1	0	1	1
Left forelimb	0	0	0	0	1	0	1	1
Right shoulder	0	0	0	0	1	0	0	0
Recov Increase in size: N=2								
Head	0	0	0	0	0	0	0	0
Right ear	0	0	0	0	0	0	0	0
Left ear	0	0	0	0	0	0	0	0
Around right eye	0	0	0	0	0	0	0	0
Right forelimb	0	0	0	0	0	0	0	0
Left forelimb	0	0	0	0	0	0	0	0
Right shoulder	0	0	0	0	0	0	0	0

Body weights: Body weights were recorded for each animal at least twice during the pre-treatment period, on the first day of treatment and then once a week until the end of the study.

There were no statistically significant differences in group mean body weights or group mean body weight gains in males or females treated with icatibant compared to vehicle treated control animals.

Food consumption: Food consumption was recorded for each animal by noting the difference between the quantity given and the remaining food left the next morning. This was estimated daily for at least 7 days before the beginning of treatment and throughout the study period. Food consumption was expressed as a percentage of the quantity given.

There were no treatment related effects on food consumption in the icatibant treated groups compared to the vehicle treated group.

Ophthalmoscopy: No ophthalmic examination was conducted in this study.

EKG: No EKG or blood pressure measurement was conducted in this study.

Hematology: Blood samples were taken from an appropriate vein. Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period, during Week 5, at Week 12 of the treatment period and at Week 21 and 27 during the recovery period. A complete hematological battery was assessed with the exception of hematocrit.

There were no statistically significant changes in the hematology parameters examined in the icatibant treated males or females compared to the vehicle control treated males.

Clinical chemistry: Blood samples were taken from a jugular vein. Prior to blood sampling all animals were deprived of food for an overnight period. Blood was collected once before the treatment period, during Week 5, at Week 12 of the treatment period and at Week 21 and 27 during the recovery period. A complete clinical chemistry battery was assessed.

There were no statistically significant differences in the clinical chemistry parameters evaluated in males or females treated with icatibant compared to those treated with the vehicle control.

Urinalysis: Urine was collected from all animals once before the treatment period, during Week 5 and then at Week 12 of the treatment period and during the recovery period. A complete urinalysis was conducted.

There were no statistically significant changes in urine parameters in the icatibant treated animals compared to the vehicle treated animals.

Gross pathology: A complete necropsy was conducted on all study animals.

Macroscopic findings were observed at injection sites (hematomas and edema), the male (testes, epididymides and prostate) and female (ovaries, oviducts and uterus) reproductive organs, thymus and liver. The findings at the injection site, thymus and liver were low in incidence and were not observed macroscopically in the recovery group animals. The findings in the male reproductive organs were not observed after the recovery period (epididymides and testes) or were low in incidence compared to the treatment period (prostate). Similarly, female reproductive organ findings were completely reversed in the ovaries and oviducts and were decreased in incidence in the uterus after the recovery period.

Observation	Icatibant Dose (mg/kg/day)							
	0		3		10		3.3 TID	
	M	F	M	F	M	F	M	F
Injection site 16 n=3								
Hematoma, diffuse	-	-	-	-	-	1	-	-
Rec. Injection site 16 n=2								
Hematoma, diffuse	-	-	-	-	-	-	-	-
Liver n=3								
Focus yellowish	-	-	-	-	-	-	1	-
Paleness	-	-	-	-	-	-	-	-
Rec Liver n=2								
Focus yellowish	-	-	-	-	-	-	-	-
Paleness	-	-	-	-	-	-	-	1
Epididymides reduced in size n=3	-	NA	2	NA	3	NA	-	NA
Rec. Epididymides reduced in size n=2	-	NA	-	NA	-	NA	-	NA
Prostate reduced in size n=3	-	NA	3	NA	3	NA	-	NA
Rec. prostate reduced in size n=2	-	NA	-	NA	1	NA	-	NA
Testes n=3		NA		NA		NA		NA
Reduced in size	-		3		3		-	
Soft	-		2		2		-	
Rec Testes n=2		NA		NA		NA		NA
Reduced in size	-		-		-		-	
Soft	-		-		-		-	
Thymus reduced in size n=3	1	-	-	-	1	1	1	-
Rec. Thymus reduced in size n=2	-	-	-	-	-	-	-	-
Ovaries, reduced in size n=3	NA	-	NA	-	NA	2	NA	1
Rec Ovaries, reduced in size n=2	NA	-	NA	-	NA	-	NA	-
Oviducts, reduced in size n=3	NA	-	NA	-	NA	2	NA	1
Rec Oviducts, reduced in size n=2	NA	-	NA	-	NA	-	NA	-
Uterus, reduced in size n=3	NA	-	NA	3	NA	3	NA	1
Rec. Uterus, reduced in size n=2	NA	-	NA	-	NA	1	NA	-

- No macroscopic finding observed
NA- Not applicable

Organ weights: The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus and thyroids with parathyroids were weighed. Absolute organ weights and organ weights relative to body weights were determined. No organ weights relative to brain weights were provided.

Decreases in reproductive organ weights (absolute and relative to body weights) were observed in males and females compared to the vehicle control treated animals through out the treatment period. After the 13-week recovery period, epididymal and prostate weights in males remained decreased but with less severity than the decreased weight during the dosing period. Testicular weights returned to normal compared to the vehicle control groups. Ovarian weights returned to normal after the 13-week recovery period as well. Adrenal weights relative to body weights were increased in males compared to the vehicle control treated animals but only slight. After the recovery period, there were no statistically significant differences in adrenal weights in the icatibant group compared to the vehicle control group.

Organ weight change relative to controls

Organ	Sex	Icatibant dose (mg/kg/day)

		3	10	3.3 TID
epididymides wt	M	-71%	-70%	-
	F	NA	NA	NA
Rec epididymides wt	M	-32%	-29%	-
	F	NA	NA	NA
epididymides rel BW	M	-71%	-68%	-21%
	F	NA	NA	NA
Rec epididymides rel BW	M	-28%	-25%	-21%
	F	NA	NA	NA
Prostate wt	M	-87%	-80%	-
	F	NA	NA	NA
Rec prostate wt	M	-	-52%	-
	F	NA	NA	NA
Prostate rel BW	M	-86%	-79%	-49%
	F	NA	NA	NA
Rec Prostate rel BW	M	-20%	-49%	-
	F	NA	NA	NA
Testes wt	M	-79%	-79%	-
	F	NA	NA	NA
Rec testes wt	M	-	-	-
	F	NA	NA	NA
Testes rel BW	M	-79%	-78%	-
	F	NA	NA	NA
Rec Testes rel BW	M	-	-	-
	F	NA	NA	NA
Ovary wt	M	NA	NA	NA
	F	-58%	-66%	-57%
Rec ovary wt	M	NA	NA	NA
	F	-	-	-
Ovary rel BW	M	NA	NA	NA
	F	-55%	-63%	-51%
Rec ovary rel BW	M	NA	NA	NA
	F	-	-	-

- No statistically significant difference compared to controls
NA- Not applicable

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

All study animals had tissues prepared for histopathological examination at the end of the treatment period. All macroscopic lesions were from all animals were microscopically examined. Reproductive organs and accessory organs (Groups 1 to 4) were microscopically examined. At the end of the recovery period, all macroscopic lesions and reproductive organs and accessory organs were microscopically examined. The thymus was not examined at the end of the recovery period. Peer review was performed for at least 20% of the slides in the control and HD groups. A complete histological battery was microscopically examined with the exception of the lachrymal gland, pharynx and seminal vesicles. All other tissues were examined.

Observation	Icatibant Dose (mg/kg/day)			
	0	3	10	3.3 TID

	M	F	M	F	M	F	M	F
Epididymides n=3								
Immature	-	NA	3(NA)	NA	3(NA)	NA	-	NA
Sloughed cells/lumen	-		-		-		-	
Oligospermia	-		-		-		-	
aspermia	-		-		-		-	
Rec.Epididymides n=2								
Immature	-	NA	-	NA	-	NA	-	NA
Sloughed cells/lumen	-		2 (1.0)		2 (1.5)		-	
Oligospermia	-		1 (4.0)		-		-	
aspermia	-		-		1 (NA)		-	
Prostate n=3								
Immature, reduced size	-	NA	3 (NA)	NA	3(NA)	NA	-	NA
Alveolar atrophy	-		-		-		-	
Rec Prostate n=2								
Immature, reduced size	-	NA	1 (NA)	NA	2 (NA)	NA	-	NA
Alveolar atrophy	-		-		1 (2.0)		-	
Testes n=3								
Immature	-	NA	3(NA)	NA	3(NA)	NA	-	NA
Peripubertal tests	-		-		-		-	
Tubuloar vacuolation	-		-		-		-	
Tubular degeneration	-		-		-		-	
Sloughed cells lumen	-		-		-		-	
Spermatid sumplasts	-		-		-		-	
Rec Testes n=2								
Immature, peripubertal	-	NA	1 (NA)	NA	1 (NA)	NA	-	NA
Tubuloar vacuolation	-		-		1 (2.0)		-	
Tubular degeneration	-		-		1 (2.0)		-	
Sloughed cells lumen	-		-		1 (2.0)		-	
Spermatid sumplasts	-		-		1 (1.0)		-	
Thymus n=3								
Lymphoid depletion	2(1.5)	1 (2.0)	1 (2.0)	-	3 (3.3)	3 (3.0)	2 (3.0)	2 (2.5)
Rec Thymus n=2								
Lymphoid depletion	-	-	-	-	-	-	-	-
Ovaries n=3								
Immature	NA	-	NA	3(NA)	NA	3(NA)	NA	3(NA)
Rec Ovaries n=2								
Immature	NA	-	NA	-	NA	-	NA	-
Uterus n=3								
Immature	NA	-	NA	3(NA)	NA	3(NA)	NA	3(NA)
Rec Uterus n=2								
Immature	NA	-	NA	-	NA	-	NA	-
Vagina n=3								
Immature	NA	-	NA	2(NA)	NA	3(NA)	NA	3(NA)
Rec Vagina n=2								
Immature	NA	-	NA	-	NA	-	NA	-

- No macroscopic finding observed
NA- Not applicable
(#)- mean severity: grade 1= minimal; grade 2= slight; grade 3= moderate; grade 4= marked; grade 5= massive

As summarize by the table above, the target organs of toxicity in the dog after treatment with icatibant are the male and female reproductive tissues and the thymus. Males treated with daily SC injections of icatibant showed immature epididymides, testes and prostate. After the 13-week recovery period, 1 of 2 males showed the testes and prostate as

immature where 1 male had recovered. No sperm was present in this animal. When males were treated twice weekly with icatibant, no affects were observed in the male reproductive organs. Females treated with once daily SC icatibant also resulted in immature ovaries, oviducts, uterus and vagina. After the 13-week recovery period, no adverse histological findings were present in female organs. Unlike the males, females treated with SC administration of icatibant twice weekly showed delayed or impaired maturation of the reproductive organs. Thymus lymphoid depletion was observed in males and females during the icatibant treatment period with either daily or twice weekly dosing of icatibant. Daily SC administration of 10 mg/kg of icatibant showed more severe lymphoid depletion in the thymus than twice weekly administration. Males and females showed no signs of thymic toxicity after the 13-week recovery period.

No NOAEL could be determined in this study in females or in males after daily SC administration of icatibant due to immature reproductive effects. In females treated with twice weekly SC doses of icatibant, no NOAEL could be determined due to immature reproductive organs. These effects were reversible upon cessation of icatibant treatment. Males treated with twice weekly doses of icatibant showed no adverse effects on reproductive organs but did demonstrate thymic lymphoid depletion.

Toxicokinetics: Venous blood samples were taken on Day 1 and in week 13 from all animals to determine icatibant levels and the metabolite M_2 (b) (4) levels. TK data are summarized below.

	Parameter	Icatibant Dose (mg/kg/day)											
		TK of Icatibant						TK of M2 Metabolite					
		3		10		3.3 TID		3		10		3.3 TID	
		M	F	M	F	M	F	M	F	M	F	M	F
Day 1	C_{max} (mcg/mL)	5.1	4.4	14.6	16.1	-	-	0.16	0.14	0.28	NC	-	-
	T_{max} (h)	0.7	1.1	0.9	1.0	-	-	3.6	2.0	3.3	NC	-	-
	AUC_{0-t} (mcg*h/mL)	13.1	11.3	44.2	41.4	-	-	0.99	0.78	1.98	NC	-	-
	AUC_{tot} (mcg*h/mL)	-	-	-	-	47.1	50.8	-	-	-	-	NC	1.52
Wk 13	C_{max} (mcg/mL)	5.1	4.8	21.8	20.6	-	-	0.19	0.24	0.23	0.32	-	-
	T_{max} (h)	0.6	0.8	0.8	0.9	-	-	3.3	3.2	4.0	3.2	-	-
	AUC_{0-t} (mcg*h/mL)	13.9	13.6	55.2	58.2	-	-	1.4	1.5	2.3	2.7	-	-
	AUC_{tot} (mcg*h/mL)	-	-	-	-	NC	NC	-	-	-	-	1.9	NC

NC- not calculated because individual values were invalid (QC rejected).
- Values not determined.

Exposure levels of icatibant increased with increased daily dose (3 to 10 mg/kg/day) in both males and females in an approximately dose-proportional manner. There was no accumulation after repeated daily SC administration of icatibant in males or females when comparing exposure levels at Day 1 to Week 13. The t_{max} for icatibant was achieved rapidly (0.6- 1 h post-dose) after SC administration in males and females. There were no PK gender differences observed after daily administration of icatibant to males

and females. After twice weekly administration of icatibant, plasma concentrations and systemic exposure levels were below the level of detection in both males and females.

The M_2 metabolite of icatibant increased in systemic exposure with increased icatibant dose in supra-proportional manner. There was no accumulation of M_2 after repeat daily SC administration of icatibant and there were no significant PK gender differences in M_2. Systemic exposure to M_2 after twice daily administration of icatibant was not detectable.

Other: To assess the sexual maturity of dogs, the testicular size (cm^3) was measured at the end of the treatment period and once every 2 weeks during the recovery period.

As shown in the table below, icatibant treatment resulted in a decrease in testicular size compared to the vehicle control animals. Administration of icatibant as daily doses resulted in a greater decrease in testicular size compared to administration of icatibant twice weekly.

%Δ in testicular size compared to controls

Treatment Period	Icatibant dose (mg/kg/day)		
	3	10	3.3 TID
End of treatment period n= 5 dogs	-87%	-81%	-31%
Rec. wk 15 n=2 dogs	-80%	-65%	NSS
Rec. wk 17 n=2 dogs	-75%	-56%	NSS
Rec. wk 19 n=2 dogs	-46%	-44%	NSS
Rec. wk 21 n=2 dogs	-54%	-51%	NSS
Rec. wk 23 n=2 dogs	NSS	NSS	NSS
Rec. wk 25 n=2 dogs	-31%	NSS	NSS

NSS- No statistically significant difference compared to vehicle controls.

Blood was sampled to determine hormone levels. Using radioimmunoassay, luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone (PROG), estradiol (ESTR) and testosterone (TESTO) levels were measured at predose and then at Weeks 4, 8, 12, 21, 23, 25 and 27.

Treatment with icatibant resulted in significant decreases in testosterone, LH and FSH levels in males compared to the vehicle control levels. These decreases coincided with the decreases in testicular volumes. Testosterone and LH levels recovered after cessation of dosing. A rebound in FSH levels was observed in males during the recovery week 23. A decrease in FSH levels was observed in females during Week 4 of treatment with icatibant compared to the vehicle control groups. However, FSH was highly variable in females throughout the study period.

Parameter	Week	Icatibant Dose (mg/kg/day)					
		3	10	3.3 TID	3	10	3.3 TID
		Males			Females		
Testo	4	-93%	-95%	-76%	NA	NA	NA
	8	blq	blq	-87%	NA	NA	NA
	12	blq	blq	-82%	NA	NA	NA
	R21	-	-	-	NA	NA	NA
	R23	-	-	-	NA	NA	NA
	R25	-	-	-	NA	NA	NA
	R27	-	-	-	NA	NA	NA

LH	4	-	blq	-	-	-	-
	8	-81%	-78%	-	-	-	-
	12	-88%	-79%	-	blq	-	-
	R21	-	-	-	-	-	-
	R23	-	-	-	-	-	-
	R25	-	-	-	-	-	-
	R27	-	-	-	-	-	-
FSH	4	-93%	-86%	-72%	-97%	-95%	-85%
	8	-	blq	-	-	-	-
	12	-	-	-	-	blq	-
	R21	-	-	-			
	R23	+500%	+433%	-	-	-	-
	R25	-	-	-	-	-	-
	R27	-	-	-	-	-	-

- No statistically significant difference compared to vehicle controls.
NA- Not applicable.
Blq-below the level of quantitiation

2.6.6.4 Genetic toxicology

Study title: Bacterial reverse mutation test

Key findings:

- Icatibant did not induce bacterial mutagenicity under the conditions tested.

Study no.: JE049-0132

Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: April, 19, 2005
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batch no. 0565984, and 98.1% pure

Methods

Strains/species/cell line: *Salmonella typhimurium* (*S. typhimurium*) strains TA1535, TA1537, TA98, TA100 and TA 102. Each strain was exposed to 5 dose-levels of icatibant (3 plates/dose-level).

Doses used in definitive study: Two definitive experiments were conducted. In the absence of metabolic activation (-S9 mix), icatibant was used at 39.06, 78.13, 156.25, 312.5, 625 and 1250 µg/plate for TA 1535, TA 100 (first and second experiment) and TA 102 (second experiment only) strains. For TA 1537, TA 98 (first and second experiment) and TA 102 (first experiment only) icatibant was assayed at 78.13, 156.25, 312.5, 625, 1250 and 2500 µg/plate in the absence of S9 mix.

In the presence of S9 mix, icatibant was used at 39.06, 78.13, 156.25, 312.5, 625 and 1250 µg/plate for TA 102 in the first experiment. For TA 1535 and TA 100 in the first experiment and TA 100 and TA 102 in the second experiment icatibant was assayed

at 78.13, 156.25, 312.5, 625, 1250 and 2500 µg/plate in the presence of S9 mix. For TA 1537, TA 98 in both experiments and TA 1535 in the second experiment, icatibant was assayed at 156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate in the presence of S9 mix.

Basis of dose selection: A preliminary dose-range finding toxicity study was conducted to establish the icatibant doses to be used for the definitive experiments. Using the test strains TA98, TA 100 and TA 102 in the presence and absence of S9 mix, 6 concentrations of icatibant (10, 100, 500, 1000, 2500 and 5000 µg/plate) were assayed. No precipitation of the drug was observed up to 5000 µg/plate. Icatibant was toxic in the preliminary assay at dose levels ≥ 1000 µg/plate in the TA 100 strain and at ≥ 2500 µg/plate in TA98 and TA 102 strains in the absence of S9 mix. In the presence of S9 mix, toxicity was observe at ≥ 1000 µg/plate in TA 102, ≥ 2500 µg/plate for TA 100 and at 5000 µg/plate for TA 98 with a significant reduction of colony counts.

Negative controls: Vehicle (water for injection)

Positive controls:
Without S9 mix:
1 µg/plate of sodium azide for TA 1535 and TA 100 strains
50 µg/plate of 9-aminoacridine for TA 1537
0.5 µg/plate of 2-nitrofluorene for TA 98
0.5 µg/plate of mitomycin C for TA 102

With S9 mix:
2 µg/plate of 2 anthramine for TA 1535, TA 1537, TA 98 and TA 100
10 µg/plate of 2-anthramine for TA 102

Incubation and sampling times: In the two definitive studies, plates were exposed to either the negative or positive controls or drug treatment in the presence or absence of S9 metabolic activation for 48 to 72 h and incubated at 37°C. At the end of the treatment period, plates were counted with an automatic plate reader.

Results

Study validity: Each concentration of icatibant, the positive controls and the negative control was assayed in triplicate for each bacterial strain tested. A complete battery of bacterial strains was examined in this study. Criteria for positive results included: a reproducible 2-fold increase for strains TA 98, TA 100 and TA 102 or 3-fold increase for strains TA 1535 and TA 1537 in the number of revertants compared with the vehicle controls, in any strain at any dose-level and/or evidence of a dose-relationship and reference to historical control data was taken into consideration when evaluating the study results. The assay methods, positive and negative controls, and the concentrations of drug used for the definitive study were adequate.

Study outcome: In the first definitive experiment, there was no increase in the number of revertants compared to the vehicle control groups for test strains TA1535, TA100,

TA102, TA 1537 and TA 98 in the presence and absence of S9 mix. Icatibant did not precipitate out of media or interfere with colony counting. Slight toxicity was observed in TA 100 at concentrations ≥ 1250 µg/plate in the absence of S9 mix (reduction of cell count by 36%) and at ≥ 2500 µg/plate in the presence of S9 mix (reduction of cell count by ~60%), and for TA 102 in the absence of S9 mix at ≥ µg/plate (reduction of cell count by ~50%).

In the second definitive study, there was no increase in the number of revertants compared to the vehicle control groups for test strains TA1535, TA100, TA102, TA 1537 and TA 98 in the presence and absence of S9 mix. Icatibant did not precipitate out of media or interfere with colony counting. Slight toxicity was observed in TA 100 in the absence of S9 mix at icatibant concentrations ≥ 625 µg/plate (reduction in colony count by 23%), for TA 1537 at ≥ 2500 µg/plate (reduction of colony count by 50%), for TA 98 in the absence of S9 mix at ≥ 2500 µg/plate (reduction of colonies by ~ 50%) and for TA 102 at ≥ 1250 µg/plate (reduction of colony count by 50%).

Taking the 2 definitive study results together and considering that this study was performed at adequate doses and under valid study methods, icatibant did not induce bacterial mutagenicity under the conditions tested.

Study title: *In vitro* mammalian chromosome aberration test in cultured human lymphocytes

Key findings:

- Icatibant did not induce chromosomal aberration formation in human lymphocytes under the experimental conditions tested.

Study no.: JE049-0133

Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: September 22, 2004
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batch no. E084 and 96.2% pure

Methods

Strains/species/cell line: Human lymphocytes

Doses used in definitive study: Two experiments were conducted. In the first experiment icatibant was assayed at 39.1, 78.1, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/mL in the presence and absence of S9 mix. In the second experiment, the icatibant concentrations were 156.3, 312.5, 625, 1250, 2500 and 5000 µg/mL in the presence and absence of S9 mix.

Basis of dose selection: The highest icatibant concentration level recommended for the assay was selected for the first experiment based on pH, osmolality and solubility. For

selection of icatibant concentrations for the second experiment, any toxicity that was indicated by a reduction of the mitotic index in the first experiment was used to adjust the icatibant doses.

Negative controls: Vehicle (culture medium)

Positive controls:
Without S9 mix:
Mitomycin C was used at 3 µg/mL (3 h treatment) or 0.2 µg/mL (continuous treatment)

With S9 mix:
Cyclophosphamide was used at 12.5 or 25 µg/mL

Incubation and sampling times: In the first experiment, lymphocyte cultures were exposed to controls and icatibant in the presence and absence of S9 mix for 3 hours. Cells were harvested 20 h post-exposure to test item initiation. In the second experiment, in the absence of S9 mix cells were exposed to icatibant concentrations or controls continuously until cell harvest. In the presence of S9 mix, cells were exposed to drug or controls for 3 hours and then rinsed and then harvested 20 h and 44 h post-exposure to test item initiation. Cells were incubated at 37°C in 5.0% CO_2 and 90% relative humidity. After cell harvest, slides were prepared for evaluation of cytotoxicity (reduction of mitotic index) and for the induction of chromosomal aberrations.

Results

Study validity: Approximately 200 metaphase spreads per treatment were scored. The tests were considered positive for chromosomal aberrations if there were statistically significant increases ($p \leq 0.05$) over concurrent controls in the percentage of cells with chromosomal aberrations without greatly exceeding a 50% reduction in growth. Review of the study methodology including the concentrations used for the definitive study, the use of the appropriate positive and negative controls, the scoring criteria and the outcomes of the positive and negative controls showed that this study was adequate.

Study outcome: There was no precipitation of drug up to the highest concentration tested (5000 µg/mL). In the absence of S9 mix and after 3 h exposure to each icatibant concentration, cytotoxicity was observed at 5000 µg/mL with a reduction in mitotic index to 33% compared to the negative controls. After 20 h of treatment in the absence of S9 mix cytotoxicity was observed at icatibant concentrations ≥ 2500 µg/mL with mitotic indices decreasing to 39%. After 44 h of treatment the mitotic index was 44% compared to the negative controls at 5000 µg/mL. In the presence of S9 mix, there was no significant reduction of mitotic indices at any concentration assayed. Therefore, there was no cytotoxicity observed in the presence of S9 mix.

In the presence and absence of S9 mix there was no increase in the frequency of structural aberrations at any of the icatibant concentrations tested. For the negative and positive controls, the frequencies of chromosomal aberration formation were within the

appropriate ranges. Under these study conditions, icatibant did not induce chromosomal aberrations in cultured human lymphocytes.

Study title: Bone marrow micronucleus test by intravenous route in rats

Key findings:
- Icatibant did not induced micronucleus formation under these study conditions.

Study no.: JE049-0135

Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: April 12, 2005
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batch no. 0565984, and 98.1% pure

Methods

Strains/species/cell line: Sprague Dawley rats; 5 rats/sex for the vehicle control, LD, MD and positive control groups and 8 rats/sex for the HD group. Rats were ~ 6 weeks old and ranged in body weight from 180.6 to 224.4 g for males and 119.6to 152.2 g for females.

Doses used in definitive study:
Males: Two IV administrations of 0 (vehicle), 3.75, 7.5, and 15 mg/kg of icatibant with a 24-hour interval between each dose
Females: Two IV administrations of 0 (vehicle), 2.5, 5, and 10 mg/kg of icatibant with a 24-hour interval between each dose

Basis of dose selection: A preliminary toxicity study was conducted to determine adequate doses of icatibant to be used for the definitive study. Three rats/sex were administered 20, 15 and/or 10 mg/kg of icatibant intravenously twice with a 24 h interval between doses. At 20 mg/kg (males only) 2/3 males died 2 hours after receiving the second dose. The remaining animal was hypoactive, showed sedation and piloerection. At 15 mg/kg (males and females), all treated animals were hypoactive and showed piloerection. Females also showed signs of sedation. One female (1/3) died 23 hours post the second treatment. In the 10 mg/kg group (females only), there were no clinical signs of toxicity and no mortality.

Negative controls: Vehicle control (0.9% NaCl, isotonic saline solution)

Positive controls: Cyclophosphamide at 1.5 mg/mL

Incubation and sampling times: All animals were administered vehicle, the icatibant doses or cyclophosphamide. Animals from the vehicle control, treatment and cyclophosphamide groups had bone marrow harvested at 24 hours post-dosing. Slides

were prepared from bone marrow cells that were harvested at sacrifice and cells were stained with Geimsa and microscopically assessed for micronuclei formation from 2000 polychromatic erythrocytes (PCEs) per animal.

Results

Study validity: Criteria for a result to be considered positive included a significant increase in the frequency of micronucleated PCE ($p< 0.05$) occurring at a minimum of 2 dose levels when compared to concurrent vehicle control and evidence of a positive dose-related response. Based on the review of the study methodology including the doses selected for male and female rats, the exposure time, the use of positive and negative controls and the outcome of these controls, this study was considered valid.

Study outcome: For males, dosed at 3.75 and 7.5 mg/kg, there were no clinical signs of toxicity observed. However, at 15 mg/kg, 2 animals died following icatibant administration. Females dosed up to 10 mg/kg IV showed no clinical signs of toxicity. However, the dose-ranging study showed that a dose of 15 mg/kg was lethal. Therefore, the dose of 10 mg/kg approximated the MTD. Males and females treated with icatibant showed similar mean values of micronucleated polychromatic erythrocytes (MPEs) and similar polychromatic erythrocyte/normochromatic erythrocytes ratios (PEs/NEs) as those observed from animals treated with the negative control. Male and female rats treated with cyclophosphamide significantly increased the MPEs compared to the vehicle controls. Vehicle treated and cyclophosphamide treated rats produced MPE counts and PE/NE ratios within the historical control ranges from the text facility.

Icatibant does not induced micronucleus formation under these study conditions.

2.6.6.5 Carcinogenicity

No carcinogenicity studies have been conducted by the sponsor to date.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Combined study of 13-week toxicity and effects on fertility and early embryonic development to implantation in rats by subcutaneous administration

Key study findings:

- HD treated females had an increase in pre-implantation loss compared to vehicle treated females.
- Untreated females paired with HD treated males showed no increase in pre-implantation loss compared to untreated females paired with vehicle control treated males.
- Icatibant had no effects on male sperm count, motility, or morphology.
- Based on no toxicity observed in male seminology and no effects on mating or fertility indices, the NOAEL for male fertility was 10 mg/kg/day. Based on an

increase of pre-implantation loss observed in HD treated females, the NOAEL for female fertility was 3 mg/kg/day. These conclusions were in agreement with the sponsor's conclusions.

Study no.: JE049-0108
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: February 17, 2005
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batches 0565984 (98.1% pure) and E084 (95.9%)

Methods

Doses: 0 (vehicle), 1, 3 and 10 mg/kg/day
Species/strain: Sprague Dawley rats
Number/sex/group or time point (main study): 24/sex/group
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in isotonic saline solution (0.9% NaCl), administered in 1.0 mL/kg and infusion rate was not applicable.
Satellite groups used for toxicokinetics or recovery: None.
Age: Males for the fertility study: 5-6 weeks old; Females: ~ 10 weeks old
Weight: males: 185 g and females: 224 g
Sampling times: None.

Unique study design or methodology (if any): The sponsor combined the 13-week repeat dose toxicity study and the effects on fertility and early embryonic development to implantation study in rats. Under this section, the reproductive toxicology study is reviewed. Male rats were given daily subcutaneous doses of icatibant for 10 weeks prior to mating, during and after mating. Female rats were given daily subcutaneous doses of icatibant for 2 weeks prior to mating, during mating and until 7 days post-coitum.

Parameters and endpoints evaluated: All animals were checked for morbidity and mortality at least twice a day and females were checked for gross BW on the first day of treatment, then twice a week until mated (or until sacrifice) and on Days 0, 4, 8, 11 and 15 post-coitum. The untreated females of the second pairing/mating were weighed on Days 0, 4, 8, 11 and 15 post-coitum. Food consumption was measured during the premating period weekly and during pregnancy over the periods of Days 0-8 and Days 8-15 post-coitum. The estrous cycle stage was assessed for each female during the pairing period. The mated females were sacrificed on Day 15 post-coitum and a macroscopic post-mortem examination of the principal thoracic and abdominal organs was performed. The ovaries and uteri were examined to determine the numbers of corpora lutea and implantations, classified as dead and live concepti and early and late resorptions. Following the observation that there were higher pre-implantation losses at 10 mg/kg/day at the day of hysterectomies, untreated, virgin females were paired with treated males from the VC and the HD groups to further assess effects of treatment. These females were sacrificed on Day 15 post-coitum and a hysterectomy was performed. The males in the LD and MD groups were sacrificed after all hysterectomies of the females had been

performed. Following the results of the second pairing/mating period, the males from the LD and HD groups were sacrificed. For all males in the fertility assessment, macroscopic post-mortem examinations of the principal thoracic and abdominal organs were performed.

Results

Mortality: All icatibant treated males survived until scheduled sacrifice. Females treated with 0, 1, 3 and 10 mg/kg/day of icatibant were sacrificed with 1/24 (sacrificed day 40), 2/24 (sacrificed days 39 and 40), 4/24 (sacrificed days 39 and 40) and 0/24 rats, respective to dose. The decision to sacrifice was based on no observed mating and not due to clinical signs of toxicity. However, each female that was sacrifice was determined to be pregnant. All remaining icatibant treated females, after the pregnancy period, survived until the scheduled sacrifice.

Following the observation that there were higher pre-implantation losses (see below) at 10 mg/kg/day at the day of hysterectomies, untreated, virgin females were paired with treated males from the VC and the HD groups to further assess effects of treatment. All untreated females that were paired with the HD treated males survived until scheduled sacrifice.

Clinical signs: Males and females treated with icatibant had no clinical signs of toxicity compared to the vehicle treated males.

Body weight: There were no statistically significant changes in group mean body weight or group mean body weight change in males treated with icatibant compared to males treated with the vehicle control. There were no statistically significant changes in group mean body weight or group mean body weight change in females treated with icatibant during the pre-mating phase or during the pregnancy period compared the vehicle control treated females. Additionally, there were no statistically significant changes in group mean body weight or group mean body weight change in the untreated females that were mated with icatibant treated males compared to untreated females mated to the vehicle control treated males.

Food consumption: There were no statistically significant differences in icatibant treated males and females in food consumption compared to the vehicle control treated animals throughout the study period.

Toxicokinetics: TK assessment was not conducted in the fertility and early embryonic development phase of this combined study.

Necropsy: One LD and 1 MD male rat showed reduced testes size. Two LD rats showed reduced epididymis size. These findings did not follow a dose-response. One female in the MD group and 1 female in the HD group had deformed kidneys. The effects observed on the kidneys were not observed in males and did not increase with dose.

Fertility parameters: There were 1, 2, 4 and 0 pairs for the dose groups 0, 1, 3 or 10 mg/kg/day, respectively, with no evidence of mating during the pairing period. However, the females delivered before sacrifice so mating had occurred. There was no effect on the estrous cycle of the females at any dose.

All control females were pregnant. There was 1 female paired with a male treated at 10 mg/kg/day that was not pregnant. There were 24, 23, 22 and 23 pregnant females in the groups administered 0, 1, 3 of 10 mg/kg/day, respectively, giving fertility indices of 100%, 96%, 92% and 100%, respectively. The mean number of corpora lutea was comparable to controls for all groups. However, the mean pre-implantation loss was higher than the controls at the HD (65 more corpora lutea than implantations vs 25 in the control group), and there were lower numbers of mean implantations and concepti. The post-implantation loss was also slightly higher in the HD group (24 implantations lost vs. 16 in the control group). There was no effects observed at 1 or 3 mg/kg/day compared to the vehicle control group. All concepti were alive.

Hysterectomy Data of Icatibant Treated Females

Parameter	**Icatibant dose mg/kg/day**			
	0	**1**	**3**	**10**
Pregnant females at term, n=24	23	21	18	23
With all dead concepti	0	0	0	0
With live concepti	23	21	18	23
Corpora lutea	393	354	301	406
No. per animal	17.1	16.9	16.7	17.7
Implantation sites	368	328	269	341
No.per animal	16.0	15.6	14.9	14.8
Preimplantation loss, total (%)	**25 (6.4)**	**26 (7.3)**	**32 (10.6)**	**65 (16.0)**
Concepti	352	308	259	317
No.per animal	15.3	14.7	14.4	13.8
Alive	100	100	100	100
Dead	0	0	0	0
Live concepti %	352	308	259	317
implantation sites	95.7	93.9	96.3	93.0
No. per animal	15.3	14.7	14.4	13.8
Postimplantation loss	16	20	10	24
% of implantation sites	4.3	6.11	3.7	7.0
No. per animal	0.7	1.0	0.6	1.0

Following the observation that there were higher pre-implantation losses at 10 mg/kg/day at the day of hysterectomies, untreated, virgin females were paired with treated males from the VC and the HD groups to further assess effects of icatibant treatment. Two untreated females paired with the treated males had no evidence of mating. However, 1 female was pregnant while the 2nd female was not. The treated males took an average of 1 day longer to mate with the untreated females than did the control males. The mean numbers of corpora lutea and implantations and the extent of pre- and post-implantation losses of the untreated females paired with males treated at 10 mg/kg/day were comparable to controls. As part of the 13-week toxicology study, icatibant treated males were evaluated for changes in sperm number, motility, and morphology. There were no

effects on these seminological parameters after daily subcutaneous treatment with icatibant for 13-weeks.

Embryofetal development

No new embryofetal development studies were submitted to the NDA. Studies conducted in the rat and the rabbit were previously reviewed under IND (b) (4)

Prenatal and postnatal development

Study title: Study for effects on pre- and post-natal development in rats by subcutaneous administration

Key study findings:

- Icatibant treatment delayed parturition at 1, 3 and 10 mg/kg/day, resulting in F_0 deaths (found dead or early sacrifice) in the 3 and 10 mg/kg/day groups.
- Clinical signs of toxicity (pallor of eyes and extremities, round back, piloerection and reddish discharge in vagina) were observed in 1 MD and in multiple HD F_0 females.
- After SC administration, icatibant and the M2 metabolite is found in rat milk.
- Based on the observed maternal toxicities including clinical signs, prolonged gestation periods for all icatibant dosage groups, F_0 spontaneous deaths and early sacrifice of icatibant treated dams, litter deaths, and necropsy findings in the MD and the HD groups, the maternal toxicity NOAEL could not be defined (< 1 mg/kg).
- The F_1 generation showed an increase in the number of pup deaths and cannabalism in the MD and HD compared to the VC groups. Therefore, the NOAEL for systemic toxicity in the F_1 generation was determined to be 1 mg/kg/day.
- The F_1 generation showed a slight decrease in air righting and hair growth at the HD compared to the VC control group. Therefore, the NOAEL for development of the F_1 generation was considered 3 mg/kg/day.

Study no.: JE049-0141
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: February 13, 2005
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Icatibant, batch no. E084, and 95.9% pure

Methods

Doses: 0 (vehicle), 1, 3 and 10 mg/kg/day
Species/strain: Sprague-Dawley rats

Number/sex/group: 24 mated females/group for the VC, LD and MD groups and 30 mated females/group for the HD group
Route, formulation, volume, and infusion rate: Subcutaneous, formulated in sterile isotonic saline solution (NaCl 0.9%), 1 mL/kg and infusion rate is not applicable.
Satellite groups used for toxicokinetics: 6 mated females/group were used for TK and milk sampling
Study design: Mated female Sprague-Dawley rats were subcutaneously dosed from Day 6 post-coitum until Day 21 post-partum. The dams were allowed to litter and rear their progeny until weaning. The HD group (10 mg/kg/day) had difficulties delivering or did not deliver at which time dosing was stopped on Day 21 post-coitum until the females delivered. Pups from F_0 dams were evaluated and then culled. After weaning, these pups (now referred to as the F_1 generation) were evaluated through sexual maturity and then mated. Evaluation of fertility and necropsy after hysterectomy of the F_1 generation was conducted.

Parameters and endpoints evaluated: For the F_0 females, morbidity and mortality, clinical signs of toxicity, BW changes, food consumption, and complete hematology, clinical biochemistry and urinalysis batteries were compared between the icatibant treated females and the VC treated females. The effects of icatibant treatment on parturition were also observed. Blood and milk were sampled and evaluated for icatibant levels in the F_0 dams. The number of implantation sites was recorded in F_0 females. Once dams had delivered, litter size and pup observations (BWs, clinical signs, pup physical and reflex development) were recorded. Litters were culled as nearly as possible to 4 pups/sex/litter. After weaning, these pups (now referred to as F_1 generation) were checked at least twice a day for mortality and morbidity, clinical signs of toxicity, changes in BW and food consumption and for sexual maturity. The animals were tested for auditory function, pupil constriction, learning and memory, spontaneous locomotor activity and the day that sexual maturity was reached was recorded. Blood and urine samples were collected from all F_1 animals before pairing, for hematological and blood biochemical investigations and urinalysis was conducted as well. Between 12 and 14 weeks, F_1 animals were paired for mating. The F_1 generation was sacrificed on Day 14 post-coitum. A macroscopic post-mortem examination was performed on all animals and for pregnant females. The numbers of corpora lutea and implantations, classification of concepti (dead or live), early and late resorptions or implantation scars were recorded.

Results

F_0 in-life: HD F_0 females and 1 MD F_0 female were either found dead of were sacrificed early due to clinical signs of toxicity or litter death. The following table summarizes the deaths, the time of death and the clinical sings of toxicity associated with these F_0 females. The sponsor stated that these clinical signs of toxicity were not directly related to icatibant administration but a result of the drug-induced delayed parturition and drug-induced difficulties with delivery, as most of the clinical signs were not observed until time of expected delivery.

F_0 dam animal no.	Death/Sacrifice	Day of Death	Observations
HD G28869	Found dead	Day 23 p.c.	• No clinical signs; 14 dead fetuses and 1 early resorption
HD G28851	Found dead	Day 24 p.c.	• Reddish vaginal discharge on days 23 and 24; 13 dead fetuses in the uterine horns
HD G28863	Early sacrifice	Day 24 p.c.	• Difficulties to deliver; pallor of extremities, round back, cold to touch, piloerection, hypokinesia, abdominal breathing and pallor of eye • 11 dead fetuses in the uterine horns
HD G28868	Early sacrifice	Day 24 p.c.	• 11 pups in bedding and 1 in vagina and 5 dead fetuses in uterine horns. • Pallor of extremities, round back, cold to touch, piloerection, hypokinesia, abdominal breathing and pallor of eye
HD G28875	Early sacrifice	Day 24 p.c.	• Difficulties to deliver. • Pallor of extremities, round back, piloerection and abdominal breathing • 6 dead pups in bedding and 1 dead fetus in the uterus
HD G28878	Early sacrifice	Day 25 p.c.	• Difficulties to deliver • Pallor of extremities, round back, piloerection, abdominal breathing and pallor of eye • 13 dead pups in the bedding and 1 dead fetus in the uterus
HD G28880	Early sacrifice	Day 27 p.c.	• Pallor of extremities, round back and piloerection • On the day prior to sacrifice, live pups in bedding but they were no longer there on day of sacrifice (cannibalism).
HD G28852	Found dead	Day 1 p.p.	• Gave birth to 7 live pups • F_0 female had pallor of extremities, round back, cold to touch, pallor of eye and eyes half-closed prior to death.
HD G28859	Found dead	Day 2 p.p.	• Gave birth to 9 live pups • No clinical signs prior to death. At necropsy, serous content in thoracic cavity, reddish contents in the vagina and 1 dead fetus in uterus
MD G28833	Early sacrifice	Day 25 p.c.	• F_0 female had pallor of extremities, round back, and piloerection • No delivery • Implantation scars were observed.

p.c.-post-coitum; p.p.- post-partum

The duration of gestation was statistically significantly longer for all groups treated with icatibant than VC treated females. Females in the LD and MD groups delivered slightly later than VC groups but survived delivery and reared their progeny until weaning. The mean number of pups born per female was comparable to the VC group. These data are provided in the following table.

During the pregnancy period, F_0 females in the HD group were found dead (2/30 females), were sacrificed due to the absence of delivery (1/30 females) or were sacrificed due to difficulties to deliver (4/30). During the lactation period, HD F_0 females were found dead (2/30) or were sacrificed during the first few days of the lactation period because of death of their litter (11/30 females). The sponsor stopped dosing of the remaining HD F_0 females to allow for delivery. However, the same delays and difficulties with delivery were observed. A total of 9 HD F_0 females delivered and reared their progeny in the HD group. No F_0 females treated with 0 or 1 mg/kg/day died or were sacrificed prior to scheduled sacrifice.

The HD F_0 females showed marked clinical signs of toxicity that were closely related to the timing of delivery. The sponsor considered these clinical signs to be associated with the late onset of delivery. Clinical signs of toxicity in HD F_0 females included pallor of eyes and pallor of extremities, reddish vaginal discharge, round back, cold to touch, piloerection and abdominal breathing. One MD F_0 female also showed signs of clinical toxicity and was not observed to deliver but implantation scars were observed at necropsy, indicating delivery had taken place. Clinical signs of toxicity were observed in several HD females during the lactation period. These females were sacrificed early.

Summary of Clinical Signs (no. during pregnancy period/no. during lactation period)

	Icatibant Dose (mg/kg/day)			
	0	**1**	**3**	**10**
Gestation				
Gestation duration (days)	21.3	21.8	22.0	23.0
Mortality				
Found dead	0/0	0/0	0/0	2/2
Decision of sacrifice	0/1	0/0	1/0	1/11
Decision to sacrifice (difficulties to deliver)	0/NA	0/NA	0/NA	4/NA
General				
Pallor of extremities	0/0	0/0	1/0	5/4
Round back	0/0	0/0	1/0	5/2
Cold to touch	0/0	0/0	0/0	2/2
Piloerection	0/0	0/0	1/0	5/0
Hypokinesia	0/0	0/0	0/0	2/0
Abdominal breathing	0/0	0/0	0/0	4/0
Reddish vaginal discharge	0/0	0/0	0/0	3/4
Pallor of eye	0/0	0/0	0/0	3/4
Eyes half-closed	0/0	0/0	0/0	0/1

NA- not applicable to lactation period.

There were no statistically significant differences between icatibant treated dams and the VC control treated dams for BWs and BW gains, food consumption, hematology, clinical biochemistry and urinalysis parameters.

Blood samples for plasma levels of the test item were collected from all groups on Day 6 and Day 17 post-coitum at 0.5, 1, 2 and 4 hours post-dose. Icatibant and M2 levels were determined using HPLC/MS MS methods. The limit of quantification of icatibant from plasma was between 27.1-38.2 ng/L.

TK Parameter	Icatibant TK			M2 TK		
	1	3	10	1	3	10
C_{max} (µg/mL)						
Day 6 p.c.	1.25	2.74	7.88	0.0884	0.218	0.406
Day 17 p.c.	0.981	3.16	7.78	0.0847	0.140	0.414
Day 4 p.p.	1.49	3.77	nm	0.113	0.160	nm
T_{max} (h)						
Day 6 p.c.	0.5	1	2	2	1	1
Day 17 p.c.	0.5	0.5	1	1	2	2
Day 4 p.p.	1	1	nm	1	1	nm
AUC (0.5-4 h) (µg*h/mL)						
Day 6 p.c.	1.08	6.21	21.3	0.117	0.423	0.941
Day 17 p.c.	1.62	5.65	20.8	0.103	0.391	1.14
Day 4 p.p.	nc	nc	nm	nc	nc	nm

p.c.: post-coitum; p.p.: post-partum, nc: not calculated, nm: not measured due to early deaths

On Day 6 post-coitum, the C_{max} for icatibant increased with increased dose in a sub-proportionate manner for all doses. However, on Day 17, C_{max} for icatibant increased in an approximately dose proportionate manner from the LD to the MD and sub-proportionately from the MD to the HD. The M2 metabolite C_{max} increased approximately in a dose proportionate manner from the LD to the MD but was sub-proportionate from the MD to the HD on Day 6. Day 17, the M2 increase was sub-proportionate for all doses. AUC levels of icatibant increased in supra-proportionately with increased dose Days 6 and 17 post-coitum. The M2 metabolite increased in an approximately dose proportionate manner on Day 6 and Day 17 and was observed at levels approximately 5-10% of the parent compound. There was no evidence of accumulation of icatibant after repeated SC administration.

For the LD, MD and HD groups, milk samples for levels of test item were planned to be collected on Day 4 post-partum at time point 0.5 and 4 hours after dosing. However, HD females either were found dead or were sacrificed prior to the scheduled milk sampling.

TK Parameter	Icatibant TK			M2 TK		
	1	3	10	1	3	10
C (µg/mL)						
1 h post-dose.	0.729	4.53	nm	Blq	0.239	nm
4.5 h post-dose.	0.216	1.11			0.084	

nm: not measured due to early deaths
blq- below the level of quantification

With increased icatibant dose, an increase in icatibant levels and M2 levels were observed in milk from lactating rats. These data show that after SC administration of icatibant, icatibant is excreted in milk.

F_0 necropsy: The pregnancy status of principle and satellite females is summarized in the follow table. All mated females in all dosage groups successfully mated. SC icatibant treatment did not adversely affect fertility indices at any dose. However, the number of HD F_0 females that successfully delivered was decreased compared to the VC control group. Only 9 HD F_0 females successfully delivered and reared their litter. At necropsy,

one HD F_0 female still had fetuses in the uterine horns, even though it had delivered one or two days earlier and had pale liver and kidneys.

	Icatibant Dose (mg/kg/day)			
	0	**1**	**3**	**10**
Number of mated females (Mating index, %)	29 (100)	30 (100)	30 (100)	30 (100)
Number of non-pregnant females	2	3	2	0
Number of pregnant females (fertility index, %)	27 (93.1)	27 (90.0)	28 (93.3)	30 (100)
Delivered	27	25	27	22
Pregnant but did not deliver	0	2*	1*	1*
Sacrificed due to difficulties to deliver	0	0	0	4
Found dead	0	0	0	4
With dead litter	1	0	0	12**
Number of principle females with a litter on day 21 post-partum	22	20	21	9
Females w/ entire liveborn litter dying and/or missing, cannibalized, culled				
Days 0-4	5	5	6	13
Days 0-21	5	5	6	13

*- marked BW gain during gestation period followed by BW loss Day 25 post-coitum and presence of only implantation scars in uterine horns suggest unnoticed delivery
**- includes 1 female which had live pups on Day 24 post-coitum but had not finished delivery, and no pups on day 24 post-coitum so could not be given a delivery date

At necropsy, the HD F_0 females showed an increase in findings compared to LD and MD groups and the VC controls. One MD female had an enlarged spleen. After inspection of the uterus, all icatibant treated females showed an increase in implantation sites with the HD F_0 having the highest increase and also showed reddish contents in uterine horns. Macroscopic findings of the vagina were observed in the HD F_0 females with fetuses inserted still inserted and reddish contents.

Summary of F_0 Necropsy Observations

	Icatibant Dose (mg/kg/day)			
	0	**1**	**3**	**10**
F_0 Females	24	24	24	30
Thoracic cavity: serous content	0	0	0	3 (10%)
Spleen: enlarged	0	0	1 (4.2%)	1 (3.3%)
Liver: Paleness	0	0	0	7 (23.3%)
Stomach: brownish focus	0	0	0	1 (3.3%)
Kidney: Paleness	0	0	0	6 (20%)
Uterus:				
Implantation sites	0	1 (4.2%)	1 (4.2%)	13 (43.3%)
Reddish contents in uterine horn	0	0	0	2 (6.7%)
Vagina:				
Fetuses inserted in vagina	0	0	0	1 (3.3%)
Reddish contents	0	0	0	4 (13.3%)

Based on the observed maternal toxicities including clinical signs(pallor of eyes and extremities, round back, piloerection), prolonged gestation periods for all icatibant dosage groups, F_0 spontaneous deaths and early sacrifice of icatibant treated dams, litter

deaths, and necropsy findings in the MD and the HD groups, the maternal toxicity NOAEL could not be defined.

F_1 physical development: Litters with live pups showed a slight decrease in the HD group compared to the VC group. At Day 4 (post-partum), pups were culled to achieve as close to 4 pups/sex/litter as possible. Due to the decrease in the number of pups in the HD group as a result of F0 deaths and losses of litters, only 12 pups were culled compared to 107 in the VC control group. A significant increase in pup deaths or missing and/or cannibalized was observed in the HD group. Additionally, pups surviving until Day 4 post-partum and then until Day 21 were significantly reduced compared to the VC group. These data are summarized in the following table.

	Icatibant Dose (mg/kg/day)			
	0	**1**	**3**	**10**
Litters w/ Live born pups	27	25	27	22
Pups delivered (total) live born Live born index (%)	338 (100)	309 (100)	337 (100)	207 (100)
Culled (total) Day 4 Cannibalized Missing Died	107 10 0 2	86 1 0 3	85 8 0 12	12 48 0 107
Pups dying, missing and/or cannibalized Day 1-4	12 (3.6%)	3 (1.0%)	20 (5.9%)	155 (74.9%)
Pups surviving 4 days (viability index, %)	326 (96.4)	306 (99.0)	317 (94.1)	51 (24.6)
Pups surviving 21 days (lactation index, %)	176 (100)	156 (99.4)	156 (100)	39 (100)

There were no macroscopic findings in pups from dams exposed to icatibant compared to the pups from dams exposed to VC. There were no statistically significant differences in pup weights/litter at Day 1 or Day 21 of litters from drug-treated dams to litters from VC treated dams. There was no difference in sex ratio between pups exposed to icatibant and pups exposed to the VC group. There were no differences in BWs, hematology, clinical biochemistry and urinalysis between F_1 animals exposed to icatibant and the VC exposed animals.

Pups exposed to icatibant showed similar reflex and physical development patterns as VC treated animals for the following: Pinna folding, tooth eruption, eye opening, auditory canal opening, surface righting and cliff avoidance. Slight decreases in the MD and HD exposed pups were observed in hair growth at Day 5 and air righting on Day 17 compared to the VC exposed pups.

	Icatibant Dose (mg/kg/day)			
	0	**1**	**3**	**10**
Live pups/litter (day1)	12.7	12.2	11.8	4.9
Hair growth day 5 (positive response, %)	100	100	98.7	94.9
Air righting day 17 (positive response, %)	92.0	93.6	91.7	82.1

F_1 behavioral evaluation: There were no differences in the F_1 generation that was exposed to icatibant and the VC exposed F_1 generation for mean age of appearance of

sexual maturity, for response to acoustic startle, pupil constriction reflex, water T-maze test or motor activity.

F_1 reproduction: The F_1 generation that was exposed to icatibant had comparable reproductive data to the VC exposed F_1 generation. These data included pairs mated, mating index, mean number of days of pairing before mating, pregnant females, fertility index, females with live concepti and gestation index.

Hysterectomy data from the F_1 generation for animals exposed to icatibant were comparable to the VC exposed F_1 generation. These data included: pregnant females alive at term, number of corpora lutea, number of implantation sites, preimplantation site loss, number of concepti, number of live concepti, number of dead concepti and post-implantation loss. There was no difference in necropsy observations made in the F_1 generation exposed to icatibant and the F_1 generation exposed to VC.

F_2 findings: The F_2 generation was not evaluated.

2.6.6.7 Local tolerance

Two local toxicity studies were conducted in rabbits using single dose administration via the subcutaneous route (study no. JE049-0142) and intrarterial/intravenous/paravenous routes (study no. JE049-0143).

Study no. JE046-0142 (non-GLP, initiated September 19, 2003) administered icatibant acetate (lot no. E084 and 96.2% pure) as a single 1.0 mL subcutaneous dose at 5, 10, 20 and 40 mg to 3 male New Zealand White rabbits/dose group (age 2-4 months old and mean BW 2.3 kg). The treatment was administered to the left flank of each animal and the vehicle (acetate buffer 0.5 M at pH 5.5) was administered to the right flank of each animal to serve as a control. Local reactions and clinical signs were checked daily for each animal. Local reactions were scored as follows:

Erythema and eshcar formation:
No erythema-0
Very slight erythema (barely perceptible)-1
Well defined erythema-2
Moderate to severe erythema-3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)-4

Edema:
No edema-0
Very slight edema (barely perceptible)-1
Slight edema (edges of area well-defined by definite raising)-2
Moderate edema (raised ~ 1mm)-3
Severe edema (raised >1mm and extending beyond area of exposure)-4

Body weight was recorded at predose and then on days 1 and 4. At the end of day 4, all rabbits were sacrificed and macroscopically examined. Skin samples were taken from the

test site (left flank) and the control site (right flank) and histopathological examination was conducted.

After subcutaneous injection up to 40 mg, there were no premature animal deaths and no clinical signs of toxicity. No changes in BW gain were observed between all dosage groups. No edema was observed at the vehicle injection site or at the icatibant treated injection sites up to the highest dose injected (40 mg). Erythema and hematoma formation were observed with similar frequency and severity at the vehicle treated injection sites (up to 3/3 with erythema score of 2 and 1/3 had a hematoma) and the 5 mg (1/3 erythema with a score of 1 and 1/3 had a hematoma), 10 mg (1/3 erythema with a score of 2 and 1/3 had a hematoma), 20 mg (1/3 had erythema with a score of 1 and no hematomas), and 40 mg (2/3 had erythema with a score of 1.5 and no hematomas) icatibant injection sites. Therefore, this finding is most likely route and vehicle dependent and not drug-related. There were no icatibant-related histopathological findings at the injections sites that showed increased incidence compared to the vehicle sites in all animals from all dosage groups.

The second study (study no. JE049-143, GLP and initiated May 4, 1995) administered a single dose of icatibant (batch no. 102) at 0.5 mg, 0.5 mg and 0.1 mg as intra-arterial (central artery of ear), intravenous (marginal vein of the ear) or paravenous (beside the marginal vein of the ear) doses, respectively, to the right ear of female albino New Zealand rabbits (4 rabbits/group; age ~ 6 months and BW ranged from 3.5 to 4.6 kg). Isotonic saline solution was administered to the left ear of each rabbit as a control via each administration route. All animals were observed for clinical signs of toxicity and the injection sites were examined 24 h post dose and 2 of the rabbits were followed for 120 h post-dose. After 24 h post dose 2/4 rabbits per group were sacrificed and the injected area was histopathologically examined. After 120 h the remaining2/4 rabbits/group were sacrificed and the injection sites were examined.

After injection of icatibant via each route of administration, no rabbits showed any clinical signs of toxicity at 24 h or 120 h post-dose. Rabbits administered a single 0.5 mg intra-arterial dose of icatibant showed similar findings as the isotonic saline administered control animals. After intravenous injection of 0.5 mg icatibant, 1/4 animals showed slight reddening at the injection site but all other animals showed no irritation at the injection site. IV injection of isotonic saline showed no injection site irritation. After paravenous injection of 0.1 mg of icatibant, there were no signs of irritation at the injection site. Based on these data, icatibant was well tolerated after intra-arterial, intravenous and paravenous administration up to 0.5, 0.5 and 0.1 mg, respectively.

Taking the two local tolerance studies together, icatibant administration was well tolerated and contributed to little injection site irritation after intra-arterial, intravenous and paravenous administration. After subcutaneous administration to rabbits, slight irritation was observed at the injection site but these findings were considered route of administration and/or vehicle dependent and not related to icatibant administration, as similar findings were observed at the vehicle control injected sites.

2.6.6.8 Special toxicology studies

Study title: H-Oic-OH: Single dose subcutaneous toxicity study in mice

(b) (4)

Key study findings:

- The sponsor stated that there were no macroscopic changes observed at the end of the 3-week observation period and the maximum tolerated dose was considered >1000 mg/kg.
- Due to the absence of a control group, the limited number of animals tested and overall poor study, no conclusions can be made on the impact of H-Oic-OH SC administration.

Study no.: JE049-0151 (92.0453)
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: July 9, 1992
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: H-Oic-OH, batch no. H006, and ~ 98.0% pure
Formulation/vehicle: H-Oic-OH dissolved in distilled water; administered SC in 10 mL/kg

Methods

Doses: 1000 mg/kg of H-Oic-OH via subcutaneous injection as a single dose. No control was used for this study.

Study design: 2 mice/sex (male BW ranged from 18-23 grams and female BW was 19 g) were administered H-Oic-OH and observed for 3 weeks post-administration for changes in behavior, changes in body weights, and then sacrificed and observed for macroscopic changes at the end of the 3-week observation period. No control group was used in this study.

Results: All animals survived after subcutaneous injection of H-Oic-OH up to 1000 mg/kg and no clinical signs of toxicity were observed throughout the 3-week post-dose follow-up period. There was observed BW loss after injection of H-Oic-OH. The sponsor stated that there were no macroscopic changes observed at the end of the 3-week observation period and the maximum tolerated dose was considered >1000 mg/kg.

Due to the absence of a control group, the limited animals tested and the poor design of this study, no conclusions can be made on the impact of H-Oic-OH SC administration. Although no animals died in this study, it is not known whether administration of H-Oic-OH causes any macroscopic or microscopic changes compared

to controls due to the absence of controls in this study and due to the long observation period post-dose that may allow for recovery from any drug-induced insults.

Study title: H-D-Tic-OH: Single-dose subcutaneous toxicity study in mice

(b) (4)

Key study findings:

- The 2 female mice dosed with 1000 mg/kg of H-D-Tic-OH via SC died within 24 hours post-dose.
- Clinical signs of toxicity included reduced motility, transiently ataxic gait, marked flank respiration and abdominal position.
- There were no clinical signs of toxicity observed in males.
- Due to the absence of a control group, the limited number of animals tested and the poor design of this study, no conclusions can be made on the impact of H-D-Tic-OH administration.

Study no.: JE049-0152 (92.0454)
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: July 9, 1992
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: H-D-Tic-OH; Batch no. 009, and ~100% pure
Formulation/vehicle: H-D-Tic-OH formulated in potato starch mucilage and administered in 10.9 mL/kg vol

Methods

Doses: 1000 mg/kg of H-D-Tic-OH via subcutaneous injection as a single dose. No control was used for this study.

Study design: 2 mice/sex (male BW ranged from 18-23 grams and female BW was 19 g) were administered H-D-Tic-OH and observed for 3 weeks post-administration for changes in behavior and changes in body weights. Those that survived until 3-weeks post-dose were sacrificed and observed for macroscopic changes. No control group was used in this study.

Results: The 2 female mice dosed with H-D-Tic-OH died within 24 hours post-dose. Clinical signs of toxicity in the 2 females that died included reduced motility, transiently ataxic gait, marked flank respiration and abdominal position, which started between 1 and 4 hours post-dose and persisted for at least 5 hours. There were no clinical signs of

toxicity observed in males. The sponsor stated that there were no changes in BW development for the surviving males throughout the 3-week observation period. Additionally, the sponsor stated that there were no macroscopic changes observed at the end of the 3-week observation period or in the animals that died within 24hours post-dose. However, there were no controls in this study with which to compared BWs or macroscopic examination of tissues.

Due to the absence of a control group, the limited number of animals tested and the poor design of this study, no conclusions can be made on the impact of H-D-Tic-OH administration. Administration of H-D-Tic-OH was toxic at 1000 mg/kg in females.

Study title: *In vitro* hemolysis test of HOE 140

Key study findings:

- This study was conducted under GLP conditions that were current as of 1982 and was not QA'd.
- Under the study conditions tested, 0.1% HOE 140 (icatibant) did not induce hemolysis of human whole blood.

Study no.: JE049-0150
Volume #, and page #: Electronic document
Conducting laboratory and location: (b) (4)
Date of study initiation: February 26, 1993
GLP compliance: Yes- By 1982 standards
QA reports: yes () no (X)
Drug, lot #, and % purity: HOE 140, lot #66 and the purity of the compound is unknown
Formulation/vehicle: NA

Methods

Doses: Negative control (physiological saline), 0.1% Icatibant and positive control (distilled water for injection)

Study design: Human normal blood from 3 donors (0.1 mL) was incubated with 1.0 mL of either negative or positive controls or icatibant at 37°C for 30 minutes. The mixture was then centrifuged and observed macroscopically for hemolytic reactions. Samples were scored as (-) no hemolysis, +1 (slight hemolysis), +2 (hemolysis red and clear), +3 (hemolysis red and turbid) or +4 (hemolysis discolored). In a second assay, whole blood was washed 3 times in saline and then the red blood cells were incubated with either negative or positive controls or icatibant at 37°C for 30 minutes. The optical density of each reaction mixture was determined.

Results: The positive and negative controls produced the expected results. The positive control induced hemolysis and the negative control did not. HOE 140 at 0.1% did not induced hemolysis under these study conditions.

Study title: Assessment of antibody responses towards icatibant in hyperimmunization studies

Key study findings:

- The sponsor concluded that hyperimmunization attempts in rat, dog and monkey, even with icatibant-protein conjugates in combination with adjuvant, did not elicit a sustained antibody response against icatibant.
- Based on the lack of information provided by the sponsor (icatibant lot and purity and detailed methods of study), no concrete conclusions can be drawn from this study report. However, based on the summary data provided by the sponsor, icatibant-thyroglobulin conjugate did not elicit a strong anti-icatibant antibody response.

Study no.: JE049-0166
Volume #, and page #: Electronic document
Conducting laboratory and location: Immunization rat and rabbit studies performed by (b) (4); Immunization dog and monkey studies performed by (b) (4)
Date of study initiation: Unknown
GLP compliance: No
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Icatibant conjugated with thyroglobulin, lot no. unknown and purity was unknown.
Formulation/vehicle: Icatibant conjugated with thyroglobulin and in adjuvant that consisted of 95% (V/V) paraffin oil, 2.4% (v/v) Tween 40, 0.1% (V/v) cholesterol and 0.01% (v/v) LPS.

Methods

Doses: NA (See under study design.)

Study design: The sponsor conducted hyperimmunization studies in the rat, dog and monkey following standard immunization protocols and in the presence of adjuvant, the animals were repeatedly exposed to icatibant which was conjugated to a carrier protein. The presence of anti-icatibant antibodies was assessed by ELISA and Biacore assays.

For rats, 6 rats were immunized with icatibant-thyroglobulin conjugate. Antigen was injected intramuscularly. The initial immunization dosed 0.12 mg of conjugate followed by 4 boosts with 0.06 mg of conjugate. A single dog was immunized with 200 µg of icatibant thyroglobulin and then followed by 5 boosts. Two Cynomolgus monkeys were hyperimmunized with icatibant-thyroglobulin at 250 µg and followed with 5 boost. One monkey showed very low titers at all test bleeds and therefore, the immunization was stopped in that animal. The second animal had blood drawn and tested for anti-icatibant antibody formation. Rabbits (15 animals) were used as a positive control due to their ability to generate antibodies against protein conjugates of small molecular entities and hyperimmunized. Blood samples were taken from each species and assayed using

ELISA for anti-icatibant IgG. Serum samples from each species were analyzed using Biacore.

Results: Hyperimmunization of rats, dogs and monkeys with icatibant-protein conjugates resulted in such a low antibody response that no specific anti-icatibant antibodies could be isolated when using the ELISA method. A single monkey showed a weak antibody response with a titer of 1:2048. The sponsor stated that rabbits produced significant amounts of anti-icatibant antibodies but these data were not provided in the study report. Using the Biacore method resulted in only a low content of icatibant-binding molecules was detected in the antiserum from rats, dog and monkey in contrast with rabbits that showed a high response. The following two summary tables of the results from the ELISA and the Biacore assays were provided by the sponsor.

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Table 6: Results of hyperimmunization studies in rat, dog, monkey and rabbit (ELISA data)

Species	No. of animals	Icatibant conjugate	Titer obtained (1:n)	Purifiable icatibant-specific antibodies?	Study/animal
Rat	6	Thyr	512	-	18931 (chapter 4.1)
	10	Thyr	2048	-	20365#
Dog	1	Thyr	512	-	8459 (chapter 4.2)
Monkey	1	Thyr	512	-	8460#
	1	Thyr	none	-	9836 (chapter 4.3)
	1	Thyr	2048	-	9837 (chapter 4.3)
Rabbit	15	Thyr	150000	+	22946 (chapter 4.4)

Data details not shown in this report.

Table 7: Results of hyperimmunization studies in rat, dog, monkey and rabbit (Biacore data)

Species	Type of study	Dilution applied	Relative response	Study/animal reference
Monkey	Hyperimmunization	1:100	350	8460#
Rat	Hyperimmunization	1:100	750	18931 (chapter 4.1)
Dog	Hyperimmunization	1:100	25	8459 (chapter 4.2)
Rabbit	Hyperimmunization	1:100	3700	22030#

Data details not shown in this report.

Study title: Acute intravenous toxicity in rats

Key study findings:

- There were no premature deaths, clinical signs of toxicity, statistically significant changes in BW gains and no macroscopic changes in the (b) (4) treated animals compared to the vehicle control treated animals.
- The minimal lethal dose was > 100 mg/kg of (b) (4) in rats.

Study no.: JE049-0155
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: August 8, 2006
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: (b) (4) batch no. 1005323 and 78.4% pure

Formulation/vehicle: (b) (4) was formulated in 0.9% NaCl for intravenous administration with a 5 mL/kg vol

Methods

Doses: 0 (vehicle control) and 100 mg/kg of (b) (4) were intravenously administered to rats as a single dose

Study design: Five Sprague-Dawley rats/sex/group were administered a single IV dose of either vehicle as the control or 100 mg/kg of the impurity. All animals were observed for clinical signs of toxicity, mortality, and changes in BW for a period of 14-days post-dose. After the 14-day period, all animals were macroscopically examined.

Results: There were no premature deaths, no clinical signs of toxicity and no statistically significant changes in BW gains in the (b) (4) treated animals compared to the vehicle control treated animals. Compared to the control treated animals there were no macroscopic changes in the (b) (4) group. The minimal lethal dose was > 100 mg/kg of (b) (4) in rats.

Study title: Bacterial reverse mutation test

Key study findings:

- The study was considered valid based on the methodology used.
- Under the experimental conditions in this study, (b) (4) up to 5000 µg/plate did not induce an increase in the number of revertants compared to the negative controls.

Study no.: JE049-0154
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b)
Date of study initiation: August 11, 2006
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: (b) (4) batch no. 1005323 and 78.4% pure
Formulation/vehicle: (b) (4) was dissolved in water

Methods

Doses: For the first mutagenicity study with and without S9 mix and using the plate incorporation method the following concentrations of (b) (4) was used: 0, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/plate. In the second study using the preincubation method the following concentrations were used with and without S9 mix: 625, 1250, 2500, 3750 and 5000 µg/plate. The maximum concentrations achieve the recommended limit concentration for this assay.

Study design: (b) (4) was tested in a dose-ranging test and then followed by 2 independent experiments in the presence and absence of metabolic activation (S9 mix). Experiments were performed using the direct plate incorporation method except for the 2nd test with S9 mix, which was performed using the preincubation method. Five strains of *Salmonella typhimurium*: TA 1535, TA 1537, TA98, TA 100 and TA102 were assayed in the 2 definitive experiments, where strains TA98, TA 100 and TA 102 were assayed in the dose-ranging study. In the dose-ranging study, single plates/concentration were assessed. In the 2 definitive studies, 3 plates/concentration with and without S9 mix were evaluated. After 48 h at 37°C, the revertant colonies were scored using an automatic counter. The potential for cytotoxicity was evaluated by the decrease in the number of revertants and or the thinning of the bacterial lawn.

Negative control was water for injection.

The positive controls without S9 mix:
1 µg/plate of sodium azide for TA 1535 and TA100 strains
50 µg/plate of 9-aminoacridine for TA 1537 strain
0.5 µg/plate of 2-nitrofluorene for TA 98 strain
0.5 µg/plate of mitomycin C for TA 102 strain

Positive controls with S9 mix:
2 µg/plate of 2-anthramine for TA 1535, TA 1537, TA98 and TA 100 strains
10 µg/plate of 2-anthramine for TA 102 strain

(b) (4) was freely soluble in water and was non-toxic in the dose-ranging assay. Therefore, the highest concentration (5000 µg/plate) was selected for the definitive studies. There was no precipitation of the drug at the doses examined.

Results: The study was considered valid based on the methodology used for each study, the criteria for evaluating the plates and the expected outcomes for the negative and the positive controls. The number of revertants in the negative control group was within the historical control data as were the positive control revertant numbers. The sponsor examined doses up to 5000 µg/plate in the 2 definitive studies, which was adequate due to the products free solubility in water, its lack of toxicity on the bacterial strains and the absence of precipitation that potentially would interfere with plate counting. Under the experimental conditions in this study, (b) (4) did not induce an increase in the number of revertants compared to the negative controls.

Study title: *In vitro* mammalian chromosome aberration test in cultured human lymphocytes

Key study findings:

- (b) (4) induced chromosome aberrations in human lymphocytes under the study conditions assayed. This is in agreement with the sponsor's conclusions.

Study no.: JE049-0156

Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: August 9, 2006
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: (b) (4) batch no. 1005323 and 78.4% pure
Formulation/vehicle: (b) (4) was dissolved in water

Methods

Doses: In the definitive study (the second experiment), the (b) (4) concentrations examined were: 0, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/mL in the absence and presence of S9 mix. The maximum concentration achieves the recommended limit concentration for this assay.

Study design: Two independent experiments (using duplicate cultures in each experiment for each concentration tested) in the presence and absence of metabolic activation (+ or –S9 mix) were conducted with different concentrations of (b) (4) to evaluate whether (b) (4) induces chromosome aberration formation in cultured human lymphocytes. The highest concentration of (b) (4) used for the first experiment was selected based on pH, osmolality and solubility. The test item was freely soluble in the vehicle (culture media) and showed no precipitation from media at 5000 µg/mL. In the first experiment, lymphocytes were exposed to controls and (b) (4) concentrations in the presence and absence of S9 mix for 3 hours and then rinsed. Cells were harvested 20 h after the beginning of treatment. In the first experiment, the (b) (4) concentrations examined were: 39.06, 78.13, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/mL.

The second experiment exposed lymphocytes to test article and controls without S9 mix continuously until harvesting of cells at 20 or 44 h post initiation of treatment. In the presence of S9 mix, cells were exposed to controls and test article for 3 h and then rinsed and incubated for 20 h post initiation of treatment and then had the cells harvested. Cells were fixed and then spread onto glass slides and scored for aberration formation. The cytotoxicity of icatibant was determined using the mitotic index (number of cells in mitosis/1000 cells examined). Analysis of 200 metaphases/concentration of test article was made with 100 metaphases/culture when possible. Only 50 metaphase cultures were analyzed when at least 10% cells with structural chromosome aberrations were observed. All metaphase analyses were performed blinded.

Negative controls: Vehicle (culture medium)

Positive controls: In the absence of S9 mix, mitomycin C was used at a final concentration of 3 µg/mL for the 3 h incubation and at 0.2 µg/mL for the continuous incubation. In the presence of S9 mix, cyclophosphamide was used at a final concentration of 12.5 or 25 µg/mL.

Results: Based on the study methodology and the negative and positive controls providing results within their respective historical control data, and based on the (b) (4) concentrations tested, the 2 studies were considered valid. Criteria for a positive result were a reproducible and statistically significant increase in the frequency of cells with structural aberrations for at least one of the dose-levels and one of the two harvest times. Reference to historical control data was also compared.

In the first experiment after the 3 h treatment period and in the absence of S9 mix, there was little reduction in the mitotic index at 5000 µg/mL (MI 96% compared to the vehicle control) and there was no increase in structural chromosome aberrations. In the presence of S9 mix, there was no reduction in MI and no increase in structural chromosomes aberration frequency compared to the vehicle control group. The positive controls induced an increase in structural chromosome aberration formation in the presence and absence of S9 mix.

After 20 and 44 h treatments, in the absence of S9 mix, there was a slight to marked decrease in mitotic index [20 hr: MI at 53% and 37% at 2500 and 5000 µg/mL of (b) (4) respectively, compared to the vehicle controls [44 h: MI at 59%, 60% and 42% at 1250, 2500 and 5000 µg/mL of (b) (4) respectively, compared to controls]. A slight, but not statistically significant, increase in the frequency of cells with structural chromosomal aberrations (- gaps) was noted at 2500 µg/mL [mean % (- gaps) 2.0] and 5000 µg/mL [mean % (- gaps) 4.0] after the 20 h treatment.

After 44 h of treatment in the absence of S9 mix, a statistically significant increase in the mean number of cells (%) with structural aberrations (-gaps) was observed at 2500 and 5000 µg/mL. However, cytotoxicity levels significantly exceeded 50% (actual value is 42%) at 5000 µg/mL. At this level of cytotoxicity, structural aberration formation may be observed as a result of indirect mechanisms unrelated to test article. At 2500 µg/mL the cytotoxicity was at 60%. Therefore, (b) (4) did increase chromosome aberration formation in this study.

Second experiment without S9 mix: Chromosome Aberration (44-h treatment)

Concentrations (µg/mL)	No. of cells scored	Structural aberrations		Cells w/ structural aberrations	
		Total no. (+ gaps)	Total no. (– gaps)	Mean % (+ gaps)	Mean % (– gaps)
0	200	8	1	4.0	0.5
1500*	200	2	2	0.5	0.5
2500[A]	200	15	15	6.5	6.5
5000[A]	200	34	21	25.0	19.0
[B]MMC (0.2 µg/mL)	100	34	28	29	26

*-The study protocol indicates that 1250 µg/mL was tested, which differs from the concentration provided in the sponsor's table.
[A]-The reviewer questions these data due to no difference between the total number of structural aberrations observed when including gaps and excluding gaps.
[B]- No control was used for the 44 hour treatment period. After 20 hours continuous treatment with MMC, an increase in structural aberrations was observed.

In the presence of S9 mix and after 3 h of treatment and 20 h harvest and after 3 h of treatment and 44 h harvest, there was no reduction of mitotic index up to 5000 µg/mL and no increase in the frequency of structural aberration formation compared to the

vehicle control group. The positive control (cyclophosphamide) reduced the MI to 35% and induced structural chromosome aberrations by 21% compared to the vehicle controls.

Based on the increased frequency of structural chromosome aberration formation observed in the absence of S9 mix at 44 h of treatment, the reviewer concludes the (b) (4) has mutagenic potential under these experimental conditions. While these data show that (b) (4) is positive for genotoxicity, the peptide class of drugs has a low genotoxic risk.

2.6.6.9 Discussion and Conclusions

In support of the proposed SC route of administration for this NDA, the sponsor conducted two 14-day SC toxicity studies in the rat, one 13-week SC toxicity study in the rat (submitted as a combined 13-week toxicity and fertility and early embryonic development study), a SC dose-ranging study in the dog, a 4-week SC toxicity study in the dog (twice weekly dosing), and two 13-week SC toxicity studies in the dog (1 with daily SC dosing and 1 with daily and TID administered twice weekly SC dosing). All studies demonstrated local injection site irritation. After 13-weeks repeat SC dosing in rats up to 10 mg/kg/day using a batch of icatibant synthesized by a new manufacturer, no systemic toxicities were observed. However, in two 14-day repeat dose SC studies using a batch of icatibant synthesized by a different (old) manufacturer, icatibant administration from 0.1 to 50 mg/kg/day resulted in significant injection site irritation at 50 mg/kg/day, decreases in prostate weights and testes weights in the 10 mg/kg/day and 50 mg/kg/day and increases in thymic weights in the HD (50 mg/kg/day) females. Histopathological findings included: epididymides (oligospermia and aspermia) and testes (hypospermia) were observed in icatibant treated groups and injection site irritation was observed in the 50 mg/kg/day group. These findings were not observed in the male reproductive organs at 1 mg/kg. The identification of a NOAEL of 10 mg/kg after 13 weeks while none was identified at 14 days may be due to adaptive responses to lower drug doses over time or differences in the impurity profiles of the differently manufactured batches. Of note, the 13 weeks study dosed to only 10 mg/kg while the 14-day study dosed to 50 mg/kg.

In dogs, SC administration of icatibant for 13-weeks (10, 30 and 100 mg/kg/day), resulted in clinical signs of toxicity included erythema of the face, injection site irritation, abnormal vocalization and several incidences of gastrointestinal disturbances (diarrhea and/or vomiting) at all doses. Macroscopic findings were observed at injection sites (hematomas and edema), the male (testes, epididymides and prostate) and female (ovaries and uterus) reproductive organs (organs all immature at all doses), thymus, liver and adrenal gland. Microscopic changes in the male (testes, epididymides, and prostate) and female (mammary gland, ovaries, uterus and vagina) reproductive organs, at the injection site (mainly HD groups but sporadic in LD and MD groups), in the adrenal gland (vacuolated cortical cell in HD males and females), liver (vacuolated hepatocyte in 1/4 males) and thymus (all doses) were observed. No NOAEL was determined for this study due to reproductive organ effects in all icatibant treated males and females, lymphoid depletion in the thymus and on injection site irritation. When altering the dosing schedule from daily administration to twice weekly administration of TID doses of icatibant in dogs (3.3 mg/kg TID twice weekly) for 13-weeks, no affects were observed in the male

reproductive organs but females treated with SC administration of icatibant twice weekly showed delayed or impaired maturation of the reproductive organs. Lymphoid depletion in the thymus was observed in both males and females after twice weekly dosing. Observed clinical signs after daily dosing of icatibant at 3 and 10 mg/kg/day included increased erythema and edema at multiple sites that increased with increased dose. With cessation of treatment erythema abated. After daily administration of icatibant at 3 and 10 mg/kg/day, the thymus (lymphoid depletion) and the male (immature testes, epididymides and prostate) and female (immature ovaries, oviducts, uterus and vagina) reproductive tissues showed toxicity as reflected by reduction in these organ weights relative to body weights, macroscopic findings of reduced size and microscopic findings of reduced size. After the 13-week recovery period, 1/2 males showed the testes and prostate as immature where 1/2 males had recovered and there were no findings in female reproductive organs suggesting these adverse effects were reversible. Hormonal analysis showed that icatibant induced a decrease of testosterone, LF and FSH in male dogs, which secondarily induced sexual immaturity.

When dosing dogs for a shorter duration (4-weeks) at 1 and 3.3 mg/kg TID as twice weekly dosing erythema was observed in low incidence in males and females at each dose and gross and microscopic findings were limited to injection site irritation (hematomas) which did not follow a dose response. Testosterone levels were significantly decreased in males treated with icatibant at 1 or 3.3 mg/kg TID twice weekly but these levels rebounded between dosing intervals. A NOAEL of 3.3 T.I.D. (10 mg/kg) administered twice weekly was determined by the reviewer based on no microscopic drug-related findings.

At 13-weeks daily SC dosing of rats showed no toxicity after administration of a newly manufactured batch of icatibant from a different site manufacturing site. However, after IV and SC administration of icatibant for 1-month or 14-days, respectively, the target organs of toxicity were the male reproductive organs, kidneys, stomach and the injection sites (using old icatibant manufacturer). Dogs administered icatibant SC daily for 13 weeks showed erythema of the face (histamine reaction), male and female reproductive organ immaturity, thymus (lymphoid depletion), adrenal (cortical cellular vacuolation), liver (vacuolated hepatocyte) and injection site irritation. After 1-month IV administration, erythema and chronic gastritis were observed in dogs. When altering the dosing schedule in dogs from daily administration to twice weekly dosing, the effects on the male reproductive organs were reduced. However, females showed immature reproductive organs.

The sponsor submitted three genetic toxicology studies in this NDA. These studies were: a bacterial reverse mutation assay (a complete bacterial strain battery), an *in vitro* mammalian chromosome aberration test, and an *in vivo* bone marrow micronucleus assay in Sprague-Dawley rats using the icatibant. The bacterial reverse mutation assay and the *in vivo* micronucleus study in rats were studies used to bridge the change in manufacturer for the drug substance. Icatibant did not induce genetic toxicity in these three studies under the conditions assayed. These results are consistent with numerous other studies previously reviewed under INDs (b) (4)

Two reproductive toxicology studies were included in this NDA to complete the reproductive toxicology battery. The sponsor combined the 13-week repeat dose toxicity study and the effects on fertility and early embryonic development to implantation study in rats. Male and female Sprague-Dawley rats were administered 1, 3 and 10 mg/kg/day of icatibant SC. HD treated females (10 mg/kg/day) had an increase in pre-implantation loss compared to vehicle treated females. Icatibant had no effects on male sperm count, motility, or morphology. Based on no toxicity observed in male seminology and no effects on mating or fertility indices, the NOAEL for male fertility was 10 mg/kg/day. Based on an increase of pre-implantation loss observed in HD treated females, the NOAEL for female fertility was 3 mg/kg/day.

In the second reproductive toxicity study submitted in this NDA, the effects of daily SC administration of icatibant on pre- and post-natal development in pregnant rats at 1, 3 and 10 mg/kg/day. Icatibant treatment delayed partuition at all doses, resulting in F_0 deaths (found dead or early sacrifice) in the 3 and 10 mg/kg/day groups. Clinical signs of toxicity (pallor of eyes and extremities, round back, piloerection and reddish discharge in vagina) were observed in 1 MD and in multiple HD F_0 females. After SC administration, icatibant and the M2 metabolite is found in rat milk. Based on the observed maternal toxicities including clinical signs, prolonged gestation periods for all icatibant dosage groups, F_0 spontaneous deaths and early sacrifice of icatibant treated dams, litter deaths, and necropsy findings in the MD and the HD groups, the maternal toxicity NOAEL could not be defined. The F_1 generation showed an increase in the number of pup deaths and cannabalism in the MD and HD compared to the VC groups. Therefore, the NOAEL for systemic toxicity in the F_1 generation was determined to be 1 mg/kg/day. The F_1 generation showed a slight decrease in air righting and hair growth compared to the VC control group. Therefore, the NOAEL for development of the F_1 generation was considered 3 mg/kg/day. The sponsor has adequately completed the reproductive toxicity battery for icatibant based on the 2 new studies submitted to the NDA and previously reviewed studies under IND (b) (4) Based on the reproductive toxicity data, icatibant should be classified as Pregnancy Category C based on the presence of embryotoxicity, increased spontaneous abortions and increased pup deaths.

Two local toxicity studies were conducted in rabbits using single dose SC administration in one study and intrarterial/intravenous/paravenous administrations in the second study. Icatibant administered via the SC route up to 40 mg in acetate buffer, pH 5.5 was comparable to the vehicle control for local irritation. In the second study, administration of icatibant up to 0.5 mg intra-arterially and intravenously and 0.1 mg paravenously resulted in little injection site irritation.

Two single-dose studies administering two unnatural, synthetic peptides that are both found in the icatibant drug substance, (b) (4) at 1000 mg/kg, SC were conducted by the sponsor. Neither study was adequately designed with the absence of control groups. No animals died after (b) (4) administration. However, after (b) (4) (2/3) females died within 24-h post-dose.

An *in vitro* hemolysis test of icatibant showed that 0.1% of icatibant did not induce hemolysis of human whole blood after incubation for 30 min where incubation with the positive control (distilled water for injection) did induce hemolysis.

In an assessment of antibody responses in rat, dog and monkey, the sponsor concluded that hyperimmunization attempts, even with icatibant-protein conjugates in combination with adjuvant, did not elicit a sustained antibody response against icatibant. Based on the lack of information provided by the sponsor (icatibant lot and purity and detailed methods of study), no concrete conclusions can be drawn from this study report. However, based on the summary data provided by the sponsor, icatibant-thyroglobulin conjugate did not elicit a strong anti-icatibant antibody response.

For safety qualification of the (b) (4) impurity, the sponsor conducted 1 acute IV toxicity study, a bacterial reverse mutation study and a chromosome aberration study. (b) (4) was negative in the Ames test but positive in the chromosomal aberration test for inducing genotoxicity. Although positive for genotoxicity in the chromosome aberration test, (b) (4) is a member of the peptide drug class which is considered to have low genotoxic risk. The acute IV toxicity study dosed rats up to 100 mg/kg. No toxicity was observed in rats. However, the duration of this study is not considered acceptable to qualify this impurity for safety. Therefore, (b) (4) has not been adequately qualified for safety.

2.6.6.10 Tables and Figures

Not applicable because no new data were submitted.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Not applicable because no new data were submitted.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Jerini US, Inc. (Jerini) submitted this NDA 22-150 for Firazyr (icatibant) 30 mg, SC for the treatment of adults (≥ 18 years old) with hereditary angioedema (HAE). The proposed dosing regimen is a single SC dose of 30 mg icatibant (0.6 mg/kg for a 50 kg human) which is associated with a systemic exposure of 4107 ng*h/mL. The potential maximum human dose is 90 mg (1.8 mg/kg for a 50 kg human) which is associated with systemic exposures of 12.3 mcg*h/mL in humans.

HAE patients have acute episodes of swelling of the hands, feet, trunk, face, gut and/or airways. Swelling of the upper airways can be fatal if not treated promptly. In Type I (quantitative deficiency) and Type II (qualitative deficiency) HAE a deficiency of the C1-esterase inhibitor (C1-INH) has been identified. C1-INH is a serine protease inhibitor that inhibits components of the complement, coagulation, fibrinolytic and kinin-releasing

systems. C1-INH inhibits kallikrein, a protease that contributes to the formation of bradykinin (BK) from the cleavage of kininogen. HAE patients have increased levels of BK. BK binds to the BK type 1 (B_1) and type 2 (B_2) receptors to trigger activation of many biochemical cascades. Two such cascades are coupled by the $G_{\alpha q/11}$ and $G_{\alpha i}$ proteins, leading to calcium mobilization and MAPK activation, respectively. Calcium mobilization and MAPK activation induces additional cellular events including cellular proliferation, excitation of neurons, vasodilatation, increased vascular permeability and smooth muscle cell contraction. These events are believed to contribute to the acute inflammation and edema observed in HAE patients.

Icatibant is a synthetic decapeptide with a similar structure to endogenous BK but contains 5 non-proteinogenic amino acids. Icatibant was shown to be a competitive B_2 receptor antagonist with similar affinity as BK in numerous *in vitro* receptor binding assays and in cell and tissue culture models. The sponsor has proposed that treatment of HAE patients with icatibant may decrease BK-induced downstream events, including vascular permeability, vasodilation and inflammation. As shown by *Han et al.*[1], C1-INH deficient mice show increased vascular permeability, similar symptomology as HAE patients, which was reversed after treatment with icatibant.

Safety pharmacology studies that were conducted under IND (b) (4) and reviewed by Dr. Choi showed effects on CNS (decreased motor activity, assumption of prone position, reduced respiration, ptosis, piloerection and mydriasis at 1-10 mg/kg IV), CVS (icatibant decreased coronary blood flow in isolated guinea pig heart, aggravated the duration of post-ischemic reperfusion arrhythmias in isolated working hearts in Wistar rats and impaired cardiodynamics) and renal systems (decreased urine output, sodium and potassium excretion, to a lesser extent for GFR, renal plasma flow and osmolar clearance in beagle dogs at 0.3 mg/kg, IV). These effects were considered the result of the partial agonistic property of icatibant on B-receptors. These effects were not observed in rats or dogs in 13-week repeat dose toxicity studies. In safety pharmacology studies submitted to this NDA, icatibant did not inhibit HERG channels up to 300 µM icatibant concentrations, did not have an effect on respiratory function or GI transit in rats after IV administration up to 10 mg/kg. Gastric acid volume and acidity were decreased in rats compared to the vehicle control treatment in rats at 10 mg/kg, IV.

Icatibant is rapidly metabolized to M1 (b) (4) and M2 (b) (4) which have been identified in mice, rats, dog, monkey and man. The major metabolite in man is M1 (>90%). M1 is formed in rat (~70%), dog (~60%) and mouse (~50%). After SC administration, the M2 metabolite (b) (4) appears rapidly (0.2 to 3 h post-dose) in rats and dogs. In rats and dogs, increases in icatibant doses from LD to MD doses (0.1 to 3 mg/kg in rats and 10 to 30 mg/kg/day in dogs) produced approximately doses proportionate increases in systemic exposures. Increases in icatibant doses from MDs to HDs resulted in super-proportionate increases in systemic exposures in both rats (increased ~128-fold instead of the expected ~50-fold) and approximately dose proportionate in dogs. Using an alternate dosing schedule in dogs (1 or 3.3 mg/kg TID of

[1] Han *et al.*, J. Clin. Invest. 109: 1057-1063, 2002 (cited by the sponsor)

icatibant twice weekly), AUC levels of icatibant and M2 increased in an approximate dose proportionate manner. The t_{max} was achieved from 0.5 to 3.0 h post-dose in rats and dogs. Neither icatibant nor M2 accumulated after repeat dosing in males and females up to 13-weeks dosing. There were no gender differences observed in rats or dogs in the systemic exposures of males and females.

Icatibant is rapidly absorbed with 100% bioavailability after subcutaneous administration. Protein binding was low in all species examined: 49% in rat, 44% in man, and ~38% in dog and monkey. Distribution studies showed that icatibant (or its metabolites) is distributed into the kidneys, liver, urinary bladder, lungs and spleen 1 hour and it still detectable in the liver, kidneys, spleen, bone marrow, adrenals and injection sites at 24-hours post SC dose in rats. Concentrations of radioactivity was detected in the milk of nursing rats with peak levels observed 7 h after administration of radiolabeled drug. Icatibant is not degraded the two main bradykinin-cleaving enzymes, angiotensin converting enzyme (ACE) and neutral endopeptidase. Neither icatibant nor the M1 or M2 metabolites are metabolized by human hepatocytes or microsomes. Additionally, icatibant is not an inducer of CYP450 isozymes. Therefore, icatibant is metabolically more stable than BK. Based on excretion studies, the primary route of elimination after SC administration of icatibant is via the kidneys in mice (~97.1%), rats ~61%) and dogs (~63%) with small amounts excreted in the feces (8.0% to 33%). Excretion was mainly renal, regardless of species and route of administration

Under IND (b) (4) acute toxicology studies conducted in the mouse, rat and dog were reviewed (Dr. Choi's review). The highest non-lethal IV dose was 30.7 mg/kg and the minimum lethal dose (MLD) was 48.3 mg/kg for mice. For rats, the highest non-lethal IV dose was 12.3 mg/kg and the MLD was 19.2 mg/kg. After IV dosing, rapid respiration, twitching and convulsions were observed prior to death. In an acute IV toxicity study, male dogs (n=2/group) were administered 0.4, 2 and 10 mg/kg icatibant. No mortality was observed in this study. However, numerous clinical signs, many suggestive of massive histamine release, were observed in the MD and HD groups. In a separate study, male dogs (n= 3/group) were administered single IV doses (infused over 5 h period) of 15, 30 and 60 mg/kg. An increase in serum CPK levels were above historical background (153 mU/mL) was noted in 1 MD animal (406 mU/mL) and two HD dogs (843 and389 mU/mL, respectively. The NOAEL in this study was 15 mg/kg. In acute SC toxicity studies, the highest non-lethal dose was 614 mg/kg and the MLD was 767 mg/kg for mice. In rats, no mortality was observed after a single SC injection of 1227 mg/kg icatibant. After SC injection, injection sites showed bluish discoloration that progressed to necrosis.

During icatibant's development, intranasal (IN), inhalation (IH), IV and SC routes of administration have been explored. Under INDs (b) (4), the main repeat dose toxicology studies investigated the intranasal (IN), inhalation (IH) and IV routes of administration with a few studies conducted using the SC route. Due to low bioavailability, no toxicity was observed in most IN toxicity studies. However, reduced testicular weights and spermiogenesis in the testes were observed in a single 1-month IN

toxicity study carried out in the rat at 125, 1250 or 12500 µg/kg. The NOAEL was considered 125 µg/kg based on these testicular findings.

In a comparative IV/SC/IN 1-month toxicity study, male rats were either intravenously (10 mg/kg/day), subcutaneously (10 mg/kg/day) or intranasally (2.5 mg/animal/day) administered icatibant. Renal tubular atrophy was observed in 7/10 rats and renal tubular necrosis in 4/10 rats treated via the IV route. Impairment in sperm maturation was observed in 8/10 rats and oligospermia in the epididymides in 7/10 rats after IV adminsitration. Tubular atrophy (4/10) and tubular necrosis (1/10) were also observed in SC treated rats. Despite similar exposure levels, oligospermia in the epididymides in 1/10 animals was the only male reproduction effect observed in SC treated animals. No control groups were included in this study. TK analysis showed the exposure (AUC_{0-4h}) to be similar between IV (20.33 µg/mL) and SC (17.3 µg*h/mL) treated animals. No adverse effects were reported in the intranasal group.

In a 1-month toxicity study, rats were intravenously administered 0.1, 1 and 10 mg/kg/day of icatibant. Renal toxicity was observed in 10/10 males and 5/10 females with areas of epithelial necrosis, areas of repair and foci of tubular atrophy at the 10 mg/kg/day dose. Irreversible tubular atrophy was present in 5/5 males and 1/5 females after a 28-day recovery period. The NOAEL was 1 mg/kg/day. In a 1-month study, dogs were intravenously administered 0.1, 0.25 and 0.8 mg/kg/day. All HD males 3/3 had swelling of flews that was attributed to histamine release. The major part of the glandular portions in the basal mucosa was missing from the stomach of 1 HD male that was reported as a relatively rare change. The NOAEL was 0.25 mg/kg/day.

Based on the previous reviews (b) (4) of the 1-month repeat dose toxicity studies in rats and dogs, the target organs toxicity in the rats were the male reproductive organs (decreased spermatogenesis and decreased weights of testes, epididymis, prostate, and/or seminal vesicles), kidneys (uneven color, increased weight, epithelial necrosis of proximal tubules, foci of tubular atrophy, increased blood urea, uric acid, creatinine, hemoglobin and epithelial cells in urine), stomach (taut and enlarged) and liver (increased wt). In the dogs, the target organs were swelling of flews (histamine release reaction) and the stomach (chronic gastritis).

In support of the proposed SC route of administration for this NDA, the sponsor conducted two 14-day SC toxicity studies in the rat, one 13-week SC toxicity study in the rat (submitted as a combined 13-week toxicity and fertility and early embryonic development study), a SC dose-ranging study in the dog, a 4-week SC toxicity study in the dog (twice weekly dosing), and two 13-week SC toxicity studies in the dog (1 with daily SC dosing and 1 with daily and TID administered twice weekly SC dosing). After 13-weeks repeat SC dosing in rats up to 10 mg/kg/day using a batch of icatibant synthesized by a new manufacturer, no systemic toxicities were observed. However, in two 14-day repeat dose SC studies using a batch of icatibant synthesized by a different (old) manufacturer, icatibant administration from 0.1 to 50 mg/kg/day resulted in significant injection site irritation at 50 mg/kg/day, decreases in prostate weights and testes weights in the 10 mg/kg/day and 50 mg/kg/day and increases in thymic weights in

the HD (50 mg/kg/day) females. Histopathological findings included: epididymides (oligospermia and aspermia) and testes (hypospermia) were observed in icatibant treated groups and injection site irritation was observed in the 50 mg/kg/day group. These findings were not observed in the male reproductive organs at 1 mg/kg. The identification of a NOAEL of 10 mg/kg after 13 weeks while none was identified at 14 days may be due to adaptive responses to lower drug doses over time or differences in the impurity profiles of the differently manufactured batches. Of note, the 13 weeks study dosed to only 10 mg/kg while the 14-day study dosed to 50 mg/kg.

In dogs, SC administration of icatibant for 13-weeks (10, 30 and 100 mg/kg/day), resulted in clinical signs of toxicity included erythema of the face, injection site irritation, abnormal vocalization and several incidences of gastrointestinal disturbances (diarrhea and/or vomiting) at all doses. Macroscopic findings were observed at injection sites (hematomas and edema), the male (testes, epididymides and prostate) and female (ovaries and uterus) reproductive organs (organs all immature at all doses), thymus, liver and adrenal gland. Microscopic changes in the male (testes, epididymides, and prostate) and female (mammary gland, ovaries, uterus and vagina) reproductive organs, at the injection site (mainly HD groups but sporadic in LD and MD groups), in the adrenal gland (vacuolated cortical cell in HD males and females), liver (vacuolated hepatocyte in 1/4 males) and thymus (all doses) were observed. No NOAEL was determined for this study due to reproductive organ effects in all icatibant treated males and females, lymphoid depletion in the thymus and on injection site irritation. When altering the dosing schedule from daily administration to twice weekly administration of TID doses of icatibant in dogs (3.3 mg/kg TID twice weekly) for 13-weeks, no affects were observed in the male reproductive organs but females treated with SC administration of icatibant twice weekly showed delayed or impaired maturation of the reproductive organs. Lymphoid depletion in the thymus was observed in both males and females after twice weekly dosing. Observed clinical signs after daily dosing of icatibant at 3 and 10 mg/kg/day included increased erythema and edema at multiple sites that increased with increased dose. With cessation of treatment erythema abated. After daily administration of icatibant at 3 and 10 mg/kg/day, the thymus (lymphoid depletion) and the male (immature testes, epididymides and prostate) and female (immature ovaries, oviducts, uterus and vagina) reproductive tissues showed toxicity as reflected by reduction in these organ weights relative to body weights, macroscopic findings of reduced size and microscopic findings of reduced size. After the 13-week recovery period, 1/2 males showed the testes and prostate as immature where 1/2 males had recovered and there were no findings in female reproductive organs suggesting these adverse effects were reversible. Hormonal analysis showed that icatibant induced a decrease of testosterone, LF and FSH in male dogs, which secondarily induced sexual immaturity.

When dosing dogs for a shorter duration (4-weeks) at 1 and 3.3 mg/kg TID as twice weekly dosing erythema was observed in low incidence in males and females at each dose and gross and microscopic findings were limited to injection site irritation (hematomas) which did not follow a dose response. Testosterone levels were significantly decreased in males treated with icatibant at 1 or 3.3 mg/kg TID twice weekly but these levels rebounded between dosing intervals. A NOAEL of 3.3 T.I.D. (10 mg/kg)

administered twice weekly was determined by the reviewer based on no microscopic drug-related findings.

At 13-weeks daily SC dosing of rats showed no toxicity after administration of a newly manufactured batch of icatibant from a different site manufacturing site. However, after IV and SC administration of icatibant for 1-month or 14-days, respectively, the target organs of toxicity were the male reproductive organs, kidneys, stomach and the injection sites (using old icatibant manufacturer). Dogs administered icatibant SC daily for 13 weeks showed erythema of the face (histamine reaction), male and female reproductive organ immaturity, thymus (lymphoid depletion), adrenal (cortical cellular vacuolation), liver (vacuolated hepatocyte) and injection site irritation. Clinical studies have shown local injection irritation effects. After 1-month IV administration, erythema and chronic gastritis were observed in dogs. When altering the dosing schedule in dogs from daily administration to twice weekly dosing, the effects on the male reproductive organs were reduced. However, females showed immature reproductive organs.

At a pre-NDA meeting held January 24, 2007 between Jerini and DPAP, Jerini was informed of the need for chronic toxicity studies (26-week in rat and 39-week in dog) to support the chronic intermittent HAE indication. Jerini indicated that these studies have been initiated as of June 2007 (dog study) and July 2007 (rat study). The final study reports are expected to be submitted to the Division in September 2008.

The sponsor submitted three new genetic toxicology studies in this NDA. These studies were: a bacterial reverse mutation assay (a complete bacterial strain battery), an *in vitro* mammalian chromosome aberration test, and an *in vivo* bone marrow micronucleus assay in Sprague-Dawley rats using the icatibant. The bacterial reverse mutation assay and the *in vivo* micronucleus study in rats were studies used to bridge the change in manufacturer for the drug substance. Icatibant did not induced genetic toxicity in these three studies under the conditions assayed. The results of these studies are consistent with numerous others that were previously reviewed under INDs (b) (4)

The sponsor has not submitted studies to evaluate icatibant's carcinogenic potential. Jerini was informed of the need for 2 carcinogenicity studies to address the carcinogenic potential of icatibant (pre-NDA meeting January 24, 2007). Jerini indicated that the carcinogenicity studies would be initiated April 8, 2008 and July 7, 2008 for mice and rats, respectively.

Under IND (b) (4) a 5-week fertility study in male mice was conducted up to 80.8 mg/kg/day SC. There were no effects on copulation rate in icatibant treated animals compared to the vehicle control group. This study did not follow current Segment 1 study guidelines. Two teratogenic studies were reviewed (1 in rats and 1 in rabbits), which were preceded by dose-ranging studies. In the dose-ranging study conducted in rats, 3 pregnant rats/group were treated with 25, 50 and 100 mg/kg/day (no vehicle control was used) of icatibant SC from Days 7 through 18 of pregnancy. Significant maternal toxicity was observed at 25 mg/kg/day and higher (slight swelling at the injection sites, blue

discoloration of skin, panting, reduced activity, abdominal position and then death) but concepti from this group were considered unremarkable with no teratogenicity observed.

In the definitive rat study, the effects of icatibant SC administration on morphological and post-natal development including fertility in Wistar rats was examined. Female rats (30 pregnant rats/group) were treated with 0 (vehicle control- 0.9% NaCl), 0.25, 2.5 and 25 mg/kg/group of icatibant SC from Days 7 through 18 of pregnancy. There were no significant major external of internal anomalies reported in pups for F_1 or F_2 generations. Although 1 or 2 fetuses in the 0.25 and 2.5 mg/kg group had multiple minor skeletal malformations or variations. Post-natal development of treated fetuses and fertility of F_1 generation were comparable to the control group. No major terata were produced in the study although 1 dam in the 25 mg/kg/day group had empty implantation sites in the uterus, and fetuses in the 25 mg/kg/day group had slightly greater numbers with less ossification of skeletons and variations of liver and kidneys than the control group (4 fetuses had lobus dexter of liver and 3 fetuses had distended pelvises in the kidney, while 1 fetus in the control group had the same findings). Therefore, icatibant was not teratogenic in rats under the conditions studied.

In a preliminary dose-ranging in rabbits, 2 pregnant rabbits/group were treated daily with SC injections up to 50 mg/kg/day of icatibant from Days 6 through 18 (no vehicle control was used). Rabbits were sacrificed on Day 29 of pregnancy and their uteri were examined. One dam in the 10 mg/kg group had only empty implantation sites in the uterus, indicating a clinically unnoticed abortion, but the second dam had 8 normally developed live fetuses. All dams in the 25 and 50 mg/kg/ groups had empty implantation sites in the uterus. Based on this study, doses of 0.1, 1 and 10 mg/kg/day were selected.

In the definitive rabbits study, Himalayan rabbits were treated with 0 (vehicle control- 0.9% NaCl), 0.1, 1.0 and 10 mg/kg/day of icatibant, SC once daily from Days 7 through 18 of pregnancy. Four unexpected abortions were produced in 2 dams each in all 3 treated groups and a premature birth in 0.1 mg/kg group, while none of these occurred in the control group. No other toxic effects were produced in the icatibant treated dams. At autopsy, light-grey depression was found on the surface of kidneys in 5-7 dams each in all 3 icatibant treated groups but only 1 dam in the control group. To determine if these effects were drug induced, the study was repeated but with 2 lower doses only and the vehicle group. The abortions or premature births were not reproduced in this study, and incidence of light grey colored kidneys in the treated groups was not increased, but it was also reported in 1 dam each in treated groups only. The depression on the kidney was due to chronic interstitial nephritis, and it was attributed to the strain of rabbits by the sponsor. Morphological examination of fetuses showed no increase in incidence of major malformations in the treated groups. Minor anomalies or variations of liver or kidneys were reported in some fetuses, but they were reported as within the normal ranges. Among the dead fetuses examined (6 in control, 4 in 1 mg/kg and 15 in the 10 mg/kg groups), 10 mg/kg had significantly more stunted (8/15) or retarded fetuses (7/15) over the control group (2/6 and 1/6, respectively). Icatibant was not teratogenic under the test conditions. All 3 doses of icatibant did not have significant toxic effects on the dams. However, 10 mg/kg was toxic to the embryo reducing the litter sizes as a result of

increased intrauterine embryofetal death rate. Icatibant treatment resulted in dose-related decreases for total implantations and total number of live fetuses as well as dose-related increases for percent of pre-implantation loss when compared to the control values.

Under the conditions tested in the rats and rabbits, icatibant did not induce major terata. However, icatibant has a potential to produce abortions/premature births, intrauterine death or resorptions.

Two reproductive toxicology studies were included in this NDA to complete the reproductive toxicology battery. The sponsor combined the 13-week repeat dose toxicity study and the effects on fertility and early embryonic development to implantation study in rats. Male and female Sprague-Dawley rats were administered 1, 3 and 10 mg/kg/day of icatibant SC. HD treated females (10 mg/kg/day) had an increase in pre-implantation loss compared to vehicle treated females. Icatibant had no effects on male sperm count, motility, or morphology. Based on no toxicity observed in male seminology and no effects on mating or fertility indices, the NOAEL for male fertility was 10 mg/kg/day. Based on an increase of pre-implantation loss observed in HD treated females, the NOAEL for female fertility was 3 mg/kg/day. These conclusions were in agreement with the sponsor's conclusions.

In the second reproductive toxicity study submitted in this NDA, the effects of daily SC administration of icatibant on pre- and post-natal development in pregnant rats at 1, 3 and 10 mg/kg/day. Icatibant treatment delayed partuition at all doses, resulting in F_0 deaths (found dead or early sacrifice) in the 3 and 10 mg/kg/day groups. Clinical signs of toxicity (pallor of eyes and extremities, round back, piloerection and reddish discharge in vagina) were observed in 1 MD and in multiple HD F_0 females. After SC administration, icatibant and the M2 metabolite is found in rat milk. Based on the observed maternal toxicities including clinical signs, prolonged gestation periods for all icatibant dosage groups, F_0 spontaneous deaths and early sacrifice of icatibant treated dams, litter deaths, and necropsy findings in the MD and the HD groups, the maternal toxicity NOAEL could not be defined. The F_1 generation showed an increase in the number of pup deaths and cannabalism in the MD and HD compared to the VC groups. Therefore, the NOAEL for systemic toxicity in the F_1 generation was determined to be 1 mg/kg/day. The F_1 generation showed a slight decrease in air righting and hair growth compared to the VC control group. Therefore, the NOAEL for development of the F_1 generation was considered 3 mg/kg/day. The sponsor has adequately completed the reproductive toxicity battery for icatibant and the data indicate that icatibant should be classified as a Pregnancy Category C based on the presence of embryotoxicity, increased spontaneous abortions and increased pups deaths.

Under IND (b) (4) icatibant treatment at 1.25 mg/kg SC for 28 days had no effect on the pituitary-testicular axis during the dosing phase (most likely due to the low icatibant dose). Interestingly, an unexpected activation, significantly increased release and synthesis of testosterone in the serum and testes, after the 8-week recovery period was observed, suggesting that these organs may have been suppressed during the 4-weeks of treatment period at a sub-threshold level.

Local tolerance studies in rabbits showed that intra-articular injection of icatibant was well tolerated. In two local irritation studies (primary skin irritation study of rabbit skin and mucosal tolerance study in rabbit eye) icatibant was considered a non-irritant at 0.5 mg/mL icatibant solution. Icatibant administered via the SC route up to 40 mg in acetate buffer, pH 5.5 was comparable to the vehicle control for local irritation. In an antigenicity study, no anti-icatibant antibodies were produced when guinea pigs were sensitized with icatibant alone in intranasal or SC route. However, when guinea pigs were sensitized subcutaneously with icatibant plus Freund's adjuvant, antibody formation was observed when challenged with icatibant-BSA conjugated. In another study, a guinea pig maximization test, 8/10 icatibant treated guinea pigs plus Freund's adjuvant had slight erythema, while the control group showed no reaction. Therefore, icatibant was a sensitizer when administered with Freund's adjuvant in 2 studies. An *in vitro* hemolysis test of icatibant showed that 0.1% of icatibant did not induce hemolysis of human whole blood after incubation for 30 min where incubation with the positive control (distilled water for injection) did induce hemolysis.

For safety qualification of the (b) (4) impurity, the sponsor conducted 1 acute IV toxicity study, a bacterial reverse mutation study and a chromosome aberration study. (b) (4) was negative in the Ames test but positive in the chromosomal aberration test for inducing genotoxicity. Although positive for genotoxicity in the chromosome aberration test, (b) (4) is a member of the peptide drug class which is considered to have low genotoxic risk. The acute IV toxicity study dosed rats up to 100 mg/kg. No toxicity was observed in rats. However, the duration of this study is not considered acceptable to qualify this impurity for safety. Therefore, (b) (4) has not been adequately qualified for safety. This impurity and others are the subject of a consult review for ONDQA and comments to the sponsor will be forthcoming from the ONDQA reviewer, Dr. Eugenia Nashed.

Based on the completion of repeat dose toxicity studies conducted in dogs and rats up to 13-weeks duration, the target organs of toxicity were the male and female reproductive organs, the injection site irritation (with possible histamine release), thymus (lymphoid depletion), adrenal (cortical cellular vacuolation) and liver (vacuolated hepatocyte). When altering the dosing schedule from daily administration to twice weekly dosing in dogs, the effects on the male reproductive organs were reduced. However, females still showed immature reproductive organs. The 13-wk SC repeat dose study in rats produced a NOAEL of 10 mg/kg using a batch of icatibant synthesized by a new manufacturer. In the 13-wk dog study, no NOAEL was defined. However, in the 4-wk dog study using an alternative dosing regimen a NOAEL of 3.3 mg/kg TID dosed twice weekly was determined (equals 10 mg/kg). The recommended clinical dose of icatibant is one 30 mg subcutaneous injection with no more than 3 injections (90 mg) per 24 hour period recommended. On an AUC basis, safety margins of 4 and 10 in the rat and dog, respectively, at a single human dose (30 mg) or 1.4 and 3.6 for the rat and the dog, respectively, at the maximum daily dose (90 mg) are observed for the parent compound at the NOAEL doses for the 13-wk rat and 4-wk dog studies, respectively. Due to the intermittent dosing (not more than 8 doses of icatibant per month) of adult HAE patients

with icatibant, the potential to observe similar toxicity as observed in animal studies is considered low. Additionally, there are currently no other approved drugs for the treatment of HAE. Lastly, the potential adverse effects of icatibant are outweighed by the risk of having a fatal HAE attack. To date, adverse effects in humans have shown local injection site irritation, which is considered monitorable.

Reproductive toxicology studies have shown that icatibant treatment dose not effect male or female fertility, or produce teratogenic effects, but may induce pre-implantation loss, spontaneous abortions, increase pup deaths and induce delayed parturition and result in difficulties in delivery. Based on the reproductive toxicity data icatibant should be classified as Pregnancy Category C.

Unresolved toxicology issues (if any): At a pre-NDA meeting held January 24, 2007 between Jerini and DPAP, Jerini was informed of the need for chronic toxicity studies (26-week in rat and 39-week in dog) and the need for 2 carcinogenicity studies to address the carcinogenic potential of icatibant to support the chronic intermittent HAE indication. To date, studies of up to only 13 weeks are available. Jerini indicated that the chronic toxicity studies have been initiated as of June 2007 (dog study) and July 2007 (rat study) and the carcinogenicity studies would be initiated April 8, 2008 and July 7, 2008 for mice and rats, respectively. In the Division's Filing Letter dated January 8, 2008, the sponsor was informed that the icatibant labeling will reflect a clinical dosing regimen that is supported by the nonclinical toxicology studies in both dose and duration; currently the sponsor has nonclinical support for subcutaneous clinical dosing for up to 13-weeks duration.

Recommendations: The nonclinical discipline recommends an approval of the application (NDA 22-150). The nonclinical data currently support a clinical dosing regimen of up to 13 weeks duration.

The following comment should be forwarded to the sponsor:
The completion of nonclinical chronic repeat dose toxicity studies (6-month in rat and 9-month in dog) via the subcutaneous route of administration is required to support chronic intermittent clinical dosing. The icatibant labeling will reflect a clinical dosing regimen that is supported by the nonclinical toxicology studies in both dose and duration. At this time you have nonclinical support for subcutaneous clinical dosing for up to 13-weeks duration. We acknowledge your submission stating that chronic toxicity studies have been initiated as of June 2007 (dog study) and July 2007 (rat study) and the carcinogenicity studies will be initiated April 8, 2008 and July 7, 2008 for mice and rats, respectively.

Suggested labeling: The review team decided not to evaluate the proposed product label at this time since the application is deemed not approvable. Therefore, labeling recommendations will not be made at this time.

Appendix/attachments: None

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/s/

Molly Shea
3/18/2008 04:45:09 PM
PHARMACOLOGIST

Timothy McGovern
3/18/2008 04:50:03 PM
PHARMACOLOGIST
I concur.

DIVISION OF PULMONARY AND ALLERGY PRODUCTS PHARMACOLOGY AND TOXICOLOGY REVIEW

Chemistry Consult #1

NDA No. 22-150 **Date of Consult:** 23 Jan 2008

Reviewer: Molly E. Shea, Ph.D. **Review Completed:** 14 Mar 2008

Information to be Conveyed to Sponsor: Yes (X), No ()

Sponsor: Jerini US, Inc., Morristown, NJ 07960

Drug:
Trade Name: Firazyr 30 mg solution for injection
Generic: Icatibant
Code name: JE049/HOE 140/S 89 0140

Chemical Name: D-arginyl-L-arginyl-L-prolyl-L[(4R)-4-hydroxyprolyl]-glycyl-L-[3-(2-thienyl)alanyl]-L-seryl-D-(1,2,3,4-tetrahydroisoquinolin-3-yl-carbonyl)-L-3aS,-7aS)-octahydroindol-2-yl-carbonyl]-L-arginine acetate

Molecular formula/molecular weight: $C_{59}H_{89}N_{19}O_{13} \cdot C_2H_4O_2$/1304.6

Drug Class: Bradykinin type 2 (B_2) receptor antagonist

Proposed Clinical Dose: For adults ≥ 18 years old: Each pre-filled syringe contains 30 mg icatibant acetate in 3 mL solution. The recommended dose of icatibant is one 30 mg subcutaneous injection per hereditary angioedema (HAE) attack. HAE patients should not exceed more than 3 injections over 24 hours, which is equivalent to 90 mg of icatibant/day. In a 50 kg adult, this is equivalent to 1.8 mg/kg or 66.6 mg/m^2 based on body surface area.

Review: Drs. Prasad Peri and Eugenia Nashed requested a safety assessment of the impurity specifications proposed in the icatibant drug substance and drug product under the paradigm of the recommendations made during the TIDES Conference 2005. Additionally, review of the qualification studies referenced in the Chemistry, Manufacturing and Controls module of the NDA (section 3.2.S.3.2.3.3, -4.2, and -4.3 (bridging toxicology) was requested.

The thresholds for the levels of impurities for peptides were announced in the TIDES Conference 2005, by Dr. Blair Fraser. These thresholds are considered as follows:

Threshold	Action
(b) (4)	Minimally identify
	Fully identify and characterize
	Fully identify, characterize and qualify for safety

In addition, the chemistry team indicated that four leachables were identified though no acceptance criteria are, as yet, provided.

Safety Assessment of Impurities in the Drug Substance and Product:

The proposed specifications for the drug substance and product organic impurities are summarized in the following tables. The specifications for all the impurities except (b) (4) [redacted] and "other individual impurities" in the drug substance exceed the acceptable qualification threshold of (b) (4) for peptides. As well, the specifications for all impurities in the drug product, including (b) (4) [redacted], exceed the acceptable qualification threshold of (b) (4) for peptides.

(b) (4)

[redacted]

[redacted] (b) (4)

A detailed review of the qualification studies for the [redacted] (b) (4) impurity is provided.

Study title: Acute intravenous toxicity in rats

Key study findings:

- There were no premature deaths, clinical signs of toxicity, statistically significant changes in BW gains and no macroscopic changes in the [redacted] (b) (4) treated animals compared to the vehicle control treated animals.
- The minimal lethal dose was > 100 mg/kg of [redacted] (b) (4) in rats.

Study no.: JE049-0155
Volume #, and page #: Electronic submission
Conducting laboratory and location: [redacted] (b) (4)
Date of study initiation: August 8, 2006
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: [redacted] (b) (4) batch no. 1005323 and 78.4% pure
Formulation/vehicle: [redacted] (b) (4) was formulated in 0.9% NaCl for intravenous administration with a 5 mL/kg vol

Methods

Doses: 0 (vehicle control) and 100 mg/kg of [redacted] (b) (4) were intravenously administered to rats as a single dose

Study design: Five Sprague-Dawley rats/sex/group were administered a single IV dose of either vehicle as the control or 100 mg/kg of the impurity. All animals were observed for clinical signs of toxicity, mortality, and changes in BW for a period of 14-days post-dose. After the 14-day period, all animals were macroscopically examined but no microscopic evaluation was conducted.

Results: There were no premature deaths, no clinical signs of toxicity and no statistically significant changes in BW gains in the [redacted] (b) (4) treated animals compared to the vehicle control treated animals. Compared to the control treated animals there were no macroscopic changes in the [redacted] (b) (4) group. The minimal lethal dose was > 100 mg/kg of [redacted] (b) (4) in rats.

Study title: Bacterial reverse mutation test

Key study findings:

- The study was considered valid based on the methodology used.

- Under the experimental conditions in this study, [redacted] (b) (4) up to 5000 µg/plate did not induce an increase in the number of revertants compared to the negative controls.

Study no.: JE049-0154
Volume #, and page #: Electronic submission
Conducting laboratory and location: [redacted] (b) (4)
Date of study initiation: August 11, 2006
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: [redacted] (b) (4) batch no. 1005323 and 78.4% pure
Formulation/vehicle: [redacted] (b) (4) was dissolved in water

Methods

Doses: For the first mutagenicity study with and without S9 mix and using the plate incorporation method the following concentrations of [redacted] (b) (4) was used: 0, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/plate. In the second study using the preincubation method the following concentrations were used with and without S9 mix: 625, 1250, 2500, 3750 and 5000 µg/plate. The maximum concentrations achieve the recommended limit concentration for this assay.

Study design: [redacted] (b) (4) was tested in a dose-ranging test and then followed by 2 independent experiments in the presence and absence of metabolic activation (S9 mix). Experiments were performed using the direct plate incorporation method except for the 2nd test with S9 mix, which was performed using the preincubation method. Five strains of *Salmonella typhimurium*: TA 1535, TA 1537, TA98, TA 100 and TA102 were assayed in the 2 definitive experiments, where strains TA98, TA 100 and TA 102 were assayed in the dose-ranging study. In the dose-ranging study, single plates/concentration were assessed. In the 2 definitive studies, 3 plates/concentration with and without S9 mix were evaluated. After 48 h at 37°C, the revertant colonies were scored using an automatic counter. The potential for cytotoxicity was evaluated by the decrease in the number of revertants and or the thinning of the bacterial lawn.

Negative control was water for injection.

The positive controls without S9 mix:
1 µg/plate of sodium azide for TA 1535 and TA100 strains
50 µg/plate of 9-aminoacridine for TA 1537 strain
0.5 µg/plate of 2-nitrofluorene for TA 98 strain
0.5 µg/plate of mitomycin C for TA 102 strain

Positive controls with S9 mix:
2 µg/plate of 2-anthramine for TA 1535, TA 1537, TA98 and TA 100 strains
10 µg/plate of 2-anthramine for TA 102 strain

(b) (4) was freely soluble in water and was non-toxic in the dose-ranging assay. Therefore, the highest concentration (5000 µg/plate) was selected for the definitive studies. There was no precipitation of the drug at the doses examined.

Results: The study was considered valid based on the methodology used for each study, the criteria for evaluating the plates and the expected outcomes for the negative and the positive controls. The number of revertants in the negative control group was within the historical control data as were the positive control revertant numbers. The sponsor examined doses up to 5000 µg/plate in the 2 definitive studies, which was adequate due to the products free solubility in water, its lack of toxicity on the bacterial strains and the absence of precipitation that potentially would interfere with plate counting. Under the experimental conditions in this study, (b) (4) did not induce an increase in the number of revertants compared to the negative controls.

Study title: *In vitro* mammalian chromosome aberration test in cultured human lymphocytes

Key study findings:

- (b) (4) induced chromosome aberrations in human lymphocytes under the study conditions assayed. This is in agreement with the sponsor's conclusions.

Study no.: JE049-0156
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: August 9, 2006
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: (b) (4) batch no. 1005323 and 78.4% pure
Formulation/vehicle: (b) (4) was dissolved in water

Methods

Doses: In the definitive study (the second experiment), the (b) (4) concentrations examined were: 0, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/mL in the absence and presence of S9 mix. The maximum concentration achieves the recommended limit concentration for this assay.

Study design: Two independent experiments (using duplicate cultures in each experiment for each concentration tested) in the presence and absence of metabolic activation (+ or –S9 mix) were conducted with different concentrations of (b) (4) to evaluate whether (b) (4) induces chromosome aberration formation in cultured human lymphocytes. The highest concentration of (b) (4) used for the first experiment was selected based on pH, osmolality and solubility. The test item was freely soluble in the vehicle (culture media) and showed no precipitation from media at 5000 µg/mL. In the first experiment, lymphocytes were exposed to controls and (b) (4) concentrations in the presence and absence of S9 mix for 3 hours and then rinsed. Cells

were harvested 20 h after the beginning of treatment. In the first experiment, the (b) (4) concentrations examined were: 39.06, 78.13, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/mL.

The second experiment exposed lymphocytes to test article and controls without S9 mix continuously until harvesting of cells at 20 or 44 h post initiation of treatment. In the presence of S9 mix, cells were exposed to controls and test article for 3 h and then rinsed and incubated for 20 h post initiation of treatment and then had the cells harvested. Cells were fixed and then spread onto glass slides and scored for aberration formation. The cytotoxicity of icatibant was determined using the mitotic index (number of cells in mitosis/1000 cells examined). Analysis of 200 metaphases/concentration of test article was made with 100 metaphases/culture when possible. Only 50 metaphase cultures were analyzed when at least 10% cells with structural chromosome aberrations were observed. All metaphase analyses were performed blinded.

Negative controls: Vehicle (culture medium)

Positive controls: In the absence of S9 mix, mitomycin C was used at a final concentration of 3 µg/mL for the 3 h incubation and at 0.2 µg/mL for the continuous incubation. In the presence of S9 mix, cyclophosphamide was used at a final concentration of 12.5 or 25 µg/mL.

Results: Based on the study methodology and the negative and positive controls providing results within their respective historical control data, and based on the (b) (4) concentrations tested, the 2 studies were considered valid. Criteria for a positive result were a reproducible and statistically significant increase in the frequency of cells with structural aberrations for at least one of the dose-levels and one of the two harvest times. Reference to historical control data was also compared.

In the first experiment after the 3 h treatment period and in the absence of S9 mix, there was little reduction in the mitotic index at 5000 µg/mL (MI 96% compared to the vehicle control) and there was no increase in structural chromosome aberrations. In the presence of S9 mix, there was no reduction in MI and no increase in structural chromosomes aberration frequency compared to the vehicle control group. The positive controls induced an increase in structural chromosome aberration formation in the presence and absence of S9 mix.

After 20 and 44 h treatments, in the absence of S9 mix, there was a slight to marked decrease in mitotic index [20 hr: MI at 53% and 37% at 2500 and 5000 µg/mL of (b) (4) respectively, compared to the vehicle controls [44 h: MI at 59%, 60% and 42% at 1250, 2500 and 5000 µg/mL of (b) (4) respectively, compared to controls]. A slight, but not statistically significant, increase in the frequency of cells with structural chromosomal aberrations (- gaps) was noted at 2500 µg/mL [mean % (- gaps) 2.0] and 5000 µg/mL [mean % (- gaps) 4.0] after the 20 h treatment.

After 44 h of treatment in the absence of S9 mix, a statistically significant increase in the mean number of cells (%) with structural aberrations (-gaps) was observed at 2500 and 5000 µg/mL. However, cytotoxicity levels significantly exceeded 50% (actual value is 42%) at 5000 µg/mL. At this level of cytotoxicity, structural aberration formation may be observed as a result of indirect mechanisms unrelated to test article. At

2500 µg/mL the cytotoxicity was at 60%. Therefore, (b) (4) did increase chromosome aberration formation in this study.

Second experiment without S9 mix: Chromosome Aberration (44-h treatment)

Concentrations (µg/mL)	No. of cells scored	Structural aberrations		Cells w/ structural aberrations	
		Total no. (+ gaps)	Total no. (– gaps)	Mean % (+ gaps)	Mean % (– gaps)
0	200	8	1	4.0	0.5
1500*	200	2	2	0.5	0.5
2500[A]	200	15	15	6.5	6.5
5000[A]	200	34	21	25.0	19.0
[B]MMC (0.2 µg/mL)	100	34	28	29	26

*-The study protocol indicates that 1250 µg/mL was tested, which differs from the concentration provided in the sponsor's table.
[A]-The reviewer questions these data due to no difference between the total number of structural aberrations observed when including gaps and excluding gaps.
[B]- No control was used for the 44 hour treatment period. After 20 hours continuous treatment with MMC, an increase in structural aberrations was observed.

In the presence of S9 mix and after 3 h of treatment and 20 h harvest and after 3 h of treatment and 44 h harvest, there was no reduction of mitotic index up to 5000 µg/mL and no increase in the frequency of structural aberration formation compared to the vehicle control group. The positive control (cyclophosphamide) reduced the MI to 35% and induced structural chromosome aberrations by 21% compared to the vehicle controls.

Based on the increased frequency of structural chromosome aberration formation observed in the absence of S9 mix at 44 h of treatment, the reviewer concludes the (b) (4) has mutagenic potential under these experimental conditions. While these data show that (b) (4) is positive for genotoxicity, the peptide class of drugs has a low genotoxic risk.

(b) (4)

(b) (4)

(b) (4)

In a later communication from Dr. Nashed, she indicated that the sponsor has not yet proposed any specification for leachables and that she will ask them to include specifications for release and stability testing and to propose adequate acceptance criteria.

Summary and Evaluation: A review of the proposed specifications of impurities in the drug substance and product concluded that most of the acceptance criteria exceed the accepted qualification threshold for peptides of (b) (4) with the exception of (b) (4) and "other individual impurities" in the drug substance. However, (b) (4) exceeds the acceptance criteria in the drug product. (b) (4) is one of the primary metabolites and, thus, is considered to be qualified for safety. The proposed acceptance criteria for impurities in the drug substance with the exception of (b) (4) and "other individual impurities" (proposed specification of (b) (4) have NOT been adequately qualified for safety based on data from the 13-week subcutaneous repeat dose toxicity study conducted in rats. Additionally, the proposed acceptance criteria for drug product impurities with the exception of (b) (4) have NOT been adequately qualified. (b) (4) has not been administered for an adequate duration in repeat

dose toxicity studies and is genotoxic in the human lymphocyte chromosome aberration study. Although resulting in a positive signal for genotoxicity, (b) (4) is a member of the peptide class which is considered to be less of a risk for genotoxicity than small molecules. Thus, the sponsor needs to conduct additional studies to qualify the drug substance and product impurities that exceed the qualification threshold of (b) (4) with the exception of (b) (4) or needs to modify the acceptance criteria to (b) (4)

The safety evaluation of any identified leachables will be conducted once the sponsor provides acceptance criteria.

Recommendation: The following impurities in the drug substance and the drug product: (b) (4) (b) (4) are not considered to be adequately qualified for safety. The sponsor should conduct an adequate qualification study (a 3-month repeat dose toxicity study in an appropriate species) that provides an appropriate margin of safety (generally 10-fold) at a NOAEL dose or modify their proposed acceptance criterion to less than (b) (4)

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/s/

Molly Shea
3/14/2008 03:30:29 PM
PHARMACOLOGIST

Timothy McGovern
3/14/2008 04:50:32 PM
PHARMACOLOGIST
I concur.

NDA Pharmacology Fileability Check List

NDA No: 22-150

Date of submission: October 22, 2007

Date of Fileability meeting: December 18, 2007

Information to Sponsor Yes (X) No ()

Date of check list: December 12, 2007

(1) On its face, is the Pharm/Tox section of the NDA organized in a manner to allow substantive review? Yes (X) No ( ) NA ( )

(2) On its face, is the Pharm/Tox section of the NDA legible for review?
Yes (X) No ( ) NA ( )

(3) Are final reports of all required and requested preclinical studies submitted in this NDA? Yes (X) No () NA ( )-

Per the pre-NDA meeting January 2007 between Jerini and the Division of Pulmonary and Allergy Products (DPAP), Jerini was notified that additional chronic repeat-dose toxicity studies in the rat (6 month) and the dog (9 month) via the clinical (subcutaneous) route of administration, and two carcinogenicity studies to address the carcinogenic potential of icatibant were required. Jerini was allowed to submit their NDA for Icatibant prior to completion of these studies due to the regulatory history of the drug. However, Jerini was told to conduct these studies as soon as possible. In addition, the chemists from DPAP notified the pharmacology review team of several impurities that will need characterization.

	Yes	No	NA
Pharmacology	(X)	( )	( )
ADME	(X)	( )	( )
Toxicology (duration, route of administration and species specified)			
acute	(X)	( )	( )
subchronic and chronic studies	(X)	(X)	()
reproductive studies	(X)	( )	( )
carcinogenicity studies	()	(X)	()
mutagenicity studies	(X)	()	( )
special studies (Impurity)	()	(X)	()
others	(X)	( )	( )

(4) If the formulation to be marketed is different from the formulation used in the toxicology studies, is repeating or bridging the studies necessary? Yes (X) No ( ) NA ()

If no, state why not?

If yes, has the applicant made an appropriate effort to repeat the studies using the to be marketed product, to bridge the studies or to explain why such repetition or bridging should not be required? Yes (X) No ( ) NA ( )

Due to a change in manufacturer in 2003 for the drug substance, the sponsor conducted bridging toxicity studies including a combined FEED and 13-week repeat dose toxicity study in rats via the subcutaneous route; an Ames test and a micronucleus test.

(5) Are the proposed preclinical labeling sections (carcinogenesis, mutagenesis and impairment of fertility, pregnancy category and overdosage) appropriate (including human dose multiples expressed in either mg/m^2 or comparative systemic exposure levels) and in accordance with 201.57? Yes () No (X).

The annotated draft labeling is in the proper SPL format. However, the sponsor does not provide appropriate human dose multiples comparisons for fertility, pregnancy category or overdosage. Additionally, a more detailed label describing the pharmacology and toxicology data and the limitations of human dosing in the absence of the nonclinical chronic repeat dose toxicology and carcinogenicity studies sections will be required.

(6) Has the applicant submitted all special studies/data requested by the Division prior to the submission including but not limited to pre-NDA discussion? Yes () No (X) NA ( )

Qualification of impurities/degradants studies have not been completed prior to submission of the NDA.

(7) On its face, does the route of administration used in the pivotal toxicity studies appear to be the same as the intended clinical route? Yes (X) No ( ) NA ( )

If not, has the applicant submitted a rationale to justify the alternative route?
Yes ( ) No ( ) NA ( )

(8) Has the applicant submitted a statement(s) that all of the toxicity studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations? Yes (X) No ( ) NA ( )

(9) Has the applicant submitted any studies or data to address any impurity or extractable issues (if any)? Yes (X) No ( ) NA ()

Studies were submitted for a single impurity (b) (4) *However, the Chemist has notified the pharmacology/toxicology of several other impurities that will need to be qualified for safety.*

(10) Are there any outstanding preclinical issues? Yes () No (X)
If yes, identify those below.

The absence of both the nonclinical chronic repeat dose toxicity studies (6 and 9 month in rat and dog, respectively) and the carcinogenicity studies was determined to not uphold filing of the Icatibant NDA due to its regulatory history. The sponsor was informed at the time of the pre-NDA (January 2007) that these studies should be completed and submitted as soon as possible. Therefore, there are no outstanding preclinical issues for this NDA.

(11) From a preclinical perspective, is this NDA fileable? Yes (X) No ( )

If no, state below why it is not.

(12) Should any additional information/data be requested? Yes (X) No ()

Comments to the Sponsor for the 74-Day Letter:

- The completion of nonclinical chronic repeat dose toxicity studies (6-month in rat and 9-month in dog) via the subcutaneous route of administration is required to support chronic intermittent clinical dosing. The Icatibant labeling will reflect a clinical dosing regimen that is supported by the nonclinical toxicology studies in both dose and duration. At this time you have nonclinical support for subcutaneous clinical dosing for up to 13-weeks duration. Provide the Division with the current status of your chronic repeat dose studies in the rat and the dog and the status of your carcinogenicity studies, also requested at the time of the pre-NDA meeting.

- As relayed to you at the pre-NDA meeting, qualify any impurity and/or degradant that exceeds the qualification threshold of (b) (4) for peptides.

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/s/

Molly Shea
12/20/2007 12:49:42 PM
PHARMACOLOGIST

Timothy McGovern
12/20/2007 01:27:42 PM
PHARMACOLOGIST
I concur.