CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-201

PHARMACOLOGY REVIEW(S)

Executive CAC
Date of Meeting: December 9, 2008

Committee:	David Jacobson Kram, Ph.D., D.A.B.T., OND IO, Member Paul Brown, Ph.D., OND IO, Member Abby Jacobs, Ph.D, OND IO, Member Sushanta Chakder, Ph.D. DGP, Alternate Member Kimberly Benson, Ph. D., Acting Pharm Tox Supervisor W. David McGuinn, Jr., M.S., Ph.D., D.A.B.T., Presenting Reviewer
Coordinator:	Sam Habet, R.Ph., Ph.D., OND IO, Senior Clinical Pharmacologist/ Science Policy Analyst (Detail)

Author of Minutes: W. David McGuinn, Jr., M.S., Ph.D., D.A.B.T.

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # :	22-201
Drug Name:	Degarelix (tradename yet undetermined)
Date of Submission:	February 14, 2008
Sponsor:	Ferring Pharmaceuticals, Copenhagen, Denmark

Background

Degarelix is a synthetic decapeptide that inhibits the GnRH receptor in the pituitary gland. This inhibition blocks the pulsatile secretion of LH and FSH. Without these signals, the gonads cannot secrete either testosterone or estradiol. Degarelix is intended to treat advanced prostate cancer. Testosterone stimulates the growth of malignant prostate derived tissues. Inhibition of testosterone secretion slows the progression of prostate cancer in some patients. This slowed progression palliates some symptoms of the disease.

Clinically, the drug is given as an initial subcutaneous loading dose of 240 mg. Subsequent doses of 80 mg are given monthly. The drug forms a subcutaneous depot and from there enters the systemic circulation slowly. Nanomolar concentrations in the blood maintain hormonal suppression throughout the dosing interval. The vehicle and route used in the two carcinogenicity studies are the same as those used clinically. In these studies the dose is given more frequently than it is clinically, once fortnightly compared to once monthly.

TWO YEAR CARCINOGENICITY STUDY IN RATS

- Species/strain: Han Wistar rats — WI(Glx/BRL/Han)IGSBR)
- Doses: daily dosing of 2, 10, and 25 mg/kg
- Vehicle: $Mannitol_{aq}$ (5% w/v)
- Route: Subcutaneous injection given fortnightly for 104 weeks
- Basis of dose selection: 13 week toxicity study in the rat

b(4)

The sponsor did not submit this protocol to the FDA for ExecCAC review prior to conducting the study.

Study Findings

The entry of the drug into the systemic circulation from the subcutaneous depot (absorption rate constant) is pharmacokinetically rate limiting. AUC increases with increasing dose but this increase is much less than dose proportional; C_{max} increases even less so. The apparent elimination half-life varies with dose and drug concentration and is usually measured as similar to or greater than the dosing interval. The value of this parameter reflects the absorption rate constant not elimination. C_{trough} values remained above the receptor binding constant in all dose groups throughout the study.

Survival among the low and mid dose animals was greater than controls. Survival among the high dose animals was comparable to controls. All treated male rats gained less weight than controls; treated females gained more. These findings are a direct result of hormonal suppression. Dosing caused dose dependant neutrophilia in female rats and mild anemia in low and mid dose male rats. Dosing was associated with some signs of mild renal and hepatic toxicity. Atrophy in the adrenal glands increased with increasing dose. Sex organs were profoundly atrophic in all treated animals.

Statistical Evaluation

The following table shows the total incidence of all benign hemangioma plus that of all malignant hemangiosarcoma.

Dose group	control				Low				Mid				High			
	HMG	HMS	Total deaths	Total incidence	HMG	HMS	Total deaths	Total incidence	HMG	HMS	Total deaths	Total incidence	HMG	HMS	Total deaths	Total incidence
male																
before day 665	0	0	5	0.0%	0	0	2	0.0%	0	0	2	0.0%	0	0	3	0.0%
day 665 to termination	0	0	4	0.0%	0	0	3	0.0%	0	0	1	0.0%	0	0	3	0.0%
Terminal necropsy	1	2	41	7.3%	1	4	45	11.1%	1	2	47	6.4%	3	1	44	9.1%
Total	1	2	50	7.3%	1	4	50	11.1%	1	2	50	6.4%	3	1	50	9.1%
Fisher's Exact for total one tail								0.150				0.380				0.220
Female																
before day 665	0	0	8	0.0%	0	0	3	0.0%	0	0	1	0.0%	0	0	5	0.0%
day 665 to termination	0	0	3	0.0%	0	0	2	0.0%	0	0	0	0.0%	0	2	3	66.7%
Terminal necropsy	0	0	39	0.0%	1	1	45	4.4%	2	2	49	8.2%	2	4	42	14.3%
Total	0	0	50	0.0%	1	1	50	4.4%	2	2	50	8.2%	2	6	50	16.0%
Fisher's Exact for total one tail								0.300				0.100				**0.009**

Dr. Karl Lin analyzed the data in the table above and determined that the result for female rats remained significant by trend analysis (table below).

Species	Sex	Combined Tumor Type	One-sided P-Values	
			Asymptotic	Exact
Rat	M	Hemangioma + Hemangioscarcoma	0.5075	0.5037
Rat	F	Hemangioma + Hemangioscarcoma	**0.0008**	**0.0013**

The Division of Drug Oncology Products added this information to the product label.

TWO YEAR CARCINOGENICITY STUDY IN MICE

- Species/strain: Male and female CD-1 mice — :CD-1 ™(ICR)BR)
- Doses: daily dosing of 0, 10 or 50 mg/kg
- Vehicle: $Mannitol_{aq}$ (5% w/v)
- Route: Subcutaneous injection given fortnightly
- Basis of dose selection: Thirteen week toxicology study in mice.

b(4)

The sponsor did not submit this protocol to the FDA for ExecCAC review prior to conducting the study.

Study findings

AUC did not increase linearly with dose on day one of dosing; the AUC at the high dose (25 fold greater than the low dose) was only 10 fold greater than the low dose AUC in males and 8 fold higher in females. The increase in C_{max} was strikingly less than dose proportional, the high dose value being only 2.1 and 1.6 fold greater than that of the low dose for females and males respectively. Again, this demonstrates that the absorption process from the subdermal depot was rate limiting. C_{trough} (just prior to the next dose) in the low dose group were on the order of k_i (1.7 nM) and considerably greater than k_i in the mid and high dose groups, thus confirming the adequacy of exposure.

Mortality was somewhat high in control females so the investigators decided to terminate this group in week in week 102. Because of this high mortality in controls, there was a statistically significant decrease in mortality in low ($p = 0.03$) and mid dose ($p = 0.026$) female mice but not in the high dose group. In males, the mortality in the high dose group was higher than in controls and this difference was significant ($p = 0.008$). Toxicities were similar to those seen in rats. Numerous tumor types decreased in incidence in treated animals due to the atrophy of the sex organs.

The following table shows the results of combining the incidence of benign hepatocellular adenoma with that of malignant hepatocellular carcinoma according to FDA criteria.

Male	control				Low dose				Mid dose				High dose			
	HCA	HCC	total tumors	dead at time point	HCA	HCC	total tumors	dead at time point	HCA	HCC	total tumors	dead at time point	HCA	HCC	total tumors	dead at time point
< 595 days				10				10				11	2			27
< 665 days	1		1	5	1		1	7	0		0	5	1		1	5
<735 days	3		3	10			0	5	3	1	4	4	4		4	10
Terminal necropsy	8	2	10	25	12	3	15	28	8	2	10	30	9	1	10	18
Percentage (total)			28%				32%				28%				28%	
totals	12	2	14	50	13	3	16	50	11	3	14	50	16	1	17	60
Female	control				Low dose				Mid dose				High dose			
	HCA	HCC	total tumors	dead at time point	HCA	HCC	total tumors	dead at time point	HCA	HCC	total tumors	dead at time point	HCA	HCC	total tumors	dead at time point
< 595 days				17				12				11				21
< 665 days	0		0	8	0		0	3			0	4	1		1	8
<735 days	1		1	25	1		1	5			0	5	2		2	7
Terminal necropsy*	0		0	0	5	1	6	30	7		7	30	5		5	24
Percentage (total)			2%				**14%**				**14%**				**13%**	
totals	1	0	1	50	6	1	7	50	7	0	7	50	8	0	8	60

Values in bold (blue) are significant by pair-wise comparison using Fisher's Exact test relative to control.

Statistical evaluation

Dr. Karl Lin analyzed the tumor data in the table above. The following table shows the main results (p-values) of his survival-adjusted trend tests on the combined tumor types. The combined incidence in dosed groups was not significantly different from control.

Species	Sex	Combined Tumor Type	One-sided P-Values	
			Asymptotic	Exact
Mouse	M	Hepatocellular Adenoma + Carcinoma	0.0872	0.0899
Mouse	F	Hepatocellular Adenoma + Carcinoma	0.0863	0.0892

Executive CAC Recommendations and Conclusions:

For both rats and mice, the Committee concurred that the studies were acceptable, and that higher doses were probably not feasible due to drug-related effects.

Rats:

- The committee concurred that the increased incidences of combined hemangioma plus hemangiosarcoma were drug related in female rats.
- Labeling should include information about the finding of hemangioma and hemangiosarcoma in female rats.

Mice:

- The committee concurred that there were no neoplasms that were clearly drug related.

David Jacobson Kram, Ph.D., D.A.B.T
Chair, Executive CAC

cc:\

/Division File, DDOP, NDA 22-201
/Team leader, S.L Verbois
/Acting team leader, K. Benson
/Reviewer, W.D. McGuinn
/PM, C. Huntley, DDOP
/D. Jacobson-Kram, OND IO
/A. Jacobs, OND IO
/P. Brown, OND IO
/S. Habet, OND IO

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/s/

Sayed Al-Habet
12/22/2008 12:55:21 PM
BIOPHARMACEUTICS

David Jacobson-Kram
12/22/2008 01:38:34 PM
PHARMACOLOGIST

MEMORANDUM

Degarelix

Date: December 22, 2008
To: File for NDA #22-201
From: John K. Leighton, PhD, DABT
Associate Director for Pharmacology
Office of Oncology Drug Products

I have examined the labeling and pharmacology/toxicology supporting reviews and memoranda provided by Drs McGuinn and Verbois and concur with their conclusions that degarelix may be approved. No additional pharmacology/toxicology studies are necessary for the proposed indication.

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/s/

John Leighton
12/22/2008 01:37:47 PM
PHARMACOLOGIST

MEMORANDUM

Date: December 12, 2008
From: S. Leigh Verbois, Ph.D.
Supervisory Pharmacologist
Division of Drug Oncology Products
To: File for NDA #22-201
Degarelix (tradename not established at the time of review)
Re: Approvability of Pharmacology and Toxicology

Non-clinical studies that investigated the pharmacology and toxicology of degarelix provided to support NDA 22201 for the treatment of patients with advanced prostate cancer and were reviewed in detail by David McGuinn, Jr., M.S., Ph. D., D.A.B.T. The supporting information included studies of subcutaneous and intravenously administered degarelix that investigated the drug's pharmacology, pharmacokinetic and ADME, safety pharmacology, general toxicology (rat, mouse, and monkey), genetic toxicity (*in vivo* and *in vitro*), carcinogenicity (rat and mouse) and reproductive toxicity in both rats and rabbits. The studies cited in the review by Dr. McGuinn consist primarily of original research conducted by the applicant.

The general pharmacology and toxicology studies submitted to the NDA demonstrate that degarelix is a gonadotropin releasing hormone (GnRH) receptor inhibitor. It binds reversibly to the pituitary GnRH receptors, thereby reducing the release of gonadotrophins and consequently testosterone. Degarelix causes well defined toxicities in reproductive organs in all species tested.

Degarelix did not cause genetic damage in standard *in vitro* assays (bacterial mutation, human lymphocyte chromosome aberration) nor in *in vivo* rodent bone marrow micronucleus tests. The carcinogenic potential of degarelix administered subcutaneously was investigated in rats and mice. Degarelix was administered subcutaneously to rats every 2 weeks for 2 years at doses of 12, 60 and 150 mg/m^2 (about 9, 45 and 120% of the recommended human loading dose on a mg/m^2 basis). Long-term treatment with degarelix at 150 mg/m^2 caused an increase in the combined incidence of benign hemangiomas plus malignant hemangiosarcomas in females. There was no statistically significant increase in tumor incidence associated with degarelix administered subcutaneously to mice every 2 weeks for 2 years at doses of 6, 30 and 150 mg/m^2 (about 5, 22 and 120% of the recommended human loading dose (240 mg) on a mg/m^2 basis). The Executive Carcinogenicity Assessment Committee, with the aid of statisticians, concurred with the assessment of these studies.

Consistent with the mechanism of action, single degarelix doses of $\geq$ 6 mg/m^2 (about 5% of the clinical loading dose on a mg/m^2 basis) caused reversible infertility in male rats. Single doses of $\geq$ 0.6 mg/m^2 (about 0.5% of the clinical loading dose on a mg/m^2 basis) caused a decrease in fertility in female rats. Reversibility of toxicity to reproductive organs was dependent on both dose and duration.

When degarelix was given to rabbits during early organogenesis at doses of 0.024 mg/m^2/day (about 0.02% of the clinical loading dose on a mg/m^2 basis), there was an increase in early post-implantation loss. Degarelix given to rabbits during mid and late organogenesis at doses of 0.072 mg/m^2/day (about 0.05% of the clinical loading dose on a mg/m^2 basis) caused embryo/fetal lethality and abortion. When degarelix was given to female rats during early organogenesis, at doses of 0.054 mg/m^2/day (about 0.036% of the clinical loading dose on a mg/m^2 basis), there was an increase in early post-implantation loss. When degarelix was given to female rats during mid and late organogenesis, at doses of 0.54 mg/m^2/day (about 0.36% of the clinical loading dose on a mg/m^2 basis), there was an increase in the number of minor skeletal abnormalities and variants. The potent abortifacient affects are of concern and have been reflected in the label.

Recommendations: I concur with Dr. McGuinn's conclusion that pharmacology and toxicology data support the approval of NDA 22-0201, degarelix. There are no outstanding nonclinical issues related to the approval of degarelix (for subcutaneous injection) for the proposed indication.

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/s/

Leigh Verbois
12/16/2008 01:28:49 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:	22-201
Serial Number:	000
Date Received by the Center:	February 14, 2008
Product:	Degarelix for injectable solution
Intended Clinical Population:	patients with prostate cancer
Sponsor:	Ferring Pharmaceuticals Inc., Copenhagen, Denmark
Documents Reviewed:	EDR - \\CDSESUB1\EVSPROD\NDA022201\0000
Review Division:	Division of Drug Oncology Products (HFD-150)
Pharmacology & Toxicology Reviewer:	W. David McGuinn, Jr., M.S., Ph. D., D.A.B.T.
Pharmacology & Toxicology Reviewer:	Robert Dorsam, Ph. D.
Pharmacology/Toxicology Supervisor:	S. Leigh Verbois, Ph. D.
Division Director:	Robert Justice, M.D.
Project Manager:	Carl Huntley, R. Ph., M.B.A.

Date of review submission to Division File System (DFS): December 11, 2008

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

This NDA is approvable for the proposed indication.

B. Recommendation for nonclinical studies

No further pharmacology or toxicology studies are needed.

C. Recommendations on labeling

Labeling will be reviewed separately.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Degarelix binds to the isolated human GnRH receptor with an affinity (k_i) of about 1.7 nM. The inhibition of the GnRH receptor prevents the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) form the pituitary gland. This results in a significant decrease in testosterone or estradiol release. The effect of this inhibition is rapid. Single subcutaneous doses of degarelix as low as 1-μg/kg caused a significant decrease in plasma testosterone in male rats six hours after the injection. The degree of testosterone suppression increases with increasing dose. This decrease in testosterone was accompanied by aspermia, derangement of sperm morphology and loss of fertility. The lack of stimulation by circulating testosterone results in atrophy in the prostate, testes and epididymides that increased in severity with increasing dose. The time to recovery of reproductive function in males also increased with increasing dose and total time of exposure. This decrease in testosterone is the desired clinical effect and the primary endpoint of the clinical study. Treatment with degarelix also caused a rapid decrease in plasma concentrations of estradiol in all species studied secondary to the inhibition of the release of LH and FSH. This decrease resulted in sporadic or infrequent estrus or complete amenorrhea depending on the dose and the total time of exposure. Recovery of reproductive function in females was somewhat faster than it was in males probably because testicular atrophy was somewhat worse than atrophy in the female sex organs after treatment. These decreases in estradiol cause weight increases in treated female animals and weight decreases in treated male animals.

Single SC doses as high as 300 mg/m^2 caused no neurological or behavioral changes in mice, but the same dose given to mice IV rapidly led to death preceded by signs of neurological toxicity including unsteady gate, hyperactivity and pallid brain tissue on gross examination.

Degarelix interacts with the baroreceptor and at high IV doses in the dog causes unusual and severe changes in arterial blood pressure that do not correlate with C_{max}. Other changes in cardiac parameters suggest the possibility of mild chronic cardiac toxicity that may affect contractility. This cardiac toxicity is not completely characterized. The implications for patients with congestive failure, frequent orthostatic hypotension, chronic renal failure or other heart related conditions remain unknown.

Red and white blood cell counts varied considerably in treated animals depending on dose, schedule and species. In most studies, male animals developed mild anemia and both males and females developed neutrophilia. Changes in other parameters suggest possible mild renal and hepatic toxicity with chronic treatment

Degarelix did not cause increases in bacterial mutations in six separate Ames assays either with or without metabolic activation. In six separate studies in L5178Y mouse lymphoma cells, degarelix caused no increase in mutations at the TK locus. In two separate *in vivo* studies, degarelix caused no increase in micronucleated immature rat erythrocytes. Thus, degarelix is not genotoxic under the conditions of standard *in vitro* or *in vivo* assays.

In a standard 24 month carcinogenicity study in rats where degarelix was given fortnightly (52 subcutaneous doses), the high dose of 150 mg/m^2 was about the same as the proposed clinical loading dose and about 3 times greater than the proposed monthly maintenance dose on a mg/m^2 basis. The mid dose was 60 mg/m^2 and the low dose was 12 mg/m^2. The incidence of benign adenoma of the pituitary gland decreased in all groups of treated females ($p < 0.02$). The incidence of benign fibroadenoma of the breast decreased in all groups of treated females ($p < 0.024$). These decreases were related to decreased stimulation of the pituitary and atrophy of both the pituitary and the mammary glands. The incidence of eosinophilic cell foci in the liver increased in low dose females ($p < 0.001$). Lastly, there was an increase in metastatic hemangiosarcoma of the mesenteric lymph node in HD females ($p < 0.04$, with a positive trend by Peto analysis $p = 0.015$). The incidence of this tumor was 8% which is within the range seen in historical controls. There was no similar finding in males. The combined incidence of all benign and malignant hemangiomas and hemangiosarcomas (16%) was significantly different from controls by pairwise comparison ($p = 0.0013$, Exact test) in the high dose group. This difference remained significant when analyzed by the asymptotic trend test ($p = 0.0008$).

In a standard 24 month carcinogenicity study in mice, treatment with degarelix at doses of 6, 30 and 150 mg/m^2 fortnightly for two years caused an increase in benign bronchio-alveolar adenoma in all groups of treated females ($p < 0.04$) when analyzed by pairwise comparison with control. When the incidence of benign bronchio-alveolar adenoma was combined with that of malignant bronchio-alveolar carcinoma the result was not statistically different from controls by pairwise comparison. The incidence of benign bronchio-alveolar adenoma in male CD-1 mice ranges from 11 to 36 %, in females it ranges from 3 to 16%. Dosing in this study also caused an increase in benign hepatocellular adenoma of the liver ($p = 0.015$) in high dose females. By trend analysis the increase in benign hepatocellular adenoma of the liver reached significance in both males ($p = 0.03$) and females ($p < 0.04$). When the incidence of benign hepatocellular adenoma was combined with that of malignant hepatocellular carcinoma the result was not significantly different from controls in males or females ($p < 0.09$) by pairwise comparison. The combined incidence of these tumors was also not statistically different from controls by asymptotic trend test ($p < 0.09$). The normal incidence of hepatocellular adenoma of the liver ranges from 2 to 33 % in male CD-1 mice and from 0 to 4% in females. The normal range for hepatocellular carcinoma ranges from 0 to 1.7 % in females and 0 to 6% in males.

Doses of 0.072 mg/m^2/day from day 6 through day 12 followed by doses of 0.18 mg/m^2/day caused significant post-implantation loss in pregnant rats (23.6 %) and a concomitant decrease in the number of live fetuses/dam. This dose caused no significant maternal toxicity and is only about 0.13% of the proposed clinical loading dose. Dosing was associated with an increase in the number of major abnormalities in the fetuses in the high dose group ($p < 0.05$) but most of these abnormalities occurred in a single litter (4 of 6). In fetuses in the mid dose group (0.54 mg/m^2/day followed by 0.18 mg/m^2/day at the schedule above) there was a statistically significant increase in a number of minor skeletal abnormalities and variants observed. These were findings generally associated with the state of ossification and were considered to be related to maternal treatment with Degarelix.

In rabbits, a daily dose of 0.024 mg/mg^2 on days 6 through 14 followed by doses of 0.072 mg/m^2 from days 15 through 27 was associated with a decrease in the number of does with implantations, the number of corpora lutea per female, the number of implantations and the number of live fetuses per female. Some of these decreases reached statistical significance in the mid-high (0.12 mg/m^2/day followed by 0.36 mg/m^2/day at the schedule above) and high dose groups particularly the number of live fetuses. Dosing was also associated with an increase in mean post-implantation loss. There was an increase in the number of fetuses with minor abnormalities in the high dose group and an increase in the incidence of major abnormalities in the mid dose group (5 in three litters) but the number of fetuses in the

high dose group was so diminished as to render any determination of teratogenicity equivocal. The high dose caused only minimal toxicity in the does (minimal decreased body weight gain). Thus, a daily dose of degarelix that was just 0.05% that of the proposed loading dose was a potent abortifacient in rabbits.

Single degarelix doses of $\geq$ 6 mg/m^2 (about 5% of the clinical loading dose on a mg/m^2 basis) caused reversible infertility in male rats. Single doses of $\geq$ 0.6 mg/m^2 (about 0.5% of the clinical loading dose on a mg/m^2 basis) caused a decrease in fertility in female rats.

When given as a relatively low IV dose in rats and monkeys, the two most often studied species in this NDA submission, the AUC increases linearly and proportionately with increasing dose and there was no evidence of accumulation. Terminal elimination half-life is about 5 hours in the monkey and 3 hours in the rat. In humans, half-life was at least twice as long. In the rat, clearance was 0.21 $\pm$ 0.04 L/kg/hr; while the volume of distribution was 0.9 $\pm$ 0.5 L/kg. Clearance and volume of distribution were less in the monkey. In humans, exposure also increased proportionally and linearly with dose after an IV dose. In healthy volunteers given a single IV dose of 1 mg of degarelix as a 1 hour infusion, clearance was 3.2 $\pm$ 0.5 L/hr and volume of distribution was 79 $\pm$ 17 L (about 1 L/kg).

Parameters derived from the toxicokinetic studies of degarelix given subcutaneously are not informative because the absorption of the drug from the subcutaneous depot is rate limiting. The terminal elimination half-life thus reflects the absorption rate constant, but in many cases this could not be determined accurately because the dosing interval was considerably shorter than five half-lives. Thus, values for clearance and volume were unusually large and variable. In almost all cases, the increase in C_{max} and AUC was non-linear and far less than dose proportional and most repeat dose studies demonstrated significant accumulation. Plots of C_{trough} demonstrated consistent exposure above the value of k_i even at low doses.

Degarelix is excreted in both urine (20 to 40%) and feces (20 to 40%) and excretion is essentially complete after 48 hours. In monkeys, total radioactivity distributed in highest amounts to excretory organs with the highest concentrations in bile, small intestine, urinary bladder, kidney, and liver respectively at 6 hours. Relatively high concentrations were found in the pituitary, prostate and testes consistent with the drugs pharmacology. Concentrations greater than that found in plasma were found in the aorta, lachrymal gland, lung, skin and vena cava. Elimination from the aorta, bile, pituitary, vena cava, prostate, kidneys and adrenals was slower than elimination from plasma. Plasma protein binding is about 90% in humans.

In vitro evidence suggests that cytochrome P450 is not extensively involved in degarelix metabolism. Most metabolism is hydrolytic at the various peptide bonds. *In vivo* evidence suggests some glucuronidation.

B. Pharmacologic activity

Degarelix is a competitive inhibitor of the human gonadotropin releasing hormone receptor (GnRH-R). The inhibitory constant for this pharmacological activity is about 1.7 nM. The toxicity profile suggests that this drug may have some weak secondary pharmacologies.

C. Nonclinical safety issues relevant to clinical use

Women who may become pregnant should avoid exposure to degarelix because it has potential to cause fetal harm at very low doses.

TABLE OF CONTENTS

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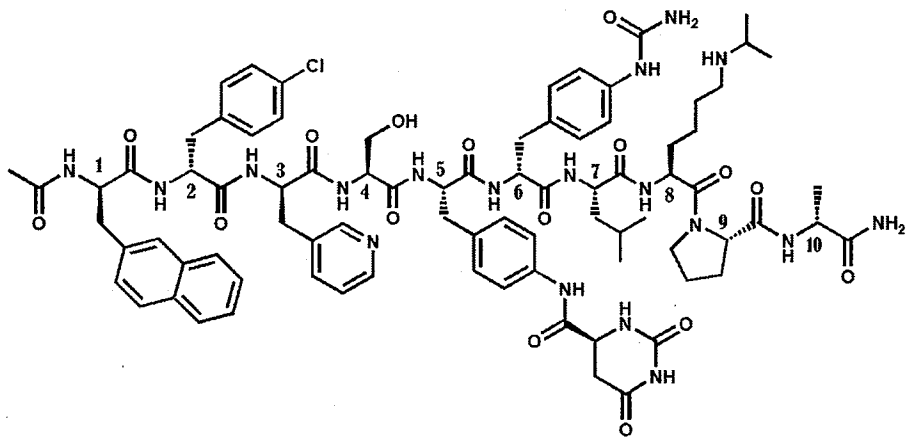
2.6 PHARMACOLOGY AND TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number:	22-201
Review number:	01
Sequence number:	000
Letter Date:	February 14, 2008
Type of Submission:	N
Information to sponsor:	Yes
Sponsor:	Ferring Pharmaceuticals, Copenhagen, Denmark
Manufacturer:	Ferring Pharmaceuticals
Reviewer name:	W. David McGuinn, Jr., M.S., Ph. D., D.A.B.T. Robert T. Dorsam, Ph. D.
Division name:	Division of Drug Oncology Products
HFD #:	150
Review completion date:	December 2, 2008

Drug:

Trade name:	Undetermined
Generic name:	Degarelix
Code name:	FE 200486
Chemical name:	D-Alaninamide, N-acetyl-3-(2-naphthalenyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridinyl)-D-alanyl-L-seryl-4-[[[(4S)-hexahydro-2,6-dioxo-4- pyrimidinyl]carbonyl]amino]-L phenylalanyl-4-[(aminocarbonyl)amino]-D-phenylalanyl-L leucyl-N6– (1-methylethyl)-L-lysyl-L-prolyl.
CAS registry number:	214766-78-6
Molecular formula	$C_{82}H_{103}N_{18}O_{16}Cl$
Molecular weight:	1632.3 Da
Structure:	



Relevant IND: 51,222

Drug class: GnRH receptor inhibitor
Proposed Mechanism: Inhibitor of the gonadotropin releasing hormone receptor

Indication: Patients with advanced prostate cancer

Clinical formulation: Degarelix for injection is a sterile powder ——— for reconstitution of the powder. The sterile powder for injection is a——— product containing degarelix (as the acetate) and mannitol. ——— b(4)

Route of administration: Depot subcutaneous injection

Dose: Loading dose of 240 mg (about 134 mg/m^2) concentration 40 mg/mL followed by a monthly dose of 80 mg (about 44 mg/m^2)

Disclaimer: Dr. Robert T. Dorsam or Dr. W. David McGuinn constructed all the tabular and graphical information unless otherwise indicated. The reviewer responsible for each study below is identified as RTD or WDM. In all tables constructed by WDM, values in bold were statistically different from controls as calculated by the investigators of the relevant study report. All human pharmacokinetic information comes from the clinical pharmacology review of this NDA by Julie M. Bullock, Pharm. D and Jun Yang, Ph.D. Dr. Karl Lin did the statistics consult for the carcinogenicity studies.

INTRODUCTION:

Degarelix or FE200486 is a linear decapeptide amide composed of seven synthetic amino acids and three naturally occurring amino acids. Five of the synthetic moieties are D-amino acids. G Jiang *et al* (*J Med Chem.* 2001 Feb 1;**44**(3):453-67) of Ferring Research Institute, have described its synthesis and initial pharmacological characterization, The drug substance is ——— as the acetate salt during manufacture. The drug product is a lyophilized powder containing mannitol that is suspended in water prior to injection. b(4)

Degarelix is a competitive inhibitor of the human gonadotropin releasing hormone receptor (GnRH-R) and is frequently referred to as a GnRH antagonist. GnRH (a.k.a. luteinizing-hormone releasing hormone or LHRH) was first characterized by AV Schally and R Guillemin in 1971 (Schally AV, *et al. J Biol Chem* 1971;**246**:7230-7236 and M Amos *et al Biochem Biophys Res Commun* 1971;**44**:205-210) for which these researchers received the 1977 Nobel Prize. Subsequently, three different types of human GnRH isoforms have been isolated (Sealfon SC, *et al. Endocr Rev* 1997;**18**:180-205). The following table (A.C.D. van Loenen, *et al. Semin Reprod Med* **20**(4):349 -364, 2002) shows that these human hormones are also decapeptides.

Name	Amino Acid Sequence									
	1 pGlu	2 His	3 Trp	4 Ser	5 Tyr	6 Gly	7 Leu	8 Arg	9 Pro	10 Gly-NH_2
Human GnRH										
GnRH-1	1	2	3	4	5	6	7	8	9	10
GnRH-II	1	2	3	4	His	6	Trp	Tyr	9	10
GnRH III	1	2	3	4	5	6	Trp	Leu	9	10

The best characterized of these is type I GnRH. [redacted] b(4)

[redacted] Type II and III GnRH remain poorly characterized. The function of multiple analogs of this hormone remains unknown. Type I GnRH remains the best understood of the three analogs. It is responsible for sex hormone signaling and is the target of Degarelix inhibition. GnRH is extremely difficult to quantify *in vivo* or from *ex vivo* samples because it is completely confined to the hypothalamic pituitary portal blood system and because its half-life is very short due to enzymatic hydrolysis (2 to 4 minutes).

Neurons of the hypothalamus deliver pulsatile secretions of GnRH through the hypothalamic-pituitary venous portal plexus to the anterior pituitary. Here it binds with its receptor located on the plasma membrane of the gonadotrophic cells of the pituitary. The receptor is a large transmembrane protein with an extracellular receptor region and an intracellular G-protein region. Most vertebrates have at least two GnRH-R proteins but only one has been clearly identified in humans and there is considerable controversy in the literature over the nature of this receptor. When GnRH binds to its receptor in gonadotrophic cells it causes the release of second messengers that include inositol-4,5-triphosphate and diacylglycerol. The release of inositol-4,5-triphosphate activates protein kinase C resulting in a release of calcium ions from intracellular pools (SS Stojilkovic *et al. Endocr Rev* 1994;15:462-499 and UB Kaiser, *Endocr Rev* 1997;18:46-70). This release of calcium causes the synthesis and secretion of the pituitary gonadotropins, LH and FSH. LH stimulates the Leydig cells of the seminiferous tubules to produce and secrete testosterone. Testosterone stimulates spermatogenesis. In females, LH triggers ovulation while FSH initiates follicular growth. Thus, pulsatile secretion of GnRH is essential for normal sexual function in both males and females. Most of the toxicology of degarelix is directly related to the disruption of LH and FSH signaling peripheral to the pituitary.

The first synthetic GnRH related drugs were receptor agonists. These drugs bind the receptor and cause the obligate release of the gonadotropins. This continuous stimulation causes hyper-secretion, but the pituitary cannot maintain this level of activity and quickly desensitizes resulting in the arrest of gonadotropin release. Receptor expression is down-regulated and signaling stops. This process is not yet completely characterized (CA McArdle *et al.* Arch Physiol Biochem. 2002 Apr;110(1-2):113-22). Thus, in men treated with synthetic GnRH agonists, gonadotropin release ceases, serum testosterone concentrations drop to castrate levels and spermatogenesis in negligible. Testosterone stimulates the growth of many prostate cancers. Decreasing testosterone by surgical or chemical castration slows the growth of some cancers, provides the patient an improvement in quality of life (palliation of some symptoms) and may result in a survival advantage in early stage disease (DG McLeod, Urology. 2003 Feb;61(2 Suppl 1):3-7).

As mentioned above, treatment with GnRH agonists results in an initial rapid and intense release of pituitary gonadotropins. This release is associated with a surge in sex hormone concentrations, which can result in an increase in the growth of tumor tissue (commonly called a disease flare) and unpleasant side effects such as hot flashes (NJ Wojciechowski, Drug Intell Clinical Pharm, 1986 Oct;20(10):746-51). Because of these unwanted side-effects, numerous investigators began searching for other GnRH analogues that might avoid the flare associated with GnRH agonists. The first two generations of new GnRH competitive inhibitors caused unacceptable anaphylactoid reactions induced by the rapid release of histamine (Phillips A et al. 1988, Hahn DW et al.1985). The sponsor developed degarelix as a treatment that would rapidly suppress the secretion of pituitary gonadotropins and thus not produce the surge in testosterone concentrations and the treatment related flare in disease.

The sponsor proposes that degarelix is indicated broadly for patients "with prostate cancer in [redacted] b(4)

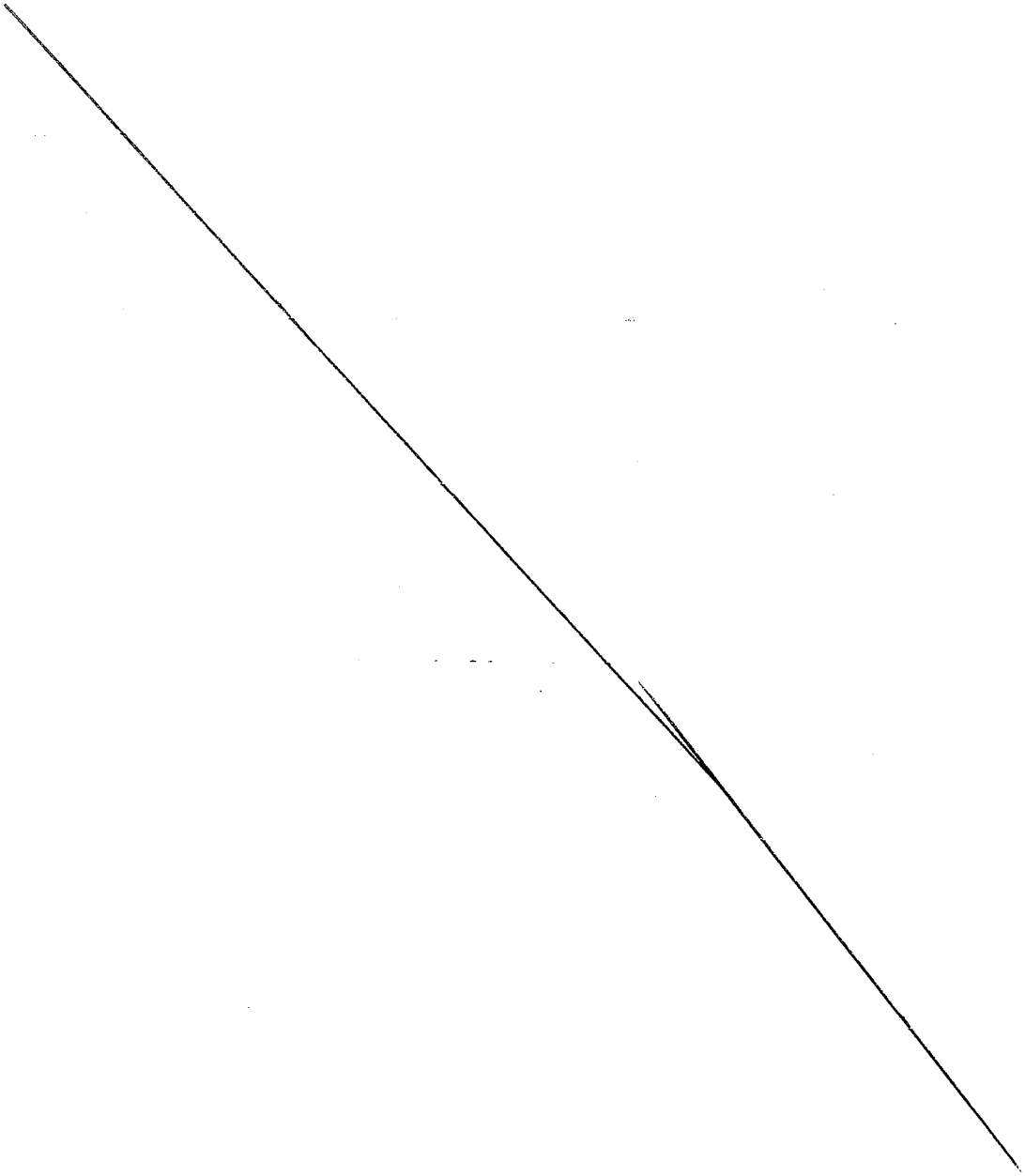
[redacted] The dose regimen used in the clinical studies that support this NDA was a starting dose of 240 mg given as two subcutaneous injections of 120 mg each (2 times 3 mL with a concentration of 40 mg/mL) followed by monthly maintenance dosing with subcutaneous injections of 80

mg (1 time 4 mL with a concentration of 20 mg/mL). The sponsor posits that after subcutaneous administration, degarelix forms a "gel-like depot" which releases the dose slowly. The primary clinical goal is to maintain testosterone concentrations below 0.5 ng/mL (1.7 nM).

Studies reviewed within this submission:

See table of contents above:

Studies not reviewed within this submission:



b(4)

3 Page(s) Withheld

✓ Trade Secret / Confidential (b4)

_____ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Pharm/Tox-1

2.6.2 PHARMACOLOGY

2.6.2.1 Summary

Degarelix binds to the isolated human GnRH receptor with an affinity (k_i) of about 1.7 nM. It displaces human GnRH from the isolated receptor with a pA_2 of 9.1 ± 1.2. Single subcutaneous doses of degarelix as low as 1-μg/kg caused a significant decrease in plasma testosterone in male rats six hours after the injection. With doses of 100 μg/kg or higher, plasma testosterone concentrations remained low for as long as 72 hours. Degarelix also suppressed plasma testosterone in mice, dogs, rats and monkeys.

In the standard battery of neurological and behavioral tests (Irwin), degarelix at single doses of 300 mg/m^2 SC caused no toxicologically significant effects in rats. Degarelix had no effect on isolated Purkinje fibers at concentrations as high as 12 μM nor did it interfere with the hERG tail current similar concentrations. In the rat, a relatively low single-doses of 18 mg/m^2 caused a small but toxicologically significant left shift in the baroreceptor reflex. This was accompanied by a decrease in the maximum attainable heart rate suggesting a decrease in cardiac responsiveness and function. This finding is consistent with the severe systolic and diastolic hypotension seen in a single dog given an IV bolus of 3 mg/kg (60 mg/m^2). This hypotension was associated with increased ST interval and occasional T-wave inversion suggesting disturbances in cardiac repolarization not associated with ion channel blockade. Lower doses, slower infusion or SC dosing caused much less pronounced hypotension sometimes preceded by episodic hypertension in the first 60 minutes after dosing. These mild changes were seen consistently across several studies but were not well characterized because the dose was well below the proposed clinical dose or the drug did not reach concentrations that could cause this toxicity due to subcutaneous depot administration or protracted IV infusion.

At a concentration of 184 μM, degarelix caused an increase in histamine from rat mast cells *in vitro* (40% above controls). This concentration is well below that of the clinical loading dose (24 mM) or the concentrations used in the subcutaneous dosing studies below (high dose typically 12 mM).

Single SC doses as high as 50 mg/kg (300 mg/m^2) had no toxicologically significant effect on respiration in the unrestrained conscious rat but this study did not measure baroreceptor reflex.

Relatively low (3 mg/kg) single subcutaneous doses caused an increase in male rat urinary output between 2.5 and 5 hours after saline challenge. This increase was not accompanied by changes in serum electrolytes or parameters of renal function.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Degarelix is a GnRH competitive inhibitor. See introduction above.

Drug activity related to proposed indication: See introduction above.

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1) PHA9816-Affinity of FE200486 for the cloned hGnRH receptor binding site – WDM

Major findings

Degarelix bound human GnRH with a k_i of 1.68 ± 0.12 nM.

Study number:	FE 200486DSPHA 9816
Conducting laboratory:	Ferring Research Ltd., Southampton, UK
Date of study initiation:	September 1998
GLP compliance:	No
QA reports:	No
Drug:	Degarelix, Batch 2008-048-1 5, and purity 98.9 % Peptide content 87%
Reference GNRH inhibitor	FE200344 Batch No. 2008-048-1 5

Methods

Cells	Cloned hGnRH receptors expressed in membranes prepared from COS-I cells
Concentrations	between 10^{-11} and 10^{-6} M

The investigators in this study expressed the human GnRH receptor in membranes of COS-I cells. They homogenized the cells, isolated the membranes and placed an aliquot in each well of a 96 well incubation plate. They then added radiolabeled ligand to the wells at varying concentrations and compared binding with a previously tested GnRH antagonist FE200344. Degarelix was tested in duplicate or triplicate at 8 concentrations between 10^{-11} and 10^{-6} M. The membranes were washed and binding was determined by scintillation counting. The values are the mean ± SEM of two experiments performed in duplicate and triplicate respectively.

Results

	Degarelix	**FE220344**
k_i (nM)	**1.68 ± 0.12**	**1.72 ± 0.25**

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2) PHA9815-Functional antagonism of the hGnRH Response by FE200486 in a reporter gene cell line – WDM

Major findings

Human GnRH was displaced from the hGnRH receptor by degarelix with a pA_2 of 9.1 ± 1.2. Thus, binding of the inhibitory ligand to the receptor occurs in the nM range.

Study number FE200486 DS PHA 9815

EDR filename	pha9815-nonclinical-data.pdf
Conducting laboratory	Ferring Research Ltd., Southampton, UK
Date of study initiation	December 1999
GLP compliance	No
QA reports	No
Drug	FE200486 Batch No. 2008-164-15 (TFA salt)
Reference compounds	
	FE200344 Batch No. 2008-048-15
	FE992003 Batch No. 2002-004-01 (Azaline B)
	FE992804 Batch No. 2002-.004-02 (Cetrorelix)
	FE992019 Batch No. 2004-162-15 (Ganirelix)
Cells	HEK293 cells (human embryonic kidney cells) expressing a cloned hGnRH receptor and a luciferase reporter gene.

Methods

The investigators incubated the cells for 5 hours at 37°C with 7 increasing concentrations of hGnRH between 10^{-11} and 10^{-6} M in the absence of test drug or in the presence of five increasing concentrations of test drug. They then quantified reporter gene activity by measuring the amount of luminescence (cps) generated. They then fitted curves to plots of cps against the log concentration of GnRH. EC_{50} values were estimated and plotted in Schild plots. They used linear regression analysis of the Schild plots to estimate the pA2 values and the slope of the regression line. The following table from the study report shows that binding was in the nanomolar range for all the GnRH inhibitors.

	FE200486	FE200344	FE992003	FE992004	FE992019
pA_2 value	9.13 ± 0.09	9.02 ± 0.08	9.12 ± 0.02	9.04 ± 0.11	9.21 ± 0.05
slope	1.20 ± 0.04	1.28 ± 0.05	1.32 ± 0.02	1.40 ± 0.15	1.19 ± 0.04

The values are the mean ± SEM of two experiments performed in duplicate.

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3) Dose-dependent suppression of plasma testosterone levels in the intact rat by the GnRH antagonist FE200486 (0.3-300 µg/kg, s.c., in 5% mannitol, 0.4 ml/kg). WDM

Major findings

Single subcutaneous doses of degarelix as low as 1-µg/kg caused a significant decrease in plasma testosterone in male rats six hours after the injection. With doses of 100 µg/kg or higher, plasma testosterone concentrations remained low for as long as 72 hours.

Study number	FE200486 DS PHA9818
EDR filename	pha9818-nonclinical-data.pdf
Conducting laboratory	[redacted] b(4)
Date of study initiation	January 1998
GLP compliance	No
QA reports	No
Drug	Degarelix, Batch No. 2008-164-1 5 and 2011-032-30, acetate salt
Methods	
Animals	Male Sprague Dawley IOPS rats
Doses	0, 0.3, 1, 3, 10, 30, 100 µg/kg (0, 1.8, 6, 18, 60, 180 and 600 µg/m^2)

N	8 per dose group
Schedule	Single dose
Route	SC
Vehicle	5% Mannitol $_{aq}$
Dose Volume	0.4 mg/mL
Blood collection	Blood was withdrawn from the tail tip. Blood was collected from hour 6 to day 3 post-treatment.
Plasma testosterone	RIA Radioimmunoassay

Results

The following table from the study report shows that six hours after a single dose of degarelix, plasma concentrations of testosterone decreased as a function of increasing dose.

Table 3: Effects on plasma testosterone levels of FE 200486 injected at 0.3-10 µg/kg, s.c. in 0.4 ml/kg 5% mannitol. Results are mean plasma testosterone levels (n = 8) ± s.e.m. * : $p < 0.05$ when compared to the control (vehicle-treated) group.

	6 hrs post treatment
Vehicle	1915.2 ± 425.9
FE200486, 0.3 µg/kg	1772.8 ± 367.7
FE200486, 1 µg/kg	564.2 ± 144.4 *
FE200486, 3 µg/kg	322.1 ± 26.2 *
FE200486, 10 µg/kg	291.4 ± 28.2 *

The following table from the study report shows that plasma testosterone concentrations remained low for as long as 72 hours after a single dose of degarelix in animals treated with doses of 100-µg/kg or greater. The control testosterone concentrations increased over this period. The investigators did not offer an explanation for this increase but it is possibly a response to stress associated with blood sampling.

Table 4 Effects on plasma testosterone levels of FE 200486 injected at 10-300 µg/kg, s.c. in 0,4 ml/kg 5 % mannitol. Results are mean plasma testosterone levels (n = 8) ± s.e.m. * : $p < 0.05$ when compared to the control (vehicle-treated) group.

	Post-injection time			
	6 hr	24 hr	48 hr	72 hr
Vehicle	815.5 ± 146.0	1684.6 ± 463.3	1951.9 ± 341.2	1539.9 ± 300.2
FE200486, 10 µg/kg	74.7 ± 10.74 *	2971.9 ± 321.84	1652.3 ± 334.98	1593.3 ± 270.44
FE200486, 30 µg/kg	79.4 ± 17.94 *	2760.3 ± 447.19	1146.4 ± 206.19	1765.2 ± 479.46
FE200486, 100 µg/kg	98.5 ± 13.94 *	58.9 ± 14.73 *	2545.5 ± 392.96	1182.8 ± 319.52
FE200486, 300 µg/kg	97.4 ± 13.90 *	23.1 ± 4.55 *	218.8 ± 156.00 *	631.3 ± 246.39

2.6.2.3 Secondary pharmacodynamics

2.6.2.4 Safety pharmacology

Neurological effects:

1) Irwin Profile Test in the Rat after Subcutaneous Administration of FE200486

Major finding

Single doses of degarelix as high as 300 mg/m^2 caused no changes in neurological signs or behavior over the 24 hours of observation.

Study number:	AA39669
Sponsor number:	200486-2372
EDR filename:	2372-Irwin.pdf
Conducting laboratory:	b(4)
Date of study initiation:	December 2006
GLP compliance:	Yes
QA reports:	Yes
Drug:	Degarelix, Batch 04D14-01, and purity 100 %
Methods:	
Animal	Male Sprague Dawley rat: RjHan: SD
Doses	0, vehicle, 0.5, 5, and 50 mg/kg (0, 0, 3, 30, 300 mg/m^2)
N	6
Schedule	Single dose
Route	SC
Formulation	Mannitol$_{aq}$ 5% w/v

Results

Mortality	None
Body weight	No changes
Temperature	No changes

Degarelix treatment caused no biologically significant changes in behavior or central and peripheral nervous system function irrespective of whether the animals were in their home cage, the open-field, or were being handled, compared to control, over 24 hours.

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2) Evaluation of effect on the autonomic nervous system following single subcutaneous administration in conscious rats of both sexes - RTD

Major findings:

A dose of 3 mg/kg degarelix produced a slight leftward shift of baroreceptor curves in both sexes. An impairment of cardiac function or the autonomic nervous system cannot be ruled out from this experiment. A maximal dose of 3 mg/kg degarelix is not a sufficiently high maximal dose to assess

toxicological effects of degarelix on this system. In addition, vehicle- and 0.03 mg/kg degarelix-treated groups were evaluated 1 hour after subcutaneous injection, whereas the highest dose in the study was evaluated 24 hours after degarelix injection.

Study no.:	FE 200486 DS PHA 9905
[redacted] study number:	980361 P
EDR filename:	pha9905-nonclinical-data.pdf
Conducting laboratory:	[redacted]
Date of study initiation:	October 1998
GLP compliance:	Yes
QA reports:	Yes
Drug:	Degarelix, Batch 2011-032-30, peptide content: 89.9% and purity 98.4%
Methods	
Doses:	Vehicle, 0.03, 0.3, and 3 mg/kg 30 mg/kg guanethidine as a positive control
Species/strain:	Rats/Wistar
N	6 per sex per group
Route	Subcutaneous
Formulation	5% mannitol in sterile water
Volume:	0.4 mL/kg for vehicle and degarelix and 1 mL/kg for guanethidine
Parameters:	Baroreflex, Arterial blood pressure response to phenylephrine, Initial heart rate decrease and secondary arterial blood pressure decrease by serotonin, Dose groups evaluated at different times, for determination of baroreflex sensitivity (groups 1 and 3: 1 hour; group 2: 2 hours; groups 4 and 5: 24 hours).

b(4)

Study design:

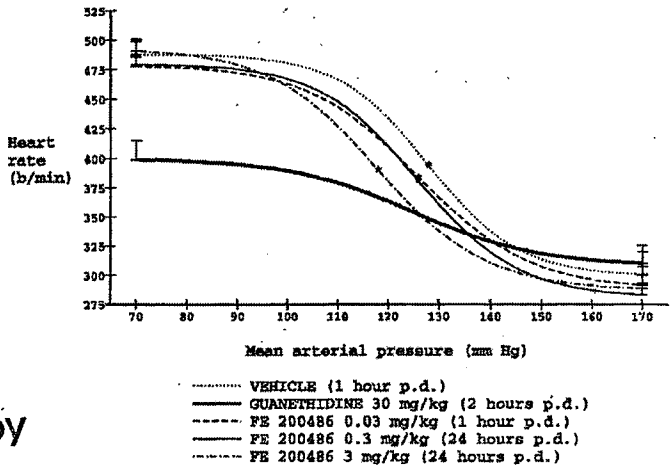
Six rats per sex per dose group received degarelix (vehicle, 0.03, 0.03 or 3 mg/kg degarelix) or guanethidine to evaluate effects on the autonomic nervous system. Drug effects on the baroreceptor reflexes were assessed, followed by evaluation of the effect of the drug on the phenylephrine-induced hypertensive response. Lastly, serotonin-initiated heart rate decrease and secondary arterial blood pressure decrease were tested in the absence and presence of drug to further assess any possible autonomic effects of degarelix.

Results:

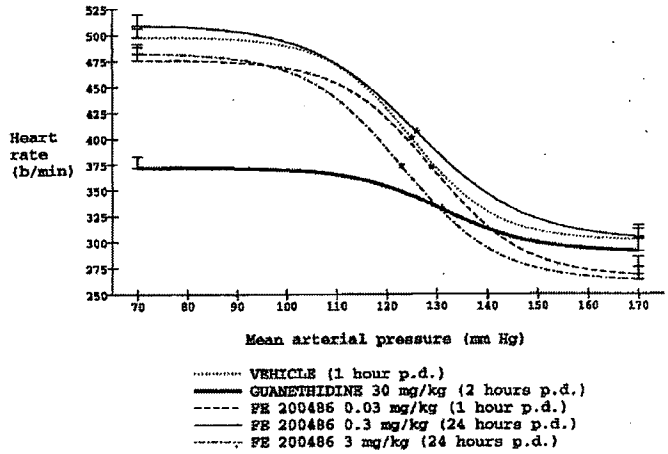
Degarelix had a marginal but dose-dependent effect at the highest dose, producing a leftward shift in the baroreflex curve in both the male and female rats. This effect, while slight, is consistent in both sexes and may have been more evident at higher doses. In addition, 3 mg/kg degarelix was evaluated 24 hours post-injection, while the vehicle was evaluated just one hour post-injection. A higher dosage and similar times-to-evaluation would clarify this potential toxicity issue.

(The graph below from the study report shows the effect of degarelix treatment on the baroreceptor response in male rats)

Effect of degarelix on the baroreceptor reflex in male rats



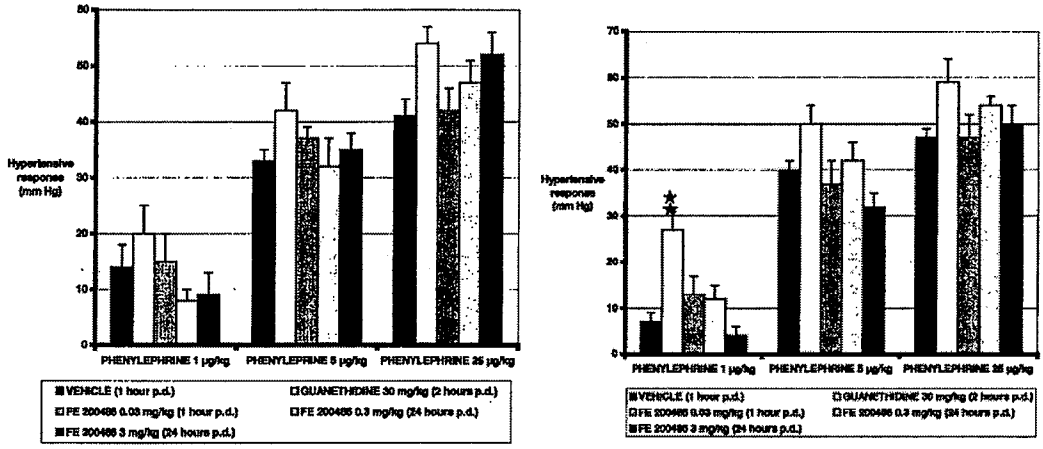
Effect of degarelix on the baroreceptor reflex in female rats



It is unclear if this is an effect on the baroreceptor reflex or if it represents toxicity directly on cardiac function. Nevertheless, the slopes of the baroreceptor curves are similar between vehicle and high-dose degarelix which suggests a responsive baroreceptor reflex. The leftward shift and slight lowering of maximum attainable heart rate (at low blood pressures) in high-dose degarelix-treated rats may indicate a decrease in the cardiac responsiveness and function.

A subsequent challenge with phenylephrine in the presence of degarelix showed that degarelix did not interfere with the hypertensive response in either male or female rats (graphs below).

(The graphs below from the study report show the phenylephrine-mediated hypertensive response is not effect by degarelix in male (left) or female (right) rats.



The bradycardic response and secondary hypotensive responses induced by doses of serotonin (3, 10 and 30 µg/kg) were unaffected by the presence of degarelix.

Cardiovascular effects:

3) Cardiovascular and Respiratory Evaluation in the Conscious Dog following Intravenous Administration - WDM

Major findings

A single IV dose of 3 mg/kg (60 mg/m^2) of degarelix caused moderate to severe systolic and diastolic hypotension between 0 to 45 minutes after dosing in a single dog. This hypotension was associated with increased ST interval and T-wave inversion, indicative of disturbances in cardiac repolarization. A dose of 1 mg/kg caused mild hypotension in the first hour after dosing suggesting that the effect is dose related. Lower doses caused no cardiac toxicity.

Study number:	FRG 046/0025 17, FE200486DSPHA0005
EDR file name	pha0005-nonclinical-data.pdf
Conducting laboratory:	[redacted] b(4)
Date of study initiation:	January 2000
GLP compliance:	Yes
QA reports:	Yes
Drug:	Degarelix, Batch 201 1-032-30, purity 87.8% peptic portion of which 97.58% is pure free peptide base
Methods:	
Animal	Beagle Dogs (fasted prior to measurements)
Doses	0, 0.03, 0.3 and 3 mg/kg (0, 0.6, 6 and 60 mg/m^2) Cross-over design, each dog received each dose with at least a 9 day interval between doses. The high dose was given to only one dog. The investigators deemed this dose too toxic so they reduced the dose to 1 mg/kg (20 mg/m^2) for the other three dogs.

N	4
Schedule	Single dose
Route	IV injection, 2 minutes
Formulation	$Mannitol_{aq}$ 5% w/v (vehicle control)
Surgical Procedure	Catheter in the thoracic aorta, recovery 12 to 13 days
Measurements	Systolic, diastolic, mean blood pressure (mm Hg), Heart rate, Electrocardiogram (lead I, II, III, aVF, aVR , aVL), Respiration, arterial blood samples
Statistical Analysis	None

Results

Over the time course of the experiment (270 minutes or 4.5 hours) the average of the mean diastolic pressure for all four dogs when they received vehicle was 85.7 ± 3.4 mm Hg and the mean systolic pressure was 155 ± 6 mm Hg. The injection of vehicle had no discernable effect on blood pressure. In these animals, mean heart rate was 92 ± 4 and respiration was 22 ± 3.

Doses of 0.03 mg/kg and 0.3 mg/kg did not effect on blood pressure, heart rate or respiration. Neither did these doses cause changes in electrocardiographic parameters (data not shown).

A dose of 3 mg/kg caused significant changes in both diastolic and systolic blood pressure. Mean heart rate was somewhat lower than in the control group and variability increased, 82 ± 23. Most of the variations in heart rate occurred between 30 and 60 minutes coincident with the major changes in blood pressure. The following table from the study report shows these fluctuations.

Effects of FE200486 (3.0 mg/kg *iv*) on blood pressure, heart rate and respiration rate in the conscious dog

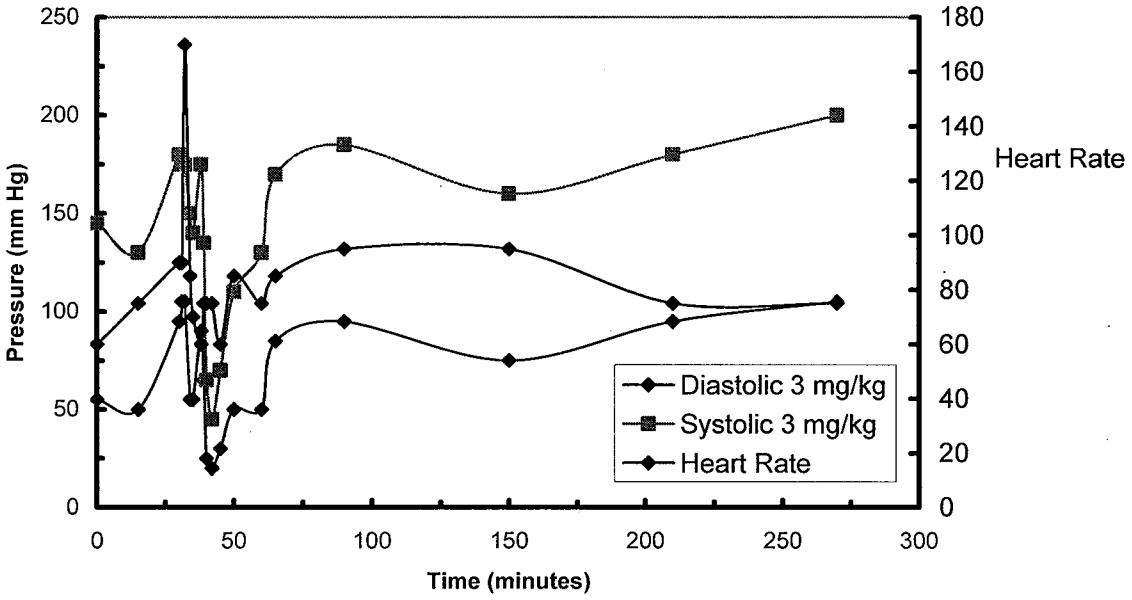
Dog no. 854M (n=1)

Treatment	Time (min)	Blood pressure			Heart rate (b/min)	Respiration rate (breath/min)
		Diastolic (mmHg)	Systolic (mmHg)	Mean (mmHg)		
FE200486 (3.0 mg/kg)	0	55	145	85	60	10
	15	50	130	77	75	11
	30	95	180	123	90	30
	31	105	175	128	90	40
	32	105	175	128	170	-
	34	55	150	87	85	30
	35	55	140	83	70	14
	38	90	175	118	60	20
	39	65	135	88	75	40
	40	25	65	38	75	-
	42	20	45	28	75	30
	45	30	70	43	60	20
	50	50	110	70	85	20
	60	50	130	77	75	20
	65	85	170	113	85	16
	90	95	185	125	95	20
	150	75	160	103	95	25
	210	95	180	123	75	30
	270	105	200	137	75	30

- Respiration rate not determined

The following graph shows that diastolic and systolic pressure was lower immediately after the dose was administered, increased sharply at about 30 minutes after dosing, then significantly decreased at around 40 minutes post dosing.

Diastolic and Systolic Pressure in One Male Dog after an IV dose of 3 mg/kg



Heart rate did not increase to compensate this hypotension which suggests that the effect is either vagal or a direct effect on cardiac contractility. The following table shows that changes in electrocardiographic parameters preceded these changes in systolic and diastolic pressure. Changes in wave form included T-wave inversion at 10 minutes and increases in ST interval between 10 and 30 minutes. The QRS interval was unaffected, suggesting delayed or disturbed repolarization.

Electrocardiography - following intravenous treatment with FE200486 at 3.0 mg/kg

Animal no.: 854M (n=1)

Time point (min/hr)	Heart rate b/min	Intervals (ms)						Amplitude (mV)						R:T ratio
		P	PR	QRS	ST	QT	QTc	P	Q	R	S	T	ST	
0 hr	82	52	92	40	162	202	236	0.19	0.74	2.29	0.42	0.13	-0.13	17.6
2 min	103	38	92	40	164	204	267	0.26	0.65	2.39	0.48	0.23	-0.13	10.4
5 min	64	40	98	38	176	214	221	0.19	0.45	2.62	0.32	0.19	-0.13	13.8
10 min	73	30	84	42	198	240	265	0.26	0.29	2.65	0.52	-0.32	-0.16	8.3
15 min	74	40	90	38	194	232	258	0.19	0.32	2.36	0.90	0.39	-0.13	6.1
30 min	87	36	90	38	168	206	248	0.16	0.55	2.87	0.48	0.26	-0.13	11.0
1.0 hour	93	44	94	40	178	218	271	0.23	0.42	3.20	0.39	0.23	-0.13	13.9
1.5 hour	100	38	94	40	160	200	258	0.23	0.68	3.07	0.42	0.13	-0.13	23.6
2.0 hour	81	34	90	40	168	208	242	0.19	0.58	2.36	0.52	0.23	-0.16	10.3
2.5 hour	86	30	88	34	176	210	251	0.10	0.32	2.68	0.16	-0.10	-0.10	26.8
3.0 hour	74	42	100	38	176	214	238	0.19	0.68	1.81	0.36	0.19	-0.16	9.5
3.5 hour	82	58	106	38	174	212	248	0.26	0.55	2.94	0.29	0.19	-0.13	15.5
4.0 hour	80	44	90	40	170	210	242	0.10	0.26	3.04	0.13	0.16	-0.06	19.0

Thus, high concentrations of degarelix within cardiac endothelium may directly decrease contractility. After consultation with the sponsor, the investigators modified the protocol and decreased the dose in the remaining three dogs, thus these experiments did not fully characterize this toxicity

In the three dogs that received 1 mg/kg, systolic and diastolic pressure decreased relative to controls and was more variable during the first hour after dosing but the changes were not statistically significant as the tables below from the study report show. Electrocardiographic parameters were unaffected.

Effects of FE200486 (1.0 mg/kg *iv*) on blood pressure, heart rate and respiration rate in the conscious dog – group mean data

Mean ± standard deviation (n=3)

Treatment	Time (min)	Blood pressure						Heart rate (b/min)		Respiration rate (breath/min)	
		Diastolic (mmHg)		Systolic (mmHg)		Mean (mmHg)					
		Mean	± sd	Mean	± sd	Mean	± sd	Mean	± sd	Mean	± sd
FE200486 (1.0 mg/kg)	0	77	17.56	130	22.91	94	19.32	100	21.79	27	5.77
	15	77	10.41	140	10.00	98	9.62	90	21.79	29	14.93
	30	72	2.89	133	14.43	92	4.19	90	21.79	22	7.64
	31	78	5.77	140	15.00	99	8.55	92	18.93	23	5.77
	32	78	2.89	138	14.43	98	6.01	97	23.09	23	n=2
	34	120	0.00	195	n=1	145	n=1	130	n=1	60	n=1
	35	93	12.58	163	30.55	117	18.56	108	22.55	28	10.41
	40	90	13.23	155	21.79	112	16.07	102	23.63	38	12.58
	45	90	18.03	152	23.63	111	19.88	103	37.86	25	12.66
	60	80	8.66	147	20.21	102	12.29	93	25.17	25	7.07
	90	72	10.41	133	20.82	92	13.88	88	22.55	23	7.64
	150	82	7.64	140	15.00	101	10.05	98	22.55	32	2.89
	210	85	0.00	143	10.41	104	3.47	88	20.21	23	7.64
	270	85	8.66	142	15.28	104	10.72	105	7.07	20	n=2

Electrocardiography - following intravenous treatment with FE200486 at 3.0 mg/kg

Animal no.: 854M (n=1)

Time point (min/hr)	Heart rate b/min	Intervals (ms)						Amplitude (mV)						R:T ratio
		P	PR	QRS	ST	QT	QTc	P	Q	R	S	T	ST	
0 hr	82	52	92	40	162	202	236	0.19	0.74	2.29	0.42	0.13	-0.13	17.6
2 min	103	38	92	40	164	204	267	0.26	0.65	2.39	0.48	0.23	-0.13	10.4
5 min	64	40	98	38	176	214	221	0.19	0.45	2.62	0.32	0.19	-0.13	13.8
10 min	73	30	84	42	198	240	265	0.26	0.29	2.65	0.52	-0.32	-0.16	8.3
15 min	74	40	90	38	194	232	258	0.19	0.32	2.36	0.90	0.39	-0.13	6.1
30 min	87	36	90	38	168	206	248	0.16	0.55	2.87	0.48	0.26	-0.13	11.0
1.0 hour	93	44	94	40	178	218	271	0.23	0.42	3.20	0.39	0.23	-0.13	13.9
1.5 hour	100	38	94	40	160	200	258	0.23	0.68	3.07	0.42	0.13	-0.13	23.6
2.0 hour	81	34	90	40	168	208	242	0.19	0.58	2.36	0.52	0.23	-0.16	10.3
2.5 hour	86	30	88	34	176	210	251	0.10	0.32	2.68	0.16	-0.10	-0.10	26.8
3.0 hour	74	42	100	38	176	214	238	0.19	0.68	1.81	0.36	0.19	-0.16	9.5
3.5 hour	82	58	106	38	174	212	248	0.26	0.55	2.94	0.29	0.19	-0.13	15.5
4.0 hour	80	44	90	40	170	210	242	0.10	0.26	3.04	0.13	0.16	-0.06	19.0

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4) Effects of FE200486 on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibers – WDM

Major finding

In dog isolated cardiac Purkinje fibers, paced at stimulation frequencies of 1 and 0.5 Hz, degarelix at 0.2, 2 and 20 µg/mL (0.12, 1.2, 12 µM) had no effects on resting membrane potential, maximum rate of depolarization, upstroke amplitude or action potential duration. Degarelix does not affect cardiac potassium ion channels under the conditions of this assay.

Study number:	DRSC1033
Sponsor number:	FE200486DSPHA0203
EDR filename	Purkinje pha0203.pdf
Conducting laboratory:	[redacted] b(4)
Date of study initiation:	January 2003
GLP compliance:	Yes
QA reports:	Yes
Drug:	Degarelix, Batch 04D14-01, and purity 100 %
Methods:	
Animal	Beagle Dogs isolated Purkinje fibers
Doses	0, 0.2, 2 and 20 µg/mL (0.12, 1.2, 12 µM)
N	4
Schedule	Single dose
Route	Tissue bath
Formulation	Mannitol $_{aq}$ 5% w/v

The following tables from the study report show the results of this experiment.

Effect of FE200486 or Vehicle on Action Potential Parameters (1 Hz)

Action Potential Parameter	FE200486 Baseline	FE200486 0.2 µg/ml	FE200486 2 µg/ml	FE200486 20 µg/ml
RMP (mV)	-89.5 ± 1.6	-87.9 ± 0.7	-87.8 ± 0.7	-88.1 ± 0.8
UA (mV)	118.4 ± 3.0	118.6 ± 2.7	118.8 ± 2.7	118.2 ± 2.0
MRD (V/s)	500.8 ± 42.8	513.6 ± 41.0	531.9 ± 39.0	529.8 ± 22.1
APD_{60} (ms)	222.4 ± 12.0	225.2 ± 15.3	226.2 ± 16.4	229.8 ± 15.8
APD_{90} (ms)	276.1 ± 15.4	275.0 ± 15.8	277.9 ± 17.0	284.4 ± 15.3

Effect of FE200486 or Vehicle on Action Potential Parameters (0.5 Hz)

Action Potential Parameter	FE200486 Baseline	FE200486 0.2 µg/ml	FE200486 2 µg/ml	FE200486 20 µg/ml
RMP (mV)	-86.2 ± 1.2	-85.6 ± 1.7	-85.2 ± 1.6	-86.6 ± 0.9
UA (mV)	114.1 ± 5.0	113.5 ± 4.7	114.5 ± 4.8	114.4 ± 3.4
MRD (V/s)	491.6 ± 54.3	494.9 ± 58.5	505.8 ± 51.7	508.3 ± 38.3
APD_{60} (ms)	250.2 ± 21.4	246.8 ± 23.3	254.5 ± 24.5	255.3 ± 24.5
APD_{90} (ms)	311.5 ± 27.3	303.5 ± 24.2	314.1 ± 25.5	317.8 ± 23.7

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5) Effect of FE200486 on HERG Tail Current Recorded from Stably Transfected HEK293 Cells – WDM

Investigators studied the effect of degarelix on HERG tail current at a concentration of 20 µg/mL (12 µM, n = 4 cells). Exposure to this concentration for about 15 min produced a residual current of 74 ± 2 % when compared to control values. This equates to a decrease in tail current of 26.2%. Vehicle produced a residual tail current of 82 ± 5% when compared to controls. Thus, there was no statistically significant difference between the vehicle and degarelix ($P > 0.05$; unpaired, 2-tailed, Student's *t*-test).

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6) Evaluation of the hemodynamic effects following subcutaneous dosing in anaesthetized dogs of both sexes - WDM

Major findings

A single subcutaneous 0.3 mg/kg (6 mg/m^2) dose caused decreases (7%) in femoral resistance at 30 minutes. A dose of 3 mg/kg caused statistically significant decreases in systolic left ventricular pressure (E_{max}: -5%), maximal dP/dt (E_{max}: -6%) and in left ventricular work (E_{max}: -10%) at 30 minutes post dose.

Study number: 980362P

Sponsor number:	FE 200486 DS PHA 9904
EDR filename	pha9904-preclinical-data.pdf
Conducting laboratory:	[redacted] b(4)
Date of study initiation:	October 1998
GLP compliance:	Yes
QA reports:	Yes
Drug:	Degarelix, Batch 2011-032-30, Peptide content: 89.9% I Purity: 98.4%.
Methods:	
Animal	Beagle Dogs
Weight	Between 1 1.2 kg and 12.8 kg on the day of the study
Doses	Vehicle then sequentially with 0.003, 0.03, 0.3 and 3 mg/kg 0, 0.06, 0.6, 6 and 60 mg/m^2 At regular 30-minute intervals
N	3 per sex
Schedule	5 sequential increasing doses
Route	Subcutaneously
Formulation	Mannitol $_{aq}$ 5% w/v
Dose volume	0.4 mL/kg
Procedures	Transducers were surgically placed in the abdominal aorta to measure arterial pressure. A side fitting attached to the arterial catheter was used to draw arterial samples for measurement of pH and blood gases, for plasma hemoglobin and for drug analysis. A second transducer was placed into the left ventricle for the measurement of left ventricular pressure. A third was placed into the pulmonary artery for the measurement of pulmonary arterial pressure. Three [redacted] probes were fitted respectively around the left renal artery, the left femoral artery and the circumflex coronary artery for the measurement of arterial flow rates. A fourth transducer was placed in the ascending aorta to measure cardiac output. b(4)
Measurements	Arterial pressure, heart rate, cardiac output and derived parameters (stroke volume and total peripheral resistance, cardiac work), left ventricular pressure (LVP) and derived parameters (systolic LVP, end-diastolic LVP, dLVP/dt(+) and dLVP/dt(-)), pulmonary arterial pressure, coronary flow and resistance, renal artery flow and resistance, femoral flow and resistance, PR, QT and QTc intervals, QRS complex and arterial pH, p02, pC02, HC03.

Results

Degarelix given as a series of subcutaneous doses of of 0.003 and 0.03 mg/kg caused no statistically significant changes in cardiovascular parameters measured for 30 minutes following dosing.

A dose of 0.3 mg/kg was associated with a statistically significant decrease in femoral resistance (E_{max}: -7%) at 30 minutes.

The highest dose, 3 mg/kg, caused statistically significant decreases in systolic left ventricular pressure (E_{max}: -5%), maximal dP/dt (E_{max}: -6%) and in left ventricular work (E_{max}: -10%) occurred at 30 minutes.

There were no other significant changes in cardiovascular parameters in this experiment.

7) Pharmacokinetic cardiovascular and respiratory evaluation in the conscious dog following subcutaneous administration – WDM

Major findings

Subcutaneous doses of 1 or 3 mg/kg caused minor changes in blood pressure and heart rate. Changes in ST amplitude and T wave morphology suggest the possibility of a minor effect on repolarization. Pharmacokinetics of degarelix after a single SC dose in this experiment was highly variable and not dose proportional.

Study number:	FRG 052/002725
Sponsor number:	FE200486DSPEIA0004
EDR filename	pha0004-amend-1-preclinical-data.pdf
Conducting laboratory:	[redacted] b(4)
Date of study initiation:	March 2000
GLP compliance:	Yes
QA reports:	Yes
Drug:	Degarelix, 94408 and 94409, purity > 99%

Methods:

Animal: Beagle dogs (used in a previous experiment of the same nature)

Doses: Nominal doses 1 and 3 mg/kg actual doses

Dog	Dose volume (ml)	Concentration (mg/ml)	Actual dose administered (mg/kg)
714	1	10	0.85
765	1	10	0.89
849	1	40	3.20
854	0.8	40	3.20

N: 1 per sex per dose group

Schedule: Single dose

Route: Subcutaneously

Formulation: Mannitol $_{aq}$ 5% w/v

Dose volume: 0.4 mL/kg

Procedures: The dogs were chronically implanted (from a previous experiment) with an arterial catheter and vascular access port. A Stethograph was fitted around the animal's thorax. Measurements were taken on days 1, 2, 4, 7, 14 and 48 post dosing.

Measurements: Respiration rate. waveform intervals (P, PR,QRS, ST, QT and QT corrected) and amplitudes (P, Q, R, S, T, ST and RT ratio), aVr, aVF and aVL

Results

Arterial BP – Blood pressure was measured in only one of the two dogs given 1.0 mg/kg. Considering the results of the IV study above (FE200486DSPHA0005), these fluctuations in the first hour of dosing are possibly dose related. The following graph from the study report demonstrates these minor fluctuations.

Pharmacokinetics The following table from the study report shows that T_{max} was highly variable and elimination was slow. C_{max} was not dose proportional. The investigators did not calculate pharmacokinetic parameters.

TABLES

Dog	714 M	854 M	765 F	849 F
Predose	<0,50	<0,50	<0,50	<0,50
0,5 h	10,0	0,58	5,10	4,16
1 h	18,6	1,45	7,66	6,71
2 h	29,0	1,79	12,7	12,8
3 h	35,5	1,91	14,5	16,7
4 h	45,3	1,86	17,6	19,6
24 h	46,2	1,29	26,6	30,3
48 h	32,8	0,78	28,4	36,4
96 h	23,2	<0,50	29,1	40,3
day 7	8,05	<0,50	9,62	24,2
day 14	3,59	<0,50	4,63	11,9
day 28	1,27	<0,50	1,92	4,43

Table I: Summary of the results. Values reported as ng FE 200486-LI/ml dog plasma.

8) Effect of FE 200486 on General Hemodynamics and Respiratory Variables in Anaesthetized Beagle Dogs – WDM

Major findings

Dosing with 1 or 3 mg/kg of degarelix IV caused a transient increase in arterial pressure in dogs at 15 minutes after dosing. This was followed by a decrease in pressure around 30 minutes (about 20 mmHg across all parameters) in both dose groups. This hypotension slowly recovered between 45 and 120 minutes. The pressure decrease was accompanied by a transient increase in contractility index. Numerous other parameters suggested an effect on contractility or repolarization but these changes did not reach statistical significance. There was no toxicologically significant effect on histamine.

In contrast to the experiment above, a dose of 3 mg/kg given as a 15 minute infusion caused significantly less cardiac toxicity than the same dose given as a rapid IV injection.

Study number: DRSC1014
Sponsor number: FE200486DSTOX0118
EDR filename tox0118-preclinical-data.pdf
Conducting laboratory: [redacted] b(4)
Date of study initiation: November 2001
GLP compliance: Yes
QA reports: Yes
Drug: Degarelix, Batch 0048262, peptide content 87.99%,

Methods:
- Animal: Beagle Dogs
- Weight: between 11.40 and 14.42 kg
- Doses: Phase 1,

Vehicle controls – 4 dogs
3 mg/kg, one dog per dosing regimen, the vehicle volume was given simultaneously with the drug volume

Dosing Regimen	Concentration (mg/ml)	Dose Volume, Test Substance (ml/kg)	Dose Volume, Vehicle (ml/kg)	Infusion Time (min)
1	0.67	4.5	0	15
2	1.5	2.0	2.5	15
3	3.0	1.0	3.5	15
4	6.0	0.5	4.0	15

Phase 2: At the Sponsor's request the dose of degarelix was reduced to 1 mg/kg and administered using 4 different dosing regimens and 4 different animals

Dosing Regimen	Concentration (mg/ml)	Dose Volume, Test Substance (ml/kg)	Dose Volume, Vehicle (ml/kg)	Infusion Time (min)
5	0.22	4.5	0	15
6	0.5	2.0	2.5	15
7	1.0	1.0	3.5	15
8	2.0	0.5	4.0	15

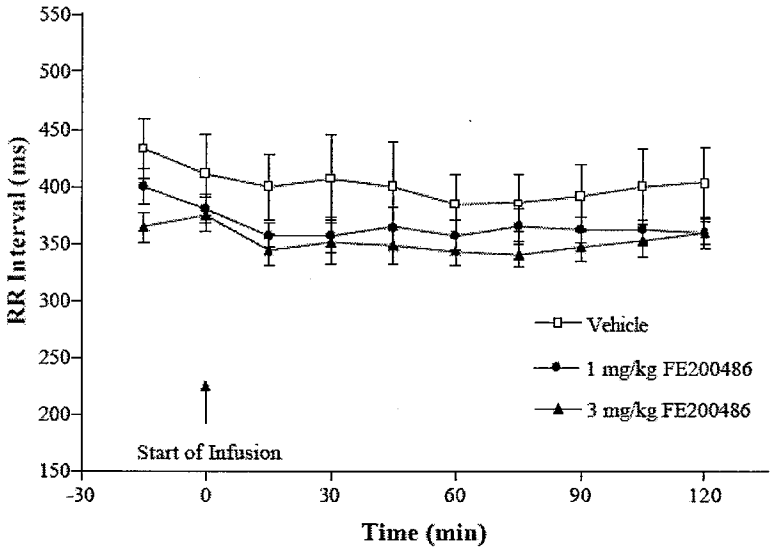
Schedule	single dose
Route	IV, 15 minute infusion
Formulation	Glucose $_{aq}$ 5% w/v
Dose volume	0.4 mL/kg
Anaesthesia	Pentobarbitone
Procedures	Transducers were surgically placed to record hemodynamic parameters
Measurements	arterial pressure, heart rate, cardiac output and derived parameters (stroke volume and total peripheral resistance, cardiac work), left ventricular pressure (LVP) and derived parameters (systolic LVP, end-diastolic LVP, dLVP/dt(+) and dLVP/dt(-)), pulmonary arterial pressure, coronary flow and resistance, renal artery flow and resistance, femoral flow and resistance, PR, QT and QTc intervals, QRS complex and arterial pH, $p0_2$, $pC0_2$, $HC0_3$.
Collection times	Stabilization 30 minutes, pre-dose recording 15 minutes, Drug infusion 15 minutes, t = 0 at start of infusion, readings at -15 min, t_0 (start of infusion) and at 15, 30, 45, 60, 75, 90, 105 and 120 min

The study report does not differentiate among the different dosing regimens in the report of the results for either phase I or phase II nor does the report state the purpose of giving the four different dosing regimens. The results below pool the data from phase I and II into a single comparative experiment with an with an N of 4 for each dose level.

Results

The following graph from the study report shows the plot of the RR interval for controls and both dosing regimens.

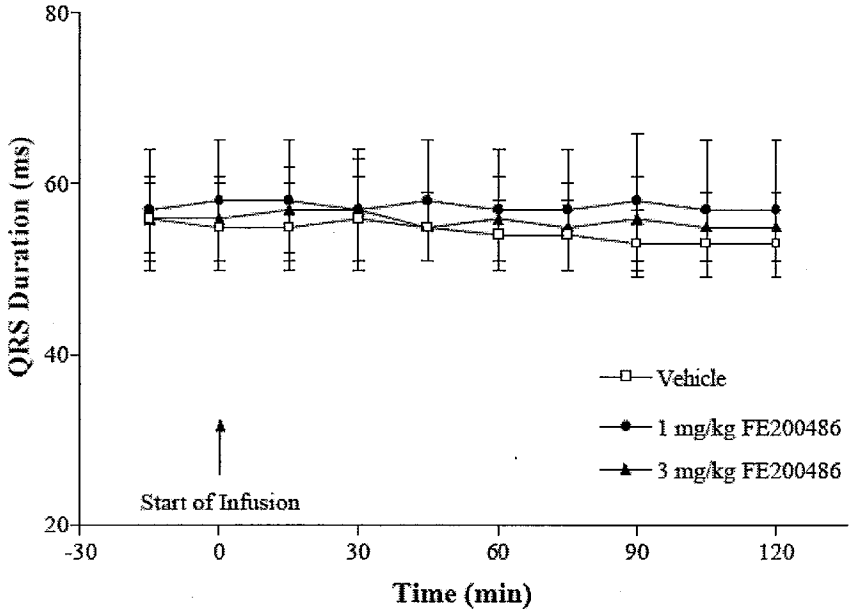
The Effect of Vehicle and FE 200486 on RR Interval in Anaesthetized Beagle Dogs



There appears to be a dose effect in that the control value is consistently higher than the values in treated dogs throughout the experiment, but this is confounded by the fact that the control value is higher at baseline. Graphs of the data for PR interval, QT interval, QTcF, QTcV and Heart Rate show similar differences between controls and treated animals suggestive of a treatment effect. The decreased RR interval in treated animals is consistent with an increase in Heart Rate. None of these parameters are significantly different from controls in the investigators analysis because of the limited sample size.

Ninety minutes after the QRS duration in the 1 mg/kg dose group was longer than that of the control group (58 ± 8 ms compared to 53 ± 4 ms, $p < 0.05$). This did not occur at any other time point and did not occur in the 3 mg/kg group.

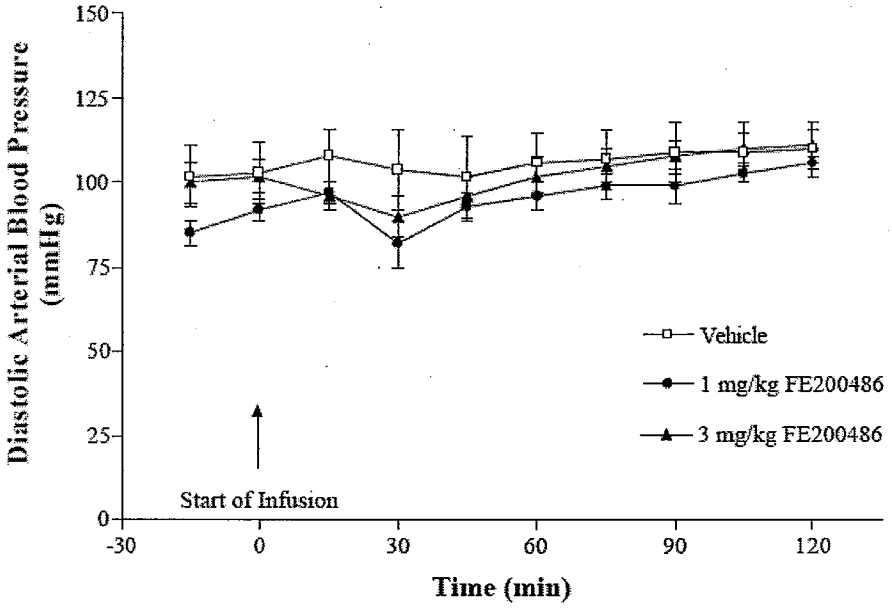
The Effect of Vehicle and FE 200486 on QRS Duration in Anaesthetized Beagle Dogs



At 30 minutes post dosing in the 1 mg/kg group there was an decrease in arterial diastolic pressure (82 ± 7 compared to 104 ± 12 mmHg in controls, $p < 0.05$). In the 3 mg/kg group, all pressure parameters were less than control between 15 and 30 minutes ($p < 0.05$). This effect was maximal at 30 min after the start of dosing when the mean, systolic and diastolic arterial blood pressures were 103 ± 6, 133 ± 8 and 90 ± 6 mmHg compared to 120 ± 12, 155 ± 15 and 104 ± 12 mmHg after vehicle, respectively.

Between 45 and 120 minutes in the low dose group, arterial systolic pressure increased above baseline ($p < 0.05$) with concomitant increase in diastolic and mean arterial pressure at 105 ($p < 0.05$) minutes and 120 minutes ($p < 0.01$). This slow increase in pressure the 30 minimum also occurred in the dogs dosed with 3 mg/kg ($p < 0.05$). The following graph from the study report demonstrates the changes in mean arterial pressure. Graph of diastolic and systolic pressure are similar.

The Effect of Vehicle and FE 200486 on Diastolic Arterial Blood Pressure in Anaesthetized Beagle Dogs



In the low dose group, left ventricular systolic pressure was above baseline at 120 minutes ($p < 0.05$). Contractility index dP/dtP^{-1} increased between 15 and 90 minutes ($p < 0.05$) when compared to vehicle. At 30 minutes post dosing $dP/dt.P^{-1}$ was 70 ± 4 compared to 55 ± 6 sec $^{-1}$. These changes were not accompanied by changes in cardiac output, stroke volume or total peripheral resistance. There were no toxicologically significant changes in respiratory parameters, blood gases, pH or pCO_2 but there was a transient increase in expiration time at 60 minutes ($p < 0.05$).

In high dose dogs, there was a significant decrease in left ventricular systolic pressure accompanied the reduction in arterial blood pressure at 15 min ($P < 0.01$) and 30 min ($P < 0.05$) after the start of dosing. Similar to the low dose group, the contractility index increased at 15 minutes (63 ± 4 compared to vehicle $51 \pm$ sec^{-1}, $p < 0.05$). Cardiac output, stroke volume and resistance were unaffected. Stroke volume increased at 120 minutes ($p < 0.05$). There were no toxicologically significant changes in arterial blood gases, pH or respiratory parameters. There was no toxicologically significant effect on histamine.

Pulmonary effects:

9) Evaluation of effect on respiration in the unrestrained conscious rat of both sexes following single subcutaneous administration – WDM

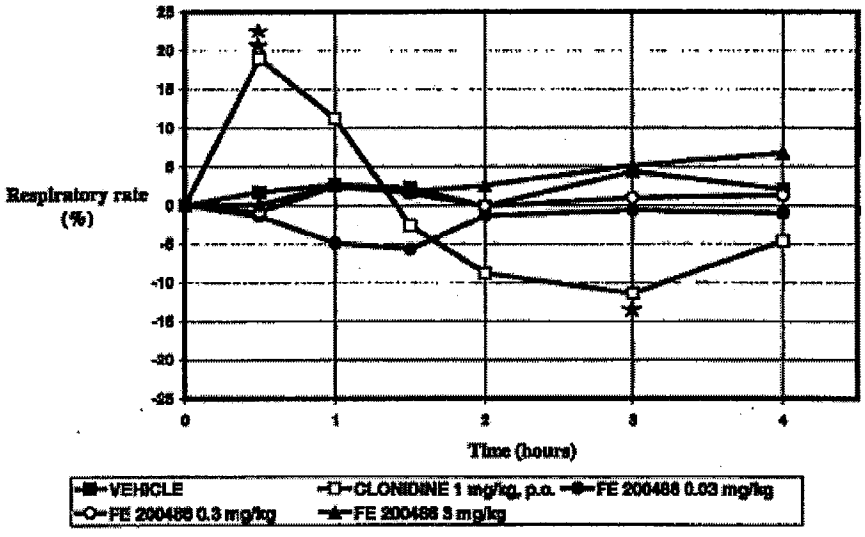
Major findings

Doses of 1 or 3 mg/kg (6 or 18 mg/m^2) had no effect on respiration in the unrestrained conscious rat.

Study number:	980360 P
Sponsor number:	FE 200486 DS PHA 9906
EDR filename	pha9906-preclinical-data.pdf
Conducting laboratory:	[redacted] b(4)
Date of study initiation:	October 1998
GLP compliance:	Yes
QA reports:	Yes
Drug:	Degarelix, Batch 20 1 1-032-30, Peptide content: 89.9%, Purity: 98.4%.
Methods:	
Animal	Male and female rats, WISTAR-DM: WI (EOPS CF).
Doses	vehicle control, positive control (1 mg/kg clonidine), 0.03, 0.3 or 3 mg/kg (0, 0.18, 1.8 or 18 mg/m^2)
N	1 per sex per dose group
Schedule	Single dose
Route	Subcutaneously
Formulation	Mannitol$_{aq}$ 5% w/v
Dose volume	10 mL/kg
Procedures	plethysmograph
Measurements	respiratory rate, peak inspiratory flow, peak expiratory flow, inspiration time, expiration time, tidal volume, airway resistance.

Results

The following graph from the study report shows that treatment with these low doses had no effect on the respiration rate of rats. Results for the other measured parameters were similar. Clonidine, the positive control, caused significant changes in respiration parameters.



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10) Respiratory function evaluation in conscious, freely moving rats by whole body plethysmography method after subcutaneous administration of FE200486 – WDM

Major findings

Single doses of degarelix given subcutaneously at levels of 0.5, 5 and 50 mg/kg (3, 30 or 300 mg/m^2) caused no significant changes in respiratory rate, tidal volume, minute volume, expiratory time, peak inspiratory flow, peak expiratory flow, relaxation time and Penh (enhanced pause). There was a small but significant change in inspiratory time in groups treated with 0.5 or 50 mg/kg.

Study number: AA18326
Sponsor number: FE200486DSPHA040
EDR filename pha0401-preclinical-data.pdf
Conducting laboratory ———— b(4)
Date of study initiation: October 1998
GLP compliance: Yes
QA reports: Yes
Drug Degarelix, Batch PPL-FE4860201B Purity > 99%, Peptide content 91.9%
Methods
Animal Male Sprague-Dawley rats: SD (IOPS RjHan)
Doses

Group/ Treatment	Dose level (mg/kg)	Dose volume (mL/kg)	Dose concentration (mg/mL)	Number of animals
A - Control	0	5	0	8
B - Low dose	0.5	5	0.1	8
C - Intermediate dose	5	5	1	8
D - High dose	50	5	10	8
E - Reference item	20	2	10	8

- Group A animals (control) received the vehicle (mannitol solution in sterile water for injection).
- Group E animals received the reference item (morphine-hydrochloride).

Schedule Single dose
Route Subcutaneously
Formulation Mannitol$_{aq}$ 5% w/v
Dose volume 5 mL/kg
Procedures Plethysmograph
Measurements Respiratory rate, peak inspiratory flow, peak expiratory flow, inspiration time, expiration time, tidal volume, airway resistance, relaxation time and Penh (enhanced pause)

Renal effects:

11) Evaluation of effect on urine output, urinary electrolyte balance and creatinine clearance in the rat of both sexes with a saline overload following single subcutaneous administration – RTD

Major findings:

- Degarelix increased male rat urinary flow rate between 2.5 and 5 hours after saline administration. This effect was evident at all degarelix doses tested (0.03, 0.3, and 3 mg/kg degarelix).
- Degarelix did not have any other effects on electrolytes or renal function under the conditions tested.
- 3 mg/kg degarelix, the maximal dose in this study, does not allow a thorough assessment of the potential renal effects that degarelix may have at higher doses.

Study no.	FE 200486 DS PHA 9903
——— study number	980364 P
EDR filename	pha9903-nonclinical-data.pdf
Conducting laboratory	———————————————
Date of study initiation:	October 1998
GLP compliance:	Yes
QA reports:	Yes
Drug:	Degarelix, Batch 2011-032-30, peptide content: 89.9% and purity 98.4%
Methods	
Doses:	Vehicle, 0.03, 0.3, and 3 mg/kg (0, 0.18, 1.8 or 18 mg/m^2) 50 mg/kg Furosemide as a positive control
Species/strain:	Rats/Wistar
Number/sex/group:	6 per sex per group
Route	Subcutaneous
Schedule	Single dose
Formulation	5% mannitol in sterile water
Volume:	0.4 mL/kg
Parameters:	2.5 and 5 hours after saline 2.5 hours – urine volume 5 hours – urinary pH urinary and plasma levels of sodium, potassium, chloride and creatinine. Also, osmolarity of plasma and urine, phosphorus and calcium.

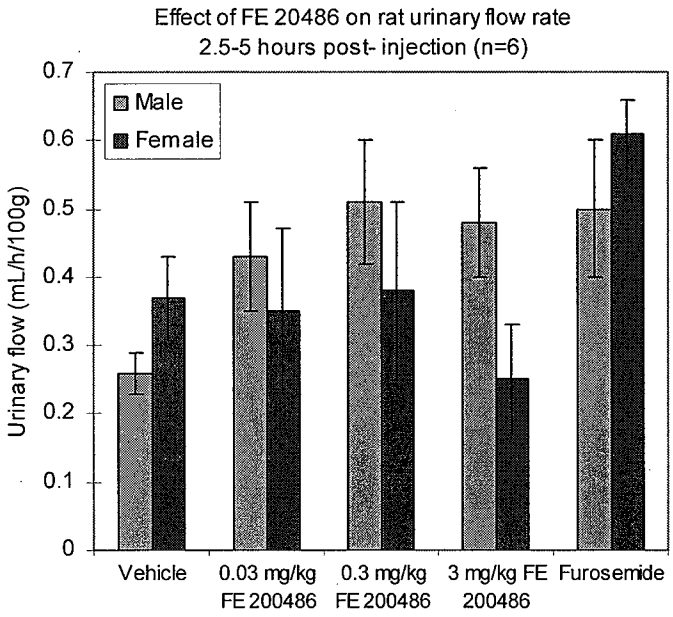
b(4)

Study design:

Six rats/sex/dose group received degarelix (vehicle, 0.03, 0.03 or 3 mg/kg degarelix) subcutaneously and then received 50 mL/kg isotonic saline solution by oral gavage. The effect of degarelix on urine volume, urine pH, electrolyte balance and creatinine clearance were evaluated at 2 and 5 hours after administration of saline.

Results:
Urinary flow rates increased at 2.5 -5 hours post-injection in male rats receiving degarelix. The lowest dose (0.03 mg/mL) increased urinary flow by 62%, while moderate (0.3 mg/kg) and high doses (3 mg/kg) increased urinary flow by 94% and 81%, respectively (See graph below). Furosemide, the positive control, increased urinary flow rate by 87%. While neither the furosemide nor the degarelix effects on

urinary flow rate are statistically significant, degarelix-dependent increases in urine production have also been noted in other species. Degarelix did not increase urinary flow rate in female rats under the same conditions.



A dose of 3 mg/kg is far lower than the recommended human dose so this experiment does not completely characterize this effect. Nevertheless, the effect is equal to or greater than that of Furosemide in both the mid and high dose groups in males.

There were no other degarelix-dependent effects on urinary pH, urinary and plasma levels of sodium, potassium, chloride and creatinine. Osmolarity of plasma and urine, phosphorus and calcium were also unaffected by degarelix within 5 hours of saline administration.

Gastrointestinal effects:

12) Evaluation of effect on intestinal transit following single subcutaneous administration in the rat of both sexes – RTD

Major findings:

Degarelix treatment caused no statistically significant decrease in intestinal transit in males and females combined. Nevertheless, the time from the injection until the rats were killed humanely was only 15 minutes. T_{max} in rats given SC doses is usually two hours or greater. This study is probably not informative.

b(4)

Study no.: FE 200486 DS PHA 9902
study number: 980363 P

EDR filename:	pha9902-nonclinical-data.pdf
Conducting laboratory:	[redacted] b(4)
Date of study initiation:	October 1998
GLP compliance:	Yes
QA reports:	Yes
Drug:	Degarelix, Batch 2011-032-30, Peptide content: 89.9%, purity 98.4%
Methods	
Doses:	Vehicle, 0.03, 0.3, and 3 mg/kg degarelix 20 mg/kg atropine as a positive control
Species/strain:	Rats/Wistar
Number/sex/group:	6 per sex per group
Route	Subcutaneous
Formulation	5% mannitol in sterile water
Volume:	0.4 mL/kg
Parameters and endpoints evaluated:	30 minutes after degarelix injection, vegetable charcoal in 2.5% carboxymethylcellulose hydrogel was administered orally and intestinal motility was assessed 15 minutes later.

Study design:

Rats received a subcutaneous injection of degarelix (vehicle, 0.03, 0.03 or 3 mg/kg degarelix) or atropine. Thirty minutes later, vegetable charcoal in 2.5% carboxymethylcellulose hydrogel was administered orally. Animals were euthanized after 15 minutes and investigators determined the total distance that the charcoal traveled in the intestine. Data are expressed as a percentage of the total length of the intestine.

Results:

Vehicle-treated rats transported the vegetable charcoal through 58.5% of the total intestine length in agreement with historical controls. Treatment with atropine, an inhibitor of intestinal motility, caused a significant 49% decrease in the rate of intestinal transit. Degarelix treatment caused no statistically significant decrease in intestinal transit in males and females combined. Nevertheless, the time from the injection until the rats were killed humanely was only 15 minutes. T_{max} in rats given SC doses is usually two hours or greater. This study is probably not informative.

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13) Study of FE 200486 vs. FE 992039 and FE 992023 (Cetrorelix) in the histamine release assay: measure of extracellular and intracellular histamine – RTD

Major findings:

- Degarelix, FE 992023 and FE 992039 each stimulated histamine release from rat mast cells.
- Degarelix stimulated the least amount of histamine release (40% of control) when compared with FE 992023 (83%) and FE 992039 (73%) under these experimental conditions.

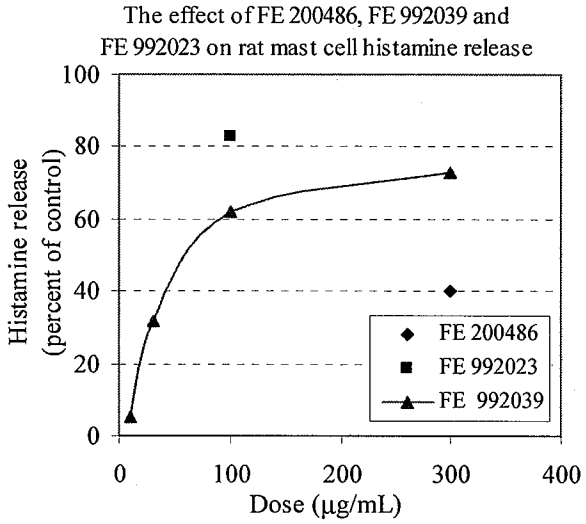
Study no.:	FE 200486 DS PHA 9813
EDR filename	pha9813-nonclinical-data.pdf

b(4)

Conducting laboratory	[redacted]		
Date of study initiation:	July 13, 1998		
GLP compliance:	Not required		
QA Report:	Yes		
Drug,	Drug	Lot #	Purity
	Degarelix	2011-032-30	98.4%
	FE 992023 (Cetrorelix) acetate	2004-184-15	98.9%
	FE 992039	2012-028-15	99.6%
	Compound 48/80	17H4151	
Methods			
Doses:	Degarelix : 300 µg/mL (184 µM)		
	FE 992023 100 µg/mL		
	FE 992039 10, 30, 100, 300 µg/mL		
	Compound 48/80		
Cells tested:	Sprague-Dawley rat peritoneal mast cells		
Number/group:	Duplicate		
Formulation:	Water (3mg/mL)		
Sampling times:	2 minutes at 37°C		

Results

FE 992039 dose-dependently stimulated histamine release from mast cells, reaching 73% of control at the highest dose (300 µg/mL). FE 992023 was more potent in stimulating histamine release, eliciting 83% histamine release at 100 µg/mL FE 992023. Degarelix also stimulated histamine release (40%), but elicited the lowest histamine response of the experimental compounds tested.



14) Effect of FE 200486 on histamine release from rat peritoneal mast cells: measure of extracellular and intracellular histamine – RTD

Major findings:

- Degarelix dose-dependently stimulated histamine release, but elicited less of a histamine response than the comparator compound, FE 992023.

Study no.:	FE 200486 DS PHA 9901
——— study:	883053 S 1-120
EDR filename	pha9901-nonclinical-data.pdf
Conducting laboratory:	———
Date of study initiation:	October 26, 1998
GLP compliance:	Not required
QA Report:	Yes

b(4)

Drug:

Drug	Lot #	Purity
Degarelix	2011-001-30	99.4%
FE 992023 (Cetrorelix) acetate	2004-184-15	98.9%
Compound 48/80	17H4151	

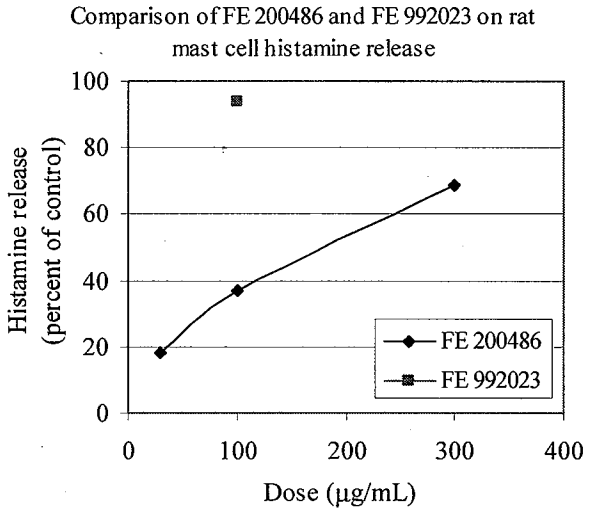
Methods

Doses:	Degarelix : 30, 100, 300 µg/mL (18.4 µM, 61.3µM, 184 µM) FE 992023 : 100 µg/mL Compound 48/80
Cells tested:	Sprague-Dawley rat peritoneal mast cells
Number/group:	Duplicate
Formulation:	Water (3 mg/mL)
Sampling times:	2minutes at 37°C

Results

Rat mast cells were treated with either degarelix or FE 992023 for 2 minutes and both extracellular and intracellular histamine were quantified. Data were expressed as a percentage of the histamine that was released from the positive control, compound 48/80.

Degarelix dose-dependently stimulated histamine release which ranged from 18% at low dose (30 µg/mL degarelix) to 69% at high dose (300 µg/mL degarelix, see graph below). Though a clear maximal effect was not achieved, the sponsor calculates the EC_{50} to be 170 µg/mL degarelix. FE 992023, the comparator compound, was more potent than degarelix, stimulating 94% histamine release at a dose of 100 µg/mL.



Pharmacodynamic drug interactions

1) Receptor Binding Profile- WDM

Major findings

The results of this study indicate that degarelix has little or no affinity for the pharmacological receptors studied.

Study number	883045 S 810/830
Sponsor number	FE200486DSPHA0203
EDR filename	pha9802-nonclinical-data.pdf
Conducting laboratory	[redacted] b(4)
Date of study initiation	January 1998
GLP compliance	No
QA reports	Yes
Drug	Degarelix, Batch 2011-001-30, and peptide content 86.4 %
Methods	
Animal	Tissues from rat, mouse, guinea-pig, chicken, calf, rabbit or cell culture depending on the assay
Doses	0 and 1µM
N	3 per assay
Schedule	Single dose
Formulation	Distilled water
Method	This experiment was a series of radioligand binding assays to determine the potential for inhibition of specific pharmacologically important receptors found on various tissues by degarelix. The results are expressed as a percent variation of control specific binding obtained in the presence of degarelix. IC_{50} values (concentration required to inhibit 50 % of specific binding) and Hill coefficients

(nH) were determined for the reference compounds by non-linear regression analysis of their competition curves. These parameters were obtained by Hill equation curve fitting. The inhibition constants (K_i) were calculated from the Cheng and Prusoff equation ($K_i = IC_{50}/(I+L/K_D)$, where L = concentration of radioligand in the assay, and K_D = affinity of the radioligand for the receptor). In all cases the concentration of degarelix was at least 150 fold higher than the normal radio-ligand.

Table 1 : FE 200486 at 1,000 nM (in distilled water solution)

Receptors	% change of specific binding
A_1	- 14
A_2	+ 13
α_1 (non-selective)	- 4
β_1	- 9
β_2	+ 4
Bombesin	- 5
B_2	- 6
CGRP	+ 7
CCK_A	- 4
CCK_B	- 5
D_1	- 6
D_2	+ 3
ET_A	- 2
ET_B	+ 5
Galanin	- 9
H_1 (central)	- 6
IP_3	- 4
Insulin	+ 26
ML_1	- 5
M (non-selective)	+ 1
NK_1	+ 16
NK_2	- 26
NK_3	- 9
NPY (non-selective)	- 3
Neurotensin	+ 8
Opiate (non-selective)	- 10
Oxytocin	- 17
PAF	+ 16
5-HT (non-selective)	+ 21
Somatostatin	- 4
Estrogen	- 4
Progesterone	+ 11
Testosterone	+ 3
VIP	- 1
V_1	+ 4
V_2	+ 7
Ca channel (L, verapamil site)	+ 9

The signs + and - indicate a stimulation and an inhibition, respectively.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

The following table (in three sections) is from the sponsor's "Pharmacology Tabulated Summary".

Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses (mg/kg) (Single dose unless specified otherwise.)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
Receptor binding profile	37 receptors	*In vitro*	1,000 nM		No or <30% inhibition of radioligand specific binding.	(Yes)	[PHAR002]
Histamine release - peritoneal mast cells	Rat SD isolated peritoneal mast cells	*In vitro*	300 µg/ml		FE200063: 40% release at 300 µg/ml (Abarelix: EC50 100 µg/ml) (Cetrorelix: 83% release at 100 µg/ml)	(Yes)	[PHAR013]
- peritoneal mast cells	Rat SD isolated peritoneal mast cells	*In vitro*	30, 100, 300 µg/ml		FE200486: EC50 170 µg/ml. (Cetrorelix: 94% release at 100 µg/ml)	(Yes)	[PHAR001]
- human skin	Human skin	*In vitro*	3, 30, 300 µg/ml		FE200436 had lower histamine releasing potency than abarelix, cetrorelix and ganirelix	-	[FSM-486-INT2003]
- cutaneous vascular permeability	Rat, Wistar-IM:WT(EOPS CF	i.d.	0, 1, 3 mg/site	8F	FE200486: No effect (Azaline B no effect) (Cetrorelix had marked effect at 0.1 mg/site)	Yes	[PHAR011]
- dog, systemic	Beagle dog	s.c.	20+20+20 or 20+40+60 mg/kg for 3 consecutive days	1M	Clinical signs (e.g. subcutaneous edema) consistent with a histamine reaction	-	[TOX0402]
CNS Primary observation test (Irwin)	Mouse NMRI	s.c.	0, 0.3, 1, 3, 10, 30	3M	0.3 mg/kg :No effect 1-10 mg/kg: Increased reactivity to touch at 15 min. 30 mg/kg: Increased reactivity to touch at 30 min & 24h; increased fear at 30 min; tremor in 1 animal at 30 min.	-	[PHAR001]

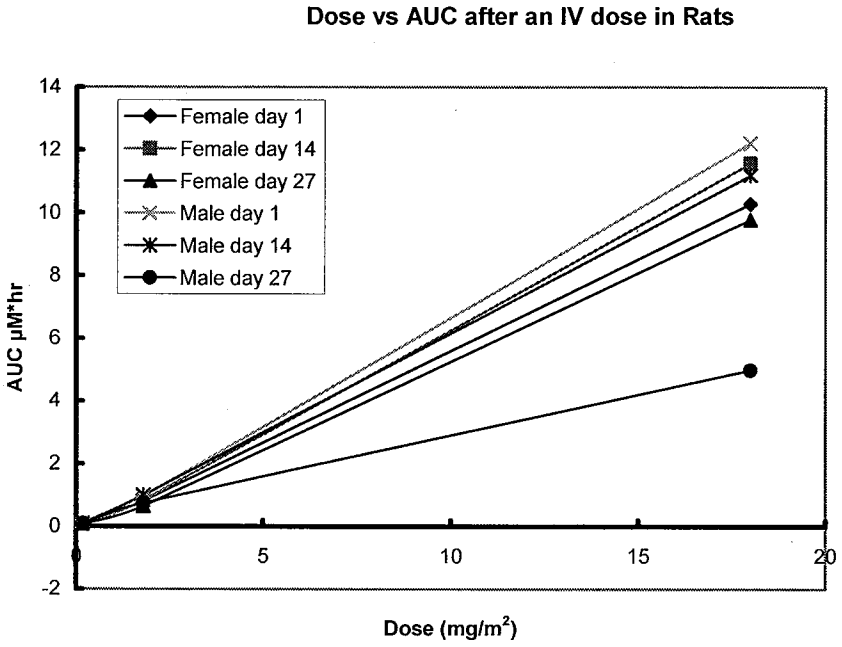
Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses (mg/kg) (Single dose unless specified otherwise.)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
CNS safety profile - Primary Irwin	Rat Rj:Wistar Han	s.c.	0, 0.03, 0.3, 3, 30	4M	0.03 mg/kg: No effect. 0.3 mg/kg: Increased reactivity to touch at 30 min; decreased muscle tone in 2/4 at 15 min. 3 mg/kg: Increased reactivity to touch and fear at 30 min; sedation at 15 & 30 min; decreased muscle tone at 15 & 30 min. 30 mg/kg: Increased reactivity to touch at 15, 30, 60 min & 24 h; increased fear at 15 min; sedation at 30 min; decreased muscle tone in 2/4 at 30 min.	-	[PHAR014]
- Activity meter			0, 0.03, 0.3, 3, (reference substances caffeine and chlorpromazine)	10M	3 mg/kg: No effect.		
- Rotating rod			0, 0.03, 0.3, 3, (reference substance diazepam)	10M	3 mg/kg: No effect.		
- Electro-convulsive shock threshold			0, 0.03, 0.3, 3, (reference substance diazepam)	15M	0.03 mg/kg: Statistically significant increase in threshold (23%); 1 death. 0.3 mg/kg: No statistically significant effect. 3 mg/kg: No statistically significant effect; 2 deaths		
CNS Irwin profile	Rat, Sprague Dawley RjHan	s.c.	0 (naive), 0 (vehicle), 0.5, 5 and 50 mg/kg	6M	No significant difference between any of the treated groups and naive or vehicle treated animals	Yes	[2372]
Cardio-vascular system	HEK293 cell transfected with hERG cDNA	*In vitro*	0, 10 µg/ml		No effect	Yes	[SHR0204]
Cardio-vascular system	Purkinje fibres	*In vitro*	0, 0.1, 2, 20 µg/ml		No effect	Yes	[PHA0103]
Cardiovascular system	Anaesthetized Dog Beagle	s.c.	0.003, 0.03, 0.3, 3 (all animals received all dose levels)	3M and 3F	No effect at any of the dose levels	Yes	[PHA0904]
Cardiovascular & respiratory	Conscious Dog Beagle	s.c.	1, 3	1M and 1F	No marked changes in: arterial pressure, heart rate, respiration rate or ECG intervals	Yes	[PHA0004]

Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses (mg/kg) (Single dose unless specified otherwise.)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
Cardiovascular & respiratory system	Conscious Dog Beagle	i.v.	0, 0.03, 0.3 (all 4 dogs), 3 (1 dog), 1 (the remaining 3 dogs)	2M and 2F	0.03 mg/kg: No effect. 0.3 mg/kg: Slight transient fall in heart rate (HR), 10 min after dose administration. 1 mg/kg: Transient increases in HR & BP approx. 4 to 15 min after dose administration. No ECG changes. 3 mg/kg: Marked hypotension in first animal treated - therefore all others treated at 1 mg/kg. Single premature QRS complex at 2.5h, and 2 premature ventricular contractions at 4 h after dosing.	Yes	[PHA0805]
Cardiovascular & respiratory system	Anaesthetized Dog Beagle	i.v.	1, 3	4M	1 mg/kg: No Notable effects. 3 mg/kg: Moderate decrease in blood pressure for 30 min and transient increase in contractility index.	Yes	[TOX0118]
	Cynomolgus monkeys	s.c.	0, 20 (three doses on three consequtive days	4M	No effect was observed during the first two applications, however the arterial blood pressure and heart rate did not decline to the expected level during the dark (sleep) period after the third administration but stayed at the levels observed at the light (wake) level.	Yes	[TOX0402]
Respiratory system	Rat, Wistar-DM:WT(EOPS CF	s.c.	0, 0.03, 0.3, 3, (method control, clonidine)	6M and 6F	No effect at any of the dose levels	Yes	[PHA9906]
	Rat, Sprague Dawley: SD(IOPS RJ Han)	s.c.	0, 0.5, 5, 50, (reference substance morphine)	8M	No effect at any of the dose levels, however a very weak but statistically significant effect on inspiratory time in 0.5 and 50 mg/kg group was observed.	Yes	[PHA0401]
Renal study with saline overload	Rat, Wistar-DM:WT(EOPS CF	s.c.	0, 0.03, 0.3, 3, (method control, furosemide)	6M and 6F	No effect at any of the dose levels	Yes	[PHA9903]
Autonomic nervous system	Rat, Wistar-DM:WT(EOPS CF	s.c.	0, 0.03, 0.3, 3 (method control, guanethidine)	6M and 6F	No effect at any of the dose levels	Yes	[PHA9905]
Intestinal transit	Rat, Wistar-DM:WT(EOPS CF)	s.c.	0, 0.03, 0.3, 3 (method control, atropine)	6M and 6F	No effect at any of the dose levels	Yes	[PHA9902]

2.6.4 PHARMACOKINETICS AND TOXICOKINETICS

2.6.4.1 Brief Summary

The following graph demonstrates that when degarelix is given to rats intravenously as a series of daily doses for 28 days, AUC increases linearly and proportionately with increasing dose except in the high dose male group after the 27th daily dose. The variation in this group is probably due experimental error. The graph also demonstrates that there is no accumulation of the drug. The doses in this experiment were significantly lower than the clinical dose.



The terminal elimination half-life in this study did not vary substantially with increasing dose or time on study. The mean value across all times and doses was 3 ± 1.5 hours. Clearance was 0.21 ± 0.04 L/kg/hr, significantly less than portal or renal blood flow; while the volume of distribution was 0.87 ± 0.46 L/kg, significantly greater than plasma volume suggesting cellular uptake.

In monkeys given daily IV doses for 28 days, the increase in AUC was less than dose proportional and nonlinear but there was no accumulation. Again the doses in these IV studies were considerably lower than the proposed clinical dose on a mg/m^2 basis. Half-life did not vary significantly with increasing dose or time on study. The mean value across all times and doses was 5 ± 2. Clearance was substantially less than that seen in the rat; the mean value was 0.070 ± 0.025 L/kg/hr. The volume of distribution was also less than that seen in the rat; the mean value was 0.5 ± 0.3 L/kg.

The following human parameters are from study CS05. When degarelix was given as a slow dose IV infusion to healthy adult men, the terminal elimination half-life increased with increasing infusion time and dose. The half-life was in all cases greater than that seen in monkeys or rats by at least a factor of 2.

	AUC (ng hr/mL)	C_{max} (ng/mL)	$T_{1/2}$ (hr)
6 µg/kg over 15 min (n = 6)	141 ± 34	38.2 ± 6.2	11.6 ± 5.2
15 µg/kg over 45 min (n = 6)	296 ± 82	58 ± 8.7	13.2 ± 1.7
30 µg/kg over 45 min (n = 6)	747 ± 120	160 ± 22	16.5 ± 1.8

Exposure increased proportionally and linearly with dose. In healthy volunteers given a single IV dose of 1 mg of degarelix as a 1 hour infusion, clearance was 3.2 ± 0.5 L/hr and volume of distribution was 79 ± 17 L (about 1 L/kg).

Parameters derived from the toxicokinetic studies of degarelix given subcutaneously are not informative because the absorption of the drug from the subcutaneous depot is rate limiting. The terminal

elimination half-life thus reflects the absorption rate constant, but in many cases this could not be determined accurately because the dosing interval was considerably shorter than five half-lives. Thus, values for clearance and volume were unusually large and variable. In almost all cases, the increase in C_{max} and AUC was non-linear and far less than dose proportional and most repeat dose studies demonstrated significant accumulation. Plots of C_{trough} demonstrated consistent exposure above the value of k_i even at most low doses.

After a single subcutaneous dose of 8.2 μg/kg (about 98.4 μg/m^2) of radiolabeled degarelix given to monkeys (1 per time point), about 10% of total radioactivity was recovered in the urine after 6 hours. Another 10% was excreted in the next 18 hours; after this urinary excretion was essentially complete with a total of 20 ± 6%. Urinary excretion in humans was 30.7% ± 4.3%, after 48 hours and was essentially complete. In the monkey study, fecal excretion of total radioactivity was essentially complete after 48 hours; total fecal excretion after 240 hours was 21 ± 22%. Total recovery of radioactivity, including cage wash, urine and feces, ranged from about 40 to 70% at 240 hours, suggesting that a significant amount of the drug remains as body burden. In these monkeys, total radioactivity distributed in highest amounts to excretory organs with the highest concentrations in bile, small intestine, urinary bladder, kidney, and liver respectively at 6 hours. Relatively high concentrations were found in the pituitary, prostate and testes consistent with the drugs pharmacology. Concentrations greater than that found in plasma were found in the aorta, lachrymal gland, lung, skin and vena cava. Elimination from the aorta, bile, pituitary, vena cava, prostate, kidneys and adrenals was slower than elimination from plasma.

In dogs, urinary excretion was about 50% while fecal excretion was about 40% of total radioactivity. Excretion was essentially complete after 48 hours. In this study in dogs, about 90% of plasma degarelix was protein-bound. The following table shows the spectrum of binding in human plasma protein.

TABLE 1: Binding of degarelix to human plasma proteins

Protein	Nominal FE200486 concentration (ng/mL)	Total concentration (C_t) of FE200486 (ng/mL)	binding (%)
Serum albumin	20	17.1	78.1
	60	50.3	77.9
	120	109	72.7
		Overall Mean	**76.3**
Gamma globulin	20	18.2	48.6
	60	46.7	46.6
	120	114	24.8
		Overall Mean	**40.0**
α_1 acid glycoprotein	20	19.6	82.5
	60	59.6	79.1
	120	116	73.0
		Overall Mean	**78.2**
High density lipoprotein	20	18.0	60.6
	60	55.1	56.9
	120	119	56.2
		Overall Mean	**57.9**
Human plasma	20	17.7	90.7
	60	54.7	90.3
	120	140	90.5
		Overall Mean	**90.5**

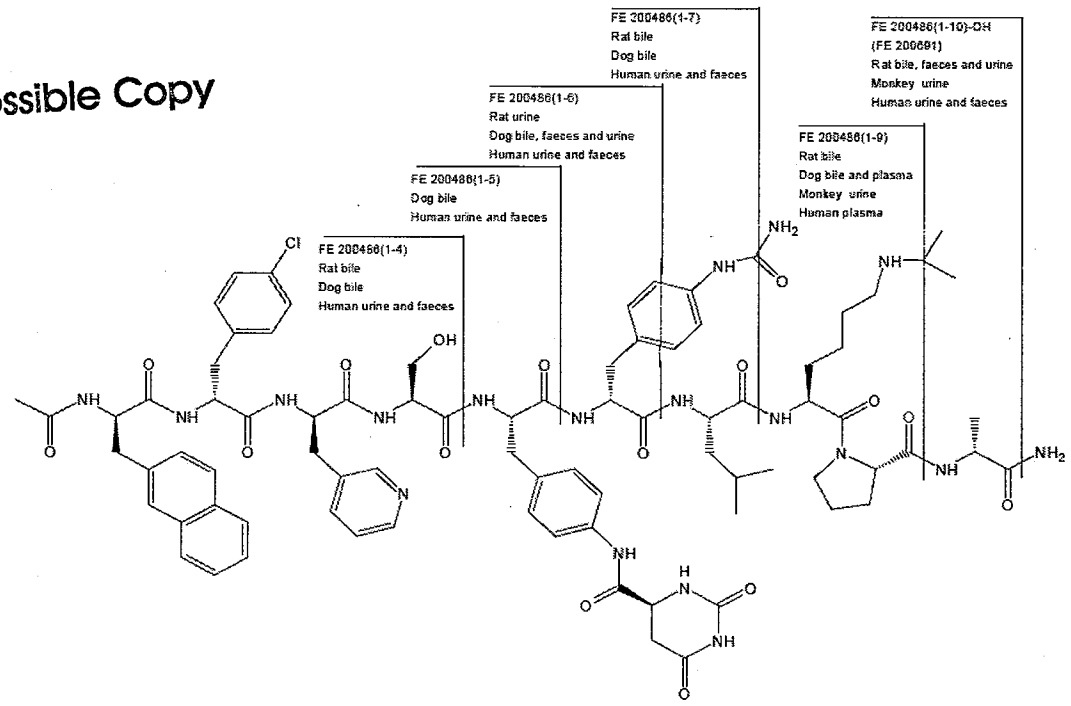
Distribution in dogs was similar to that in monkeys. In the dog study, about 97% of the radioactivity in urine was parent compound. In feces about 45% was parent, 26% was metabolite I-6, and 23% was metabolite M1. Plasma contained parent compound (78%) and metabolite M2a (18%).

In vitro, human hepatocytes oxidized degarelix to five different metabolites. One metabolite, degarelix (I-9), was the most abundant metabolite and was likely a product of proteolysis. The most abundant oxidative metabolite was degarelix-Ox-I, with oxidation occurring at the D-2 Nal amino acid. The second most abundant metabolite was degarelix-Ox-II, with oxidation occurring on the D-3Pal amino acid. Total metabolite concentration was less than 1 % of the parent compound in this *in vitro* system. Cytochrome P450s are not likely a major route for degarelix metabolism in humans. Glucuronidated-degarelix was found in the bile (45% of total) of dogs along with metabolites M1 (20.2 %), and degarelix (I-6) (17%).

The sponsor provided the following schematic diagram to illustrate the possible metabolic degradation of degarelix in various species.

2.6.5.11 Pharmacokinetics: Possible Metabolic Pathways

Test Article: degarelix



2.6.4.2 Methods of Analysis

The following studies validating the various methods of analysis were reviewed only from the sponsor's "Pharmacokinetics Written Summary." They were not reviewed individually.

1 Page(s) Withheld

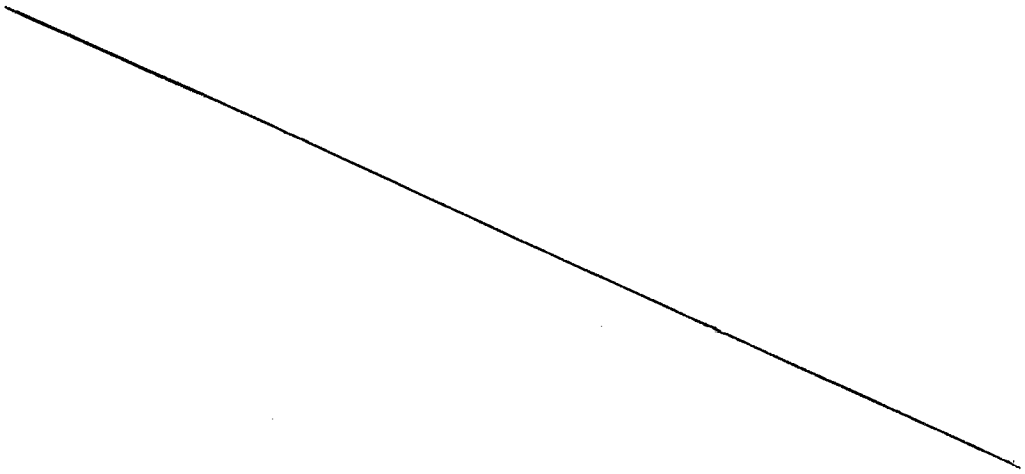
✓ Trade Secret / Confidential (b4)

_____ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Pharm/Tox- 2



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Portions of the following narrative are from the sponsor's "Pharmacokinetics Written Summary" with modification.

Radioimmunoassay (RIA)

Radioimmunoassay (RIA) was initially used to quantify degarelix in rat, dog and monkey samples. Study QFD-141 validated that this method. The lower limit of quantitation (LLOQ) was 500 pg/mL for rat serum and plasma, 250 pg/mL for monkey serum, 500-pg/ml for monkey plasma, 500 pg/ml for rabbit serum, 500-pg/ml for dog serum/plasma and 100 pg/ml for human plasma. The between run precision, expressed as coefficient of variation at the LLOQ, was between 5.9 and 10.5 % for plasma between 6.9 % and 17.5 % for the serum samples. At the upper limit of quantitation (ULOQ) the coefficient of variation was between 9.8 % and 21.0 % for plasma samples from all studied species and between 9.5 % and 17.3 % for the serum samples. The between-run accuracy (mean values) of the method at the LLOQ was between 106 % and 123 % for plasma samples from all studied species and between 103 % and 109 % for the serum samples. At the ULOQ the accuracy was between 88.2 % and 111 % for plasma samples from all studied species and between 90.9 % and 107 % for the serum samples.

Studies AR 576-151 and AR 576-152 extended the ULOQ of this RIA method from 4 pg degarelix/mL plasma to 20 pg/mL plasma. The LLOQ was 0.5 ng/mL for all three species. The mean inter-occasion accuracy was between 88.2% and 123% for rat plasma, between 99% and 106% for dog plasma, and between 102% and 117% for monkey plasma. The mean inter-occasion precision (expressed as coefficient of variation, CV) was between 4.6% and 20.7% for rat plasma, between 3.4% and 15.1% for dog plasma and between 3.1% and 21.0% for monkey plasma. Study TNO7041 validated RIA method for determining anti-degarelix antibodies in rat samples. The LLOQ was 1.0 ng/mL corresponding to 52 ng/mL in undiluted serum samples. Study SR-DCB-0020.01 validated new software for the RIA method.

The sponsor developed a liquid chromatography with tandem mass spectrometry (LC-MS/MS) method for detecting degarelix in biological samples. Study FRG/055 validated this technique in rat samples. Study FRG/050 validated it in dog samples and study FRG/049 validated it in monkey samples. This method used———————— as sample preparation technique. The mean inter-occasion accuracy for the determination of degarelix in rat plasma was 97.2% at 1 ng/mL (LLOQ), 101.1% at 3 ng/mL, 103.7% at 9 ng/mL and 105.2% at 45-ng/mL. The mean inter-occasion precision for the

b(4)

determination of degarelix in rat plasma was 12.1 % at 1 ng/mL, 8.1 % at 3 ng/mL, 5.2 % at 9 ng/mL and 7.0 % at 45 ng/mL. The mean inter-occasion accuracy in dog plasma was 103 % at 0.5 ng/mL (LLOQ), 100.9% at 1.5 ng/mL, 99.2% at 6 ng/mL and 98.7% at 45 ng/mL. The mean inter-occasion precision for degarelix in dog plasma was 10.9% at 0.5 ng/mL, 6.3% at 1.5 ng/mL, 6.3% at 6 ng/mL and 4.5% at 45 ng/mL. The mean inter-occasion accuracy for degarelix in monkey plasma was 103.6% at 0.5 ng/mL (LLOQ), 98.0% at 1.5 ng/mL, 95.0% at 6 ng/mL and 99.3% at 45 ng/mL. The mean inter-occasion precision for degarelix in monkey plasma was 10.8% at 0.5 ng/mL, 5.7% at 1.5 ng/mL, 4.5% at 6 ng/mL and 3.1% at 45 ng/mL.

Study AR576149 extended the ULOQ in rat plasma samples to 90 ng/mL again with ———— ———— The mean inter-occasion accuracy for degarelix in rat plasma was 105.5% at 0.5 ng/mL (LLOQ), 91.8% at 1.5 ng/mL, 93.5% at 50 ng/mL and 95.2% at 90 ng/mL. The mean inter-occasion precision for degarelix in rat plasma was 13.6% at 0.5 ng/mL, 6.5% at 1.5 ng/mL, 4.1% at 50 ng/mL and 3.1% at 90 ng/mL. b(4)

Studies MVR-PD-0007.01 and MVR-PD-0007.02 validated a change in the sample preparation method from ———————————. The mean inter-occasion accuracy for degarelix in rat plasma after this change was 106% at 1 ng/mL (LLOQ), 107% at 3 ng/mL, 109% at 100 ng/mL and 110% at 175 ng/mL. The mean inter-occasion precision for the determination of degarelix in rat plasma was 11% at 1 ng/mL, 5% at 3 ng/mL, 4% at 100 ng/mL and 3% at 175 ng/mL. b(4)

Study IR301586 validated the LC-MS/MS method for mouse plasma. The mean inter-occasion accuracy for degarelix in mouse plasma was 104.6% at 1 ng/mL (LLOQ), 100.1% at 2 ng/mL, 99.2% at 20 ng/mL and 103.2% at 325 ng/mL. The mean inter-occasion precision for degarelix in mouse plasma was 12.4% at 1 ng/mL, 11.8% at 2 ng/mL, 7.0% at 20 ng/mL and 13.4% at 325 ng/mL.

Study FRG/075 cross validated the two analytical techniques for the determination of degarelix, radioimmunoassay and liquid chromatography with tandem mass spectrometry.

Scintillation counting was used for measurement of total radioactivity for the isolation of degarelix metabolites. Measurement and identification of metabolites in plasma, urine, bile and feces were done by liquid chromatography with radiochemical detection (LC-RAD) and LC-MS/MS.

2.6.4.3 Absorption

1) ^{3}H-FE 200486 absorption, distribution, metabolism and excretion (ADME) in the dog after single doses - RTD

Major findings:

- 50% of degarelix administered subcutaneously is excreted into the urine, while 40% is excreted into the feces. The peak excretion of degarelix in the urine occurs between 6 and 24 hours post injection, while fecal excretion occurs in the first 48 hours.
- Peak plasma concentrations achieved 2 hours post-injection and decreased to trace amounts in the plasma after 24 hours. When injected subcutaneously, C_{max} is 6.7-ng equiv/g in males and 5.5 ng equiv/g in females, whereas IV injection C_{max} is 19.7 in males and 21.2 in females. Degarelix is 90% protein-bound in the first 24 hours after injection.
- Degarelix distributes to the bile, pituitary glands, vena cava, aorta, lungs and kidneys in the first 24 hours post-injection. Ten days post-injection, degarelix is at greatly reduced levels, but is higher in the aorta, bile, kidneys and pituitary gland relative to other tissues.
- In the urine, 97% of all radiolabeled compound was parent degarelix. Feces contained parent compound (45%) and the metabolites degarelix (I-6) (26%) and M1 (23%). Plasma contains degarelix (78%), but also M2a (18%).
- Degarelix-glucuronide was found in the bile (45% of total radiolabel) along with metabolites M1 (20.2 %), and degarelix (I-6) (17%).

Study no.:	FRG 072/014388		
EDR filename	frg072-nonclinical-data.pdf		
Conducting laboratory			
Date of study initiation:	April 23, 2001		
GLP compliance:	Yes		
QA Report:	Yes		
Drug:	Drug	Lot #	Purity
	^{3}H-Degarelix	424-075-050	>98%
	Degarelix	2011-032-30	98%
	FE 200691	D-0397	
	Degarelix(1-4)	E-0521	
	Degarelix(1-6)	E-0522	
	Degarelix(1-7)	E-0523	
	Degarelix(1-9)	E-0524	
	Degarelix(4-10)	E-0525	

b(4)

Methods

Doses	3 µg/kg [^{3}H]-Degarelix
Species	Dog/ Beagle
N	Group A : 2 per sex
	Group B : 2 per sex
	Group C : 3 per sex
Route	Group A : Subcutaneous
	Group B : Intravenous
	Group C : Subcutaneous

Formulation:	5% w/v ethanol : water (1:1)
Infusion rate:	0.4 - 0.5 ml formulation/kg bodyweight and 3 µg/kg bodyweight
Age:	12 - 18 months
Weight:	10 - 15 kg at time of dose
Sampling times:	Group A – single subcutaneous injection – blood plasma concentrations -15 and 30 minutes and 1, 2, 3, 4, 6, 8, 12, 24, 48, 96, 168 and 240 hours post injection. Urine - 0-6, 6-24, and at 24-hour intervals to 10 days after dose administration. Feces – 24 hour intervals up to 10 days post-injection. Tissue distribution examined at 10 days. Group B – single intravenous injection – blood plasma concentrations - monitored for 5, 10, 15 and 30 minutes and 1, 2, 3, 4, 6, 8, 12, 24, 48, 96, 168 and 240 hours up to 10 days. Group C – single subcutaneous injection – tissue distribution monitored in a male and female at 2, 8 and 24 hours.

Subcutaneous injection (Group A)

Clinical observations

Increased water intake and urine production were noted for a male and female dog Group A (subcutaneous), and for a male in Group B (intravenous).

Group	Animal ID	Increased water intake	Increased urine volume	Times noted
3 µg/kg SC	2M	X	X	up to 144 hrs post-inj.
	3F	X	X	48-72 hrs.
3 µg/kg IV	5M		X	24-48 hrs.

Excretion of radioactivity:

The peak excretion of degarelix in the urine occurred at 6 to 24 hours after subcutaneous injection of 3 mg/kg ^{3}H-degarelix. Urinary excretion accounted for approximately 50% of the total peptide amount. Excretion of degarelix into the feces occurs primarily 24-48 hours post-injection. Ultimately, 35-40% of total injected peptide was excreted in the feces. The total mean recovery of radioactivity in urine and feces from males was 94.3%, and 88.7% from females.

The following tables from the study report below documents the amount of degarelix excreted into the urine and feces over time from both male and female dogs that received subcutaneous injections.

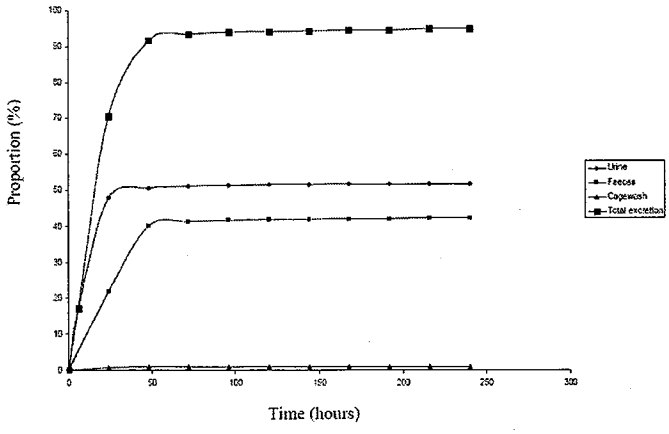
Time (hours)	Animal number and sex 1M	2M	Mean	± sd
Urine				
0 - 6			17.3	14
6 - 24			30.7	12
24 - 48			2.8	0.1
48 - 72			0.6	0.1
72 - 96			0.2	0.1
96 - 120			0.1	0.0
120 - 144			0.1	0.0
144 - 168			0.1	0.0
168 - 192			<0.1	0.0
192 - 216			<0.1	0.0
216 - 240			<0.1	0.0
Total urine	50.7	52.9	51.8	1.6
Cage washes				
0 - 24			0.8	0.4
24 - 48			0.1	0.0
48 - 72			<0.1	0.0
72 - 96			<0.1	0.0
96 - 120			<0.1	0.0
120 - 144			<0.1	0.0
144 - 168			<0.1	0.0
168 - 192			<0.1	0.0
192 - 216			<0.1	0.0
216 - 240			<0.1	0.0
Total cage washes	1.2	0.6	0.9	0.4
Faeces				
0 - 24			22.0	1.1
24 - 48			17.6	11
48 - 72			1.0	0.9
72 - 96			0.5	0.1
96 - 120			0.1	0.0
120 - 144			0.1	0.0
144 - 168			0.1	0.0
168 - 192			0.1	0.1
192 - 216			0.2	0.1
216 - 240			0.1	0.1
Total faeces	47.7	35.5	41.6	8.6
Total recovery	99.6	89.0	94.3	7.5

Time (hours)	Animal number and sex 3F	4F	Mean	± sd
Urine				
0 - 6			<0.1	<0.1
6 - 24			48.0	0.8
24 - 48			2.2	0.2
48 - 72			0.3	0.1
72 - 96			0.3	0.1
96 - 120			0.1	0.0
120 - 144			0.1	0.1
144 - 168			0.1	0.1
168 - 192			<0.1	0.0
192 - 216			<0.1	0.0
216 - 240			<0.1	0.0
Total urine	50.2	51.5	50.9	0.9
Cage washes				
0 - 24			0.5	0.2
24 - 48			0.1	0.1
48 - 72			<0.1	0.0
72 - 96			<0.1	0.0
96 - 120			<0.1	0.0
120 - 144			<0.1	0.0
144 - 168			<0.1	0.0
168 - 192			<0.1	0.0
192 - 216			<0.1	0.0
216 - 240			<0.1	0.0
Total cage washes	0.3	0.7	0.5	0.3
Faeces				
0 - 24			17.8	12.4
24 - 48			18.7	13.6
48 - 72			0.5	0.2
72 - 96			0.3	0.1
96 - 120			0.1	0.1
120 - 144			0.1	0.0
144 - 168			0.1	0.1
168 - 192			<0.1	0.0
192 - 216			<0.1	0.0
216 - 240			<0.1	0.0
Total faeces	36.3	38.3	37.3	1.4
Total recovery	86.8	90.5	88.7	2.6

b(4)

The following graph from the sponsor illustrates urine and fecal excretion of degarelix over time.

Mean cumulative excretion of radioactivity (total % dose) following a single subcutaneous dose of ^{3}H-FE 200486 to male dogs (Group A)

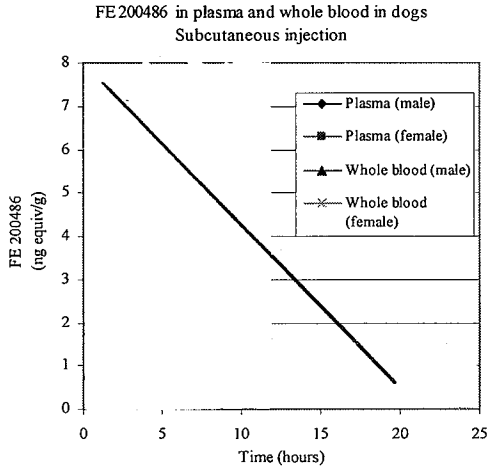


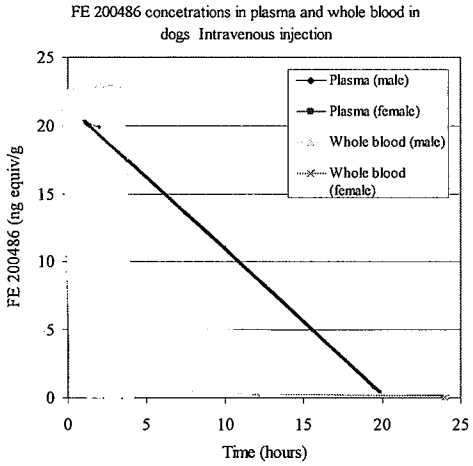
There was less than 0.1% of radioactivity found at the dose site.

Degarelix levels in blood and plasma

In plasma, the measured T_{max} for degarelix was 2 hours in both sexes. C_{max} was 6.7-ng/g in males and 5.5-ng/g in females. In whole blood, the measured T_{max} was also 2 hours; C_{max} values were lower than those measured in plasma indicating incomplete partition across the RBC membrane. Concentrations of degarelix in the whole blood are 3.6-ng/g in males and 3.0 ng/g in females (left graph).

Concentrations of degarelix after IV injection reached 19.7-ng equiv/g in male plasma, compared with 6.7 ng equiv/g when degarelix was injected subcutaneously. Concentrations in whole blood were less than those found in the plasma post-injection. All data is compiled in the Table below.





b(4)

Degarelix levels in plasma and whole blood after subcutaneous versus intravenous injection

	Dog subcutaneous injection				Dog intravenous injection			
	plasma		whole blood		Plasma		Whole blood	
Time (hours)	Male	Female	Male	Female	Male	Female	Male	Female
0.08								
0.17								
0.25								
0.5								
1								
2								
3								
4								
6								
8								
12								
24								
48								
96								
168								
240								

b(4)

The two tables below from the study report show the plasma pharmacokinetic parameters for both subcutaneous injection and intravenous injection. The C_{max} for subcutaneous injections in males is 6.7 ng equiv/g after 2 hours, whereas intravenous injection attains a C_{max} of 24.4 ng equiv/g at the time of injection. Interpretation of the AUC data has been cautioned (see footnote below tables), nevertheless, there appears to be no difference in AUC_t between the subcutaneous and intravenous routes of administration. The elimination half-life for degarelix, ranging from 93 hrs to 153 hours, appears to vary among gender and dosing groups with no apparent trend. The long half-life of degarelix is evident in all groups and is in agreement with data presented in other non-clinical studies.

Plasma total and non-volatile radioactivity pharmacokinetic data following a single subcutaneous dose of ^{3}H-FE 200 486 to dogs (Group A)

Sex	Dog no.	C_{max} (ng equiv/g)	T_{max} (hr)	AUC_t (ng equiv.h/g)	AUC (ng equiv.h/g)	λ_z (hours^{-1})	$t_{½}$ (hours)
Male	1M	7.7	2	63.8	65.5	0.0044	158.8
	2M	5.6	2	50.5	51.8	0.0048	144.0
	mean	6.7	2	57.2	58.7	0.0046	150.7[a]
Female	3F	6.2	2	45.5	46.2	0.0079	87.3
	4F	4.8	2	39.8	40.8	0.0069	99.9
	mean	5.5	2	42.7	43.5	0.0074	93.7[a]

Data from dogs 1M, 2M and 4F did not meet the acceptance criteria defined in Data processing and values for AUC, λ_z and $t_{½}$ must be viewed with caution
[a] Calculated from ln2/(mean rate constant)

Pharmacokinetic parameters derived from plasma total radioactivity concentrations in dog administered single intravenous bolus doses of 3.0 µg ^{3}H-FE200486/kg body weight

Sex	Dog no.	C_0 (ng equiv/g)	AUC_t (ng equiv.h/g)	AUC (ng equiv.h/g)	λ_z (hours^{-1})	$t_{½}$ (hours)
Male	5M	22.5	46.8	47.7	0.0087	79.8
	6M	26.2	53.5	55.5	0.0051	135.3
	mean	24.4	50.2	51.6	0.0069	100.5[a]
Female	7F	25.1	57.4	59.2	0.0055	126.6
	8F	26.6	50.9	52.7	0.0055	126.6
	mean	25.9	54.2	56.0	0.0055	126.0[a]

Data from dogs 6M, 7F and 8F did not meet the acceptance criteria defined in Data processing and values for AUC, λ_z and $t_{½}$ must be viewed with caution
[a] Calculated from ln2/(mean rate constant)

Tissue Distribution of Degarelix

Animals received a single subcutaneous injection of 3 mg/kg degarelix and tissues were taken from male and female dogs at 2, 8, 24 and 240 hours after injection. The largest distribution of radioactivity was found in the bile likely accounting for peptide excretion, and pituitary glands which are the target organ for degarelix. The vena cava and aorta show the next highest distribution in the first 24 hours of exposure to the drug. At the 2 hours time point, there are high levels of radioactivity in the plasma and kidneys though this decreases by 8 hours. A full battery of tissues is in included below.

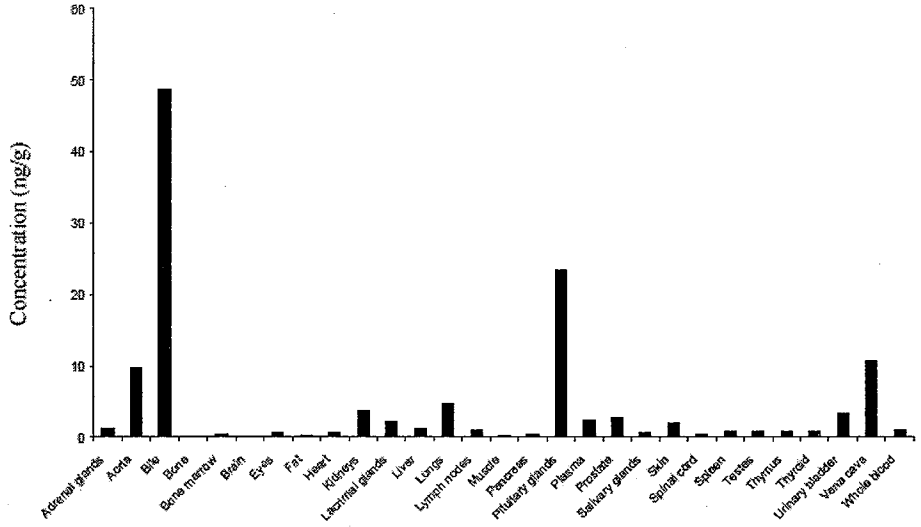
The Table below was sorted so that tissues with higher degarelix levels at 8 hours post-injection are found at the top of the table)

Tissue Distribution of [^{3}H]-Degarelix after subcutaneous injection in male dogs

Tissue	Sacrifice time			
	2 hours	8 hours	24 hours	10 days
Bile	14.3	48.7	53.7	0.027
Pituitary glands	12.5	23.4	12.8	0.067
Vena cava	6.6	10.7	4.07	0.014
Aorta	5.49	9.74	7.58	0.121
Lungs	4.69	4.79	0.944	0.004
Kidneys	8.14	3.79	1.05	0.028
Urinary bladder	2.62	3.39	0.306	0.004
Prostate	0.961	2.79	0.405	0.005
Plasma	6.29	2.42	0.286	0.008
Lacrimal glands	1.91	2.27	0.711	0.013
Skin	1.75	2.07	0.492	0.018
Liver	2.64	1.28	0.318	0.018
Adrenal glands	2.55	1.15	0.233	0.01
Lymph nodes	2.34	1.06	0.231	0.014
Whole-blood	3.57	0.97	0.188	0.006
Thyroid	1.96	0.877	0.18	0.014
Testes	1.03	0.852	0.172	0.004
Spleen	2.18	0.817	0.124	0.006
Thymus	1.85	0.77	0.418	0.015
Salivary glands	1.23	0.72	0.115	0.002
Heart	1.12	0.696	0.081	0.004
Eyes	0.547	0.577	0.151	0.003
Pancreas	1.16	0.49	0.096	0.004
Spinal cord	0.42	0.44	0.122	0.003
Bone marrow	1.08	0.376	0.115	0.009
Fat	1.12	0.332	0.149	<0.002
Muscle	0.494	0.274	0.052	0.003
Bone	0.104	0.078	0.016	<0.003
Brain	0.123	0.044	0.017	0.002

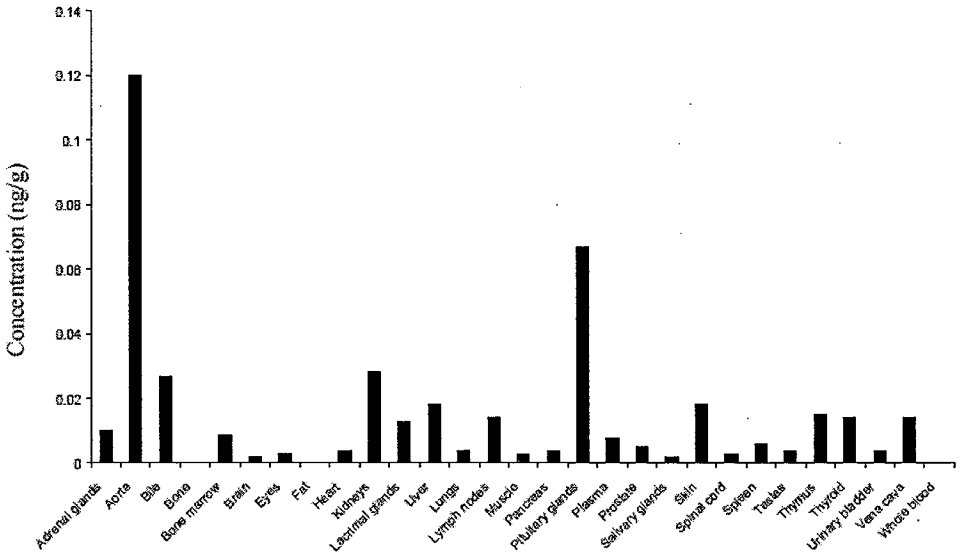
The following graph from the study report illustrates that the aorta, bile, pituitary glands and vena cava are the major sites for distribution after 8 hours.

Concentrations of radioactivity in tissues 8 hours after subcutaneous administration of ^{3}H-FE 200 486 in male dogs (Group C)



The sponsor's graph below identifies the aorta, bile, kidneys and pituitary gland as the sites with greatest distribution after 10 days. The actual amount of degarelix is low in the body after 10 days, as is evident by the small scale on the y-axis.

Concentrations of radioactivity in tissues 240 hours after subcutaneous administration of ^{3}H-FE 200 486 in male dogs (Group A)



The following table from the study report shows that degarelix is moderately protein-bound. Protein binding decreases at with time probably because the drug is metabolized to shorter chain peptides that bind less avidly to plasma proteins.

Proportions of plasma protein binding of radioactivity following intravenous administration of ^{3}H-FE 200 486 to dogs as determined by ultracentrifugation

Time (hours)	Animal number (sex)	
	6 (M)	8 (F)
0.25	90.2	93.0
2	92.0	92.8
8	91.0	92.0
24	85.9	87.4

Results are expressed as a percentage of the total sample radioactivity

Excretion

Urine

Urine samples from male and female dogs were analyzed for metabolites using HPLC which showed 97% of degarelix was unmetabolized, and approximately 2.0% was degarelix (I-6).

Feces

The following table from the study report shows that the feces contained approximately 45% unmetabolized degarelix, 30 % degarelix (I-6), and 22 % M1.

Proportions of components in faeces extracts (0 - 48 hours) determined using HPLC system 2 following a single subcutaneous dose of ^{3}H-FE 200 486 (3 µg/kg) to male and female dogs (Group A)

Results are expressed as % sample radioactivity

Component	Typical retention time (min)	Male	Female
M1	40	23.1	21.7
M2	41	26.6	33.0
FE 200 486	47	44.6	40.6
Others		<0.1	<0.1
Total extracts		94.2	95.3
Unextractable		5.8	4.7
Total		100	100

Bile

The following table from the study report illustrates that parent degarelix makes up 10% of the total recovered radioactivity. In addition, degarelix (I-6) is 18% of recovered radioactivity, and glucuronide-conjugated degarelix is 48% of the total recovered radioactivity.

Neither dog nor human microsomes conjugated glucuronide to degarelix *in vitro* after 60 minutes of incubation (see below). Nevertheless, the table below from the study report shows that between about 44 and 49% of the radioactivity is present in bile as the glucuronide conjugate of degarelix (M3).

Proportions of components in bile (24-hour sample) determined using HPLC system 2 after a single subcutaneous dose of ^{3}H-FE 200 486 (3 µg/kg) to male and female dogs

Results are expressed as % sample radioactivity

Component	Typical retention time (min)	Male	Female
M1	40	20.2	21.1
M2	41	17.4	18.5
M3	45	44.7	49.2
FE 200 486	47	10.6	10.7
Others		7.1	0.5
Total		100	100

Samples analysed were taken twenty four hours after a dose administration and were considered to be representative of other time points
Others Represents areas on the radiochromatogram which sum above zero but which contain no discrete areas of radioactivity
M2 was shown to correspond to FE 200 486 (1-6)
M3 was shown to be FE 200 486-glucuronide

Plasma

At 2, 8 and 24 hours after dosing, 2/3rd of the total recovered radioactivity in plasma is degarelix, while M2a represents 20% and M4 comprises 3-12% of total degarelix metabolites.

Proportions of components in plasma determined using HPLC system 2 after a single intravenous dose of ^{3}H-FE 200 486 (3 µg/kg) to male dogs

Results are expressed as % sample radioactivity and ng equivalents/g sample

Component	Typical retention time (min)	Time (hours)					
		2		8		24	
		%	ng/g	%	ng/g	%	ng/g
M2a	43	17.5	0.79	27.1	0.33	24.4	0.05
FE 200 486	47	77.6	3.49	68.1	0.82	65.2	0.12
Others		4.9	0.22	4.8	0.06	10.4	0.02
Total		100	4.5	100	1.2	100	0.19

Others Represents areas on the radiochromatogram which sum above zero but which contain no discrete areas of radioactivity

2.6.4.4 Distribution

1) The Disposition of Radioactivity in the Cynomolgus Monkey Following Single Subcutaneous Administration of [3H]-FE 200486 – RTD

Major findings:

- About 10% of injected [^{3}H]-degarelix was recovered in the urine after 6 hours. Another 10% was excreted in the next 18 hours; after this urinary excretion was essentially complete with a total of 20 ± 6%. After 48 hours fecal excretion of total radioactivity was essentially complete; total fecal

excretion after 240 hours was 21 ± 22%. Total recovery of radioactivity was less than 45% at 240 hours. These numbers are based on relatively few animals

- Total radioactivity distributed in highest amounts to excretory organs with the highest concentrations in bile, small intestine, urinary bladder, kidney, and liver at 6 hours.
- Relatively high concentrations were found in the pituitary consistent with the drugs pharmacology.
- Concentrations greater than that found in plasma distributed to the aorta, lachrymal gland, lung, skin and vena cava
- Relatively high concentrations were found in prostate and testes
- Elimination from the aorta, bile, pituitary, vena cava, prostate, kidneys and adrenals was slower than elimination from plasma.

Study no.:	DRSC1040
Sponsor study No.:	FE 200486DSTOX9905
EDR file name	drsc1040-nonclinical-data.pdf
Conducting laboratory	[redacted] b(4)
Date of study initiation:	November 17, 2003
GLP compliant:	Yes
QA report:	Yes
Drug:	[^{3}H]-Degarelix, Batch 524-185-040, purity 97.3% Degarelix, Batch PPL-FE4860001-PS-0313, purity 91.8%

Methods

Doses:	8.2 µg/kg degarelix
Species:	Cynomolgus monkeys
Number:	4 males (one monkey per time point)
Route:	Subcutaneous
Formulation:	5% mannitol (w/v) in water
Volume:	0.5 mL/kg
Age:	3- 4 years old
Weight:	4.21 kg to 7.58 kg
Sampling times:	6, 24, 48 and 240 hours
Tissues examined:	Adrenals, aorta, bile, bone marrow, brain, eyes, fat, heart, kidneys, lachrymal glands, large intestine (including contents), liver, lungs, lymph nodes, muscle, pituitary, prostate gland, salivary glands, skin, small intestine (and contents), spleen, stomach (including contents), testes, thyroid, urinary bladder, vena cava.

Results

The following table from the study report shows the excretion of degarelix as a function of time.

Recovery of radioactivity from male Cynomolgus monkeys following a single subcutaneous administration of [^{3}H]-FE 200 486 at a target dose level of 8.2 µg/kg

Sample	Collection time (h)	Per cent of adminisitered dose					
		001M	002M	003M	004M	Mean	±SD
Urine	6					10.0	1.2
	24					11.0	5.1
	48					2.0	0.9
	72					0.5	n.a.
	96					0.4	n.a.
	120					0.4	n.a.
	144					0.2	n.a.
	168					0.1	n.a.
	192					0.2	n.a.
	216					0.2	n.a.
	240					0.1	n.a.
	subtotal	11.4	22.2	26.3	19.1	19.7	6.3
Faeces	6					1.0	1.2
	24					8.3	8.9
	48					21.1	1.5
	72					6.5	n.a.
	96					2.0	n.a.
	120					1.9	n.a.
	144					0.3	n.a.
	168					0.2	n.a.
	192					0.1	n.a.
	216					0.1	n.a.
	240					0.0	n.a.
	subtotal	0.2	9.8	22.2	50.2	20.6	21.7
Cage wash	*					2.7	3.9
Methanol wash	*					0.6	0.4
Cage debris	*					0.6	0.7

b(4)

* - Time of sacrifice: 001M – 6 h
002M – 24 h
003M – 48 h
004M – 240 h

n.c. - not collected
n.a. - not applicable
SD - standard deviation

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On Original

The following table from the study report shows the tissue distribution of degarelix in monkeys as a function of time.

Mean tissue concentrations of radioactivity following a single subcutaneous administration of [^{3}H]-FE 200 486 at a target dose of 8.2 µg/kg to male Cynomolgus monkeys

Sample	Concentration (ng equivalents/g)			
	001M (6 h)	002M (24 h)	003M (48 h)	004M (240 h)
Adrenals	4.07	1.01	0.430	0.060
Aorta	11.9	12.0	6.41	0.120
Bile	733	218	36.1	0.209
Blood	n.s.	0.420	0.040	0.010
Bone marrow	2.16	0.590	0.180	0.020
Brain	0.070	0.010	BLQ	BLQ
Eyes	1.00	0.360	0.050	0.010
Fat	1.36	0.640	0.160	0.010
Heart	4.07	0.730	0.170	0.020
Kidneys	19.6	8.41	2.50	0.470
Lachrymal glands	5.43	1.52	0.120	0.020
Large intestine	102	137	136	0.690
Liver	13.2	2.74	1.08	0.060
Lungs	15.6	2.67	0.400	0.040
Lymph nodes	3.67	1.02	0.180	0.050
Muscle	0.830	0.140	0.020	0.000
Pituitary	25.7	33.7	25.1	0.490
Plasma	4.58	0.650	0.070	0.010
Prostate gland	7.40	0.570	0.470	BLQ
Salivary glands	2.06	0.420	0.110	0.020
Skin	5.28	0.770	0.180	0.120
Small intestine	25.8	8.58	1.50	0.060
Spleen	3.33	0.680	0.210	0.040
Stomach	0.670	0.900	0.210	0.020
Testes	3.29	0.600	0.210	BLQ
Thyroid	2.55	0.570	0.160	0.080
Urinary bladder	19.4	2.03	1.71	BLQ
Vena cava	8.49	8.43	3.66	0.030

The times in brackets indicates the time of sacrifice.
n.s. – no sample
BLQ – below the limit of quantification

2.6.4.5 Metabolism

1) *In vitro* metabolism of FE 200486 in human liver microsomes - RTD

Major findings

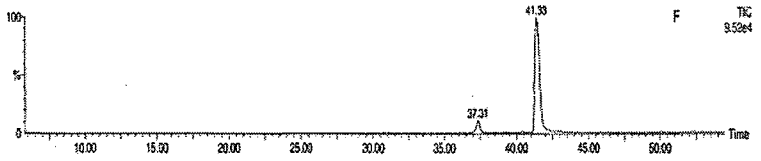
- Five oxidation products of degarelix were identified. One metabolite, degarelix (I-9), was the most abundant metabolite and was likely a product of proteolysis.
- The largest oxidative metabolite is degarelix Ox-I, with oxidation occurring at the D-2 Nal amino acid. The second largest metabolite is degarelix-Ox-II, with oxidation occurring on the D-3Pal amino acid.
- Metabolites represent < 1 % of the parent compound in this *in vitro* system. Cytochrome P450s are not likely a major route for degarelix metabolism in humans.

Study no.:	IAP-0193-00
EDR filename	iap-0193-00-nonclinical-data.pdf
Conducting laboratory	Ferring Pharmaceuticals
Date of study initiation:	August 8, 2001
GLP compliance:	No
QA report:	No
Drug,	Degarelix, batch 4869801, purity Peptide content 84.5%

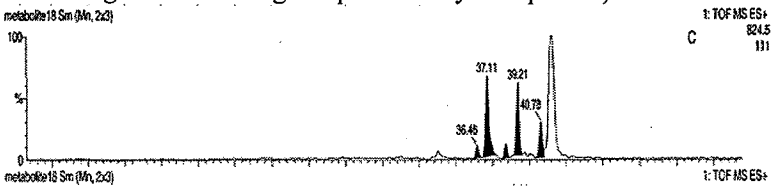
Methods

40 µM Degarelix was incubated in a 37°C water bath for varying times (0, 5, 10, 20, 40 and 60 min.) in the presence of NADPH regenerating system (NGS) and pooled human liver microsomes. The samples were analyzed for metabolic products using liquid chromatography (LC) using ultraviolet (UV) and mass spectrometry (MS) detection.

Two peaks, with retention times of 37.35 and 41.4 min. (degarelix parent compound), were present in the sample at "Time 0." The following chromatogram from the study report demonstrates the retention of these compounds.



Six peaks, one of which is parent compound, were produced after 60 minutes incubation with liver microsomes. (The following is a chromatogram provided by the sponsor)

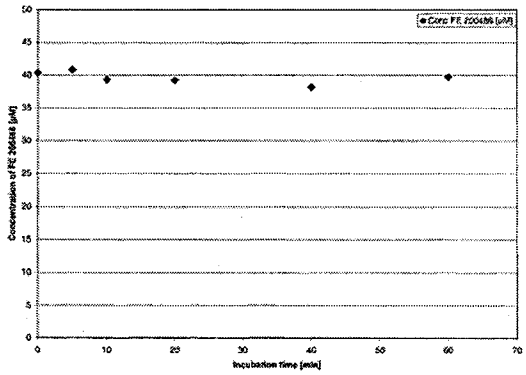


Time (min)

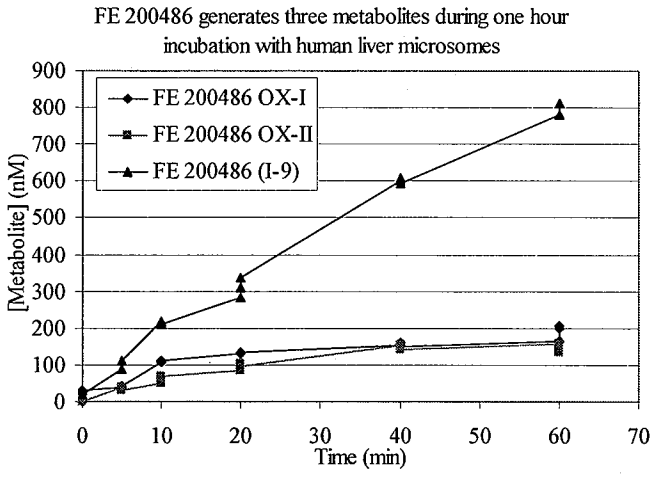
The concentration of degarelix remains essentially constant throughout the 60 minutes incubation with liver microsomes at 37°C, illustrating low levels of biotransformation by liver microsomes.

(See graph from the study report)

Concentration of degarelix in the presence of liver microsomes



The concentrations of degarelix and three major metabolites: degarelix Ox-I, degarelix Ox-II, and degarelix (1-9), were measured during 60 minutes incubation with liver microsomes.
The concentration of each metabolite increases over time and yields a maximal concentration of 208 nM for degarelix Ox-I, 149 nM for degarelix Ox-II, and 812 nM for degarelix (1-9).



Based on mass spectra showing proton adduct data, degarelix Ox-I and degarelix Ox-II represent oxidative products resulting from marginal amounts of cytochrome 450 metabolism of the parent peptide degarelix. Degarelix (I-9) is not a product of cytochrome 450 and is likely a product of proteolysis from the liver microsome preparation.

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2) Study on stability of FE 200486 when incubated *in vitro* in human plasma at 37°C during 60 minutes – RTD

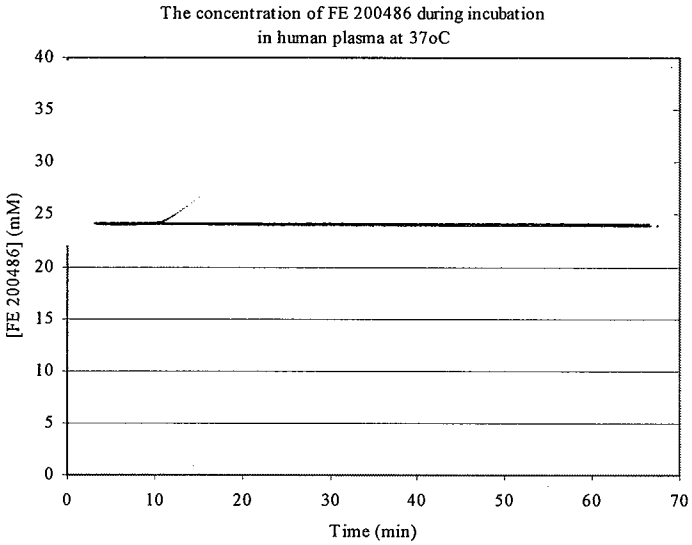
Major findings:

- The concentration of degarelix did not measurably decline from an initial value of 29 µM after it was incubated with freshly prepared human plasma for 60 minutes at 37 C. Thus, degarelix is not labile in human plasma *in vitro*.

Study no.:	IAP—216-00
EDR filename	iap-0216-00-nonclinical-data.pdf

Conducting laboratory	Ferring Pharmaceuticals
Date of study initiation:	April 23, 2001
GLP compliance:	No report
QA report:	No
Drug	Degarelix, batch 4869801, Peptide content 84.5%

The stability of degarelix (29 μM) was tested in human plasma at 37°C for a time course that spanned 60 minutes (0, 5, 10, 20, 30, 40, 50, 60 minutes). After the plasma proteins were precipitated, the supernatants were analyzed for degarelix proteolytic products by LC using UV and mass spectrometry. The concentration of degarelix remained constant in human plasma throughout the duration of the study.



b(4)

Degarelix suspended in stop solution yielded a peak at 39.83 min, which is found in samples that are incubated in human plasma after 0 minutes (second tracing below) and 60 minutes (third tracing). The plasma preparations yielded an additional peak at 47 minutes which is present in both 0 and 60 minute tracings, and which do not contain degarelix products. The degarelix peptide is therefore stable in human plasma.

The extraction efficiency appears acceptable since the amount of degarelix extracted from the plasma content (second tracing) is comparable to the 29 mM standard in stop solution (in top tracing).

(The following chromatograms are supplied by the sponsor)

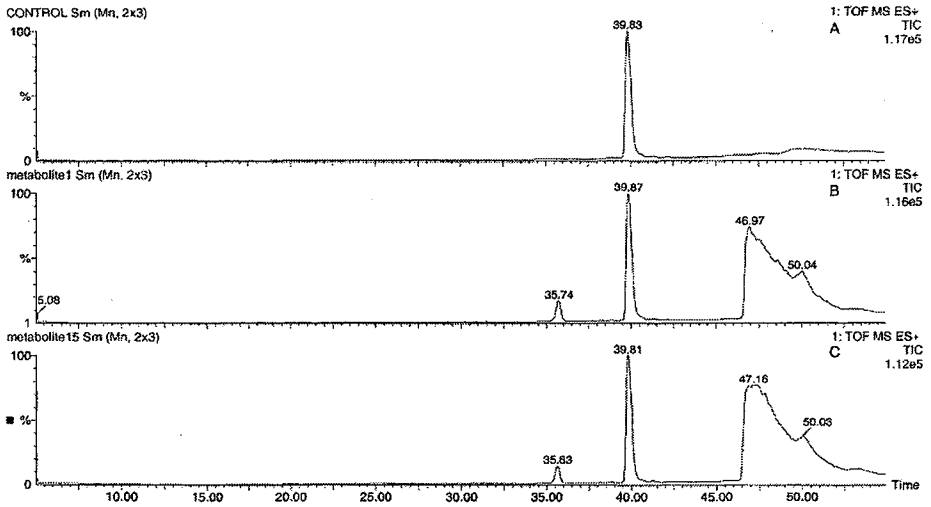


Figure 2. LC/MS (total ion current) chromatograms of 29 µM FE 200486 in (A) stop solution, (B) human plasma after incubation at 37°C for 0 min and (C) human plasma after incubation at 37° for 60 min.

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3) Investigation on possible *in vitro* glucuronidation of FE200486 in dog and human liver microsomes treated with alamethicin - RTD

Major findings:

- Degarelix does not undergo glucuronidation after a 60 minute incubation with human or dog liver microsomes in the presence of the glucuronidation cocktail: UDPGA, alameticin, MgCl, NADPH and D-Saccharic acid-1,4-lactone.

Study no.:	AR-DCB-0010.01
EDR filename	ar-dcb-001001-nonclinical-data.pdf
Conducting laboratory	Ferring Pharmaceuticals, Copenhagen Denmark
Date of study initiation:	April 20, 2005
GLP compliance:	No
QA report:	No
Drug,	Degarelix, batch 004862 #PPL-FE486 0001, Peptide content 91.77%

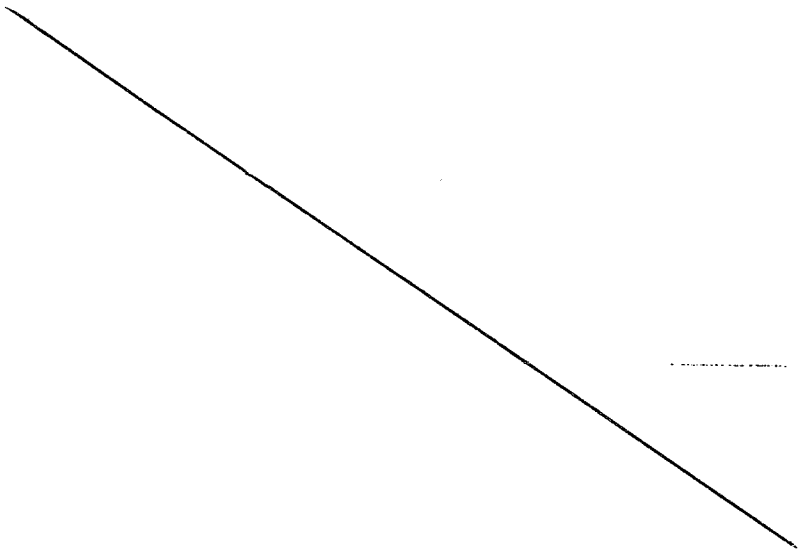
Approach:

This study investigates whether degarelix is modified by glucuronic acid in dog and human liver microsomes. Various concentrations of degarelix (1µM, 10 µM, 50 µM, 100 µM, 150 µM, 1.5 mM, 7.5 mM and 15 mM degarelix) were incubated with liver microsomes, alameticin for permeability, MgCl, NADPH, D-Saccharic acid-1,4-lactone and UDPGA. The concentration of degarelix used in this study, 100 µM degarelix, is orders of magnitude above physiologic concentrations and is therefore acceptable. Samples were taken at 0, 30 and 60 minutes during the reaction and analyzed by LC-MS and LC with tandem MS for detection of degarelix that has been modified by glucuronidation.

Results:

Experiments showing the expected glucuronidation of 7-ethoxycoumarin in both dog and human liver microsomes were successfully performed to validate the detection of glucuronidation by LC-MS.

Pooled dog liver microsomes (DLM) were incubated with 100 µM degarelix for 60 minutes at 37°C but did not lead to any glucuronidation of the peptide. The only metabolite detected was a previously identified truncated peptide named degarelix (I-9) which is the product of proteolysis from the microsome. Similarly, degarelix was incubated with human liver microsomes and did not lead to glucuronidation of the peptide as evaluated from neutral-loss scan by LC/MS/MS for an expected 176 Da loss (see chromatograms from the study report below).



b(4)

Figure 15. Neutral loss LC-MS/MS analysis of samples representing incubation of 100µM FE200486 in DLM, measuring loss of 176Da. Trace A represents a 60min incubation sample, trace B represents a 30min incubation sample and trace C represents a 0min incubation sample.

2.6.4.6 Excretion

See above

2.6.4.7 Pharmacokinetic drug interactions

See above

2.6.4.8 Other Pharmacokinetic Studies

None

2.6.4.9 Discussion and Conclusions

See above in summary

2.6.4.10 Tables and figures to include comparative TK summary

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2.6.5 PHARMACOKINETICS TABULATED SUMMARY

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Study	species	schedule	Route	Gender	Dose Number	doses mg/kg	doses mg/m_2	C_{max} ng/g	C_{max} µM	AUC ng*h/ml	AUC µM*hour	T_{max} hour	$t_{1/2}$ hours	Clearance mL/kg/h	Vb mL/kg
FRG 072/014388	Dog	single	SC	male	single	3 mg/kg	60	6.7	0.004	58.7	0.036	2	150.7		
frg072-nonclinical-data.pdf				female		3 mg/kg	60	5.5	0.003	43.5	0.027	2	93.7		
			IV	male		3 mg/kg	60	24.4	0.015	51.6	0.032	0	100.5		
				female		3 mg/kg	60	25.9	0.016	56	0.034	0	126		
FE200486DSTOX0112	Rats	once per 2 weeks	SC	male	1	0.5	3	86	0.053	1530	0.94	2	77		
[redacted] 455806		for 13 weeks			6	0.5	3	81	0.05	2134	1.31	2	85		
tox0112-nonclinical-data-1.pdf				male	1	5	30	129	0.079	17352	10.63	2	416		
TOX0112					6	5	30	115	0.07	/	/	4	506		
				male	1	50	300	166	0.102	37104	22.73	4	227		
					6	50	300	348	0.213			48	466		
				female	1	0.5	3	95	0.058	1464	0.9	2	74		
					6	0.5	3	86	0.053	2125	1.3	2	52		
				female	1	5	30	150	0.092	11785	7.22	2	223		
					6	5	30	131	0.08	52962	32.45	4	844		
				female	1	50	300	208	0.127	32017	19.61	4	272		
					6	50	300	321	0.197	183486		8	581		
FE200486DSCAR0101	Rat	per 2 weeks	SC	male	1	2	12	143	0.088	7445	4.56	2	169	269	65537
		for 26 weeks		male	1	10	60	159	0.097	29039	17.79	2	348	344	172912
car0101-nonclinical-data.pdf				male	1	25	150	212	0.13	58744	35.99	2	382	426	234549
				male	13	2	12	186	0.114	20389	12.49	8	237	144	49799
				male	13	10	60	299	0.183	130739	80.09	4	473	187	124401
				male	13	25	150	553	0.339	511448	313.33	8	2130	270	449894
				male	26	2	12	124	0.076	21651	13.26	12	139	139	44686
				male	26	10	60	316	0.194	93712	57.41	8	195	195	84563
				male	26	25	150	699	0.428	397263	243.38	8	215	215	238563
				male	39	2	12	137	0.084	23650	14.49	4	119	119	32181
				male	39	10	60	371	0.227	189049	115.82	8	161	161	145886
				male	39	25	150	672	0.412	239476	146.71	12	218	218	108919
				male	52	2	12	184	0.113	29400	18.01	8	103	103	32316
				male	52	10	60	370	0.227	256240	156.98	8	131	131	130657
				male	52	25	150	621	0.38	335654	205.63	24	178	178	104626
FE200486DSTOX0111	Mice	per 2 weeks	SC	male	1	1	3	413	0.253	3771	2.31	2	95.37	265	36482
		for 13 weeks		male	6	1	3	167	0.102	4580	2.81	2	214	294	67449
TOX0111				male	1	10	30	452	0.277	17885	10.96	2	215	559	173523
				male	6	10	30	379	0.232	26635	16.32	2	147	469	79746
tox0111-nonclinical-data-1.pdf				male	1	100	300	881	0.54	48654	29.81	2	96.32	2055	285609
				male	6	100	300	2605	1.596	163525	100.18	12	141	754	125166
				female	1	1	3	184	0.113	2589	1.59	2	32	386	17787
				female	6	1	3	280	0.172	2604	1.6	2	232	446	128745
				female	1	10	30	387	0.237	35172	21.55	2	799	284	327937
				female	6	10	30	396	0.243	64056	39.24	2	528	406	118869
				female	1	100	300	731	0.448	47316	28.99	8	335	2113	1021134
				female	6	100	300	4855	2.974	274797	168.35	8	61	374	32017
FE200486DSTOX0401	Rat	per 2 weeks	SC	male	1	10	60	207	0.127	12800	7.84	4			
		for 26 weeks		female	1	10	60	200	0.123	14300	8.76	2			
TOX0401				male	1	50	300	267	0.164	27800	17.03	2			
				female	1	50	300	287	0.176	26800	16.42	2			
tox0401-nonclinical-data.pdf				male	1	100	600	296	0.181	25100	15.38	4			
				female	1	100	600	219	0.134	29500	18.07	4			
				male	7	10	60	327	0.2	34200	20.95	4			
				female	7	10	60	309	0.189	30400	18.62	8			
				male	7	50	300	499	0.306	87400	53.54	4			
				female	7	50	300	432	0.265	80300	49.19	4			
				male	7	100	600	649	0.398	122000	74.74	24			
				female	7	100	600	533	0.327	99100	60.71	8			
				male	13	10	60	254	0.156	35500	21.75	8			
				female	13	10	60	223	0.137	38100	23.34	12			
				male	13	50	300	509	0.312	110000	67.39	24			
				female	13	50	300	688	0.421	114000	69.84	24			
				male	13	100	600	733	0.449	179000	109.66	24			
				female	13	100	600	605	0.371	138000	84.54	24			

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Study	species	schedule	Route	Gender	Dose Number	doses mg/kg	doses mg/m_2	C_{max} ng/g	C_{max} µM	AUC ng*h/ml	AUC µM*hour	T_{max} hour	$t_{1/2}$ hours	Clearance mL/kg/h	Vb mL/kg
FE200486DSTOX0126	Monkey	Monthly	SC	male	1	0.5	5	92	0.056	10519	6.44	1	189	65	18141
tox0126-nonclinical-data.pdf		for 12 months		female	1	0.5	5	112	0.069	10551	6.46	1	226	67	27798
				male	1	5	50	276	0.169	102003	62.49	24	221	49	15625
TOX0126				female	1	5	50	139	0.085	38546	23.61	16	300	130	56082
				male	1	50	500	1106	0.678	288028	176.46	24	177	174	44340
				female	1	50	500	923	0.565	275309	168.66	24	287	198	83901
				male	3	0.5	5	46	0.028	5583	3.42	1	236	95	36291
				female	3	0.5	5	69	0.042	8718	5.34	1	227	91	26757
				male	3	5	50	201	0.123	47933	29.37	24	260	135	39150
				female	3	5	50	134	0.082	\	\	14	\	214	\
				male	3	50	500	837	0.513	412056	252.44	48	289	332	59596
				female	3	50	500	1475	0.904	382794	234.51	36	295	287	59970
				male	7	0.5	5	35	0.021	4885	2.99	1	256	130	47215
				female	7	0.5	5	47	0.029	7495	4.59	1	251	125	36468
				male	7	5	50	131	0.08	\	\	24	\	179	\
				female	7	5	50	114	0.07	\	\	24	\	229	\
				male	7	50	500	1034	0.633	395520	242.31	48	315	279	67183
				female	7	50	500	1652	1.012	318008	194.82	48	252	320	77464
				male	13	0.5	5	42	0.026	12891	7.9	1	162	84	9088
				female	13	0.5	5	58	0.036	7097	4.35	1	263	105	37716
				male	13	5	50	204	0.125	88058	53.95	48	243	166	46827
				female	13	5	50	124	0.076	62716	38.42	24	264	199	30349
				male	13	50	500	773	0.474	\	\	24	\	317	\
				female	13	50	500	1831	1.122	179184	109.77	24	288	221	121150
FE200486DSCAR010	Mice	per 2 weeks	SC	male	1	2	6	294	0.18	4290	2.63	2	26	466	17752
455832		for 104 weeks		male	13	2	6	391	0.24	11470	7.03	2	127	195	35455
				male	26	2	6	293	0.18	10844	6.64	2	182	224	59036
car0102-nonclinical-data.pdf				male	39	2	6	506	0.31	10458	6.41	2	43	192	11988
				male	52	2	6	303	0.186	11335	6.94	2	104	188	28229
				male	1	10	30	409	0.251	27099	16.6	4	456	369	243025
				male	13	10	30	759	0.465	98819	60.54	2	393	218	121054
				male	26	10	30	523	0.32	95808	58.7	2	462	245	163468
				male	39	10	30	651	0.399	75058	45.98	4	258	214	79172
				male	52	10	30	246	0.151	44206	27.08	4	131	278	52553
				male	1	50	150	482	0.295	43487	26.64	2	275	1150	455486
				male	13	50	150	1419	0.869	157298	96.37	2	135	376	72222
				male	26	50	150	900	0.551	218146	133.64	96	102	262	38478
				male	39	50	150	2204	1.35	178587	109.41	12	118	314	53207
				male	52	50	150	1004	0.615	115705	70.88	48	171	506	124662
				female	1	2	6	292	0.179	,4177	2.56	2	51	479	34924
				female	13	2	6	526	0.322	9256	5.67	2	92	227	31102
				female	26	2	6	327	0.2	6902	4.23	2	85	301	36691
				female	39	2	6	811	0.497	9304	5.7	2	44	216	13738
				female	52	2	6	239	0.146	10317	6.32	2	207	240	71729
				female	1	10	30	652	0.399	16265	9.96	2	191	615	169095
				female	13	10	30	754	0.462	80474	49.3	2	338	234	116823
				female	26	10	30	561	0.344	71564	43.84	2	261	229	84886
				female	39	10	30	680	0.417	64923	39.77	2	135	193	37759
				female	52	10	30	331	0.203	46904	28.73	4	156	276	62147
				female	1	50	150	614	0.376	33403	20.46	2	208	1497	450019
				female	13	50	150	1460	0.894	184942	113.3	48	79	291	33049
				female	26	50	150	1550	0.95	306188	187.58	96	77	175	19408
				female	39	50	150	1550	0.95	198287	121.48	48	151	303	65957
				female	52	50	150	985	0.603	200108	122.59	48	525	508	384729

Continued

Study	species	schedule	Route	Gender	Dose Number	doses mg/kg	doses mg/m_2	C_{max} ng/g	C_{max} µM	AUC ng*h/ml	AUC µM*hour	T_{max} hour	$t_{1/2}$ hours	Clearance mL/kg/h	Vb mL/kg
99-3396	Monkeys	Single	SC	male	Single	0.03	0.3	84.7	0.052	1374	0.84				
FE 200486DSTOX9905				male		0.3	3	235	0.144	11437	7.01				
tox9905-nonclinical-data.pdf				male		3	30	421	0.258	31932	19.56				
				male		30	300	3219	1.972	229596	140.66				
				female		0.03	0.3	77.6	0.048	1863	1.14				
				female		0.3	3	208	0.127	11189	6.85				
				female		3	30	402	0.246	30712	18.82				
				female		30	300	3783	2.318	287583	176.18				
595/602	Rats	Daily for	IV	female	Day 1	0.03	0.18	-	-	115	0.07		1.11	261	419
FE200486DSTOX0122		4 weeks		male	Day 1	0.03	0.18	-	-	149	0.09		1.67	202	487
tox0122-nonclinical-data.pdf				female	Day 1	0.3	1.8	-	-	1319	0.81		2.88	227	946
				male	Day 1	0.3	1.8	-	-	1586	0.97		3.18	189	867
				female	Day 1	3	18	-	-	16782	10.28		4.47	179	898
				male	Day 1	3	18	-	-	19942	12.22		3.48	150	971
				female	Day 14	0.03	0.18	-	-	134	0.08		7.4	224	2395
				male	Day 14	0.03	0.18	-	-	161	0.1		5.02	187	1353
				female	Day 14	0.3	1.8	-	-	1352	0.83		2.97	222	950
				male	Day 14	0.3	1.8	-	-	1638	1		3.61	183	954
				female	Day 14	3	18	-	-	18879	11.57		2.63	159	604
				male	Day 14	3	18	-	-	18289	11.2		1.94	164	460
				female	Day 27	0.03	0.18	-	-	108	0.07		1.21	277	484
				male	Day 27	0.03	0.18	-	-	138	0.08		1.6	217	501
				female	Day 27	0.3	1.8	-	-	1055	0.65		2.64	284	1083
				male	Day 27	0.3	1.8	-	-	1218	0.75		1.73	246	616
				female	Day 27	3	18	-	-	15961	9.78		3.11	188	844
				male	Day 27	3	18	-	-	8152	4.99		3.58	165	853
595/604	Monkey	Daily for	IV	female	Day 1	0.25	2.5	-	-	3938	2.41		10.9	63	999
FE200486DSTOXO120		4 weeks		male	Day 1	0.25	2.5	-	-	3712	2.27		2.39	67	232
tox0120-nonclinical-data.pdf				female	Day 1	0.8	8	-	-	13760	8.43		4.03	58	338
				male	Day 1	0.8	8	-	-	15812	9.69		3.57	51	261
				female	Day 1	2.5	25	-	-	19265	11.8		4.14	130	775
				male	Day 1	2.5	25	-	-	27080	16.59		5.89	92	784
				female	Day 27	0.25	2.5	-	-	4816	2.95		7.3	52	547
				male	Day 27	0.25	2.5	-	-	4312	2.64		2.46	58	206
				female	Day 27	0.8	8	-	-	16561	10.15		4.34	48	302
				male	Day 27	0.8	8	-	-	17777	10.89		3.61	45	234
				female	Day 27	2.5	25	-	-	27492	16.84		4.08	91	536
				male	Day 27	2.5	25	-	-	27481	16.84		4.69	91	616
595/605	Monkey	Daily for	IV	female	Day 1	0.025	0.25	-	-	297	0.18		7	100	1030
FE200486DSTOX0115		2 weeks		male	Day 1	0.025	0.25	-	-	405	0.25		3.6	80	410
tox0115-nonclinical-daat.pdf				female	Day 1	0.175	1.75	-	-	1870	1.15		6.1	110	940
				male	Day 1	0.175	1.75	-	-	3696	2.26		9.5	50	680
				female	Day 1	1.25	12.5	-	-	11429	7		5.1	110	810
				male	Day 1	1.25	12.5	-	-	22492	13.78		6.8	70	620
				female	Day 14	0.025	0.25	-	-	422	0.26		6.7	80	780
				male	Day 14	0.025	0.25	-	-	535	0.33		6.6	70	510
				female	Day 14	0.175	1.75	-	-	1933	1.18		7	120	1110
				male	Day 14	0.175	1.75	-	-	4590	2.81		7.6	40	490
				female	Day 14	1.25	12.5	-	-	8317	5.1		4.3	150	960
				male	Day 14	1.25	12.5	-	-	16353	10.02		6.3	90	850
44565	Rats	Daily for	IV	female	Day 1	0.05	0.3	-	-	112	0.07		0.96	450	615
FE200486DSTOX0109		14 days		male	Day 1	0.05	0.3	-	-	98	0.06		0.96	510	707
tox0109-nonclinical-data.pdf				female	Day 14	0.05	0.3	-	-	227	0.14		1.56	220	496
				male	Day 14	0.05	0.3	-	-	236	0.14		1.49	210	457
				female	Day 1	0.35	2.1	-	-	988	0.61		1.41	350	719
				male	Day 1	0.35	2.1	-	-	898	0.55		1.32	390	744
				female	Day 14	0.35	2.1	-	-	1656	1.01		1.67	210	511
				male	Day 14	0.35	2.1	-	-	1732	1.06		1.51	200	440
				female	Day 1	2.5	15	-	-	6470	3.96		1.82	390	1014
				male	Day 1	2.5	15	-	-	8608	5.27		1.87	290	783
				female	Day 14	2.5	15	-	-	13677	8.38		1.28	180	338
				male	Day 14	2.5	15	-	-	15062	9.23		1.57	170	375

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On Original

2.6.6 TOXICOLOGY

2.6.6.1 Overall Toxicology Summary

General toxicology:

Single IV doses of 100 mg/kg (300 mg/m^2) were rapidly lethal to mice. Death appeared to result from neurological toxicity. A single subcutaneous dose of degarelix as high as 100 mg/kg (600 mg/m^2) caused no lethality in rats. Treated females weighed more than controls. In male rats, degarelix caused the kidney, liver, prostate, seminal vesicles, testes and epidiymus to decrease in weight, while the thymus increased in weight by 68% compared to control. In female rats, the ovaries and uterus with cervix significantly decreased in weight. In the monkey, a single subcutaneous dose of 30 mg/kg (360 mg/m^2) caused males to gain significantly less weight than controls. This high dose caused splenomegaly in males and atrophy of the sex organs in males and females.

Two weeks of daily dosing with doses as high as 3 mg/kg (18 mg/m^2) in male and female rats caused no dose related mortality. All doses levels caused significant atrophy of the sex organs in both males and females. Changes in clinical chemistry parameters (increased cholesterol), decreases in organ weight and hepatic perilobular vacuolization suggest the possibility of mild to moderate liver and kidney damage. An increase in alkaline phosphatase and deoxypyridinoline suggests the possibility of minor changes in bone metabolism. Plasma LH, testosterone and estradiol concentrations were reduced to castrate levels in males and females at all doses. These parameters did not fully return to normal by 10 weeks after the last dose. In monkeys treated daily for two weeks, a dose of 36 mg/m^2/day for two weeks caused injection site damage in all animals tested. This dose also caused atrophy of the sex organs in both males and females. At this dose level there was some indication of elevated urinary deoxypyridinoline levels in both sexes suggesting bone resorption. Dosing at 0.3 mg/kg/day (1.8 mg/m^2/day) caused similar but less severe changes. These changes were not completely reversible after 8 weeks recovery.

In mice, 300 mg/m^2 given fortnightly for 13 weeks (7 SC doses) was a putative LD_{10}. Males gained less weight than controls throughout the study while females weight more than control. Sex organs were profoundly atrophic in both males and females. Both males and females showed signs of splenomegaly and adrenal hypertrophy in males. Organ weights also indicated the possibility of atrophy in the heart, kidneys and salivary glands of both sexes and the pituitary adrenal glands in females. The weight changes in hematopoietic organs were corroborated by microscopic changes. There was no microscopic damage in the heart but there was some damage to the renal capsule.

The same dose and schedule caused no mortality in rats. Female rats treated with doses as high as 300 mg/m^2 gained more weight than controls as a function of increasing dose while body weight in males was unaffected. In degarelix-treated male rats, glucose, cholesterol and creatinine were increased even at low dose (3 mg/m^2). In LD females, alkaline phosphatase, cholesterol and triglycerides increased with increasing dose. In MD (30 mg/m^2) and HD females glucose levels increased and albumin decreased significantly. Degarelix treatment at all dose levels significantly decreased the size, weight and appearance of the epididymis, testes, prostate, and testis in males, and the ovary and uterus of females. Kidneys and liver decreased in weight in males, while the pituitary and adrenal gland weights decreased in females. The thymus increased in weight in both male and female rats with degarelix-treatment.

In a 26 week study of subcutaneous degarelix in rats, 13 fortnightly doses of degarelix as high 600 mg/m^2 caused no dose related mortality in rats. All male dose groups gained less weight than controls but all female groups gained more. RBC decreased in dosed males (<7%) but increased in females (as much as 6%). MCH and MCV decreased in both sexes. White cell count, neutrophils, monocytes and APTT increased with dose. The percent of lymphocytes decreased with dose. Pituitary and adrenal weights decreased with dose in females. Cholesterol and globulin increased; the latter

shifting the A/G ratios significantly lower. The decrease in relative liver weight was greater in males (30% < controls) than females correlated with decreased ALT and AST. There was also possibly some dose dependant kidney damage; relative kidney weight decreased with dosing particularly in females while creatinine increased in males. Alkaline Phosphatase increased with increasing dose to as much as 236% in females. Sex organs were severely atrophied in both males and females consistent with the primary pharmacology of the drug.

In a study of subcutaneous degarelix in monkeys dosed monthly for a year (7 doses in interim kill group and 13 in the main study) one HD male in poor condition was killed humanely on day 245 primarily because of neurological (ataxia) and gastrointestinal (diarrhea). On examination this animal had interstitial pneumonia and lesions in the stomach. No other monkeys showed these signs so it is not clear that this animals condition was dose related.

In this monkey study, all dosed monkeys showed signs of injection site damage but there were few other clinical signs. Male monkeys lost a significant amount of weight relative to controls. High dose males weighed as much as 32% less than controls and did not gain weight over the six month recovery period. Treated females had body weights comparable to controls throughout the study but were frequently amenorrhic. There were minor sporadic variations in arterial blood pressure. Hematological changes were transient and small. Changes in clinical chemistry parameters included elevations in serum gamma glutamyl transferase (GGT) in treated females, mild elevations in cholesterol in HD males and elevations in creatinine in HD females. Testosterone and estradiol decreased with increasing dose. Sex organs in males and females were atrophic in males and females by six months and sperm count was very low. In some treated monkeys degarelix appeared to retard epiphyseal closure.

In a four week study of IV dosing, twenty-eight daily doses of degarelix of 18 mg/m^2 caused no mortality in rats. Lower doses of 0.18 and 1.8 caused little toxicity. High dose males weighed significantly less than controls or other treatment groups while female rats gained more weight than controls as a function of increasing dose. Concentrations of testosterone in males were below those considered equivalent to castration in humans in mid dose rats and below the level of quantitation in high dose rats. Male rats had mild anemia without changes in MCV and both sexes had dose related elevations in white cell counts. Creatinine (to 85% in males), BUN (to 120% in males) and cholesterol (to 85% in males) were elevated in both sexes as a function of increasing dose. Alkaline phosphatase was elevated up to 25% in females. AST was mildly elevated up to 35% in females. Urine volume was increased and pH was decreased at the end of the study. Sex organs were atrophic in both sexes. Thymus and spleen weights were elevated as a function if increasing dose. There was aggregation of Kupffer cells in the liver and macrophages in the spleen. This correlated with the finding of drug in Kupffer cells by immunological assay in high dose males and PAS positive staining in both Kupffer cells and splenic macrophages. This implies that these cells may be responsible for clearing the drug. There were microscopic signs of toxicity in the kidneys in high dose males and females.

Genetic toxicology:

Degarelix did not cause increases in bacterial mutations in six separate Ames assays either with or without metabolic activation. In six separate studies in L5178Y mouse lymphoma cells, degarelix caused no increase in mutations at the TK locus. In two separate *in vivo* studies, degarelix caused no increase in micronucleated immature rat erythrocytes. Thus, degarelix is not genotoxic under the conditions of standard *in vitro* or *in vivo* assays.

Carcinogenicity:

In a standard 24 month carcinogenicity study in rats where degarelix was given fortnightly (52 subcutaneous doses), the high dose of 150 mg/m^2 was about the same as the proposed clinical loading dose and about 3 times greater than the proposed monthly maintenance dose on a mg/m^2 basis. The mid dose was 60 mg/m^2 and the low dose was 12 mg/m^2. A parallel toxicokinetic study demonstrated that degarelix accumulated to steady state values about week 24 and that exposure was roughly dose proportional. Overall mortality of treated animals was less than that of controls. The high dose males weighed 24% less than controls at the end of the study. Treated females weighed significantly more than controls throughout the study depending on dose. The incidence of benign adenoma of the pituitary gland decreased in all groups of treated females ($p < 0.02$). The incidence of benign fibroadenoma of the breast decreased in all groups of treated females ($p < 0.024$). The incidence of eosinophilic cell foci in the liver increased in low dose females ($p < 0.001$). This preneoplastic finding is consistent with mild hepatic damage seen in males and females. Lastly, there was an increase in metastatic hemangiosarcoma of the mesenteric lymph node in HD females ($p < 0.04$, with a positive trend by Peto analysis $p = 0.015$). The incidence of this tumor was 8% which is within the range seen in historical controls. There was no similar finding in males. The combined incidence of all benign and malignant hemangiomas and hemangiosarcomas (16%) was significantly different from controls by pairwise comparison ($p = 0.0013$, Exact test) in the high dose group. This difference remained significant when analyzed by the asymptotic trend test ($p = 0.0008$).

In a standard 24 month carcinogenicity study in mice, treatment with degarelix at doses of 6, 30 and 150 mg/m^2 fortnightly for two years caused an increase in benign bronchio-alveolar adenoma in all groups of treated females ($p < 0.04$) when analyzed by pairwise comparison with control. When the incidence of benign bronchio-alveolar adenoma was combined with that of malignant bronchio-alveolar carcinoma the result was not statistically different from controls by pairwise comparison. The incidence of benign bronchio-alveolar adenoma in male CD-1 mice ranges from 11 to 36 %, in females it ranges from 3 to 16%. Dosing in this study also caused an increase in benign hepatocellular adenoma of the liver ($p = 0.015$) in high dose females. By trend analysis the increase in benign hepatocellular adenoma of the liver reached significance in both males ($p = 0.03$) and females ($p < 0.04$). When the incidence of benign hepatocellular adenoma was combined with that of malignant hepatocellular carcinoma the total incidence was between 28 and 32% in all males groups including controls and 2, 14, 14 and 13% in female controls, low dose, mid dose and high dose groups respectively. These results were not significantly different from controls in males or females ($p < 0.09$) by pairwise comparison. The combined incidence of these tumors was also not statistically different from controls by asymptotic trend test ($p < 0.09$). The normal incidence of hepatocellular adenoma of the liver ranges from 2 to 33 % in male CD-1 mice and from 0 to 4% in females. The normal range for hepatocellular carcinoma ranges from 0 to 1.7 % in females and 0 to 6% in males.

Reproductive toxicology:

Doses of 0.072 mg/m^2/day from day 6 through day 12 followed by doses of 0.18 mg/m^2/day caused significant post-implantation loss in pregnant rats (23.6 %) and a concomitant decrease in the number of live fetuses/dam. This dose caused no significant maternal toxicity and is only about 0.13% of the proposed clinical loading dose. Dosing was associated with an increase in the number of major abnormalities in the fetuses in the high dose group ($p < 0.05$) but most of these abnormalities occurred in a single litter (4 of 6). In fetuses in the mid dose group there was a statistically significant increase in a number of minor skeletal abnormalities and variants observed. These were findings generally associated with the state of ossification and were considered to be related to maternal treatment with degarelix.

In rabbits, a daily dose of 0.024 mg/mg^2 on days 6 through 14 followed by doses of 0.072 mg/m^2 from days 15 through 27 was associated with a decrease in the number of does with implantations, the number of corpora lutea per female, the number of implantations and the number of live fetuses per female. Some of these decreases reached statistical significance in the mid-high and high dose groups particularly the number of live fetuses. Dosing was also associated with an increase in mean post-

implantation loss. There was an increase in the number of fetuses with minor abnormalities in the high dose group and an increase in the incidence of major abnormalities in the mid dose group (5 in three litters) but the number of fetuses in the high dose group was so diminished as to render any determination of teratogenicity equivocal. The high dose caused only minimal toxicity in the does (minimal decreased body weight gain). Thus, a daily dose of degarelix that was just 0.05% that of the proposed loading dose was a potent abortifacient in rabbits.

Single doses of 1 mg/kg or five fortnightly doses of degarelix caused complete lose of fertility in male rats. Fertility returned in most animals between 10 to 14 weeks after the end of dosing demonstrating pharmacological reversibility of the effects of degarelix in male rats.

Special toxicology:

None reviewed

2.6.6.2 Single-dose toxicity

1) Acute Intravenous Toxicity to the Mouse - WDM

Major Findings:

A single IV dose of 100 mg/kg of degarelix was lethal to 3 of 5 male mice and 3 of 3 female mice. Doses of 30 mg/kg were not lethal but were associated with acute clinical signs of distress including partially closed eyelids, cold extremities and hunched posture. Dosing caused a significant decrease in the size of sex organs in males and females seen at necropsy (day 29).

Study number	FE200486DSTOX9902
[redacted] study number	20786
EDR filename	tox9902-nonclinical-data-1.pdf
Conducting laboratory	[redacted] b(4)
Date of study initiation	October 2001
GLP compliance	Yes
QA reports	Yes
Drug	Degarelix, Lot 2011-032-30, 97.58% in terms of peptide; peptide content 87.8%

Methods

Animal: male and female mice (IRC)

Doses

Group	Treatment	Dosage†(mg/kg/ Bodyweight)	Dosage*(mg/kg/ Bodyweight)	Dosage#(µmol/kg/ Bodyweight)	Mortality M	Mortality F
1	Vehicle	0	0	0	5	5
2	FE 200486	30	34.2	18.5	5	5
3	FE 200486	100	114	61.6	5	5

† Dosage in terms of test item supplied (test item has an FE 200486 content free base of 87.8 % correction factor of 1.14 applied).

* Dosage in terms of FE 200486 content free base

\# Dosage in terms of µmol/kg (FE 200486 content (free base) – 87.8 % (0.54 µmol FE 200486/mg).

0: Denotes control animals in the above and following Tables

Schedule	Single dose
Route	IV tail vein
Dose volume	2 mL/kg
Formulation	Mannitol $_{aq}$ 5% w/v (vehicle control)
Mortality	Twice daily
Clinical signs	Twice daily
Bodyweight	Days 1 (prior to dosing), 8, 15, 22 and 29 (or at death).
Necropsy	Day 29
Histopathology	Not done

Results

Mortality	at 100 mg/kg two out of five males and three out of three females died within 10 minutes of dosing. To avoid killing the remaining two females the investigators dosed these animals with 40 and 50 mg/kg in an informal deviation from the protocol. These doses were not lethal to these animals.
Clinical signs	A dose of 30 mg/kg caused partially closed eyelids, cold extremities, hunched posture and slight bruising at the injection site. The two males that survived a dose of 100 mg/kg were lethargic and showed signs piloerection, waddling unsteady gait, abnormal respiration and pallid extremities. All signs but the injection site damage resolved within 7 days.
Body weight	No toxicologically significant changes
Necropsy	In two of the three dead male mice the brain tissue was pallid Four males at 30 mg/kg and two males had grossly atrophic prostrate glands, seminal vesicles, testes and epididymides. In the 30 mg/kg group two females had small uteruses and three had small ovaries.

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2) Acute Subcutaneous Toxicity to the Rat - RTD

Major findings:

- Rats (n = 5/sex/group) were administered vehicle, 30 or 100 mg/kg degarelix SC.
- No mortality was observed throughout the study.
- Degarelix caused loss of hair at the site of injection in 5 of 20 rats, while swelling at the site of injection was found in most rats at the moderate (30 mg/kg) and high doses (100 mg/kg) of degarelix.
- Changes in body weight were unremarkable in male rats that received degarelix, while female rat weights increased 15-19% at both moderate and high doses of degarelix.
- In male rats, degarelix caused the kidney, liver, prostate, seminal vesicles, testes and epidiymus to decrease in weight, while the thymus increased in weight by 68% compared to control. In female rats, the liver, lungs, spleen and thymus grew larger, while the ovaries and uterus with cervix significantly decreased in weight.
- Degarelix targeted the reproductive organs of both male and female significantly at both 30 and 100 mg/kg degarelix.

Study no.:	FRG 031/994150.AC,
Sponsor study number	FE200486DSTOX9903
EDR filename	tox9903-nonclinical-data.pdf
Conducting laboratory	[redacted]
Date of study initiation:	July 22, 1999
GLP compliance:	Yes
QA report:	Yes
Drug:	Degarelix, batch 2011-032-30, peptide content 87.8%

b(4)

Methods

Doses:	0, 30, and 100 mg/kg degarelix
Species/strain:	Albino CD Sprague-Dawley rat/[redacted]:CD BR strain
Doses	0, 30, and 100 mg/kg
N	5 per sex per group
Route	Subcutaneous

b(4)

Formulation:	5% w/v mannitol in water
Infusion rate:	2 mL/kg bodyweight, no rate noted
Age:	11 weeks
Weight:	426 to 448 g (male) and 277 to 309 g (female)
Necropsy:	28 days post injection

Observations and times:

Mortality: Twice daily

Clinical signs: Throughout the day of injection, and then twice daily

Body weights: Prior to injection, and weekly thereafter

Gross pathology: The following organs were dissected and observed at sacrifice:

Adrenals	Kidneys	Prostate
Brain	Liver	Seminal vesicles
Epididymides	Ovaries	Testes
Heart	Lungs	Spleen
Thymus	Uterus with cervix	

Organ weights: The organs listed directly above were removed on Day 29, fixed in 10% formalin and weighed. The organs were dissected free of fat. Testes and epididymides were fixed in Bouins' solution and transferred to 70% alcohol.

Histopathology: Not done

Results

Mortality: None.

Clinical signs:

Number of rats in group of 5 showing signs

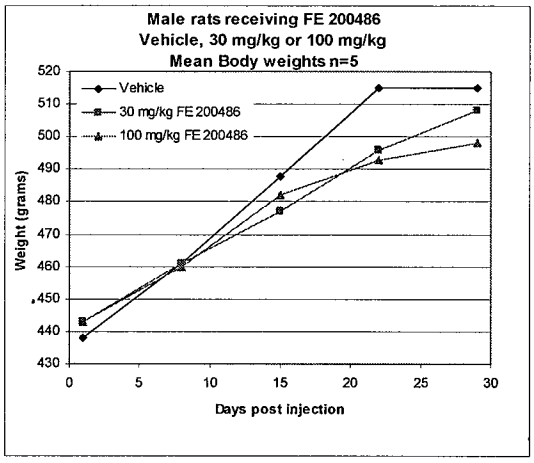
Signs of reaction	Dose (mg/kg)					
	0	0	30	30	100	100
	Male	Female	Male	Female	Male	Female
Lump on dose site	0	0	3	4	5	5
Hair loss	0	0	1	2	1	1

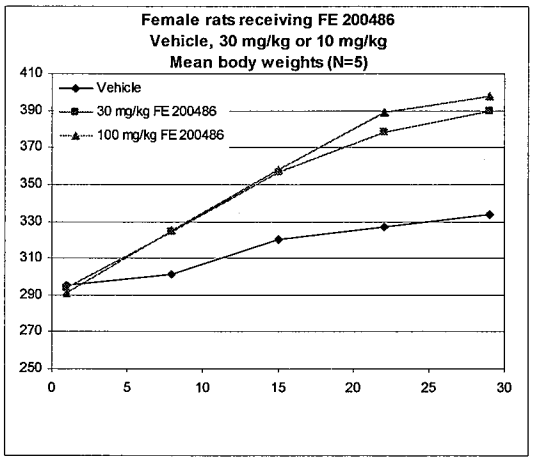
No other clinical signs were noted.

Body weights:

Male rats dosed with 30 mg/kg degarelix experienced a 3.7% and 1.4% decrease in weight compared to vehicle on days 22 and 29, respectively. Male rats dosed with 100 mg/kg had a 4.2% and 3.3% drop in weight (compared to control) on days 22 and 29, respectively; therefore weight loss in male rats was dose-dependent.

Female rats that were injected with 30 mg/kg or 100 mg/kg degarelix experienced an increase in body weight. On days 22 and 29 post injection, female rats dosed with 30 mg/kg degarelix showed an approximately 15% increase in weight gain while females dosed with 100 mg/kg degarelix experienced a 19% gain in weight.





Gross pathology:
Gross pathological evaluation found that the prostate, seminal vesicles, testes and epididymides were smaller in male rats that received either 30 mg/kg or 100 mg/kg degarelix compared with vehicle. Similarly, females rats that received either dose of degarelix had smaller ovaries and uterus compared with the vehicle group.
Pale swelling was noted at the site of injection for all rats receiving degarelix and 3 out of 5 rats (both male and female) had an enlarged thymus in both 30 mg/kg and 100 mg/kg dosing groups.

Organ weights:

Mean organ weight changes
Male rats

	Kidneys	Liver	Prostate	Sem. Ves.	Thymus	Epididymides	Testes
Vehicle	100	100	100	100	100	100	100
30 mg/kg Degarelix	88	98	14	15	128	25	24
100 mg/kg Degarelix	85	89	12	14	168	24	24

Mean organ weight changes
Female rats

	Liver	Lungs	Spleen	Thymus	Uterus with cervix	Ovaries
Vehicle	100	100	100	100	100	100
30 mg/kg Degarelix	117	113	123	193	27	42
100 mg/kg Degarelix	132	116	132	177	28	40

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3) A Single-dose Toxicity Study in Cynomolgus Monkeys with a 2-Week or 39-Week Recovery Period – RTD

Major findings:

- 30 mg/kg degarelix, the highest dose tested, caused reversible swelling at the site of injection in both sexes.
- Decreases in the body weight in male monkeys compared with the control group, an effect which lasted through the latter 2/3 of the study.
- Decreased urinary pH of male monkeys at 2 and 9 weeks post-injection.
- Primary end-organ toxicities were lymphatic (decreased WBC, enlarged spleens) and reproductive organs (testes, prostate, epididymus, uterus and ovaries) and occurred at > 0.03 mg/kg, the lowest dose administered.
- The increase in exposure (plasma AUC) with increasing dose was non-linear. The half-life extends fairly dramatically between the two lowest doses tested and could not be determined at higher concentrations.
- The observed effects from 0.3 and 3 mg/kg degarelix are reversible, while the effects of 30 mg/kg are more persistent. TK data illustrates that undetermined factors are preventing the full exposure of the monkey to both the 3 mg/kg and 30 mg/kg degarelix doses.

Study no.:	99-3396
Sponsor Study No.:	FE 200486DSTOX9905
EDR file name	tox9905-nonclinical-data.pdf
Conducting laboratory	[redacted] b(4)
Date of study initiation:	July 12, 1999
GLP compliance:	Yes
QA Report:	Yes
Drug:	Degarelix, Batch # 2011-032-30, 97.58% pure
Methods	
Doses:	0.03, 0.3, 3, or 30 mg/kg (0.36, 3.6, 36 or 360 mg/m^2)
Species:	Cynomolgus monkeys
Age:	3- 9 years old
Weight:	Males – mean: 3.3 kg, range 2.4 – 4.6 Females – mean: 3.0 kg, range 2.4 - 4.1
N:	6 per sex per dose group for 0, 3, and 30 mg/kg degarelix. Three from each group will be sacrificed at 2 weeks post- injection, and the remaining 3/sex will remain until 40 weeks. 3 per sex per dose for 0.03 and 0.3 mg/kg degarelix, to be evaluated at Week 2.
Route:	Subcutaneous
Formulation:	5% mannitol in sterile water
Volume:	1 mL/kg
Infusion rate:	Not specified
Sampling times:	Necropsy was performed at 2 weeks and 40 weeks to assess recovery. Toxicokinetics, hormone assays, clinical pathology were each performed at time points that are outlined by the sponsors in the Table below.

				Number of Animals															
				Toxicokinetics[b]		Hormone Assays[c]				Clinical Pathology				Necropsy				Microscopic Pathology	
	Doses[a]			Predose, 0.5, 1, 3, 6, 12, 24, 48, 96 & 168 hours		Predose & Week 2		Weeks 4, 8, 12, 16, 20, 26, 30, 34 & 39		Predose & End of Week 2		Weeks 6, 13, 27 & 40		Termination Week 2		Recovery Week 40			
Group	Dose (mg/kg)	Volume (mL/kg)	Conc. (mg/mL)	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
1	0	1	0	6	6	6	6	3	3	6	6	3	3	3	3	3	3	6	6
2	0.03	1	.03	3	3	3	3	-	-	3	3	-	-	3	3	-	-	3	3
3	0.3	1	0.3	3	3	3	3	-	-	3	3	-	-	3	3	-	-	3	3
4	3.0	1	3.0	6	6	6	6	3	3	6	6	3	3	3	3	3	3	6	6
5	30.0	1	30.0	6	6	6	6	3	3	6	6	3	3	3	3	3	3	6	6

[a]Doses are expressed in terms of the pure peptide free base. The test article contained 87.8% peptidic portion of which 97.58% was pure free base FE 200486.

[b]Toxicokinetic samples were collected predose, and 0.5, 1, 3, 6, 12, 24, 48, 96 and 168 hours after dosing.

[c]Hormonal assays of lutenizing hormone (LH), follicle-stimulating hormone (FSH), testosterone (males only) and estradiol (females only) were performed at the designated intervals.

Observations and times

Mortality:	Observed twice daily
Clinical signs:	Pre-test and weekly after injection
Body weights:	Once pre-test, twice on weeks 1 and 2, then once weekly
Food consumption:	Inspected daily, and scored by a scale of 1 (none eaten) through 5 (completely consumed)
Ophthalmoscopy:	Once pre-test, then at 2 and 32 weeks
Hematology:	Twice pretest, at the end of week 2 and at Weeks 6, 13, 27 and 40
Clinical chemistry:	Twice pretest, at the end of week 2 and at Weeks 6, 13, 27 and 40
Urinalysis:	Twice pretest, Week 2, and at Weeks 9, 13, 26 and 39
Gross pathology:	Week 2 and Week 39
Organ weights:	Week 2 and Week 39
Histopathology:	Adequate Battery
Peer review:	Yes
Toxicokinetics:	Monitored predose and at 0.5, 1, 3, 6, 12, 24, 48, 96 and 168 hours after dose administration

Results

Mortality: None.

Clinical signs:

In males receiving 30 mg/kg degarelix, swelling at the site of injection occurred in 2/6 at Week 1 then decreased to 1/6 at Week 2. The swelling was reversible and returned to control levels for the duration of the study. All 6/6 females monkeys that received 30 mg/kg degarelix showed swelling at the site of injection in Weeks 1 and 2. As swelling subsided, only 2/6 females had swelling over Weeks 3 and 4, and 1/6 females had swelling in Weeks 5-12. The swelling did not recur. Only high-dose (30 mg/kg) degarelix caused swelling at the site of injection.

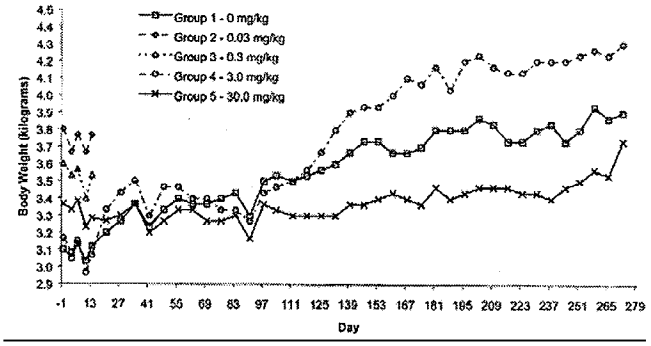
Periodically throughout the study, 1/3 monkeys that received 3 or 30 mg/kg degarelix had a noted laceration or abrasion with exudate or scab. These appeared to be isolated events that did not last more than two weeks in any group and was therefore reversible. While these events were not frequent, they appeared with greater frequency than in the control group for both sexes.

Body weights:

Body weights for male Cynomolgus monkeys were marginally higher in the 0.3 mg/kg degarelix dose group, while the highest dose (30 mg/kg) of degarelix decreased male body weight. Though these differences are not statistically significant, the trend remains consistent from Day 110 until the end of the study.

(the following graph is from the study report)

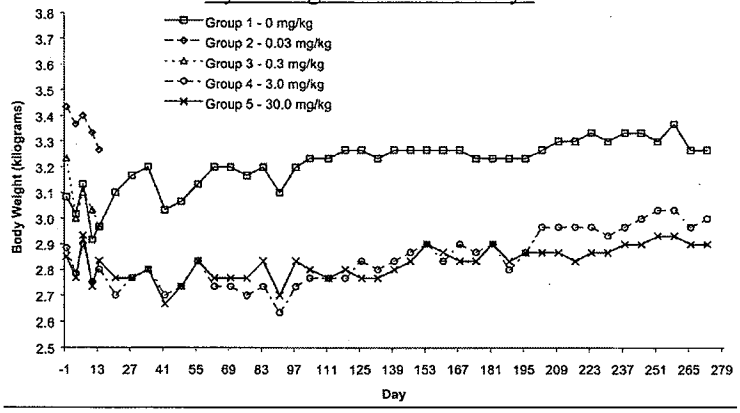
The effect of single dose Degarelix on body weights of male monkeys



The weights of female monkeys that received degarelix were lower in both 3 mg/kg and 30 mg/kg dose groups; however these groups also began the study with a lower mean weight. The weights of individual female monkeys did not differ with degarelix treatment.

(the following graph is from the study report)

The effect of single dose Degarelix on body weights of Cynomolgus female monkeys



Food consumption: No toxicologically significant effects

Ophthalmoscopy: No toxicologically significant effects

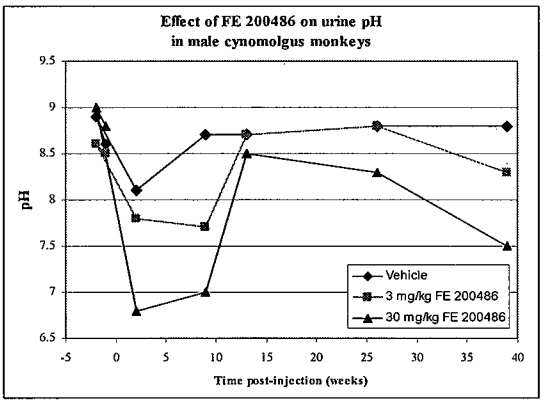
Hematology:

White blood cell (WBC) counts decreased in 6 (of 6) males two weeks post-injection for vehicle-treated (0 mg/kg), 3 mg/kg and 30 mg/kg degarelix groups. The decrease in WBC counts did not

continue after week 2 in the population and therefore this is likely an acute and reversible effect that is associated with the injection or vehicle. A similar effect is evident in female monkeys.

Clinical chemistry: No toxicologically significant effects

Urinalysis:



Gross pathology:

		Males Degarelix dose (mg/kg)					Females Degarelix dose (mg/kg)				
		0	0.03	0.3	3	30	0	0.03	0.3	3	30
Week 2 observations	Total mice per group	3	3	3	3	3	3	3	3	3	3
Injection site	Discolored			1	2	2	1		1	2	3
	Thickened					2					3
Liver	Discolored								2		1
Skin	Discolored										1
	Scab					1					
Mesentary/Perito	Nodules										1
Week 39 observations	Total mice per group	3	--	--	3	3	3	--	--	3	3
Spleen	Enlarged		--	--		2	2	--	--		
Tail	Abrasion		--	--		1	1	--	--		
Mesentary/Perito	Nodules		--	--				--	--		1

Organ weights:

- Decreased testicular weight at 3 mg/kg (Week 2) and 30 mg/kg (Week 39). Reversibility at high dose cannot be determined.
- Prostate decreased in weight at 2 weeks for $\geq$ 0.3 mg/kg doses of degarelix. Finding at 3 mg/kg Degarelix was partially reversible at 39 weeks, but 30 mg/kg dosage lead to a further decrease in prostate weight at 39 weeks.
- Decreased epididymal weight (30 mg/kg) at Week 39.
- Increased thymic weight following dose $\geq$ 0.3, 3.0 and 30 mg/kg at Week 2. Variability in the data at Week 39 does not allow for assessment of reversibility

Organ weight from male Cynomolgus monkeys at 2 weeks and 39 weeks after single injection with degarelix
Percent of control

FE 200486 dose (mg/kg)	Monkey ID	Testes		Prostate		Epididymus		Thymus	
		2 week	39 week	2 week	39 week	2 week	39 week	2 week	39 week
Vehicle	1131/M 1133/M 1134/M								
	mean	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	std. dev.	21.7	90.1	18.3	60.4	8.5	59.9	100.0	114.6
0.03 mg/kg	2130/M 2131/M 2132/M								
	mean	150.0	--	85.3	--	122.7	--	133.3	--
	std. dev.	129.9	--	13.6	--	60.8	--	57.7	--
0.3 mg/kg	3130/M 3131/M 3132/M								
	mean	87.5	--	67.3	--	89.8	--	233.3	--
	std. dev.	43.3	--	32.9	--	29.6	--	57.7	--
3 mg/kg	4132/M 4134/M 4135/M								
	mean	25.0	118.5	72.0	82.9	85.0	96.1	200.0	75.0
	std. dev.	21.7	64.2	16.0	27.7	26.2	43.6	100.0	0.0
30 mg/kg	5130/M 5133/M 5135/M								
	mean	125.0	14.8	79.3	38.8	108.4	43.0	200.0	200.1
	std. dev.	43.3	12.8	30.6	22.0	31.6	24.9	100.0	114.6

b(4)

- Decreased uterine weight was observed at 3 mg/kg.
- Decreased ovarian weight at ≥0.03 mg/kg. Partially reversible at dose of 0.03 and 0.3 mg/kg.

Organ weight from female Cynomolgus monkeys at 2 weeks and 39 weeks after single injection with degarelix
Percent of control

FE 200486 dose (mg/kg)	Monkey ID	Uterus		Ovaries		Thymus	
		2 week	39 week	2 week	39 week	2 week	39 week
Vehicle	mean	100.0	100.0	100.0	100.0	100.0	100.0
	std. dev.	40.3	5.9	17.6	56.9	0.0	0.0
0.03 mg/kg	mean	130.8	--	78.4	--	133.3	--
	std. dev.	23.2	--	20.7	--	57.7	--
0.3 mg/kg	mean	92.3	--	54.9	--	100.0	--
	std. dev.	12.2	--	9.0	--	0.0	--
3 mg/kg	mean	96.9	80.2	56.9	142.3	166.7	133.3
	std. dev.	13.8	19.3	12.2	29.0	57.7	57.7
30 mg/kg	mean	95.4	60.4	70.6	88.5	100.0	133.3
	std. dev.	9.6	48.2	51.3	43.7	0.0	57.7

b(4)

Histopathology:
The reproductive organs of both male and female monkeys appear to be the main target for degarelix, with the following effects still present at Week 39 at both 3 mg/kg and 30 mg/kg doses.

Necropsy at Week 2 Microscopic findings			Males FE 20486 dose (mg/kg)					Females FE 20486 dose (mg/kg)				
Organ	Finding	Extent	0	0.03	0.3	3	30	0	0.03	0.3	3	30
		Number examined	3	3	3	3	3	3	3	3	3	3
Colon	Increased lumenal germ cells	Minimal				1	1					
		Slight		2		1						
		Moderate			2							
Epididymus	Atrophy	Slight	1			1						
		Moderate			2							
		Marked			1	2	1					
	Absence of sperm	Present										
Heart	Myocardial inflammatory foci	Minimal	1	1	1		2					
Injection site	Subcut. Granulomatous inflammation +/- hemorrhage	Slight									1	
		Moderate				2	1				1	
		Marked					2					3
	Histocytic infiltration of lower dermis	Slight										1
		Moderate										2
Lacrimal glands	Lymphcytic foci	Minimal	1	1		1	1		2	1	1	2
		Slight		2	1			3				1
		Moderate										1
		Marked			1							
Liver	Inflammatory foci with associated hepatocytic degen.	Minimal	1	1	1	1	1		2		3	2
Lungs	Ductal hyperplasia	Minimal				2	2					
		Slight					1					
	Secretory activity	Minimal						3	2		2	3
		Slight							1	3	1	
Mammary glands	Mediastinal LN	Minimal	1		2	1	1		1			
	Pigment-filled macrophages	Slight		1		2		2			3	2
		Moderate	1				1	1	2	3		1
Prostate	Acinar expansion	Minimal				1						
		Slight				2	3					
		Moderate			2							
Seminal vesicles	Atrophic epithelium	Present			1	2	1					
Spleen	Reduced follicular lymphocytes	Slight					2		1	1		2
Testes	Depletion of elongating spermation	Slight	2	1			1					
		Moderate		2	1	1	1					
		Marked			1							
		Severe	1		1	2	1					
	Depletion of round spermatids	Minimal	2	1			1					
		Slight		1			1					
		Moderate		1	2	1						
		Marked	1									
		Severe			1	2	1					
	Depletion of spermatocytes	Minimal		2								
		Slight		2								
		Moderate	1									
		Marked										
		Severe										
	Spermatid retention	Slight			2							
		Marked				1						
Thymus	Cysts	Present		1	1	1					1	3
Uterus	Early proliferative phase	Present						1	1	3	3	2
	Surface endometrial desquamation and hemorrhage	Present							1	3	3	1
	Cervix: mucosal crypts dilated with mucus	Present										1

Necropsy at Week 39 Microscopic findings			Males FE 20486 dose (mg/kg)			Females FE 20486 dose (mg/kg)		
Organ	Finding	Extent	0	3	30	0	3	30
		Number examined	3	3	3	3	3	3
Epididymus	Atrophy	Slight			1			
		Marked	1		2			
	Absence of sperm	Present	1		3			
Injection site	Subcutaneous: Histiocytic infiltrate	Minimal			1			
		Moderate			2			1
		Marked						1
Lacrimal glands	Lymphocytic foci	Minimal	2	2	2		2	3
		Slight	1		1	1	1	
Ovaries	Follicular atresia	Moderate					2	2
Prostate	Atrophic Epithelium	Present	1		2			
Salivary gland	Lymphoid cell accumulation	Minimal	1	1	2			1
Seminal vesicles	Atrophic Epithelium	Present	0	0	2			
	Sarcocysts	Minimal		1				
		Present		1	1			
Spleen	Expansion of pulp	Slight			2	2		1
Testes	Depletion of elongating spermatids	Severe	1	1	3			
	Depletion of round spermatids	Marked		1				
		Severe	1		3			
	Depletion of spermatocytes	Moderate		1				
		Marked	1		1			
		Severe	1		3			
Uterus	Early proliferative phase	Present						2
Vagina	Thin non-cornified epithelium	Moderate						1

Toxicokinetics:

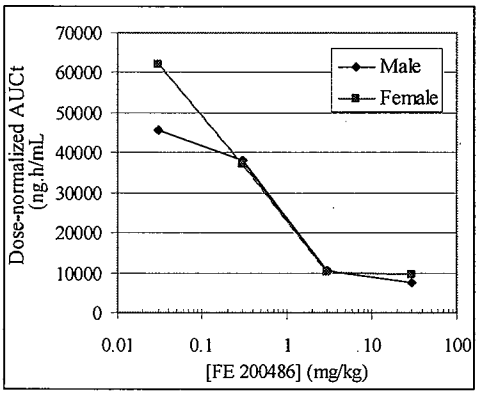
Increasing doses of degarelix led to a non-linear increase in drug exposure, with no differences between sexes. Apparent T_{max} occurred between 1-3 hours for 0.03 and 0.3 mg/kg doses, and 12-48 hours for 3 and 30 mg/kg doses. The terminal half-life increased with increasing the dose from 0.03mg/kg to 0.3 mg/kg, and could not be determined at higher doses. The investigators suggested that the decreased relative exposure and longer half-life for 3 mg/kg and 30 mg/kg doses could be due to increased clearance, or because of a possible precipitation of drug at the site of injection followed by slow solubility over time. The most likely reason is saturation of the absorption process at the injection site.

(The following table was provided by the sponsor, with an addition of half-life data found in the text)

Dose level mg/kg	Cmax (ng/mL) Males	Cmax (ng/mL) Female	AUCt (ng.h/mL) Males	AUCt (ng.h/mL) Female	Half-life (hr)
0.03	84.7	77.6	1374	1863	17.5 to 26.3
0.3	235	208	11437	11189	43.5 to 71.1
3	421	402	31932	30712	not determined
30	3219	3783	229596	287583	not determined

Dose normalization illustrates the non-linearity of drug exposure with escalating doses of degarelix.

Dose normalized AUC data for Cynomolgus monkeys treated with Degarelix.



Dose level mg/kg	AUCt (ng.h/mL) Males	AUCt (ng.h/mL) Female
0.03	45800	62100
0.3	38123	37297
3	10644	10237
30	7653	9586

Appears This Way
On Original

2.6.6.4 Repeat-Dose Toxicity

1) 13 Week Toxicity Study in Mice with Subcutaneous Administration – WDM

Major Findings:

A dose of 100 mg/kg given every two weeks for seven doses was a putative LD_{10} in mice (2 of 20 deaths prior to scheduled necropsy in the main group, 3 of 42 in the TK group). Male high dose mice weighed significantly less than controls throughout the study, mid and high dose females weighed more than controls throughout the study. Changes in hematological and clinical chemistry parameters were minor. Organ weights indicated profound atrophy of the sex organs that correlated with gross and microscopic findings. Organ weights also indicated the possibility of atrophy in the heart, kidneys and salivary gland of both sexes. Splenic weights indicated hypertrophy in both sexes. Pituitary weights in females suggested atrophy. Adrenal weights increased in males and decreased in females. Changes in hematopoietic organs were corroborated by microscopic changes. There was no microscopic damage in the heart but there was some damage to the renal capsule.

Study number	FE200486DSTOXO111
study number	20786
EDR filename	tox0111-nonclinical-data-1.pdf
Conducting laboratory	[redacted] b(4)
Date of study initiation	October 2001
GLP compliance	Yes
QA reports	Yes
Drug	Degarelix, Batch 0048262#PPL-FE4860001, > 99 % purity Peptide content 87.99 %

Methods	
Animal	Male and female CD-1 mice — CD-ITM(ICR)BR
Doses	0, 1, 10 or 100 mg/kg, (0, 3, 30 or 300 mg/m^2) b(4) Expressed as 100% peptide content Groups 1, 2, 3 and 4 respectively
N	10 (+21 satellite animals for TK) per sex per group with replacement
Schedule	Fortnightly (7 total doses)
Route	Subcutaneous injection
Dose volume	10 mL/kg
Formulation	Mannitol $_{aq}$ 5% w/v (vehicle control)

Observations	
Mortality	Twice daily
Clinical signs	Twice daily
Body Weight	Weekly
Food cons	Weekly
Hematology	Prior to necropsy
Clinical chemistry	Prior to necropsy
Necropsy	After week 13