

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
22-074

PHARMACOLOGY REVIEW(S)



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:	22-074
SERIAL NUMBER:	N-000
DATE RECEIVED BY CENTER:	11/1/2006
PRODUCT:	Somatuline®Autogel® injection
INTENDED CLINICAL POPULATION:	Treatment of acromegaly
SPONSOR:	Beaufour Ipsen Pharma
DOCUMENTS REVIEWED:	Vol. 1-92
REVIEW DIVISION:	DMEP (HFD-510)
PHARM/TOX REVIEWER:	Dylan Dalin Yao, Ph.D.
PHARM/TOX SUPERVISOR:	Karen Davis Bruno, Ph.D.
DIVISION DIRECTOR:	Mary Parks, M.D.
PROJECT MANAGER:	Jennifer Johnson

Date of review submission to Division File System (DFS): August 20, 2007

TABLE OF CONTENTS

EXECUTIVE SUMMARY	4
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW	8
2.6.1 INTRODUCTION AND DRUG HISTORY.....	8
2.6.2 PHARMACOLOGY.....	10
2.6.2.1 Brief summary	11
2.6.2.2 Primary pharmacodynamics.....	12
2.6.2.3 Secondary pharmacodynamics.....	18
2.6.2.4 Safety pharmacology	22
2.6.2.5 Pharmacodynamic drug interactions.....	25
2.6.3 PHARMACOLOGY TABULATED SUMMARY.....	26
2.6.4 PHARMACOKINETICS/TOXICOKINETICS.....	28
2.6.4.1 Brief summary	28
2.6.4.2 Methods of Analysis	28
2.6.4.3 Absorption.....	29
2.6.4.4 Distribution	31
2.6.4.5 Metabolism	34
2.6.4.6 Excretion	35
2.6.4.7 Pharmacokinetic drug interactions.....	36
2.6.4.8 Other Pharmacokinetic Studies.....	36
2.6.4.9 Discussion and Conclusions	36
2.6.4.10 Tables and figures to include comparative TK summary.....	37
2.6.5 PHARMACOKINETICS TABULATED SUMMARY.....	38
2.6.6 TOXICOLOGY	41
2.6.6.1 Overall toxicology summary.....	41
2.6.6.2 Single-dose toxicity	48
2.6.6.3 Repeat-dose toxicity.....	50
2.6.6.4 Genetic toxicology	71
2.6.6.5 Carcinogenicity	97
2.6.6.6 Reproductive and developmental toxicology.....	257
2.6.6.7 Local tolerance.....	262
2.6.6.8 Special toxicology studies.....	264
2.6.6.9 Discussion and Conclusions	264
2.6.6.10 Tables and Figures.....	266
2.6.7 TOXICOLOGY TABULATED SUMMARY	266

OVERALL CONCLUSIONS AND RECOMMENDATIONS..... 271
APPENDIX/ATTACHMENTS (P/T RECOMMENDED LABELING) 271

**APPEARS THIS WAY
ON ORIGINAL**

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Approval (AP), pending acceptance of recommended labeling changes.

Based on the results of nonclinical pharmacology and toxicology studies, Pharmacology/Toxicology recommends approval of the NDA for Somatuline® Autogel® for the indication of treatment of acromegaly.

B. Recommendation for nonclinical studies

No additional nonclinical studies are required.

C. Recommendations on labeling

Recommended labeling changes have been appended to this review.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Drug development background:

Under _____, a clinical formulation of _____ (long acting lanreotide acetate, 30 mg, in a microparticle formulation, MPF) has been tested in a 26-week dog toxicity study where it was administered i.m. every two weeks. Severe intramuscular granulomatous inflammation was observed at all dose levels (≥ 1 mg/kg, well below the human dose of 30 mg then proposed). Other toxicity studies generally examined daily or twice daily s.c. administration of the active drug product (immediate release formulation, IRF) rather than MPF, including a 26-week rat and a 24-month dog studies at doses of microgram levels (200, 1000 or >1000 $\mu\text{g}/\text{kg}$ for the rat and 8, 40 or 120 $\mu\text{g}/\text{kg}$ for the dog). Prominent target organ toxicity was the s.c. injection sites with dose-dependent skin inflammatory reaction, partially reversible after 4-week treatment-free period. Miscellaneous dose-dependent mild changes in clinical pathology were not associated with histologic findings. The NOAELs in both rat and dog studies were $<$ the human exposure based on body surface area comparison.

The sponsor has changed the formulation of lanreotide to remove _____ in NDA 22-074 reviewed here. The clinical formulation is a supersaturated lanreotide acetate solution dissolved in _____, and packed as prefilled syringes at strengths of 60, 90 and 120 mg without any _____

For the general toxicology assessment, two pivotal chronic toxicity studies with the therapeutic formulation (Somatuline® Autogel®) dosed subcutaneously once every 14-day for 26 weeks in the rat and dog were provided for bridging to the previous available toxicity data. In both species at higher dose levels, Somatuline® Autogel® did not produce new toxicities or worse toxicity findings compared to the previous findings with either IRF or MPF lanreotide under _____

_____ The local tissue reactions at the injection sites with chronic inflammatory changes, including fibrosis and granuloma formation, were major histopath findings with Somatuline® Autogel®, which is likely resulted from the depot formation of the drug compound and its irritation to the subcutaneous tissues. The drug induced reduction of growth rate (decreased body weight and decrease in weight of some organs) appeared to be related to the pharmacological activity. In the rat, the NOAEL on local tissue reaction may achieve <47X the proposed maximum human exposure (at 120 mg, AUC=3619 ng/ml.h); and the NOAEL on systemic toxicity was ~156X the maximum human exposure. In the dog, the NOAEL for local toxicity was <2X the maximum human exposure while the NOAEL for systemic toxicity was about 5X exposure multiple that at the human maximum dose. It appears that the dog is more sensitive to lanreotide autogel than does the rat in the toxicity testing.

The standard battery of genotoxicity tests were conducted, including a reverse mutation assay in bacteria, a cultured mammalian cell assay, a test for chromosome aberrations in human lymphocytes and an in vivo test for micronucleated erythrocytes in mice. In addition, the ability of lanreotide to induce gene mutations in the lacZ transgene in liver and bone marrow tissue from MutaMice was also assessed. These genotoxicity tests are considered acceptable, and the results from these assays demonstrate that lanreotide has no genotoxic potential.

104-week life-time carcinogenicity bioassays with daily subcutaneous administration were performed in rats and mice. In the SD rats, the study design (dose selection and dosing regimen) is deemed suboptimal. Statistically significant increases in cutaneous and subcutaneous tumors of fibrous connective tissues at injection sites at the high dose 0.5 mg/kg/day were observed, however the tumor findings might not be relevant to humans undergoing monthly injections. In the CD-1 mice, the study was considered adequate although the daily dosing regimen likely limited systemic exposure. The study was positive for cutaneous and subcutaneous tumors of fibrous connective tissues at the injection sites at the high dose 30 mg/kg/day. Fibrosarcomas in both genders and malignant fibrous histiocytoma in males were increased at the high dose which

produces 3 times the maximum clinical exposure. Based on the frequency of dosing in mice relative to therapeutic use, the tumors observed may not be clinically relevant.

Reproductive toxicity of lanreotide (IRF) was assessed _____ and all the study reports were resubmitted with the current NDA (#22-074). Lanreotide (IRF) reduced female fecundity in rats and rabbits at doses less than the maximum human exposure. The reduction in female fertility likely to be an expected side effect in light of the pharmacological activity of the drug (inhibition of GH secretion). The fertility of males was unaffected by the treatment, though seminiferous tubule atrophy/degeneration was observed. In the rats and rabbits, embryofetal toxicity studies revealed that lanreotide is not teratogenic in pregnant animals. Using the therapeutic formulation under the current NDA (Somatuline® Autogel®), the rat subcutaneous seg 1 and seg 2 studies showed that s.c. dosing once every 2 weeks at 4, 10 and 20 mg/animal/ injection to male and female SD rats, from pre-mating through mating and until sacrifice (males) or until Day 7 post-coitum (females), 20 mg/animal/injection was poorly tolerated at the injection sites in all males. At 4 and 10 mg, lower body weight gain and reduced FC in both genders and lower ovulation and implantation parameters in females were observed and considered to be drug-related effects. In males, none of the fertility parameters were affected. NOAEL for female fertility was <4 mg/animal/injection, a fraction of the proposed maximum human dose.

In the specific local tolerance studies with repeated dosing in NZW rabbits, once every 4-week subcutaneous or intramuscular dosing of the therapeutic formulation at 10 mg/animal in 4 injection sites during a 98-day period induced irreversible induration, transient erythema and edema at injection sites. The induration was resulted from drug component deposition and its consequent tissue reaction, including granuloma formation and chronic inflammatory infiltration of macrophages and lymphocytes. Both s.c. and i.m. routes showed similar tissue reactions. The lesions persisted up to 98 days following the very first dosing. Longer term reversibility was not assessed in these studies.

B. Pharmacologic activity

Lanreotide acetate is a synthetic analogue of somatostatin with high potency and selectivity for somatostatin receptors 2 and 5, with potent activity inhibiting GH release in basal and stimulated conditions. Somatuline® Autogel® is a longer acting formulation intended to be administered once every 4 weeks for chronic treatment of patients with acromegaly.

C. Nonclinical safety issues relevant to clinical use

Based on nonclinical data, the local tolerance of the drug at the site of injection is a safety concern. While longer term reversibility was not specifically assessed in the NZW rabbits and cutaneous indurations were not recovered after 98 days since the very first injection with the therapeutic formulation, and the drug will be

administered chronically to patients, an extra attention should be paid on the local tissue reaction and/or its complications.

**APPEARS THIS WAY
ON ORIGINAL**

**APPEARS THIS WAY
ON ORIGINAL**

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW**2.6.1 INTRODUCTION AND DRUG HISTORY**

NDA number: 22-074
Review number: #1
Sequence number: 000
Date/type of submission: Nov. 1, 2006/NDA
Information to sponsor: Yes () No (X)
Sponsor and/or agent: Beaufour Ipsen Pharma
24 rue Erlanger
75016 Paris
France

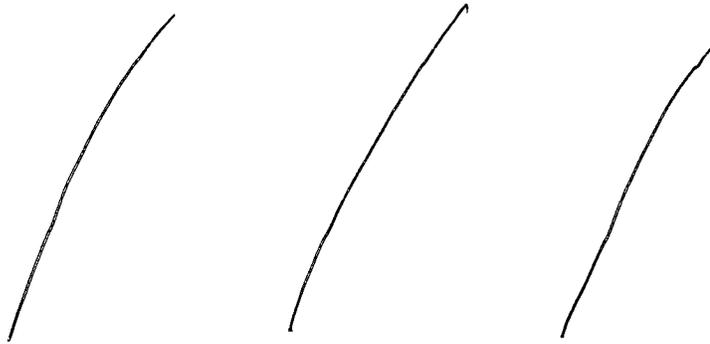
Manufacturer for drug substance: Ipsen Pharma Biotech
Parc d'Activités du Plateau de Signes
Chemin Départemental n°402
83870 Signes
France

Reviewer name: Dylan Dalin Yao, Ph.D.
Division name: Division of Metabolism and Endocrinology
Products
HFD #: 510
Review completion date: July 10, 2007

Drug:

Trade name: Somatuline® Autogel® (Lanreotide acetate) injection 60, 90 and 120 mg
Generic name (list alphabetically): Lanreotide AUTOGEL
Code name: none
Chemical name: [cyclo S-S]-3-(2-naphthyl)-D-alanyl-L-cysteinyl-L-tyrosyl-D-tryptophyl-L-lysyl-L-valyl-L-cysteinyl-L-threoninamide, acetate
CAS registry number: 108736-35-2
Molecular formula: ————— (CH₃COOH)_x, where
x=1.6 to 3.4
Molecular weight: MW 1096.34

Structure:



Relevant INDs/NDAs/DMFs:

Lanreotide: _____
INDs 53,993, _____
Sandostatin/Octreotide: NDA 21-008;
INDs 41,361; _____
DMF #8974

Drug class:

Somatostatin analogue

Intended clinical population:

Treatment of acromegaly

Clinical formulation:

Lanreotide AUTOGEL is provided in three strengths (60, 90 or 120 mg) as sterile, ready to use, pre-filled syringes containing the same lanreotide supersaturated bulk solution of 24.6% w/w lanreotide base.

Table 1. Unit Composition of Lanreotide AUTOGEL

Name of Ingredient	Unit Composition (mg)			% w/w	Function	Reference to Quality Standard
	60 mg	90 mg	120 mg			
<i>Drug substance</i> (Lanreotide base) Equivalent to weighed lanreotide acetate ^{1,2}	79.8	116.4	155.5	(24.6)	Somatostatin analogue	In-house (See DMF #8974)
<i>Excipient</i> Water for injection ²	186.2	271.6	363.0	QS. to 100	Solvent	USP / Ph.Eur.
Total mass	266	388	518.5			
Injected mass³						
Delivered dose (lanreotide base)	60	90	120			

- 2 For a lanreotide base concentration in the supersaturated solution of 24.6 %w/w
- 3 For the 60 and 90 mg doses, the loss due to retained material in the syringe is _____
For the 120 mg dose, the loss due to retained material in the syringe is _____

Route of administration: Deep s.c. injection at 4-week intervals

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

Studies reviewed within this submission: Refer to "Table of Contents" on page 2.

Studies not reviewed within this submission: Most of resubmitted non-clinical studies that have been reviewed and documented under _____ are not reviewed again.

**APPEARS THIS WAY
ON ORIGINAL**

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Drug development background: Lanreotide was first developed as an immediate release formulation (IRF) to assess the preclinical pharmacologic properties of the peptide, therefore, most preclinical studies included in this application, including toxicology and TK, utilized the IRF data. Lanreotide was first developed for commercial use as a micro-particle formulation (MPF) administered every 7, 10 or 14 days (depending on GH and IGF-1 response) and has been marketed in about 50 countries since 1994, according to the sponsor.

— Subsequently the AUTOGEL formulation was developed to further extend the duration of drug release allowing for dosing once every 4 weeks,

Lanreotide possesses a high affinity for binding to somatostatin receptors isolated from animals as well as cloned human receptors. Lanreotide has essentially no affinity for other bioreceptors although it does bind to the mu morphine receptor isolated from guinea pig brain. The characteristics of this binding suggest that the drug may possess morphine antagonism activity.

Lanreotide was demonstrated to inhibit GH release from cultured anterior pituitary cells. The drug also inhibited both basal and stimulated GH release in in vivo experiments with rats, sheep and monkeys. Its duration of action was longer than that of somatostatin, most likely due to reduced metabolic degradation. Lanreotide also inhibited GH secretion by GH secreting tumor cells in rats. These results support the proposed application of the drug in the treatment of acromegaly.

Lanreotide showed a significant interaction with estradiol, leading to an inhibitory effect on prolactin secretion. This could be related to the inhibitory effect of the peptide on the development of prolactin secreting cells.

SC administered lanreotide was less active than either somatostatin or octreotide in inhibiting glucose stimulated insulin secretion. Lanreotide was, however, equipotent to octreotide and somatostatin in inhibiting insulin-induced glucagon release.

Repeated doses of lanreotide increased gall bladder weight suggesting that the drug, like octreotide, may increase the risk of gall stone formation in some patients. The effects of lanreotide on the GI tract were similar to those produced by both somatostatin and octreotide.

Lanreotide did not induce any central nervous system or cardiovascular adverse effects.

In in vivo experiments, intradermal injections of lanreotide and somatostatin caused significant, dose-dependent inflammatory cutaneous reactions at the sites of injection.

However the doses of lanreotide and somatostatin which demonstrated a proinflammatory effect were 100 times greater than the effective doses of the positive control. This effect may at least be partially explained by the mast cell prodegranulant effect observed in vitro at high doses (10^{-5} M).

High doses of lanreotide produced antidiuretic effects in water loaded rats and this effect may be mediated through release of vasopressin since it was not observed in rats with diabetes insipidus.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Lanreotide acetate is a synthetic analogue of somatostatin with high potency and selectivity for somatostatin receptors 2 and 5, with potent activity inhibiting GH release in basal and stimulated conditions. Somatuline® Autogel® is a longer acting formulation intended to be administered once every 4 weeks for chronic treatment of acromegaly.

Drug activity related to proposed indication: Pharmacology studies submitted to this NDA included in vitro assays to evaluate binding affinity and selectivity of lanreotide to somatostatin receptors, and effect on GH release and cell proliferation in vitro. In vivo studies provided data on inhibitory effect on basal and stimulated GH secretion in rats and monkeys, and anti-proliferative effect on rat pituitary tumor cell line. Secondary pharmacodynamics on insulin and glucagon secretion, on the digestive system, on gallbladder emptying and the pancreas, were also assessed.

Receptor binding studies:

In vitro binding of lanreotide to somatostatin receptors

- In most rat tissues lanreotide exhibited binding affinity equivalent to that of somatostatin-14 (Report No. 23014 Ph 49R). The notable exception was cerebral cortex receptors for which lanreotide had no measurable affinity. On the other hand, somatostatin-14 exhibited similar binding affinity for all tissues studied (scanned Table 2.6.2-1).

Table 2.6.2-1: *In Vitro* Inhibitory IC_{50} Values for Lanreotide and Somatostatin-14 for [^{125}I -Tyr 11] Somatostatin Binding Sites in Several Tissues Isolated from Rats

Test Agent	IC_{50} , nM			
	Pancreas	Anterior Pituitary	Adrenal Cortex	Cerebral Cortex
Lanreotide	0.39	1.2	0.26	>10000
Somatostatin-14	0.37	0.91	0.27	0.53

- Lanreotide, octreotide and somatostatin-14 exhibited equivalent binding affinities for anterior pituitary membrane receptors and somatostatin exhibited the same affinity for cerebral cortex (Report No. 23014 Ph 55P). Both octreotide and lanreotide had reduced affinity for cerebral cortex receptors but the affinity of octreotide was more than six fold greater than that of lanreotide (scanned Table 2.6.2-2).

Table 2.6.2-2: In vitro IC₅₀ Values for Lanreotide, Octreotide and Somatostatin-14 for [¹²⁵I-Tyr¹¹] Somatostatin Binding Sites in Rat Cerebral Cortex and Anterior Pituitary.

Test Agent	IC ₅₀ nM (95% Confidence Limits)	
	Cerebral Cortex	Anterior Pituitary
Somatostatin-14	1.12 (0.8-1.5)	1.17 (0.5-1.8)
Octreotide	165.3 (47.6-282.9)	0.73 (0.2-1.7)
Lanreotide	>1000	0.35 (0.3-0.9)

- Lanreotide had similar potency and selectivity to that of octreotide for binding the cloned human somatostatin receptors (scanned Table 2.6.2-3) (Report No. 23014 Ph 166)

Table 2.6.2-3. Potency and Selectivity of Octreotide and Lanreotide from Cloned Human Receptors

Peptide	K _i ± SEM (nM)				
	Receptor 1	Receptor 2	Receptor 3	Receptor 4	Receptor 5
Octreotide	870 ± 18	0.44 ± 0.07	26.8 ± 7.68	5030 ± 2000	9.24 ± 1.95
Lanreotide	1707 ± 418	0.72 ± 0.08	97.8 ± 14.5	1717 ± 187	9.97 ± 2.58

*K_i: Inhibition Constant

Effect of lanreotide on GH release and cell proliferation (in vitro and in vivo)

- Lanreotide was equipotent to octreotide and somatostatin-14 when added to the culture medium containing GH released by cultured anterior pituitary cells (scanned Table 2.6.2-4) (Report 23014 Ph 55P)

Table 2.6.2-4: Inhibitory Effects of Somatostatin-14, Octreotide, and Lanreotide on In Vitro Secretion of Growth Hormone by Cultured Rat Anterior Pituitary Cells

Test Substance	Inhibitory EC ₅₀ on GH Secretion [nM (95% Confidence Limits)]	Replicates
Somatostatin-14	0.07 (0.01-0.1)	20
Octreotide	0.014 (0-0.02)	3
Lanreotide	0.017 (0.01-0.02)	4

*EC₅₀: Concentration required to inhibit GH secretion by 50%

- When dosed either i.v. or s.c. to pentobarbital anesthetized rats lanreotide attenuated both basal and stimulated (D-Ala²-GRF, 10 µg/kg) GH release (Report No. 23014 Ph 65). Lanreotide was more potent than somatostatin regardless of route of dosing and its effect persisted longer than did the effect of somatostatin. The inhibitory effects of both lanreotide and somatostatin were dose related (summarized in scanned Tables 2.6.2-5 and 2.6.2-6).

Table 2.6.2-5: Inhibition of Basal Growth Hormone Release by Somatostatin and Lanreotide in Anesthetized Rats

Dose (mcg/kg)	Percent reduction of plasma growth hormone (relative to controls) at various time intervals after injection of somatostatin or lanreotide			
	0.25 hr	0.5 hr	0.75 hr	1.25 hr
Intravenously administered lanreotide				
0.1	0	0	0	0
1.0	55.34 ³	49.49 ²	12.74	0
3.0	78.12 ³	51.60 ¹	0	0
10.0	79.51 ³	82.30 ³	57.70 ¹	50.51
ED ₅₀ (mcg/kg)	1.12	1.66	8.52	9.88
Intravenously administered somatostatin				
10	0	0	ND	ND
30	37.60 ¹	0	ND	ND
100	5.60	0	ND	ND
300	57.21	0	11.24	ND
900	80.25 ²	0	0	ND
ED ₅₀ (mcg/kg)	320	-	-	-
Subcutaneously administered lanreotide				
0.1	0	53.65 ¹	ND	ND
1.0	77.70 ²	79.54 ²	43.40	72.72 ²
10	77.65 ¹	85.59 ²	91.88 ²	91.82 ²
ED ₅₀ (mcg/kg)	0.92	0.053	1.37	0.065
Subcutaneously administered somatostatin				
0.1	33.38 ¹	40.64 ¹	ND	ND
1.0	0	0	ND	ND
10	61.56	29.14	44.52	0
25	92.18 ³	40.14	0	26.74
50	0	0	0	0
100	81.36 ³	79.65 ³	0	0
ED ₅₀ (mcg/kg)	76.55	77.26	-	-

¹p <0.05 ²p <0.01 ³p <0.001 ND - Not determined

Best Possible Copy

Table 2.6.2-6: Inhibition of Stimulated¹ Growth Hormone Release by Somatostatin and Lanreotide in Anaesthetised Rats

Dose (mcg/kg)	Percent reduction of plasma growth hormone (relative to controls) at various time intervals after injection of somatostatin or lanreotide				
	0.25 hr	0.5 hr	0.75 hr	1 hr	1.5 hr
Intravenously administered lanreotide					
1.0	30.55	0	19.31	ND	ND
3.0	81.52 ²	45.81	0	ND	ND
10	96.75 ³	84.52 ³	72.00 ³	ND	ND
ED ₅₀ (mcg/kg)	1.68	3.71	6.92	-	-
Intravenously administered somatostatin					
10	0	0	0	ND	ND
30	0	0	0	ND	ND
100	22.72	0	0	ND	ND
300	0	0	ND	ND	ND
900	88.06 ²	6.21	ND	ND	ND
ED ₅₀ (mcg/kg)	560	-	-	-	-
Subcutaneously administered lanreotide					
0.1	0	0	0	ND	ND
1.0	62.72 ³	46.21 ²	0	0	0
10	99.65 ³	98.69 ²	99.45 ³	95.52 ²	69.30 ²
ED ₅₀ (mcg/kg)	0.83	1.08	3.18	3.34	5.27
Subcutaneously administered somatostatin					
0.1	6.54	0	0	ND	ND
1.0	33.46	0	0	ND	ND
10	72.97 ⁴	0	12.63	0	19.45
25	68.81 ⁴	40.37 ²	0	0	18.96
50	77.29 ³	0	0	0	0
100	95.24 ²	0	28.92	37.00	0
ED ₅₀ (mcg/kg)	3.46	-	-	-	-

¹D-Ala2-GRF, 10 mcg/kg was administered subcutaneously at the indicated time intervals after the administration of somatostatin or lanreotide

²p < 0.05

³p < 0.01

⁴p < 0.001

ND - Not determined

- When lanreotide, octreotide and somatostatin were s.c. dosed to anesthetized rats (Report No 23014 Ph 134), lanreotide and octreotide had similar inhibitory effects on GH levels, and both were somewhat more potent than somatostatin (scanned Table 2.6.2-7)

Best Possible Copy

Table 2.6.2-7: Comparative Inhibitory Effects of Lanreotide, Octreotide and Somatostatin on Basal and ¹D-Ala²-GRF Stimulated GH Release in Anaesthetised Rats

Treatment Group (mcg/kg)	Percent Reduction in Plasma GH Concentration		
	0.25 hr	0.5 hr	0.75 hr
Effect on basal GH			
Lanreotide			
1.0	91.02 ²	71.88 ²	0
2.5	85.90 ²	95.23 ²	87.73 ¹
10	86.05 ²	97.67 ²	87.21 ¹
Somatostatin			
1.0	0	20.83	0
10	79.95 ¹	38.97	0
Octreotide			
1.0	95.96 ²	92.29 ¹	52.13
10	89.53 ¹	92.67 ¹	85.34
Effect on stimulated GH			
Lanreotide			
1.0	60.55 ²	28.20	5.42
2.5	96.30 ³	92.52 ³	66.20 ²
10	97.12 ³	97.44 ³	99.80 ³
Somatostatin			
1.0	41.85 ²	46.88 ¹	20.32
10	46.65 ³	27.70	9.80
Octreotide			
1.0	99.04 ³	99.23 ³	92.73 ³
10	98.97 ³	98.30 ³	99.84 ³

¹p ≤ 0.05

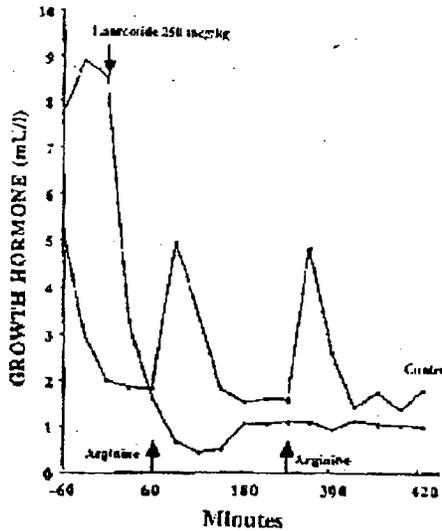
²p ≤ 0.01

³p ≤ 0.001

Best Possible Copy

- The effects of lanreotide on basal and arginine-stimulated GH release in cyno monkeys were similar to that observed in rats (Report No. 23014 Ph 10). Cyno monkeys were sedated with ketamine, and s.c. dosed with lanreotide (10 or 250 µg/kg) then challenged with arginine infusion (20 ml of 12.5% arginine HCl over 20 min). Lanreotide reduced plasma levels of GH somewhat more rapidly than the spontaneous reduction in the sedated monkeys, and completely blocked arginine-induced stimulation of GH release at the 60 and 240 min post dose (scanned Figure 2.6.2-1).

Figure 2.6.2-1: Effect of Lanreotide on Basal and Stimulated GH Secretion in a Sedated Cynomolgus Monkey



Best Possible Copy

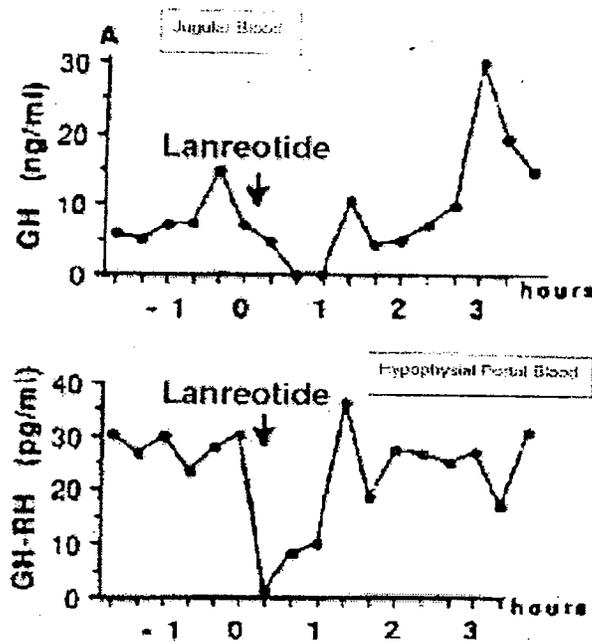
- Antisecretory activity of lanreotide was assessed in GH hypersecreting rats (report No. 23014 Ph 119R), the effect was shown by reduced mean tumor weight and mean concentration of plasma GH, and increased mean receptor density (scanned Table 2.6.2-8)

Table 2.6.2-8: Effects of Lanreotide and Octreotide on Growth, Tumor Proliferation, Receptor Density and Plasma Growth hormone in GH Hypersecreting Rats

Treatment group	Mean body weight gain (g ± SD)	Mean tumour weight (dg)	Mean Plasma GH (ng/ml)	Mean receptor density (mmol drug bound/mg protein)
Saline	82.5 ± 17.2	45.5 ± 19.4	978.9 ± 624.8	88.8 ± 37.7
Lanreotide	71.1 ± 11.4	32 ± 20.1	816 ± 306.5	90.1 ± 63.9
Octreotide	52.8 ± 36.5	27.6 ± 29.3	534.1 ± 471.2	121.7 ± 109.3

- Lanreotide and somatostatin significantly reduced cellular proliferation when measuring DNA production with cultured cells in the presence or absence of the drugs but no dose-response relationship (Report No. 23014 Ph 105).
- Lanreotide also acts at the level of GH-releasing hormone (GH-RH) in sheep when 1 mg of lanreotide was i.v. dosed and plasma hormones monitored for 3 to 5 hrs (Report No. 23014 Ph 90) (scanned Figure 2.6.2-2)

Figure 2.6.2-2: Effect of Intravenous Administration of Lanreotide on Hypophysial Portal Blood Concentration of GH-RH and Jugular Blood Concentrations of GH



Best Possible Copy

2.6.2.3 Secondary pharmacodynamics

Effects of lanreotide on insulin and glucagon secretion, on digestive system, on gallbladder emptying, on the pancreas and on bioreceptors were assessed.

Effects on insulin and glucagon secretion:

- Lanreotide did not significantly inhibit glucose-stimulated insulin release but somatostatin-14 and octreotide did in rats receiving s.c. doses of the drugs 10 min before the i.v. glucose challenge (scanned Table 2.6.2-9) (Report No. 23014 Ph 132)

**APPEARS THIS WAY
ON ORIGINAL**

Table 2.6.2-9: Effect of Lanreotide, Somatostatin, and Octreotide on Glucose Stimulated Insulin Secretion in Rats

Treatment (mcg/kg, s.c.)	Serum Insulin (mcU/ml ± SD)	Percent Inhibition
Control	89.85 ± 20.79	---
Lanreotide		
30	67.70 ± 32.37	24.46
300	63.80 ± 28.72	29.00
Somatostatin		
300	34.51 ± 13.91 ¹	61.16 ¹
Octreotide		
10	66.54 ± 15.50	25.95
30	38.80 ± 12.73 ¹	56.82 ¹
300	20.41 ± 8.82 ¹	77.28 ¹

p < 0.001

- Lanreotide, octreotide and somatostatin all attenuated the pancreatic release of glucagon in insulin challenged rats when the drugs were dosed s.c. or i.v. then challenged with i.v. insulin (1.5 U/kg) (scanned Table 2.6.2-10) (Report No. 23014 Ph 133)

Table 2.6.2-10: Effect of Lanreotide, Somatostatin, and Octreotide on Insulin Stimulated Glucagon Secretion in Rats.

Treatment (mcg/kg, s.c.)	Serum Glucagon (pg/ml ± SD)	Percent Inhibition
Control	295 ± 57	---
Lanreotide		
10	169 ± 57 ²	42.71 ²
30	183 ± 27 ²	37.97 ²
Somatostatin - 14		
10	212 ± 37 ¹	28.14 ¹
30	173 ± 19 ¹	41.35 ²
Octreotide		
10	150 ± 26 ³	49.15 ³
30	187 ± 57 ¹	36.61 ¹

p < 0.05

p < 0.01

p < 0.001

Effects on the digestive system:

- Gastric emptying in rats: Both s.c. dosed lanreotide and somatostatin increased gastric emptying of the liquid meal but no effect on gastric emptying of the solid meal following s.c. dosed lanreotide (10, 80, 200 or 500 µg/kg) or somatostatin (200 µg/kg) (Report No. 236-120).
- Effect on gastric secretions in rats: Both s.c. dosed lanreotide (10, 80, 200, or 500 µg/kg) and somatostatin (200 µg/kg) exhibited a dose-related antisecretory effect

Best Possible Copy

as evidenced by significantly reduced volumes of gastric secretions (↓free acidity, total acidity and buffer capacity) at all dose levels tested (Report No. 236-120).

- Effect on gastrointestinal and colonic motility in dogs: i.v. dosed lanreotide (0.1, 1, or 10 µg/kg) reduced gastric motility in fasting or fed dogs and increased jejunal motility in fasted but not fed animals. Colonic activity was increased at highest dose tested (scanned Table 2.6.2-13) (Report No. 23014 Ph 103 R).

Table 2.6.2-13: Mean Gastric, Jejunal, or Colonic Motor Indices (g.min/hr) During Two Hours Following Administration of Lanreotide to Fasting or Postprandial Beagle dogs (n=4).

Treatment (mcg/kg)	Mean motor index (g.min/hr)		
	Gastric	Jejunal	Colonic
Fasting animals			
Control	8.3 ± 1.3	5.2 ± 1.1	7.9 ± 1.1
Lanreotide			
0.1	8.4 ± 1.2	6.4 ± 1.1 ¹	7.8 ± 1.1
1.0	4.5 ± 1.4 ²	6.6 ± 1.1 ¹	7.8 ± 2.1
10	0.6 ± 0.4 ²	10 ± 1.8 ²	16.3 ± 6.1 ²
Postprandial animals			
Control	5.6 ± 1.0	7.5 ± 0.8	15.1 ± 1.9
Lanreotide			
0.1	5.4 ± 1.0	7.7 ± 1.0	14.4 ± 2.2
1.0	3.7 ± .09 ²	7.6 ± 1.0	15.4 ± 1.5
10	2.4 ± 0.8 ²	7.5 ± 1.2	12.5 ± 4.4 ²

¹p < 0.05

²p < 0.01

Effects on gall bladder emptying in the mouse:

- Lanreotide at 10, 80, 200, or 500 µg/kg/d s.c. given to mice for 5 days increased gall bladder weight and the effect was generally dose-related with statistically significant increase at 80 and 200 µg/kg (Report No. 236-120). Somatostatin at s.c. dose of 200 µg/kg also caused a non-significant increase in gall bladder weight.

Effects on the pancreas:

- Lanreotide and somatostatin suppressed pancreatic exocrine secretion in surgically-induced pancreatitis (Report No. 23014 Ph 69). When s.c. dosed immediately prior to ligation of common bile/pancreatic duct, lanreotide was ~500X more potent than somatostatin in inhibiting ligation-associated elevation in plasma α-amylase concentrations. When dosed after ligation (1.5 hr later), neither drug inhibited α-amylase secretion (scanned Table 2.6.2-14).

Best Possible Copy

Table 2.6.2-14: Inhibition of Ligation Induced Increases in α -Amylase in Rats.

Treatment (mcg/kg)	Percent Inhibition Relative to Controls after Subcutaneous Drug Administration					
	0.75 hr	1 hr	1.25 hr	1.5 hr	3 hr	6hr
Lanreotide						
0.1	3.17	14.13	3.43	ND	ND	ND
0.3	0	33.58	27.31	ND	ND	ND
1.0	52.03	89.04	87.12	82.90	20.19	0
ED ₅₀	95.41	0.35	0.41	-	-	-
Somatostatin						
100	0	0	0	ND	ND	ND
200	89.93	77.85	51.37	ND	ND	ND
300	65.21	74.22	76.09	84.02	0	0
ED ₅₀	~ 179	180	202	-	-	-

ND - Not determined

Effects on bioreceptors:

- Lanreotide exhibited selectivity for the mu receptor and its binding constant was identical to that of morphine (Report No. 23014 Ph 62). Lanreotide's binding to the delta and kappa receptors was ~15-20% that of morphine (scanned Table 2.6.2-15). Parallel binding experiments in the presence and absence of sodium chloride showed that the affinity of pure opiate agonist is markedly decreased in the presence of sodium. The binding affinity of pure agonists (morphine, morphiceptin, DADLE, and EKC) was clearly reduced by the presence of sodium while that of the opiate antagonist (naloxone) remained unaffected (scanned Table 2.6.2-16) (Report No. 23014 Ph 60).

Table 2.6.2-15: *In Vitro* Binding of Lanreotide and Morphine to Guinea Pig Forebrain Opiate Receptor Subtypes

Receptor	Ki (nM)		
	Radio Ligand	Lanreotide	Morphine
Mu	[³ H] DAGO ¹ or [³ H] naloxone	3.41 ± 0.08	3.6 ± 0.4
Delta	[³ H]DADLE ¹	392 ± 43	48 ± 3.7
Kappa	[³ H]EKC ¹	199 ± 84	34 ± 6.4

DAGO=[D-Ala²,Gly-ol³]enkephalin; DADLE=[D-Ala²,Gly-Leu³]enkephalin; EKC=ethylketocyclazocine

Best Possible Copy

Table 2.6.2-16: Effect of Sodium on the *In Vitro* Binding Affinity of Lanreotide and Opioid Compounds

Compound	Ki, nM		+NaCl/ -NaCl ratio	Pharmacologic Class
	-NaCl	+NaCl		
Lanreotide	14 ± 2	99 ± 27	7.1	Agonist/antagonist
Morphine	3.7 ± 0.5	128 ± 14	35	Agonist
Morphiceptin	156 ± 35	4752 ± 1247	30	Agonist
DADLE	14 ± 2.9	568 ± 108	41	Agonist
EKC	0.58 ± 0.08	11 ± 0.3	19	Agonist
Cyclazocine	0.10 ± 0.007	0.40 ± 0.08	4	Agonist/antagonist
Levallorphan	0.20 ± 0.07	0.47 ± 0.07	2.3	Agonist/antagonist
Naloxone	0.91 ± 0.10	0.68 ± 0.01	0.75	Antagonist

2.6.2.4 Safety pharmacology

Neurological effects:

Lanreotide was tested in mice at s.c. doses up to 500 µg/kg. There was no evidence of lanreotide mediated effects on grossly observable behavior, spontaneous motor activity or skeletal muscle tone, or on body temperature. The 500 µg/kg dose did cause a marginal increase in the duration of pentobarbital sleeping time. Lanreotide did not exhibit anticonvulsant activity in the supramaximal electroshock test but a higher percentage (10-40%) of dosed mice died after administration of electroshock. But no deaths in the control group were seen. Lanreotide did not significantly affect the time to onset or lethality of pentylenetetrazole seizures (Report No. 23014 Ph 114a).

Lanreotide was also tested for analgesic activity in the phenylbenzoquinone writhing and hotplate tests in mice. The drug was without statistically significant effect in the writhing test in doses up to 500 µg/kg. In the hotplate test, both lanreotide and somatostatin significantly prolonged the pain responses in mice. Lanreotide (10, 80, 200, and 500 µg/kg, s.c.) caused 30, 62, 59, and 54%, respectively, increases in response time 3 hrs after administration. The tested dose of somatostatin (200 µg/kg) caused a 22% increase in response time 6 hrs after administration. The positive control, morphine (5 mg/kg) more than doubled response time 15 minutes after administration and caused a 33% increase in response time 3 hrs after administration (Report No. 23014 Ph 114c).

Lanreotide was also found to be without effects on the cortical EEG of conscious rabbits when given i.v. doses at 20 or 80 µg/kg and measured at 1, 5, 30, and 60 min post dose (Report No. 910258).

Cardiovascular and pulmonary effects:

The HERG tail current (I_{kr}) was measured in human embryonic kidney cells (HEK-293) stably transfected with HERG-1 cDNA, lanreotide at 10⁻⁵ M produced no statistically significant inhibition of HERG tail current (Report No. 910258).

Lanreotide was tested for adverse effects on cardiac action potential in isolated

Best Possible Copy

canine Purkinje fibers at concentrations of 10^{-7} , 10^{-6} and 10^{-5} M, no statistically significant effect on action potential parameters under either normal (1 Hz) or low (0.33 Hz) stimulation rates was recorded. Neither early nor delayed after-depolarization was observed at any concentration (Report No. 20040085PECM).

Lanreotide (5, 20, or 80 $\mu\text{g}/\text{kg}$) was i.v. dosed to normotensive, anesthetised rats and the effects of the drug on BP, HR and ECG were observed. In addition, effects of the drug on cardiovascular responses to adrenalin, noradrenalin, isoprenaline and acetylcholine were monitored. Lanreotide (and somatostatin 80 $\mu\text{g}/\text{kg}$) produced transient, non-dose dependent hypotensive responses immediately after injection. Somatostatin caused bradycardia of about 10 min duration but lanreotide did not. Lanreotide did not affect the BP response to the standard agents tested (Report No. 145-275).

Lanreotide at 20 or 80 $\mu\text{g}/\text{kg}$ was i.v. infused to anesthetised dogs over periods of two min. Cardiovascular parameters monitored included arterial BP, HR, femoral and arterial flow, respiration rate and amplitude, ECG, and blood gases (PO_2 , PCO_2 , HCO_3) and pH. Lanreotide caused no significant changes in BP or venous or arterial flow. At 80 $\mu\text{g}/\text{kg}$, a transient bradycardia was noted for one min. This change was not observed at 20 $\mu\text{g}/\text{kg}$. Lanreotide did not induce any ECG, blood gases or pH changes. Both doses of lanreotide caused transient (one min) decreases in respiratory amplitude but there were no changes in respiratory rate (Report No. 910259).

When anesthetised dogs were infused with lanreotide at total dose of 5000 $\mu\text{g}/\text{kg}$ over six hrs duration with cardiovascular parameters monitored, including aortic pressure, ECG, left ventricular pressure, left end diastolic pressure, $+\text{dp}/\text{dt}$, cardiac output, contractile force, HR and total peripheral resistance (each parameter was measured at five min intervals throughout the experiment), minimal and transient changes observed in all parameters were within normal biological limits and could not be attributed to the dosing of lanreotide (Report No. 23014 Ph 52).

In six conscious, radio-telemetry instrumented dogs, lanreotide was i.v. infused at 1, 3 and 10 mg/kg over a 24 hrs. No statistically significant change in arterial BP, HR, QRS complex duration and QTc was observed. No arrhythmia attributable to lanreotide was observed at any dose level. The NOEL was 3 $\text{mg}/\text{kg}/24$ h and the NOAEL was 10 $\text{mg}/\text{kg}/24$ h on cardiovascular parameters, respectively (Report No. 20030465PCC).

Two groups of conscious male and female beagle dogs (3/s/g) were i.m. dosed with lanreotide at 0.43 or 4.3 mg/kg after instrumented for telemetric transmission of arterial BP and ECG (lead II). Telemetric measurements (15 second periods at 15 min intervals) were initiated 24 hrs prior to treatment and continued for 14 days after dosing. In addition, ECG leads I, II, III, aVR, aVL, and aVF were monitored, under restraint conditions, one hr after dosing then on post dose days 3, 7, and 14. Administration of lanreotide at either dose was without effect on arterial BP. Neither dose produced significant disturbances in ECG leads I, II, III, aVR, aVL or aVF. Similarly, there were no clinical signs attributable to the administration of lanreotide (Report No. 980274P).

Renal effects:

Food and water deprived (18 hrs) rats were loaded with an oral dose of 25 ml/kg and a subcutaneous dose of 3 ml/kg of physiological saline. Two experiments were conducted. In the first, animals were also s.c. dosed with 80, 200 or 500 µg/kg of lanreotide or 200 µg/kg of somatostatin and in the second both drugs were dosed at 200 or 500 µg/kg. Urine was collected for 6 hrs and in the first experiment it was analyzed for volume, uric acid, sodium, potassium, chloride. In the second experiment urine was analyzed for calcium, and creatinine and blood was analyzed for sodium, potassium, calcium, urea, and creatinine (Report No. 275-170).

In the first experiment, 500 µg/kg lanreotide significantly elevated the sodium/potassium ratio but somatostatin was without effect on this parameter. In the second experiment lanreotide significantly reduced urinary volume and caused increased serum concentrations of urea but neither dose of somatostatin had a similar effect.

The effects of lanreotide on renal clearance of dextrose and insulin were studied in anesthetised rats. In the first study, i.v. infusion of lanreotide at 10, 30, 100, or 300 µg/kg/20 min had no effect on urine flow, urine osmolality, or free water clearance. In the second study, an initial decrease in glomerular filtration rate (GFR) by an average of 22% at 150 µg/kg lanreotide was not reproducible while continued infusion of lanreotide was given and GFR returned to control values. The sponsor explains that the initial decrease in GFR may have been associated with a brief (10 min) hypotensive response to initial infusion of the drug (Report No. 23014 Ph 164).

The antidiuretic effect of lanreotide, initially observed in water loaded rats, was studied in the diabetes insipidus rat. The antidiuretic effects of s.c. dosed lanreotide (200 and 400 µg/kg) were confirmed in normal rats undergoing water diuresis. In contrast, neither single nor repeated doses of lanreotide affected urine osmolality, urine volume, or free water clearance in diabetes insipidus rats. The results of this experiment indicate that lanreotide-stimulated release of vasopressin may play a role in the renal response of the rat to the drug (Report No. 23014 Ph 165).

Gastrointestinal effects:

Gastric emptying in rats: Both s.c. dosed lanreotide and somatostatin increased gastric emptying of the liquid meal but no effect on gastric emptying of the solid meal following s.c. dosed lanreotide (10, 80, 200 or 500 µg/kg) or somatostatin (200 µg/kg).

Effect on gastric secretions in rats: Both s.c. dosed lanreotide (10, 80, 200, or 800 µg/kg) and somatostatin (200 µg/kg) exhibited a dose-related antisecretory effect as evidenced by significantly reduced volumes of gastric secretions (↓ free acidity, total acidity and buffer capacity) at all dose levels tested.

Effect on gastrointestinal and colonic motility in dogs: i.v. dosed lanreotide (0.1, 1, or 10 µg/kg) reduced gastric motility in fasting or fed dogs and increased jejunal motility in fasted but not fed animals. Colonic activity was increased at highest dose tested.

Effect of lanreotide on the autoimmune system (in vitro and in vivo) (Report No. 23014 Ph 114b):

Suspensions of mast cells collected from the peritoneal cavity of groups of 16 fasted female rats were preincubated for 10 min at 37°C, and lanreotide, somatostatin (both at 10^{-7} , 10^{-6} , and 10^{-5} M), compound 48/80 (0.5 µg/ml, a positive control) or saline (20 µl) was added and left in contact with the cells for 10 min. Released histamine was determined in the supernatant with a fluorometric method using spectrofluorimeter. Lanreotide at 10^{-7} and 10^{-6} M did not induce the release of histamine from the rat peritoneal mast cells in vitro, while at 10^{-5} M it exerted a significant prodegranulant effect. Equivalent doses of somatostatin exerted a similar action.

A histamine assay was performed on the supernatant with mast cell culture in another in vitro study, lanreotide and somatostatin did not potentiate immune degranulation. At low doses a slight protective effect by lanreotide was observed.

In the in vivo experiment, groups of six non-fasted male rats received intradermal injections of lanreotide, somatostatin (both at 80, 200 and 500 µg/kg), the compound 48/80 (0.5, 2.5 and 6.25 µg/kg; a positive reference in the test) or vehicle (physiological saline). Simultaneously, 1 ml of a solution containing 5 mg of Evans Blue in physiological saline was given via the penile vein. After 30 min the animals were sacrificed and the stain content of the papules formed was measured spectrophotometrically. Lanreotide caused a significant, dose-dependent inflammatory cutaneous reaction at the site of injection; however, the change in staining of the papules, expressing an increase in capillary permeability, was not statistically significant.

Somatostatin had a similar activity at the same doses but with a greater and more significant effect on staining. Doses of lanreotide and of somatostatin, which demonstrated a proinflammatory effect, were 100 times greater than the effective doses of compound 48/80.

2.6.2.5 Pharmacodynamic drug interactions

n/a

**APPEARS THIS WAY
ON ORIGINAL**

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Type of Study	Test System	Method of Administration	Testing Facility	Study Number	Location Section
Primary Pharmacodynamics					
Tissue selectivity of BM somatostatin analogues on growth hormone release in rats	Rat tissues (anterior pituitary, adrenal cortex, pancreas, cerebral cortex)	In vitro	Biomeasure, USA	23014 Ph 49R	4.2.1.1
Differential binding of somatostatin agonists to somatostatin receptors in brain and adenohypophysis	Rat (cerebral cortex and pituitary tissues)/Adult male Sprague Dawley (200-250g)	In vitro		23014 Ph 55P	4.2.1.1
Sub-type selectivity of peptide analogues for all five cloned human somatostatin receptors (hsstr 1-5)	Genomic fragments of human SSTR 2, 3 and 5 or cDNA clones of human SSTR 1 and 2A subcloned into the pRc/CMV mammalian expression vector	In vitro		23014 Ph 166	4.2.1.1
Sub-type selectivity of octreotide and lanreotide for all five cloned human somatostatin receptors (hsstr 1-5)	Genomic fragments of human SSTR 1, 2, 3, 4 and a cDNA clone for human SSTR 5 subcloned into the pCMV mammalian expression vector	In vitro	Biomeasure, USA	Supplement to 23014 Ph 166	4.2.1.1
Comparative effect of lanreotide and somatostatin on growth hormone release	Rat/ Male Sprague Dawley (260-300g)	s.c.	Biomeasure, USA	23014 Ph 65	4.2.1.1
Comparative effect of lanreotide, somatostatin and octreotide (Sandostatin) on growth hormone release	Rat/ Male Sprague Dawley (250-300g)	s.c.	Biomeasure Inc.	23014 Ph 134	4.2.1.1
Effect of lanreotide on growth hormone levels	Cynomolgus Monkey/ 2 male; 2 female	s.c.		23014 Ph 10	4.2.1.1
Comparison of antiproliferative and antisecretory effects of lanreotide, octreotide on GH hypersecreting rats	Rat/ 30 female Wistar Furch	s.c.		23014 Ph 119R	4.2.1.1

Type of Study	Test System	Method of Administration	Testing Facility	Study Number	Location Section
Effects on GH ₁ cell proliferation assessed by fluorimetry	Rat/ GH ₁ pituitary tumour cell line	In vitro		23014 Ph 105	4.2.1.1
Effect on growth-hormone-releasing hormone secretion	Sheep/ 12 male Merino (40-45kg)	i.v.		23014 Ph 90	4.2.1.1
Effect on prolactin and pituitary adenomas	Rat/ Female Wistar (200g)	i.m.		23914 Ph 2R	4.2.1.1
Secondary Pharmacodynamics					
Effect of lanreotide and somatostatin on glucose-stimulated insulin secretion	Rat/ Male Sprague Dawley (200-250g)	i.v./s.c.	Biomeasure Inc.	23014 Ph 58	4.2.1.2
Effect of lanreotide, somatostatin and octreotide on glucose-stimulated insulin secretion	Rat/ Male Sprague Dawley (250-300g)	s.c.	Biomeasure Inc.	23014 Ph 132	4.2.1.2
Effect of lanreotide and somatostatin on insulin-stimulated glucagon secretion	Rat/ Male Sprague Dawley (200-250g)	i.v./s.c.	Biomeasure Inc.	23014 Ph 61	4.2.1.2
Effect of lanreotide, somatostatin and octreotide on insulin-stimulated glucagon secretion	Rat/ Male Sprague Dawley (250-270g)	s.c.	Biomeasure Inc.	23014 Ph 133	4.2.1.2
Gastric emptying of liquids	Rat/ Male Sprague Dawley (200g)	s.c.		236-120	4.2.1.2
Gastric emptying of solids	Rat/ Male Sprague Dawley (200g)	s.c.		236-120	4.2.1.2
Gastrointestinal transit with charcoal	Rat/ Male Sprague Dawley (200g)	s.c.		236-120	4.2.1.2
Effect of gall bladder emptying	Mouse/ Male CD ₁ (approx. 20g)	s.c.		236-120	4.2.1.2
Hyposecretion by ligature of the pylorus	Rat/ Male Sprague Dawley	s.c.		236-120	4.2.1.2
Effects on intestinal transit in the rat	Rat/ Male Wistar (300-350g)	s.c.		23014 Ph 116R	4.2.1.2
Comparison of lanreotide and somatostatin on pentagastrin-stimulated gastric acid secretion	Rat/ Female Sprague Dawley	s.c.	Biomeasure Inc.	23914 Ph 70	4.2.1.2
Effect on the gastric acid secretion in the conscious rat carrier of a chronic gastric canula. Comparison with octreotide.	Rat/ Male Wistar	s.c.		23014 Ph 114d	4.2.1.2
Effect of lanreotide on water transport in the jejunum in basal and stimulated (PGE1) states	Rat/ Male Wistar (250-300g)	s.c.		23014 Ph 104 R-a	4.2.1.2
Effect of lanreotide, octreotide and somatostatin on transport of water in the jejunum in basal and stimulated (PGE1) states	Rat/ Male Wistar (250-300g)	s.c.		23014 Ph 104 R-b	4.2.1.2

Best Possible Copy

Type of Study	Test System	Method of Administration	Testing Facility	Study Number	Location Section
Effect of lanreotide, octreotide and somatostatin on jejunal hypersecretion induced by intraluminal infusion of cholera toxin	Rat/ Male Wistar (250-300g)	s.c.	/	23014 Ph 104 R-c	4.2.1.2
Effect on gastrointestinal and colonic motility	Dog/ 4 Female Beagle (12-15kg)	i.v.	/	23014 Ph 103R	4.2.1.2
Comparison of somatuline and somatostatin on ligation induced pancreatitis in rats	Rat/ Male Sprague Dawley (250-300g)	s.c.	Biomeasure Inc.	23014 Ph 69	4.2.1.2
Effect of somatostatin analogues on caerulein-induced pancreatic hyperplasia	Rat/ Male Sprague Dawley (200-225g)	s.c.	Biomeasure Inc.	23014 Ph 9	4.2.1.2
In vitro receptor binding profile of BIM-23014 C (Somatuline/lanreotide)	Rat or Guinea pig tissue/ Males	In vitro	Biomeasure Inc.	23014 Ph 62	4.2.1.2
In vitro binding interactions of Somatuline (lanreotide) with opioid receptors	Guinea Pig forebrains/ Males (200-300g)	In vitro	Biomeasure Inc	23014 Ph 60	4.2.1.2
Safety Pharmacology					
Neuropharmacology profile in the mouse	Mouse/ Male CD ₁ (20-22g)	s.c.	/	23014 Ph 114a	4.2.1.3
Effect of spontaneous motor activity in the mouse	Mouse/ Male CD ₁ VAF (20g)	s.c.	/	23014 Ph 114a	4.2.1.3
Investigation of a motor incapacitation or myorelaxant effect	Mouse/ Male CD ₁ VAF (22g)	s.c.	/	23014 Ph 114a	4.2.1.3
Effect of basal body temperature in the mouse	Mouse/ Male CD ₁ VAF (22g)	s.c.	/	23014 Ph 114a	4.2.1.3
Interaction with hypothermic and piosis effects of tetrabenazine	Mouse/ Male CD ₁ VAF (22g)	s.c.	/	23014 Ph 114a	4.2.1.3
Investigation of anticholinergic activity in the mouse: interaction with the effects of oxotremorine	Mouse/ Male CD ₁ VAF (20g)	s.c.	/	23014 Ph 114a	4.2.1.3
Investigation of potentiation of a subliminal dose of pentobarbitone	Mouse/ Male CD ₁ VAF (20g)	s.c.	/	23014 Ph 114a	4.2.1.3
Investigation of antagonism of installed pentobarbital-induced narcosis	Mouse/ Male CD ₁ VAF (20g)	s.c.	/	23014 Ph 114a	4.2.1.3
Interactions with supramaximal electric shock	Mouse/ Male CD ₁ VAF (22g)	s.c.	/	23014 Ph 114a	4.2.1.3
Interaction with the convulsant effects of pentylenetetrazol	Mouse/ Male CD ₁ VAF mice (22g)	s.c.	/	23014 Ph 114a	4.2.1.3
Effect of lanreotide on pain of a chemical origin	Mouse/ Male CD ₁ VAF 20-25g	s.c.	/	23014 Ph 114c	4.2.1.3
Effect of lanreotide on pain of thermal origin	Mouse/ Male CD ₁ VAF 25-27g	s.c.	/	23014 Ph 114c	4.2.1.3
Type of Study	Test System	Method of Administration	Testing Facility	Study Number	Location Section
Effect of lanreotide on the electrocorticogram	Rabbit/ Male New Zealand (2.4-2.9kg)	i.v.	/	910258	4.2.1.3
Effect of lanreotide on HERG tail current	HEK-293 cells, stably transfected with HERG-1	In vitro	/	20030464 PEHP	4.2.1.3
Effect of lanreotide on cardiac action potential	Isolated Purkinje fibres - Dog/ Beagle (2 males)	In vitro	/	20040085 PECM	4.2.1.3
Action of lanreotide on the cardiovascular system	Rat/ Male Sprague Dawley (290g)	i.v.	/	145-275	4.2.1.3
Cardiovascular and respiratory studies in dogs	Dog/ Beagle (3 males, 3 female) (9-15kg)	i.v.	/	910259	4.2.1.3
Cardiovascular studies in dogs	Dog/ Beagle (3 male, 3 female) (8-12kg)	i.v.	/	23014 Ph 52	4.2.1.3
Effect on blood pressure, heart rate and ECG in dogs.	Dog/ Beagle (3 females/3 males) (11.35-15.90kg)	i.v.	/	20030465PCC	4.2.1.3
Cardiovascular studies in dogs	Dog/ Beagle (6 male, 6 female) (10.4-13.8kg)	Im.	/	980274 P	4.2.1.3
Effects of lanreotide on diuresis	Rat/ Male Wistar (approx. 200g)	s.c.	/	275-170	4.2.1.3
Effect of lanreotide on renal clearance	Rat/ Male Wistar-Kyoto (356-394g); Rat/ Male Long Evans (400-460g)	i.v.	/	23014 Ph 164	4.2.1.3
Antidiuretic activity of lanreotide	Rat/ Male Wistar-Kyoto (WKY); Rat/ Male diabetes incipidus (DI) Harlan Sprague-Dawley; Rat/ Male Long Evans (LE) Harlan Sprague-Dawley	s.c.	/	23014 Ph 165	4.2.1.3
Investigation of a proinflammatory cutaneous effect at the site of injection: anaphylactic cutaneous reaction	Rat/ Male Sprague Dawley VAF (180-200g)	i.d.	/	23014 Ph 114b	4.2.1.3
Investigation of an inherent prodegranulant effect	Rat/ Sprague Dawley - Mast cells	In vitro	/	23014 Ph 114b	4.2.1.3
Interaction with immune degranulation	Rat/ Sprague Dawley - Mast cells	In vitro	/	23014 Ph 114b	4.2.1.3
Pharmacodynamic/ Drug Interactions					
None					

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The absorption, distribution, metabolism and excretion of lanreotide have been studied in SD and Wistar rats and Beagle dogs after i.v., s.c. and i.m. administration. TK data after s.c. administration in mice were also provided.

Lanreotide IRF was used to assess the preclinical pharmacology and TK, mainly in rats. As a consequence, most preclinical studies, including TK studies in mice, rats and dogs, were conducted with IRF, dosed either by s.c injection or as an i.v. infusion as reviewed under → TK data with lanreotide MPF were also assessed. TK data with lanreotide Autogel® in a single s.c. dosing in dogs and 26-week chronic toxicity studies in rats and dogs were provided.

The results of these studies revealed that lanreotide is promptly absorbed from subcutaneous or intramuscular injection sites. TK behavior is generally linear after subcutaneous, intramuscular or intravenous administration except at high doses where absorption is somewhat reduced. Deviations from linearity were observed in repeated dose studies at the higher doses (dose-related increases in serum half-life) and may have been reflections of residual absorption of the higher doses. Despite this deviation from linearity, there was no evidence of significant bioaccumulation after repeated dosing, even at the higher doses.

After absorption the drug is quickly distributed to mainly the liver, kidney and pancreas, and eliminated as unchanged lanreotide with some metabolites through biliary and secreted into the duodenum, and then undergoes extensive metabolism within the gastrointestinal tract (more than 50% of excreted radioactivity was due to products of lanreotide metabolism). The drug is extensively metabolized in rats and dogs with numerous, as yet to be identified, polar metabolites being observed. One metabolite has been tentatively identified as des-Thr-lanreotide and is produced in both rats and dogs. Lanreotide and/or metabolites are reabsorbed from the gastrointestinal tract.

Lanreotide and its metabolites are primarily excreted via feces with urinary elimination playing a smaller role. Lanreotide products contained in the feces are highly metabolized whereas urine contains primarily unchanged drug.

The metabolism of lanreotide in humans has not been addressed.

2.6.4.2 Methods of Analysis

Most TK studies employed unlabeled lanreotide IRF and serum concentrations of the drug were determined by radioimmunoassay (RIA). Radio-labeled lanreotide ($[^{14}\text{C}]$ and $[^3\text{H}]$) was employed in assessment of distribution, metabolism and excretion. Radioactivity was detected by liquid scintillation counting and autoradiography. HPLC

coupled with radioactivity or Liquid Chromatography/Mass Spectrometry detection techniques were used to separate and identify lanreotide metabolites.

2.6.4.3 Absorption

TK with IRF and MPF lanreotide:

Lanreotide was promptly absorbed ($T_{max} = 0.5-1.3$ h for the IRF and 1 h for the MPF) from either s.c. or i.m. site. TK behavior is generally linear after dosing except at HD where bioavailability is somewhat reduced. The data of major TK studies with either IRF or MPF lanreotide (not specified in the individual studies) are summarized in the table below:

Rept Number	Species (# of Animals)	Route	Dose $\mu\text{g}/\text{kg}$ (or specified)	C_{max} ng/ml (or $\mu\text{g}/\text{L}$)	T_{max} Hrs (or specified)	AUC_{0-24} $\text{ng}\cdot\text{ml}\cdot\text{h}$ (or specified)	$T_{1/2}$ Hrs (or specified)	GLP Status
92/PKS/39	Male Wistar rats (n=6)	IM Single	6.0 mg/kg	22.45 $\mu\text{g}/\text{L}$	0.042 days	60.31 $\mu\text{g}\cdot\text{L}\cdot\text{h}$	3.42 days	Non-GLP
92/PKS/12	Male Wistar rats (n=4)	SC Single	30	5.27 $\mu\text{g}/\text{L}$	0.56	11.22 $\mu\text{g}\cdot\text{L}\cdot\text{h}$	1.16	Non-GLP
			100	11.66 $\mu\text{g}/\text{L}$	1.06	43.11 $\mu\text{g}\cdot\text{L}\cdot\text{h}$	1.73	
			300	25.36 $\mu\text{g}/\text{L}$	1.33	132.59 $\mu\text{g}\cdot\text{L}\cdot\text{h}$	3.30	
		IV Single	30			19.84 $\mu\text{g}\cdot\text{L}\cdot\text{h}$	1.82	
			100			94.26 $\mu\text{g}\cdot\text{L}\cdot\text{h}$	2.09	
			300			216.95 $\mu\text{g}\cdot\text{L}\cdot\text{h}$	6.56	
95/PKE/09	Male SD rats (n=6)	SC Single	80	6.807	0.50	24.5	1.32	GLP
			200	22.076	0.75	80.4	4.27	
			2000	82.047	1.025	676	13.76	
		IV Single	80			36.4	0.98	
			200			106.9	3.15	
			2000			1400.4	9.68	
95/PKE/07	Male SD rats	SC Repeat 6 days	80	9.455	0.5	20.32	3.77	GLP
			200	18.457	0.517	44.46	9.73	
			2000	202.939	0.567	590.35	15.39	
95/PKE/07	Male Beagle dogs (n=6)	IV Single	50	10.456	22.028	155.920	2.340	GLP
			100	15.094	16.917	283.386	4.348	
			200	34.260	22.028	594.132	4.468	
92/PKS/44	Dog (n=4)	IM Single	3 mg/kg	23.25 $\mu\text{g}/\text{L}$	0.042 days	101.52 $\mu\text{g}\cdot\text{L}\cdot\text{day}$	3.47 days	GLP
95/PKE/06	Male Beagle dogs (n=6)	IV Single	80			63	2.9	GLP
			200			138	4.9	
			2000			2600	21.4	
		SC Single	80	31.8	0.50	51.1	3.3	
			200	88	0.42	142	4.6	
			2000	650	0.56	1420	23.3	
92/PKS/42	Dog (n=5)	SC Single	200	75.18 $\mu\text{g}/\text{L}$	0.43	77.01 $\mu\text{g}\cdot\text{L}\cdot\text{h}$	10.0	Non-GLP

95/PKE/08	Beagle dogs N=6 (3/s/g)	SC, twice daily for 11 doses	80 µg/kg/d	23.236	0.417	33.90	4.454	GLP
-----------	----------------------------	------------------------------	------------	--------	-------	-------	-------	-----

TK with the therapeutic formulation of lanreotide (Autogel):

Single s.c. dosing in rats

Rats receiving single dose s.c. lanreotide autogel at 5, 10 and 15 mg/animal showed continuous exposure to lanreotide during the entire 14 day interval at all dose levels tested, indicating the prolonged release nature of the autogel formulation. TK parameters were similar for male and female rats at the three doses (scanned Figure 2.6.4-1 and Table 2.6.4-11).

Figure 2.6.4-1. Mean (±SD) Serum Concentrations of Lanreotide in Rats after a Single s.c. Dose of Lanreotide Autogel

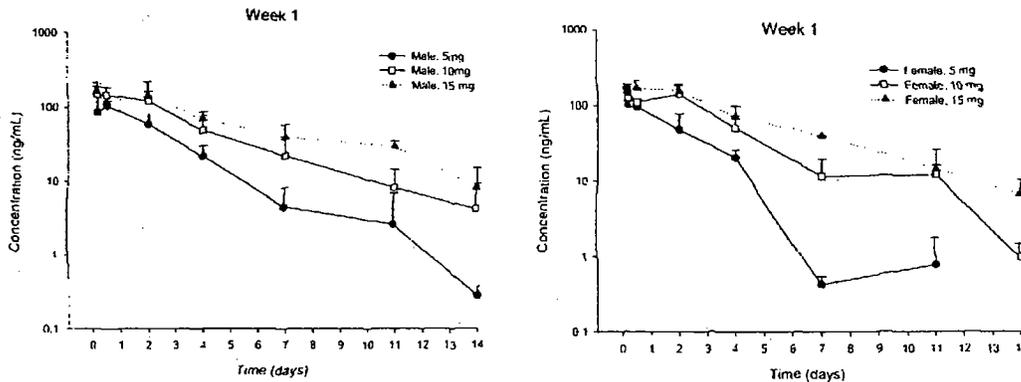


Table 2.6.4-11. Selected Pharmacokinetic Values after a Single s.c. Dose of Lanreotide Autogel in Rats

Parameter	5 mg/animal		10 mg/animal		15 mg/animal	
	M	F	M	F	M	F
T _{1/2} day	0.5	0.167	0.167	2	0.167	0.5
C _{max} ng/ml	106.279	169.370	149.764	140.042	165.126	169.353
C _{max} /D	21.256	33.874	14.976	14.004	11.008	11.290
AUC _t ng/ml/day	284.3	244.5	597.5	557.7	809.2	814.4
AUC _t /D	56.9	48.9	59.8	55.8	53.9	54.3
MRT day	2.45	1.71	3.31	3.21	4.44	3.83

Repeated s.c. dose TK with lanreotide autogel in rats and dogs:

Lanreotide autogel provides continuous exposure in both species for the entire 2-week dose interval during 26-week treatment period (see relevant repeat dose tox study reviews).

In rats, after the first s.c. dosing at 5, 10 and 15 mg/animal, an increase of C_{max} and AUC was observed at increasing doses, but the exposure values were higher than expected linear manner, except for the 5 mg dose group in male rats. The sponsor

Best Possible Copy

explains that the increased exposure is expected to be due to the interaction of lanreotide with circulating antibodies (scanned Table 2.6.4-14).

Table 2.6.4-14. Selected Pharmacokinetic Values after Repeat Dose s.c. Lanreotide Autogel in Male and Female Rats (13 Doses at 14 Day Intervals)

Parameter	5 mg/animal		10 mg/animal		15 mg/animal	
	M	F	M	F	M	F
T _{max} (day)	0.5 ¹ 0.2 ²	0.5 ²	0.5 ²	2 ²	4 ²	11 ²
C _{max} (ng/ml)	221.3 ¹ 755.1 ²	1620.2 ²	1373.1 ²	4293.9 ²	2054.8 ²	3698.5 ²
C _{max} /D	44.2 ¹ 151.0 ²	324.0 ²	137.3 ²	429.4 ²	137.0 ²	246.6 ²
C _{ss} (ng/ml)	50.3 ¹ 336.6 ²	675.7 ²	703.8 ²	2351.3 ²	1015.7 ²	2351.2 ²
AUC _t (ng/ml/day)	704.2 ¹ 4712.9 ²	9459.5 ²	9853.3 ²	32917.9 ²	14220.2 ²	32916.5 ²
AUC _t /D	140.8 ¹ 942.6 ²	1891.9 ²	985.3 ²	3291.8 ²	948.0 ²	2194.4 ²

¹ Parameters obtained from mean serum level profiles of rats with non-specific binding (NSB) < 10%

² Parameters obtained from mean serum level profiles of rats with NSB > 10%

C_{ss}: Mean serum concentration at steady state

In dogs, repeated dosing of 60, 120 and 360 mg/animal at 14-day intervals produced a moderate accumulation of lanreotide in males and females with an accumulation factor between 1.60 and 3.15, which was interpreted by sponsor in relation with the long half life of lanreotide autogel. In male dogs without anti-lanreotide antibodies, AUC seemed to increase proportionally with increasing dose level. In female dogs without anti-lanreotide antibodies, the increase in AUC was less than dose proportional at the highest dose (scanned Table 2.6.4-19).

Table 2.6.4-19. Pharmacokinetic Values of Repeat Dose s.c. Lanreotide Autogel in Male and Female Dogs (Mean ± SD) (13 Doses at 14 Day Intervals)

Parameter	60 mg/animal		120 mg/animal		360 mg/animal	
	M	F	M	F	M	F
T _{max} day*	0.167	0.167	0.167	0.334	0.167	0.500
C _{max} ng/ml	63.244 ± 18.5	79.374	142.439 ± 15.1	138.577 ± 38.502	316.435 ± 59.718	210.366 ± 14.8
C _{max} /D	1.05 ± 0.308	1.323	1.187 ± 0.1260	1.155 ± 0.321	0.879 ± 0.166	0.584 ± 0.0412
AUC _t ng/ml/day	313 ± 86.8	389	694 ± 206	748 ± 173	1711 ± 162.9	1236 ± 319
AUC _t /D	5.21 ± 1.45	6.49	5.78 ± 1.720	6.23 ± 1.44	4.75 ± 0.453	3.43 ± 0.887
AUC _{t ratio}	1.00	1.00	2.22	1.92	5.47	3.18
Rac _(AUC)	1.76	2.51	3.15	2.55	3.04	1.60

*Median

2.6.4.4 Distribution

Tissue distribution was studied in albino and pigmented rats after s.c. or i.v. dosing of ¹⁴C-lanreotide up to 168 hrs post dosing.

After s.c. administration a rapid overall distribution was observed followed by a slower elimination associated with a wide distribution of the radioactivity to peripheral tissues.

Best Possible Copy

The highest radioactivity concentration in tissue (relative to blood concentrations) was found in liver, kidney, pancreas and lung (refer to scanned tables below). Melanin binding of the radiolabeled components was very low.

Organ distribution after s.c. dosing:

Species:	Rat				
Gender (M/F)/Number of animals:	5 M Albino				
Feeding condition:	Fed				
Vehicle/Formulation:	Solution, 0.9% saline with ¹⁴ C-lanreotide				
Method of Administration:	s.c.				
Dose (mg/kg):	2mg/kg				
Radionuclide:	¹⁴ C				
Specific Activity:	50.2 mCi/mg				
Sampling time:	1, 4, 8, 24 and 168 hours post dose				
	Total radioactivity mcg equiv/g				
Tissues/organs	<u>T(1) 1 h</u>	<u>T(2) 4 h</u>	<u>T(3) 8 h</u>	<u>T(4) 24 h</u>	<u>T(5) 168 h</u>
Adrenal gland	0.33	1.43	3.54	2.93	1.23
Blood	0.59	0.81	1.23	1.78	1.30
Bone marrow	0.27	1.87	4.15	6.98	1.13
Kidney	1.17	2.29	3.78	4.58	1.84
Lachrymal gland	NM	3.17	5.47	4.69	1.33
Liver	1.63	2.23	3.42	3.96	1.66
Pancreas	0.99	8.40	19.19	7.08	1.52
Preputial gland	NP	1.96	NP	5.39	NP
Prostate gland	0.18	1.36	NP	4.62	1.86
Rectum	NP	0.55	3.09	3.19	1.52
Salivary gland	0.39	2.64	4.40	4.73	1.69
Spleen	NM	1.45	3.13	3.82	1.48
Stomach wall (glandular)	0.41	1.26	4.07	4.10	1.31
Thymus	0.32	0.97	2.12	3.17	1.29
Whole blood*	0.18	0.43	0.68	0.99	0.95

Species:	Rat		
Gender (M/F)/Number of animals:	5 M Pigmented		
Feeding condition:	Fed		
Vehicle/Formulation:	Solution, 0.9% saline with ¹⁴ C-lanreotide		
Method of Administration:	s.c.		
Dose (mg/kg):	2mg/kg		
Radionuclide:	¹⁴ C		
Specific Activity:	50.2 mCi/mg		
Sampling time:	2, 24 and 168 hours post dose		
	Total radioactivity mcg equiv/g		
Tissues/organs	<u>T(1) 2 h</u>	<u>T(2) 24 h</u>	<u>T(3) 168 h</u>
Adrenal gland	0.81	5.00	2.55
Blood	0.50	2.57	1.75
Bone marrow	0.63	5.82	1.83
Kidney	1.3	4.38	3.13
Lachrymal gland	NM	3.64	1.90
Liver	2.46	5.07	2.91
Pancreas	4.54	11.43	2.27
Preputial gland	NP	6.87	NP
Prostate gland	0.51	4.85	2.08
Rectum	0.88	NP	1.88
Salivary gland	1.60	3.88	2.18
Spleen	0.64	3.38	2.38
Stomach wall (glandular)	0.84	2.91	1.98
Thymus	0.61	4.42	2.15
Whole blood*	0.26	1.58	1.18

Best Possible Copy

Species:	Rat		
Gender (M/F)/Number of animals:	48 M		
Feeding condition:			
Vehicle/Formulation:	Solution, 0.9% saline with ¹²⁵ I-lanreotide		
Method of Administration:	s.c.		
Dose (mg/kg):	40 mcg animal		
Radionuclide:	¹²⁵ I		
Specific Activity:	1 mCi ¹²⁵ I-lanreotide/40 mcg unlabelled lanreotide		
Sampling time:	5, 10, 15, 30, 45 and 60 min after lanreotide administration		
	% of administered dose		
	<u>T(1) 30 min</u>	<u>T(2) 45 min</u>	<u>T(3) 60 min</u>
Tissues/organs			
Plasma*			
Jejunum	2.7 ± 0.83	6.78 ± 1.19	11.40 ± 2.94
Duodenum	6.69 ± 1.85	11.95 ± 2.96	7.9 ± 2.55
Ileum	0.28 ± 0.15	2.32 ± 1.09	4.23 ± 1.66
Liver	17.16 ± 2.28	1.79 ± 0.38	1.53 ± 0.34
Kidney	0.25 ± 0.05	3.78 ± 1.30	2.75 ± 0.93
Stomach content	20.21 ± 4.49	14.31 ± 3.04	7.23 ± 5.51
Caecum content	0.16 ± 0.06	0.43 ± 0.14	1.80 ± 0.44
Large intestine content	0.10 ± 0.01	0.25 ± 0.10	1.31 ± 0.48
Whole blood	8.67 ± 1.60	4.81 ± 1.30	3.29 ± 0.90
	Cl 1 corresponds to 30min after administration	Cl 2 corresponds to 45min after administration	Cl 3 corresponds to: 60min after administration = last measurement.

After i.v. dosing radioactivity was also widely distributed following a similar pattern. Secondary peaks in radioactivity were observed during the first 24 hrs in blood and some tissues such as liver, kidney, pancreas, spleen and thymus. The appearance of these secondary concentration peaks is probably reflective of the fact that lanreotide and/or its metabolites undergo enterohepatic circulation.

Organ distribution after i.v. dosing:

Species:	Rat				
Gender (M/F)/Number of animals:	5 M Albino				
Feeding condition:	Fed				
Vehicle/Formulation:	Solution, 0.9% saline with ¹⁴ C-lanreotide				
Method of Administration:	i.v.				
Dose (mg/kg):	2mg/kg				
Radionuclide:	¹⁴ C				
Specific Activity:	50.2 mCi /mg				
Sampling time:	0.5, 4, 8, 24 and 168 hours post dose				
	Total radioactivity mcg equiv./g				
Tissues/organs	<u>T(1) 0.5 h</u>	<u>T(2) 4 h</u>	<u>T(3) 8 h</u>	<u>T(4) 24 h</u>	<u>T(5) 168 h</u>
Adrenal gland	1.06	2.14	1.42	1.93	1.02
Blood	0.65	0.85	0.66	1.16	0.92
Bone marrow	1.12	2.90	2.30	3.22	0.77
Kidney	2.76	2.68	1.94	2.33	1.20
Lachrymal gland	1.42	0.35	2.51	NP	0.94
Liver	4.38	2.88	1.79	2.24	1.19
Pancreas	5.21	14.62	10.37	2.89	0.98
Preputial gland	NP	2.72	1.72	4.29	1.03
Prostate gland	NP	2.28	1.86	2.61	0.88
Rectum	0.72	2.09	NP	3.01	NP
Salivary gland	1.42	2.13	2.58	2.43	1.02
Spleen	1.38	2.40	1.56	2.21	0.93
Stomach wall (glandular)	1.46	2.27	2.08	1.67	0.85
Thymus	0.49	1.15	1.01	2.26	0.90
Whole blood*	2.87	0.82	0.72	0.60	0.68

A study performed in pregnant rats after subcutaneous administration of ¹⁴C-lanreotide indicates presence of metabolites of lanreotide in placenta amniotic fluid and fetal tissue.

Best Possible Copy

No unchanged lanreotide was found. Radioactivity distribution was similar between pregnant and non-pregnant rats.

Binding of ^{14}C -lanreotide to serum proteins was assessed by in vitro studies. The results show that lanreotide is mildly bound to serum protein with similar binding percentages in the different species evaluated. Mean results of lanreotide serum protein binding were 76 to 82% in mice, 74 to 82% in rats, 84 to 85% in dogs, and 79 to 83% in humans.

2.6.4.5 Metabolism

Metabolism in Rats:

Mass balance and metabolism studies were performed by i.v. or s.c. administration of labeled di- ^3H -lanreotide in intact or bile duct cannulated rats. In intact animals 40 to 67% of the radioactivity was retained in the carcass after 72 hrs with the feces containing 11-19% and urine 7-8%. This high retention in the carcass might attribute to the extensive metabolism and probable incorporation of ^3H to the intermediary metabolic pathways or possible incorporation to the surrounding water. In cannulated rats 55% and 29% of the dose after i.v. and s.c. administration, respectively, was excreted in the bile.

Chromatographic analysis of bile, feces and urine revealed that lanreotide is rapidly and extensively metabolized in the gastrointestinal tract and that more than 50% of excreted radioactivity was due to products of lanreotide metabolism.

After s.c. administration of ^{14}C -lanreotide in rats, 25% of the administered radioactivity was excreted in feces, and 5% in urine. Radioactivity in expired air accounted for 29-37%, and 35-50% of radioactivity was found in the carcass. Studies in lactating rats confirmed that unchanged lanreotide and a metabolite were excreted in milk.

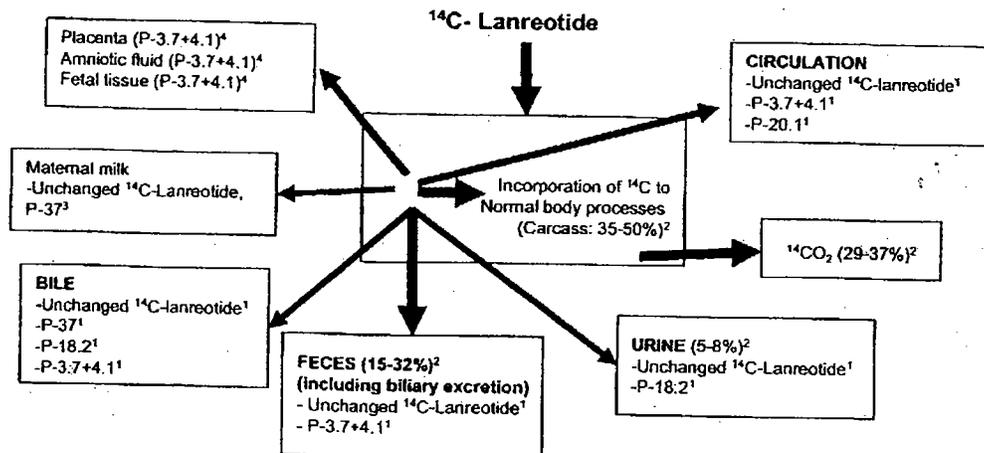
Studies using ^{14}C -lanreotide in rats confirmed that a high percentage (>40% at 24h) of radioactivity excreted in bile, feces and urine was in the form of metabolic derivatives. A total of 15 different metabolites were detected in bile, one of the metabolites (P-18.2) was identified as Des-Thr-lanreotide and represented more than 10% of the radioactivity after s.c. administration. Unchanged lanreotide accounted for 24 and 32% of the dose excreted in the bile after s.c. (0-24 hr) and i.v. (0-8h) administration respectively. Urine contained mostly unchanged lanreotide. The absence of some metabolites in bile as compared to feces suggest intestinal metabolism. In pregnant rats, unchanged ^{14}C -lanreotide was not found in amniotic fluid and fetal tissue and the polar ^{14}C -lanreotide-derived metabolites were the most abundant radioactive components in these tissues and placenta. They also appeared in maternal kidney samples, before appearing in plasma samples, suggesting that they might be formed by kidney metabolism. In addition metabolite P-37, at present unidentified, was present in plasma.

In plasma samples of lactating rats, P-18.2 metabolite was detected at 6 hr post dose. Other polar metabolites as P-3.7+4.1 were also quantifiable and two more peaks (P-20.1 and P-37.0) appeared in plasma although at non-quantifiable levels. While unchanged

lanreotide was excreted in maternal milk, metabolite P-37.0 was the most abundant component between 6 and 24 hrs.

A summary of lanreotide metabolism in the rat is shown in Figure 2.4-1.

Figure 2.4-1. Metabolism of Lanreotide in the Rat



Metabolism in Dogs

Metabolism studies in dogs were performed after ^{14}C -lanreotide administration by i.v. and s.c. route. Approximately 35% of the administered radioactivity was excreted in feces as unchanged lanreotide. During the first 12 hrs after i.v. administration in dogs, >50% of the dose was excreted in bile mainly as unchanged lanreotide and P-37 (major biliary metabolite). Metabolic transformation in the gut accounted for 13-14% of the dose. Unchanged lanreotide was the unique component detected in plasma supernatants after protein precipitation, up to 1 hr (i.v.) and 2 hr (s.c.) after administration.

The metabolism of lanreotide in humans has not been addressed.

2.6.4.6 Excretion

After s.c. or i.v. administration of ^{14}C -lanreotide rats excreted 17 and 25%, respectively of administered radioactivity in their feces. An additional 5% of administered radioactivity was recovered from urine regardless of route of administration. Fecal excretion of lanreotide and metabolites is more prominent in dogs as evidenced by the fact that it accounted for approximately 60% of administered lanreotide radioactivity administered either s.c. or i.v. Urinary excretion accounted for about 5%. Consistent with excretion of lanreotide into the bile by both rats and dogs, fecal elimination is a significant pathway of elimination for both rat and dog.

Extensive biliary excretion of ^{14}C -lanreotide and/or metabolites has been observed after i.v. administration of the drug to bile duct cannulated dogs. About 28% of the administered dose was recovered in bile within 2 hrs of injection and 51% and 56% were recovered within 6 and 12 hrs, respectively. The transfer of ^{14}C -lanreotide in lactating rats was also determined following a single subcutaneous dose of 2 mg/kg on Day 10/11

after parturition. Total radioactivity was detected in milk, confirming that drug-related material is secreted in this fluid. Total radioactivity in plasma reached the highest levels at 6 h and 24 h post-dose, with mean values of 0.700 and 0.79 µg equiv/ml, respectively. The highest concentration of total radioactivity in milk was noted at 6 h post-dose with a mean value of 3.402 µg equiv/ml. Radioactivity was still measurable at 72 h post-dose in both plasma and milk with mean values of 0.318 and 0.213 µg equiv/ml, respectively. The rate of elimination of total radioactivity was similar in both matrices. The mean milk: plasma concentration ratios were 0.7, 4.9, 1.3 and 0.7 at 1, 6, 24 and 72 h, respectively.

In summary, in both rats and dogs fecal excretion of ¹⁴C-lanreotide is the main excretion route, total radioactivity excreted was 29 % and 60% respectively. Urinary excretion is a minor route, 5 % of total radioactivity is recovered in urine in both animal species.

2.6.4.7 Pharmacokinetic drug interactions

A study was conducted in rats to investigate if lanreotide dosed subcutaneously at 120 µg/kg interferes with the pharmacokinetics of Cyclosporin A (CsA) when the latter is dosed orally (20 mg/kg) or i.v. (20 mg/kg at 12 µg/kg/min over 4-5 hr). No changes were observed in the trough levels of CsA when the drug was co-administered with lanreotide. This finding suggests that lanreotide neither interferes with the binding of CsA to red blood cells nor alters the metabolism of CsA as observed with other drugs. It may be concluded that co-administration of lanreotide, which may inhibit myointimal hyperplasia in organ transplants, could be employed without significantly altering the immunosuppressive activity of CsA. No other interaction studies were conducted.

2.6.4.8 Other Pharmacokinetic Studies

None

2.6.4.9 Discussion and Conclusions

The results of lanreotide's ADME with provided studies show the followings:

- After repeated subcutaneous administration of the Autogel® formulation every 14 days in animals sustained lanreotide levels are obtained, demonstrating exposure to the drug during the entire dosing interval.
- After a single intramuscular dose of Autogel® formulation in dogs, half-lives of 14, 36 and 23 days were obtained for doses of 60, 90 and 120 mg/dog, respectively. Bioavailability was 84, 93 and 91%, respectively. Peak levels were obtained during the first day of dosing and a low burst effect was noted.
- After subcutaneous or intramuscular administration of the IRF formulation, lanreotide is quickly absorbed. Bioavailability in rats is between 67 and 75% at doses of 80 and 200 µg/kg and decreases to 57% at 2000 µg/kg. In dogs bioavailability was 83, 103 and 57% at doses of 80, 200 and 2000 µg/kg, respectively.

- Mean half-life with lanreotide IRF is around 1 and 3 hrs after i.v. dosing in rats at 80 and 200 µg/kg. In dogs the mean half-life after an i.v. infusion at doses between 50 and 200 µg/h for 24 h ranged from 2.3 to 4.5 hrs. Dose dependent TK parameters of lanreotide are linear except at high doses.
- After subcutaneous administration of labeled lanreotide, a rapid overall distribution was observed followed by a slower elimination associated with a wide distribution of the radioactivity to peripheral tissues. A second peak observed after i.v. administration may reflect enterohepatic circulation of radioactive fragments of the peptide. Placental transfer was observed in pregnant rats indicating that the drug is able to crossing blood-placenta barrier.
- Lanreotide is mildly serum protein bound in all species. In vitro mean results were: 76-82% in mouse, 74-82% in rat, 84-85% in dogs and 79-83% in humans.
- The primary route of elimination of lanreotide in rats and dogs is biliary (feces) while urinary excretion is a minor secondary route.
- Lanreotide is rapidly and extensively metabolized. The results of urine, feces and bile indicate that more than 50% of the excreted radioactivity in rats was due to product metabolism. Urine contained mostly unchanged lanreotide. The absence of metabolites compared to feces suggest intestinal site of metabolism.
- 10% of the radioactivity that appears in bile has been identified as des-Thr-lanreotide. Unchanged lanreotide accounted for 24% and 32% of the dose excreted in bile after subcutaneous or intravenous administration, respectively. In lactating rats unchanged lanreotide is excreted in milk but the most abundant radioactive component is the P-37.0 metabolite.
- During the first 12h after i.v. administration in dogs, >50% of the dose was excreted in bile mainly as unchanged lanreotide and P-37 (major biliary metabolite). Metabolic transformation in the gut accounts for 13-14% of the dose. Unchanged lanreotide was the unique component detected in plasma supernatants after protein precipitation, up to 1 hr (i.v.) and 2 hr (s.c.) after dosing.

2.6.4.10 Tables and figures to include comparative TK summary

Please see the individual study

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Study	Duration	Species	Route	Dose/Formulation	Sex	AUC _{0-∞} ¹ (ng/ml).h	AUC _{0-∞} ² (ng/ml).day	AUC _{0-∞} ³ - 28 days ³ (ng/ml).day	Exposure/ AI49 Ratio ⁴	Dose (mg/kg) Ratio ⁵
A93-52030-149		Man	deep s.c.	120 mg Autogel	(male)	-	-	148.5	-	-
					(female)	-	-	153.1	-	-
27707 TSR	8 Weeks	Rat	s.c.	10 mg/animal every 14 d Autogel	(male)	-	3264	6528	44.0	32.8
					(female)	-	4364	8728	57.0	40.7
				20 mg/animal every 14 d Autogel	(male)	-	5134	10268	69.1	69.3
					(female)	-	13602	27204	177.7	86.9
				30 mg/animal every 14 d Autogel	(male)	-	5926	11852	79.8	102.6
					(female)	-	21681	43362	283.2	128.7
27708 TSC	8 Weeks	Dog	s.c.	300 mg animal every 14 d Autogel - (Day 43)	(male)	-	1343	2686	18.1	48.3
					(female)	-	1182	2364	15.4	48.4
				600 mg animal every 14 d Autogel - (Day 43)	(male)	-	4201	8402	56.6	101.8
					(female)	-	2174	4348	28.4	113.0
				900 mg animal every 14 d Autogel - (Day 43)	(male)	-	4953	9906	66.7	154.7
					(female)	-	7940	15880	103.7	186.7
28223 TCR	26 weeks	Rat	s.c.	5 mg/animal every 14 d Autogel - (Week 13)	(male)	-	1460.3	2920.6	19.7	15.3
					(female)	-	5744.0	11488	75.0	20.3
				5 mg/animal every 14 d Autogel - (Week 25)	(male)	-	4712.9	9425.8	63.5	13.3
					(female)	-	9459.5	18919	123.6	18.2
				10 mg/animal every 14 d Autogel - (Week 13)	(male)	-	9411.7	18823.4	126.8	31.0
					(female)	-	19636.7	39273	256.5	41.0
				10 mg/animal every 14 d Autogel - (Week 25)	(male)	-	9853.3	19706.6	132.7	25.8
					(female)	-	32917.9	65836	430.0	35.7
				15 mg/animal every 14 d Autogel - (Week 13)	(male)	-	11881.3	23762.6	160.0	48.9
					(female)	-	17337.6	34675	226.5	61.2
				15 mg/animal every 14 d Autogel - (Week 25)	(male)	-	14220.2	28440.4	191.5	41.8
					(female)	-	32916.5	65833	430.0	54.3

Study	Duration	Species	Route	Dose/Formulation	Sex	AUC _{0-∞} ¹ (ng/ml).h	AUC _{0-∞} ² (ng/ml).day	AUC _{0-∞} ³ - 28 days ³ (ng/ml).day	Exposure/ AI49 Ratio ⁴	Dose (mg/kg) Ratio ⁵
28224 TCC	26 Weeks	Dog	s.c.	60 mg animal every 14 d Autogel - (Week 13)	(male)	-	226.0	452	3.0	8.2
					(female)	-	238.0	476	3.1	7.3
				60 mg animal every 14 d Autogel - (Week 25)	(male)	-	313.0	626	4.2	8.2
					(female)	-	389.0	778	5.1	7.3
				120 mg animal every 14 d Autogel - (Week 13)	(male)	-	517.0	1034	7.0	17.6
					(female)	-	620.0	1240	8.1	15.8
				120 mg animal every 14 d Autogel - (Week 25)	(male)	-	694.0	1388	9.3	18.2
					(female)	-	748.0	1496	9.8	14.9
				360 mg animal every 14 d Autogel - (Week 13)	(male)	-	1391.0	2782	18.7	51.0
					(female)	-	1758.0	3476	22.7	30.1
				360 mg animal every 14 d Autogel - (Week 25)	(male)	-	1711.0	3422	23.0	49.9
					(female)	-	1236.0	2472	16.1	50.8
77006	104 weeks	Mouse	s.c.	0.5 mg/kg/day IRF - (Week 26)	(male)	131	5.46	152.8	1.0	9.0
					(female)	131	5.46	152.8	1.0	7.1
				0.5 mg/kg/day IRF - (Week 52)	(male)	111	4.63	129.5	0.9	9.0
					(female)	123	5.13	143.5	0.9	7.1
				0.5 mg/kg/day IRF - (Week 104)	(male)	155	6.46	180.8	1.2	9.0
					(female)	203	8.46	236.8	1.5	7.1
				1.5 mg/kg/day IRF - (Week 26)	(male)	325	13.54	379.2	2.6	27.1
					(female)	565	23.54	659.2	4.3	21.4
				1.5 mg/kg/day IRF - (Week 52)	(male)	342	14.25	399.0	2.7	27.1
					(female)	320	13.33	373.3	2.4	21.4
				1.5 mg/kg/day IRF - (Week 104)	(male)	298	12.42	347.7	2.3	27.1
					(female)	855	35.63	997.5	6.5	21.4
				5 mg/kg/day IRF - (Week 26)	(male)	1504	62.67	1754.7	11.8	90.2
					(female)	2842	118.42	3315.7	21.7	71.2

Best Possible Copy

Study	Duration	Species	Route	Dose/Formulation	Sex	AUCs ¹ (ng/ml).h	AUCs ² (ng/ml).day	AUCs - 28 days ³ (ng/ml).day	Exposure/ A149 Ratio ⁴	Dose (ng/kg) Ratio ⁵
77006	104 weeks	Mouse	s.c.	5 mg/kg/day	(male)	1465	61.04	1709.2	11.5	90.2
				IRF - (Week 52)	(female)	1613	67.21	1881.8	12.3	71.2
				5 mg/kg/day	(male)	1003	41.79	1170.2	7.9	90.2
				IRF - (Week 104)	(female)	2184	91.00	2548.0	16.6	71.2
				10 mg/kg/day	(male)	3794	158.08	4426.3	29.8	180.4
				IRF - (Week 26)	(female)	8109	337.88	9460.5	61.8	142.3
				10 mg/kg/day	(male)	3494	145.58	4076.3	27.5	180.4
				IRF - (Week 52)	(female)	2417	100.71	2819.8	18.4	142.3
				10 mg/kg/day	(male)	1530	63.75	1785.0	12.0	180.4
				IRF - (Week 104)	(female)	4009	167.04	4677.2	30.5	142.3
				30 mg/kg/day	(male)	8811	367.13	10279.5	69.2	541.3
				IRF - (Week 26)	(female)	12356	514.83	14415.3	94.2	427.0
30 mg/kg/day	(male)	7242	301.75	8449.0	56.9	541.3				
IRF - (Week 52)	(female)	14745	614.38	17202.5	112.4	427.0				
77005	104 weeks	Rat	s.c.	0.1 mg/kg/day	(male)	69.8	2.9	81.5	0.5	1.8
				IRF - (Week 26)	(female)	57.6	2.4	67.2	0.4	1.4
				0.1 mg/kg/day	(male)	129.7	5.4	151.3	1.0	1.8
				IRF - (Week 52)	(female)	103.7	4.3	121.0	0.8	1.4
				0.1 mg/kg/day	(male)	94.31	3.9	110.0	0.7	1.8
				IRF - (Week 104)	(female)	53.97	2.2	63.0	0.4	1.4
				0.2 mg/kg/day	(male)	153.90	6.4	179.6	1.2	3.6
				IRF - (Week 26)	(female)	137.50	5.7	160.4	1.0	2.8
				0.2 mg/kg/day	(male)	173.60	7.2	202.5	1.4	3.6
				IRF - (Week 52)	(female)	177.90	7.4	207.6	1.4	2.8
				0.2 mg/kg/day	(male)	152.50	6.4	177.9	1.2	3.6
				IRF - (Week 104)	(female)	129.60	5.4	151.2	1.0	2.8
				0.5 mg/kg/day	(male)	508.30	21.2	593.0	4.0	9.0
				IRF - (Week 26)	(female)	397.00	16.5	463.2	3.0	7.1
				0.5 mg/kg/day	(male)	426.30	17.8	497.4	3.3	9.0
				IRF - (week 52)	(female)	315.20	13.1	367.7	2.4	7.1
				0.5 mg/kg/day	(male)	323.10	13.5	372.0	2.5	9.0
				IRF - (Week 104)	(female)	351.70	14.7	410.3	2.7	7.1

Study	Duration	Species	Route	Dose/Formulation	Sex	AUCs ¹ (ng/ml).h	AUCs ² (ng/ml).day	AUCs - 28 days ³ (ng/ml).day	Exposure/ A149 Ratio ⁴	Dose (ng/kg) Ratio ⁵
77314	14 days	Mouse	s.c.	10.0 mg/kg every 24h	(male)	2909.83	121.24	3394.8	22.9	180.4
				IRF	(female)	2365.35	96.56	2759.6	18.6	142.3
				30.0 mg/kg every 24h	(male)	6168.59	257.02	7196.7	48.5	360.9
				IRF	(female)	2780.13	115.84	3243.5	21.2	284.7
77004	13 Weeks	Mouse	s.c.	0.5 mg/kg every 24h	(male)	203.57	8.48	237.5	1.6	9.0
				IRF	(female)	146.09	6.09	170.4	1.1	7.1
				0.5 mg/kg every 12h	(male)	351.83	6.33	354.3	2.4	18.0
				IRF	(female)	252.95	10.54	390.2	3.9	14.2
				1.0 mg/kg every 24h	(male)	433.2	18.05	505.4	3.4	18.0
				IRF	(female)	1397.45	58.23	1630.4	10.6	14.2
800036	13 weeks	Mouse	s.c.	10.0 mg/kg every 24h	(male)	2780.26	115.84	3243.6	21.8	180.4
				IRF	(female)	2609.82	108.74	3044.8	19.9	142.3
				30.0 mg/kg every 24h	(male)	9337.44	389.06	10893.7	73.4	541.3
				IRF	(female)	12999.63	541.65	15166.2	99.1	427.0
60.0 mg/kg every 24h	(male)	22276.85	928.20	25989.7	175.0	1082.6				
	IRF	(female)	23596.25	983.18	27529.0	179.8	554.0			
2-4-R50-85	6 weeks	Rat	s.c.	0.004 mg/kg/day ⁶	(male)	1.225	0.05	1.4	0.01	0.1
				IRF	(female)	1.225	0.05	1.4	0.01	0.1
				0.04 mg/kg/day ⁶	(male)	12.25	0.51	14.3	0.1	0.7
				IRF	(female)	12.25	0.51	14.3	0.1	0.6
				0.2 mg/kg/day ⁷	(male)	80.4	3.35	93.8	0.6	3.6
				IRF	(female)	80.4	3.35	93.8	0.6	2.8
20404 TSR	14 Days Cont. Inf.	Rat	i.v.	1 mg/kg/day	(male)	-	429.7	859.4	5.8	18.0
				IRF	(female)	-	473.6	947.2	6.2	14.2
				5 mg/kg/day	(male)	-	2109.8	4219.6	28.4	90.2
				IRF	(female)	-	1604.2	3208.5	21.0	71.2
				20 mg/kg/day	(male)	-	7372.0	14744.0	99.3	360.9
				IRF	(female)	-	5553.6	11107.2	72.5	284.7

Best Possible Copy

Study	Duration	Species	Route	Dose/Formulation	Sex	AUC _{0-∞} ¹ (ng/ml).h	AUC _{0-∞} ² (ng/ml).day	AUC _{0-∞} - 28 days ³ (ng/ml).day	Exposure/ A149 Ratio ⁴	Dose (mg/kg) Ratio ⁵
77003	13 Weeks	Rat	s.c.	0.1 mg/kg every 12h	(male)	128.79	5.37	300.5	2.0	3.6
				IRF	(female)	73.56	3.07	171.6	1.1	2.8
				0.5 mg/kg every 24h	(male)	525.57	21.90	613.2	4.1	9.0
				IRF	(female)	356.41	14.85	415.8	2.7	7.1
				0.5 mg/kg every 12h	(male)	624.4	26.02	1456.9	9.8	18.0
				IRF	(female)	335.28	13.97	782.3	5.1	14.2
18091	26 weeks	Rat	s.c.	1.0 mg/kg every 24h	(male)	662.57	27.61	773.0	5.2	18.0
				IRF	(female)	749.57	31.23	874.4	5.7	14.2
				0.1 mg/kg every 12h ⁶	(male)	44.46	1.85	103.7	0.7	3.6
				IRF	(female)	44.46	1.85	103.7	0.7	2.8
				0.5 mg/kg every 12h ⁷	(male)	295.175	12.30	688.7	4.6	18.0
				IRF	(female)	295.175	12.30	688.7	4.5	14.2
2-2-851-85	6 weeks	Dog	s.c.	1 mg/kg every 12h ⁸	(male)	590.35	24.60	1377.5	9.3	36.1
				IRF	(female)	590.35	24.60	1377.5	9.0	28.5
				0.004 mg/kg/day ¹⁰	(male)	2.6	0.1	3.0	0.02	0.1
				IRF	(female)	2.6	0.1	3.0	0.02	0.1
				0.04 mg/kg/day ¹⁰	(male)	25.6	1.1	29.8	0.2	0.7
				IRF	(female)	25.6	1.1	29.8	0.2	0.6
802856 & 829002	45 Days Cont. Inf.	Dog	i.v.	0.2 mg/kg/day ¹¹	(male)	142.0	5.9	165.7	1.1	3.6
				IRF	(female)	142.0	5.9	165.7	1.1	2.8
				0.4 mg/kg/day ¹²	(male)	30210.1	1258.8	783.2	5.3	7.2
				IRF	(female)	30210.1	1258.8	783.2	5.1	5.7
				4 mg/kg/day ¹³	(male)	30210.1	12587.5	7832.2	52.7	72.2
				IRF	(female)	30210.1	12587.5	7832.2	51.2	56.9
23171 RSH		Rat	s.c.	10 mg/kg/day ¹³	(male)	755253	31468.9	19580.6	131.9	180.4
				IRF	(female)	755253	31468.9	19580.6	127.9	142.3

Study	Duration	Species	Route	Dose/Formulation	Sex	AUC _{0-∞} ¹ (ng/ml).h	AUC _{0-∞} ² (ng/ml).day	AUC _{0-∞} - 28 days ³ (ng/ml).day	Exposure/ A149 Ratio ⁴	Dose (mg/kg) Ratio ⁵
8391	26 Weeks	Dog	i.m.	1-1.6 mg/kg/14days ³	(male)	-	33.8 - 54.1	67.7 - 108.3	0.5 - 0.7	2.1
				MPP	(female)	-	33.8 - 54.1	67.7 - 108.4	0.4 - 0.7	1.6
				3.35-4.98mg/kg/14days ¹¹	(male)	-	113.4 - 168.5	226.7 - 337.0	1.5 - 2.3	6.4
				MPP	(female)	-	113.4 - 168.5	226.7 - 337.0	1.5 - 2.2	5.1
				6.26-9.95mg/kg/14days ³	(male)	-	211.8 - 336.7	423.7 - 673.4	2.9 - 4.5	12.8
				MPP	(female)	-	211.8 - 336.7	423.7 - 673.4	2.8 - 4.4	10.1
20563 MAS		Mouse	i.v.	6.25 mg/kg	(male)	-	-	-	468.7*	4.0
				IRF	(female)	-	-	-	402.1*	3.2
				12.5 mg/kg	(male)	-	-	-	937.4*	8.1
				IRF	(female)	-	-	-	804.2*	6.4
				25.0 mg/kg	(male)	-	-	-	1874.8*	16.1
				IRF	(female)	-	-	-	1608.4*	12.7
434/184		Rat	s.c.	0.1 mg/kg every 12h ⁸	(male)	44.46	1.9	103.74	0.7	3.6
				IRF	(female)	44.46	1.9	103.74	0.7	2.8
				0.3 mg/kg every 12h ⁸	(male)	133.38	5.6	311.22	2.1	10.8
				IRF	(female)	133.38	5.6	311.22	2.0	8.5
				1.0 mg/kg every 12h ⁸	(male)	590.35	24.6	1377.48	9.3	36.1
				IRF	(female)	590.35	24.6	1377.48	9.0	28.5
434/169		Rat	i.m.	3 mg/kg every 2 weeks ¹⁴	(male)	-	36.67	73.33	0.5	3.9
				MPP	(female)	-	36.67	73.33	0.5	3.1
				10 mg/kg every 2 wks ¹⁴	(male)	-	122.22	244.43	1.6	12.9
				MPP	(female)	-	122.22	244.43	1.6	10.2
				30 mg/kg every 2 wks ¹⁴	(male)	-	366.65	733.30	4.9	38.7
				MPP	(female)	-	366.65	733.30	4.8	30.5
23171 RSH		Rat	s.c.	4mg/animal every 2wks ¹⁵	(male)	-	338.80	677.60	4.6	10.6
				Autogel	(female)	-	305.60	611.20	4.0	12.9
				10mg/animal every 2wks ¹⁵	(male)	-	847.00	1694.00	11.4	27.9
				Autogel	(female)	-	764.00	1528.00	10.0	33.1
				20mg/animal every 2wks ¹⁶	(male)	-	1622.00	3244.00	21.8	85.1
				Autogel**	(female)	-	-	-	-	-

Best Possible Copy

Study	Duration	Species	Route	Dose/Formulation	Sex	AUC _{ss} ¹ (ng/ml).h	AUC _{ss} ² (ng/ml).day	AUC _{ss - 28 days} ³ (ng/ml).day	Exposure/ A149 Ratio ⁴	Dose (mg/kg) Ratio ⁵
18891		Rabbit	s.c.	0.05 mg/kg every 12h IRF	(female)	-	-	-	-	1.4
				1 mg/kg every 12h IRF	(female)	-	-	-	-	28.5
				2.5 mg/kg every 12h IRF	(female)	-	-	-	-	71.2
					(female)	-	-	-	-	
26691		Rabbit	s.c.	0.05 mg/kg every 12h IRF	(female)	-	-	-	-	1.4
				0.225 mg/kg every 12h IRF	(female)	-	-	-	-	6.4
				1 mg/kg every 12h IRF	(female)	-	-	-	-	28.5
					(female)	-	-	-	-	
18991		Rat	s.c.	0.05 mg/kg every 12h ⁷ IRF	(female)	25.40	1.1	59.26	0.4	1.4
				1 mg/kg every 12h ⁸ IRF	(female)	590.35	24.6	1377.48	9.0	28.5
				2.5 mg/kg every 12h ⁸ IRF	(female)	1475.88	61.5	3443.71	22.5	71.2
					(female)	-	-	-	-	
26791		Rat	s.c.	0.05 mg/kg every 12h ⁷ IRF	(female)	25.40	1.1	59.26	0.4	1.4
				0.225 mg/kg every 12h ⁸ IRF	(female)	100.02	4.2	233.39	1.5	6.4
				1 mg/kg every 12h ⁸ IRF	(female)	590.35	24.6	1377.48	9.0	28.5
					(female)	-	-	-	-	

- AUC at steady state in (ng/ml)*h units after s.c. or i.m. repeated administration. When lanreotide is administered as i.v. continuous infusion (i.v. cont. inf.), AUC value corresponds to total AUC.
- AUC in (ng/ml)*day. If original data is reported as (ng/ml)* h units, this value has been recalculated.
- AUC over 28 days, assuming even release over time.
- Exposure ratio in animals compared to 120mg lanreotide Autogel in man (based on AUC_{ss-28 day} values).
- Dose ratio compared to 120 mg lanreotide (Autogel) in humans (males 1.552 mg/kg, females 1.967 mg/kg)
- Estimated from study 95/PKE/09 (D=0.08 mg/kg/day)
- Estimated from study 95/PKE/09 (D=0.2 mg/kg/day)
- Estimated from study 95/PKE/07 (D=0.1 mg/kg every 12 h)

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

Single-dose

The single-dose toxicity of lanreotide was assessed in mice and rats with IRF via i.v. and s.c. routes as have been reviewed under [redacted], and summarized in section 2.6.6.2 below. The i.v. LD₅₀ was 120 - 135 mg/kg in mice and of > 48 mg/kg in rats; the s.c. LD₅₀ was >1200 mg/kg in mice and of >1500 mg/kg in rats. Commonly seen clinical signs in both mice and rats included reduced body weight gain, decreased activity, prostration, abnormal gait, decreased muscle tone, edema and scab formation at injection sites, etc. No histopathology has been performed on these single dose studies. A single-dose toxicity of the therapeutic formulation (autogel) has not been investigated.

Repeated Dose

Previously, repeated dose chronic toxicity has been assessed with either lanreotide IRF or MPF in a 26-week rat, a 26-week dog and a 24-month dog studies. These studies have been reviewed under [redacted]. In summary of these studies, chronic toxicity of lanreotide in rats and dogs was predominantly demonstrated at the injection

Best Possible Copy

sites, in which the slow release formulation (MPF) with — induced severe foreign body granulomatous inflammation in the injected muscles in dogs; while the immediate release formulation (IRF) also induced local irritation and inflammation at s.c. injection sites with mixed inflammatory cell infiltration in rats. Other drug-induced changes included adrenal cortex atrophy, vacuolation and necrosis at high dose >1000 µg/kg/d in rats (a fraction of the MRHD 120 mg); male reproductive organ atrophy/inflammation and mild findings in liver and kidney at 40 and 120 µg/kg/d in dogs (a fraction of the MRHD 120 mg). None of the NOAELs (based on injection site tissue reaction) provides exposure multiples for the — human dose of 30 mg every 7 or 14 days — because of the presence of injection site inflammation.

In order to bridge the previously available chronic toxicity data with the IRF or MPF lanreotide in rats and dogs, the currently submitted NDA included two pivotal chronic toxicity studies with the therapeutic formulation (Somatuline® Autogel®) with a once every 14-day s.c. dosing regimen in these two species.

In the rat study, Lanreotide Autogel was s.c. dosed once every 14 days for 26 weeks (total of 13 dosing) at doses of 5, 10 or 15 mg/animal using pre-filled syringes at 7 rotating injection sites on the dorsal area (roughly each site received 2 injections during the study). No reversibility observation was performed. The dose levels equal to approximately 20, 40, and 60 mg/kg/14-d based on mean body weight of 250 g (or 1.4, 2.8, and 4.3 mg/kg/day). Animals tolerated the HD with clinical signs predominantly observed at the injection sites at 10 and 15 mg groups, including skin nodules and inflammatory reactions. Major findings include the following:

- The mean body weights of both M and F were statistically lower in all treated groups when compared to the C from week 2 until the end of the study. The mean body weights approximately decreased 20% in males and 15% in females compared to that of control, at all dose-levels, which appeared to be a pharmacologically mediated effect.
- Very slight decreases in protein and albumin levels were observed in week 25 in treated males and/or females when compared to the C. These changes were not associated with any histopath findings and were possibly related to the decreased FC and/or pharmacologic activity.
- Predominant treatment-related findings were limited to the injection sites. Macroscopically, nodules and scabs were observed at the injection sites in most animals treated and notably at 10 and 15 mg. Histopathologically, these changes were generally dose-related and characterized by granuloma formation, vasculitis and intimal thickening of mainly small and mid-size arteries at most injection sites in the treated groups. They were consistent with the depot formation of the drug compound, which has sometimes shown an inflammatory response/fibrosis and sometimes formed abscesses and/or ruptured at 15 mg. This was noted with a low incidence at 5 mg and with a high incidence at 10 and 15 mg. The subcutaneous granulomas and granulomatous inflammation were slight to marked in severity.

- Based on the injection site granulomatous reaction, the NOAEL was considered to be <5 mg/animal/14 days (5 mg treated animals showed a lower incidence in granuloma formation, AUC < 7086 ng/ml.d or 170076 ng/ml.h, about < 47X the maximum human exposure). However, since no apparent systemic toxicity was noted up to the HD, 15 mg is considered to be the NOAEL in general. At this dose level, the AUC values were 14,220 ng/ml.day in males and 32,917 ng/ml.day in females, after the 13th dosing (or 23,569 ng/ml.day or 565,656 ng/ml.h, sex combined), which is about 156-fold the maximum human exposure (3619 ng/ml.h at 120 mg).
- Systemic exposure values determined following the 7th and 13th dosing were much higher than the level of initial dosing, which could be resulted from anti-lanreotide antibody formation leading to higher non-specific binding, however, the possibility of drug accumulation could not be ruled out.

In the dog study, Lanreotide Autogel was s.c. dosed once every 14 days for 26 weeks (total of 13 dosing) at dose levels of 60, 120 or 360 mg/animal/injection at 26 pre-defined injection sites (2 sites for each dosing occasion, i.e. a half of each full dosage was given into 1 site) in the dorsum and flanks. No reversibility observation was performed (but the injection sites with earlier dosing can be considered as recovery observation). There were no drug-related mortality and other clinical signs except local tissue reaction at the injection sites (yellowish nodules) in all drug treated animals and diarrhea in most treated animals. Major findings include the following:

- Body wt in males decreased during the first 8 weeks of treatment (↓ 7.4, 9.6 and 10% at LD, MD, and HD, respectively, vs. C); by the end of treatment, body weights in males were still slightly lower compared to C (↓3, 12, and 4%, respectively). No significant wt changes were seen in treated females.
- No marked findings in EKG, hematology and serum chemistry parameters.
- Injection site tissue reactions were the major histopathologic findings at all dose levels tested, including the presence of infiltration of the subcutaneous tissue by macrophages and/or granulomatous inflammation. Overall, severity of granulomatous inflammation increased with the dose from ≥ 120 mg and decreased with the age of lesion (indicative of partial reversibility). Minimal to slight changes in the kidney (cortical tubular vacuolation) and lung at ≥ 120 mg were noted.
- NOAEL for injection site tissue reaction was <60 mg/animal/14-d (AUC < 351 ng/ml.d or 8424 ng/ml.h), ~2-fold the maximum human exposure; while the systemic NOEL is 120 mg/animal/14-d (AUC = 721 ng/ml.d or 17304 ng/ml.h), which is about 5-fold the maximum human exposure.

Comparing the two 26-week bridging toxicity studies s.c dosed with Lanreotide Autogel bi-weekly in rats and dogs to previously conducted chronic toxicity studies under — with IRF (rats) or MPF (dogs) lanreotide, the study outline and toxicity findings are summarized in the following table:

NDA#	22-074			
Species	Rat	Dog	Rat	Dog
Dosing duration	26 wks	26 wks	26 wks	26 wks
Recovery	4 wks	4 wks	none	none
Formulation	IRF	MPF (slow release)	Autogel (slow release)	Autogel (slow release)
Dosing regimen	b.i.d. S.C.	Bi-wkly, I.M.	Bi-wkly S.C.	Bi-wkly S.C.
Dose levels	0, 0.2, 1 and 2 m/k/d	0, 1.62, 4.98, 9.95 m/k/14-d	0, 5, 10, 15 m/animal/14d (~20, 40, 60 m/k/14-d or 1.4, 2.8, 4.3 m/k/d)	0, 60, 120, 360 m/animal/14-d (~6, 12, 36 m/k/14-d)
Mortality	none	none	none	none
Signs	Skin, local @ MD & HD	unremarkable	Skin, local @ MD & HD	Skin, local @ all dose levels
Body wt	↓@MD (22% M, 32% F) & HD (25%M, 28% F)	unremarkable	↓ ~15% to 20% at all dose levels, M & F	Slightly ↓ in M, no effect in F
Cli. hematology	Miscellaneous changes @ MD & HD, e.g ↑ Hb, PCV, RBC, PLT	unremarkable	unremarkable	unremarkable
Serum chem.	Miscellaneous changes @ MD & HD, e.g. ↑ALT, AST	unremarkable	↓ T. protein & albumin	unremarkable
EKG	n/a	Not performed	n/a	unremarkable
Histopath	<u>Inj. Site:</u> inflam. reaction & fibrosis @≥1 m/k/d Not reversed. <u>Thymus:</u> lymphoid atrophy @2 m/k/d	<u>Inj. Site:</u> chronic inflam. reaction w/ granulomas, @ all doses; Partially reversed	<u>Inj. Site:</u> dose-related chronic inflam. reaction w/ granulomas & fibrosis @ all doses; ↑zymogen in pancreas @ all doses	<u>Inj. Site:</u> dose-related chronic inflame. reaction w/ granulomas @ all doses. <u>Kidney:</u> tubular vacuolation, min. @ 360 mg <u>Lung:</u> lymph cell infiltrates, min. @≥120 mg
NOAEL for inj. site reactions	0.2 m/k/d	<1.62 m/k/14-d	< 1.4 m/k/d	< 6 m/k/14-d
NOAEL for general tox	0.2 m/k/d	9.95 m/k/14-d	4.3 m/k/d	12 m/k/14-d
AUC @ NOAELs	n/a	n/a	< 7087 ng/ml.d (local tox) =23,569 ng/ml.d (general tox)	<351 ng/ml.d (local tox) =721 ng/ml.d (general tox)

In summary, the 6-month chronic tox studies with the therapeutic formulation (lanreotide autogel) in rats and dogs at much higher dose levels (a few folds) did not produce new toxicities nor worse tox findings compared to previously conducted similar tox tests with either IRF or MPF lanreotide under — The local tissue reactions at the injection sites with chronic inflammatory changes including fibrosis and granuloma formation were major histopath findings. In the rat, the NOAEL on local reaction may achieve <47X the proposed maximum human exposure; and the NOAEL on systemic toxicity was ~156X the maximum human exposure. In the dog, the NOAEL for local toxicity is < 2-fold the maximum human exposure and the NOAEL for general toxicity is

about 5-fold that at the human maximum dose. It appears that the dog is more sensitive to lanreotide autogel than does the rat in the toxicity testing.

Genetic toxicology:

The standard battery of genotoxicity tests were conducted between 2000 and 2002. These studies included a reverse mutation assay in bacteria in the absence and presence of metabolic activation, a cultured mammalian cell assay, a test for chromosome aberrations in human lymphocytes and an in vivo assay for micronucleated erythrocytes in mice. In addition, the ability of lanreotide to induce gene mutations in the lacZ transgene in liver and bone marrow tissue from MutaMice was also assessed. The standard battery of genotoxicity tests conducted are considered valid, and the results from these assays demonstrate that lanreotide has no genotoxic potential.

Previously, the mutagenic and clastogenic potential of lanreotide have been studied in both in vitro and in vivo models with the IR formulation (without under between 1987 and 1993, among them, no genotoxic potential of lanreotide was observed.

Carcinogenicity: 104-week life-time carcinogenicity bioassays with daily subcutaneous administration in rats and mice were conducted.

Sprague Dawley rats were administered subcutaneous doses of vehicle (2 control groups) or lanreotide acetate at 0.1, 0.2, and 0.5 mg/kg once daily for 104 weeks. The rationale for the dose selection and daily dose regimen was not specified, and the protocol was not previously assessed by the Executive CAC. The high dose achieved only a fraction (1/10) of the maximum human exposure based on AUC values. The major neoplastic finding was a drug-related, statistically significant increase in the incidence of cutaneous/subcutaneous fibrous connective tissue tumors at the injection sites, including fibrosarcoma and malignant fibrous histiocytoma, in both male and female animals treated at the high dose of 0.5 mg/kg/day. The incidence of both tumors also exceeds the historical range. A statistically significant increase in the incidence of malignant lymphoma was observed in the high dose treated males (4.28%); however, the incidence of this tumor fell within the range of historical data (0.91 to 6%), hence it is not considered a drug-related finding. No drug effect on malignant lymphoma was seen in female rats. The local tumorigenesis may be attributed to the frequency of injection of the drug thereby leading to subcutaneous inflammation and local tissue hyperplasia.

In the mouse study, CD-1 mice were administered daily subcutaneous doses of vehicle (2 control groups) or lanreotide acetate at 0.5, 1.5, 5, 10, and 30 mg/kg for 104 weeks. The protocol was not previously assessed by the Executive CAC. The high dose of 30 mg/kg/day induced higher mortality and premature termination of the animals in Weeks 87 (males) and 97 (females) due to dermal lesions at injection sites. This indicates that the 30 mg/kg/day dose level exceeded the maximum tolerated dose (MTD). The AUC at the high dose was 3X that at the maximum human dose. Subcutaneous fibrosarcoma (both genders) and malignant fibrous histiocytoma (males) at injection sites were observed in animals treated at 30mg/kg/day with statistical significance; the incidence of

these tumors exceeds the testing lab's historical values.

The Executive CAC (meeting held on June 26, 2007) recommended and concluded the followings:

Rat:

- The Committee felt that the dosing regimen was suboptimal in that the high frequency of injections in animals (daily) compared to the clinical regimen (once every 4 weeks) resulted in local toxicity which likely precluded attainment of adequate systemic exposure. The Committee noted that the dosing frequency likely contributed to the injection site tumors.
- The Committee agreed that the study showed increased cutaneous and subcutaneous tumors of fibrous connective tissues at injection sites at the high dose, but felt that they might not be relevant to humans undergoing monthly injections.

Mouse:

- The Committee agreed that the study was adequate although, as above, the daily dosing regimen likely limited systemic exposure.
- The Committee agreed that the study was positive for cutaneous and subcutaneous tumors of fibrous connective tissues at the injection sites at the high dose. Fibrosarcomas in both genders and malignant fibrous histiocytoma in males were increased at the high dose which produces 3 times the maximum clinical exposure. Based on the frequency of dosing in mice relative to therapeutic use, the tumors observed may not be clinically relevant.

Reproductive toxicology:

The following resubmitted reproductive tox studies (two seg I in rats, two seg II in rats and rabbits) with either IRF or MPF of lanreotide acetate have been reviewed under _____ and summarized in the scanned table below:

- Fertility study by the subcutaneous route in the rat (seg I) (434/184)
- Fertility study by the intramuscular and subcutaneous routes in the rat (seg I) (434/169)
- BIM 23014 – Dose range finding study by the subcutaneous route in the pregnant rat (18991)
- Teratology study by subcutaneous route in the rat (seg II) (26791 or 434/099)
- BIM 23014 – Dose range finding study by subcutaneous route in the pregnant rabbit (18891)
- BIM 23014 – Teratology study by subcutaneous route in the rabbit (seg II) (26691 or 434/098)

Seg.	Study Title (as was in sponsor's document)	Species	Treatment duration	Dose µg/kg (or specified)	NOAEL
	Dose-range finding, sc	Rat,	Twice daily sc from Day 6-15 of gestation	100, 2000, 5000 µg/kg/d	= 100 µg/kg/d
	Dose-range finding, sc	Rabbit	Twice daily sc from Day 6-15 of gestation	100, 2000, 5000 µg/kg/d	= 100 µg/kg/d
I	Fertility study by the intramuscular and subcutaneous routes in the rat	Rat	10 wks before mating for males, 2 wks before mating for females and during mating, gestation and lactation, once every 2 wks.	3, 10 or 30 mg/kg/2 wk	Maternal <3 mg/kg/2wk Fetal = 3 mg/kg/2wk
I & II	Fertility study by the subcutaneous route in the rat	Rat	10 wks before mating for males, two weeks before mating, and during mating, gestation and lactation for females, twice daily.	200, 600 or 2000 µg/kg/d	Maternal <200 µg/kg/d F1 = 600 µg/kg/d F2 < 600 µg/kg/d
II	Teratology study by subcutaneous route in the rat	Rat	Twice daily from Days 6-15 of gestation for mated female rats	100, 450 or 2000 µg/kg/d	Maternal = 100 µg/kg/d Fetal < 100 µg/kg/d
II	Teratology study by subcutaneous route in the rabbit	Rabbit	Twice daily from Days 6-18 of gestation for mated female rabbits	100, 450 or 2000 µg/kg/d	Maternal = 100 µg/kg/d Fetal < 100 µg/kg/d

In summary of the above tabulated studies, rat and rabbit maternal toxicity consists of decreased body weight gain and injection site inflammation. Treated male rats in a fertility study had seminiferous tubule atrophy/degeneration at doses less than proposed maximum human exposure, but without fertility damage. The similar histologic change was also observed in a 24-month dog toxicity study. This suggests impaired spermatogenesis which probably would not affect male fertility in rodents, but the significance to humans is unclear. In the female rats at dose levels less than that of clinical exposure, decreased implantation sites, decreased fetal survival and increased incidence in skeletal variations (numbers of ribs) were observed. Gestation length was significantly increased at dose level less than maximum human exposure.

In the rabbit, statistically significant, dose related increase in resorptions (7, 4, 27, and 88% at 0, 0.1, 0.45, and 2 mg/kg/d, respectively) and reduced implantation sites were observed. In the rat a trend suggesting a treatment related increase in resorptions was observed but not significant, one HD (2 mg/kg/day) female had six early resorptions and no viable fetuses. A statistically significant decrease in live fetal numbers were seen in 0.45 and 2 mg/kg/d treated rabbits (73%, 13% respectively compared to 93% control). Only 2/12 rabbit dams delivered viable fetuses dosed at 2 mg/kg/day compared to 17/17 in control group. This severe reduction in live birth limited the evaluation of malformations/variations for dose dependency.

In addition to the resubmitted reprotox study reports summarized above, a rat s.c. fertility study (23171 RSR) using the therapeutic formulation (lanreotide autogel) was performed. In this study, lanreotide was s.c. dosed every 2 weeks at 4, 10 and 20 mg/animal/injection to male and female SD rats, from pre-mating through mating and until sacrifice (males) or until Day 7 post-coitum (females). 20 mg/animal/injection was poorly tolerated at the

injection sites in all males. At 4 and 10 mg, lower body weight gain and FC in both genders and lower ovulation and implantation parameters in females were considered to be drug-related effects. In males, none of the fertility parameters were affected. NOAEL for female fertility was <4 mg/animal/injection, a fraction of the proposed maximum human dose.

Local toxicity:

Specific local tolerance studies with the lanreotide Autogel formulation have been conducted in rabbits following repeated subcutaneous or intramuscular administration.

In the preliminary single dose local tolerance studies in the rabbit, monkey and minipig as outlined in the scanned table below, masses or nodules were seen subcutaneously at all injection sites in the three species, corresponding to large cavities surrounded by a fibrotic capsule with inflammatory reaction. The minipig and the monkey appeared more sensitive than the rabbit (macroscopic and microscopic observations).

Table 2.6.6-15: Study Design for a Local Tolerance Study with Subcutaneous Administration of Lanreotide to Rabbit, Monkey and Minipig.

Species	Sex and Number of Animals	Group Number	Treatment	Dose Volume
Rabbit	2 males	1	PLC	1.5 ml
	2 males	2	lanreotide Autogel	220 mcl
	2 males	3	BIM23014-PLGA	1.5 ml
Monkey	1 male	NA	PLC	1.5 ml
			lanreotide Autogel	220 mcl
			BIM23014-PLGA	1.5 ml
Minipig	1 female	NA	PLC	1.5 ml
			lanreotide Autogel	220 mcl
			BIM23014-PLGA	1.5 ml

In the specific local tolerance studies with repeated dosing in NZW rabbits, once every 4-week subcutaneous or intramuscular dosing of lanreotide autogel formulation at 10 mg/animal in 4 injection sites during a 98-day period (each site receives one injection of the 4 dosing) induced irreversible induration, transient erythema and edema at injection sites. The induration was resulted from drug component deposition and its consequent tissue reaction, including granuloma formation and chronic inflammatory infiltration of macrophages and lymphocytes. Both s.c. and i.m. routes showed similar tissue reactions. The lesions persisted up to 98 days following the very first dosing. Longer term reversibility was not assessed in these studies.

2.6.6.2 Single-dose toxicity

Single dose toxicity of lanreotide was studied with IRF after administration by the i.v. and s.c. routes in mice and rats, and has been reviewed under —, as briefly tabulated below. Lanreotide does not pose a marked acute toxicity effect by subcutaneous route in mice and rats. The half of lethality dose (LD₅₀) was ≥ 1200 mg/kg with s.c. dosing and 120 to 135 mg/kg with i.v. dosing in mice; LD₅₀ ≥ 48 mg/kg with i.v. dosing

and was not defined with s.c. dosing in rats (however, reportedly $LD_{50} \geq 1500$ mg/kg via s.c. in a non-GLP study).

Lanreotide Autogel was not tested for acute toxicity with single dose.

Acute Toxicity Studies (all were GLP studies):

STUDY (DATE)	SPECIES (No.)	DOSES /DESIGN	RESULTS
Single dose LD_{50} study (March, 1987)	NMRI mouse (5m & 5f)	BM 23014 (Batch 21-171-230C) 800 μ g/kg Single dose i.v. Observed for 14 d	All animals survived, no clinical signs were observed. LD_{50} was not established
Single dose i.v. tox study (Feb. 1988)	CD-1 mouse (5/s/g)	BIM-23014C (Lot# 31-84) 0, 30, 100, 150 or 180 mg/kg Single i.v. infusion over 5 min Observed for 14 d	$LD_{50} = 144$ mg/kg in males $LD_{50} = 125$ mg/kg in females Miscellaneous clinical signs were observed at all dose levels: decreased muscle tone, dyspnea, partial ptosis, chromaturia, hypersensitivity to touch, body weight \downarrow , decreased activity, abnormal gait, decreased responses of pinna and cornea, increased pain response, tremors and prostration.
Single dose i.v. tox study in mice (Jan. 1991)	CD-1 mouse (5/s/g)	BIM-23014C (Lot# 90K-518) 0, 30, 100, 120, 135 or 150 mg/kg Single i.v. infusion over 5 min Observed for 14 d	$LD_{50} \geq 120$ mg/kg and ≤ 135 mg/kg Miscellaneous clinical signs seen at all dose levels include: dyspnea, body weight \downarrow , eye twitching, decreased activity, abnormal gait, convulsion and prostration.
Single dose s.c. tox study in mice (March, 1987)	NMRI mouse (5m & 5f)	BM-23014 (Batch 21-171-230C) 800 μ g/kg single dose s.c. Observed for 14 d	All animals survived, no clinical signs were found. LD_{50} was not established
Single dose s.c. tox study in mice (March, 1988)	CD-1 mouse (5m & 5f)	BIM-23014C (Lot# 31-84) 0, 600, 900 or 1200 mg/kg Single dose s.c. Observed for 14 d	$LD_{50} \geq 1200$ mg/kg Miscellaneous clinical signs in all treated animals include: decreased activity and muscle tone, abnormal gait, ptosis and prostration, excessive grooming, necrosis and sloughing of the skin at injection sites
Single dose i.v. tox study in rats (March, 1988)	SD rat (5/s/g)	BIM-23014C (Lot# 31-84) 0, 3, 6, 24, 48, 60 and 75 mg/kg Single dose i.v. infusion over 5-10 min Observed for 14 d	$LD_{50} \geq 48$ mg/kg (groups of 60 and 75 mg/kg were excluded as infusion accident occurred). Clinical signs were seen at all dose levels include: decreased activity, abnormal gait, decreased muscle tone; discolored, necrotic and missing tails of the distal portion.
Single dose s.c. tox study in rats (March, 1987)	Wistar rat (5m & 5f)	BM-23014 (Batch 21-171-230C) 800 μ g/kg single dose s.c. Observed for 14 d	No mortalities, no clinical signs. LD_{50} was not established
Single dose s.c. tox study in rats (April 1988)	SD rat (5M & 5F/g)	BM-23014C (Lot # 31-84) 0 or 1500 mg/kg single dose s.c. observed for 14 days	No mortalities. Clinical signs: \downarrow activity, prostration, abnormal gait and stance, \downarrow muscle tone, edema and scab at injection sites.

2.6.6.3 Repeat-dose toxicity

Repeat-dose toxicity of lanreotide (IRF) has been assessed in mice, rats and dogs via s.c., i.v., and/or i.m. routes. These studies have been reviewed previously under and summarized in Section 2.6.6.1 under "General toxicity".

Toxicity profiles of the therapeutic formulation (lanreotide autogel) were evaluated in two pivotal chronic studies with subcutaneous dosing (administered every 14 days for 26 weeks) in rats and dogs, respectively, in association with local tolerance test as reviewed below.

Study title: Twenty-six Week Toxicity Study by Repeated Subcutaneous Injection Every 14 Days in Rats (Lanreotide Autogel)

Key study findings: Lanreotide Autogel was s.c. dosed once every 14 days for 26 weeks (total of 13 dosing) to SD rats at doses of 5, 10 or 15 mg with 7 rotating injection sites on the dorsal area (roughly each site received 2 injections during the study). No reversibility observation was performed.

- The mean body weights of both M and F were statistically lower in all treated groups when compared to the C from week 2 until the end of treatment. The mean weight approximately decreased 15 – 20 % in males and females at all dose levels compared to that of C, which appeared to be a pharmacologically mediated effect.
- Very slight decreases in protein and albumin levels were observed in week 25 in treated males and/or females when compared to controls. These changes were not associated with any histopath findings and were possibly related to the decreased FC.
- The major treatment-related findings were limited to the injection sites. Macroscopically, nodules and scabs were observed at the injection sites in most animals treated and notably at 10 and 15 mg. Histopathologically, these changes were generally dose-related and characterized by granuloma formation, vasculitis and intimal thickening of mainly small and mid-size arteries at most injection sites in the treated groups. They were consistent with the depot formation of the drug compound, which has sometimes shown an inflammatory response/fibrosis and sometimes formed abscesses and/or ruptured at 15 mg. This was noted with a low incidence at 5 mg and with a high incidence at 10 and 15 mg. The subcutaneous granulomas and granulomatous inflammation were slight to marked in severity.
- Based on the injection site granulomatous reaction, the NOAEL was considered to be <5 mg/animal/14 days (where 5 mg has shown low incidence of granuloma formation). However, since no apparent systemic toxicity was noted up to the HD, 15 mg is considered to be the NOAEL in general. At this dose level, the

AUC values were 14,220 ng/ml.day in males and 32,917 ng/ml.day in females, after the 13th dosing (or 23569 ng/ml.day, sex combined).

- Systemic exposure values determined following the 7th and 13th dosing were much higher than the level of initial dosing, which could be resulted from anti-lanreotide antibody formation leading to higher non-specific binding, however, the possibility of drug accumulation could not be ruled out.

Study no.: 28223 TCR

Volume #, and page #: Vol A3.37, pages 1 to 1028

Conducting laboratory and location:

Date of study initiation: November 23, 2004

GLP compliance: yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Lanreotide Autogel, batch # 04K2205, and purity not specified (mean peptide content of — in each syringe)

Methods

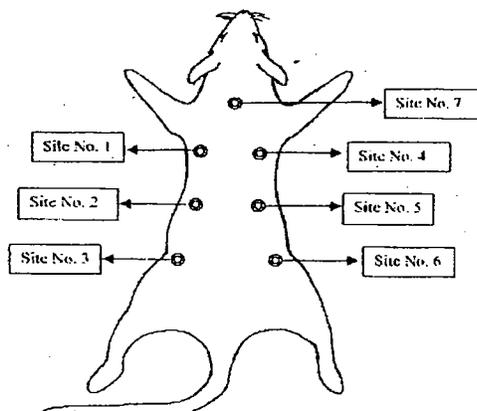
Doses: 0, 5, 10, 15 mg/animal/14 days

Species/strain: Rat/SD

Number/sex/group or time point (main study): 20/s/g

Route, formulation, volume, and infusion rate: s.c. injection in the dorsum and flanks using pre-filled syringes fitted with a single use needle. Each animal received 13 injections every 14 days over the treatment period on 7 rotated injection sites as shown below. Control animals received the vehicle only which is a sterile isotonic saline solution (0.9% NaCl), batch # SIP133, FVE13B and FVI08A.

injections on days 1 and 99	: site No. 1. (thoracic left).
injections on days 15 and 113	: site No. 2. (dorsal left).
injections on days 29 and 127	: site No. 3. (flank left).
injections on days 43 and 141	: site No. 4. (thoracic right).
injections on days 57 and 155	: site No. 5. (dorsal right).
injections on days 71 and 169	: site No. 6. (flank right).
injection on day 85	: site No. 7 (scapular).

Injection sites

Satellite groups used for toxicokinetics or recovery: 12/s/g for TK
 Age: ~6 weeks at initiation of dosing
 Weight: 180 to 217 g for males and 125 to 179 g for females
 Sampling times: animals were sacrificed on completion of the study following a 14 hrs fasting.
 Unique study design or methodology: none

Observations and times:

Mortality: once a day during treatment including weekends

Clinical signs: once a day

Body weights: once pretest, once on the first day of dosing, and once a week during the first 13 weeks of dosing period and then once every 4 weeks during rest of the study.

Food consumption: once a week during the first 13 weeks of dosing period and then once every 4 weeks during rest of the study.

Ophthalmoscopy: once pretest and once at the end of the dosing period performed on 10 animals/sex/group from the C and HD.

EKG: n/a

Hematology: collected in Week 25 from 10 animals/s/group

Clinical chemistry: collected in Week 25 from 10 animals/s/group

Urinalysis: in Week 25 from 10 animals/s/group (overnight 14 hrs collection in metabolism cages)

Gross pathology: at necropsy

Organ weights: see histopath table

Histopathology: see histopath table

Results

Mortality: No drug-related mortality (1 F in the 5 mg group was moribund sacrificed on Week 8 was considered not drug-related, and the cause was not identified).

Clinical signs: Nodules (reported as nodosities) at all injection sites were observed in all the animals treated at the dose-levels of 10 or 15 mg. At 5 mg, nodules were observed in

all the animals for the 6 first injection sites and in 13/19 M and 17/20 F at the last injection site. These nodules were observed up to the end of the study. The nodules were associated with scabs in most of the animals. Abscesses were observed at some injection sites with a dose-related frequency (2 animals at 5 mg; 6 animals at 10 mg and 11 animals at 15 mg) as summarized in the scanned table below. One single abscess was generally observed per animal, except in females given dose of 15 mg, where 2 abscesses were observed in 4 F out of 10 presenting abscesses.

Frequency of abscesses at the injection site
in rats treated with LANREOTIDE AUTOGEL

Dose-level (mg)	0		5		10		15	
	M	F	M	F	M	F	M	F
Number of animals with abscesses	0	0	1	1	2	4	1	10
Total number of abscesses	0	0	1	1	2	4	1	14

Body weights: The mean body weights of both M and F were statistically lower in all treated groups when compared to the C from the second week and until the end of treatment.

The mean body weight decrements (%) comparing to that of the C at the end of dosing are summarized in the following table:

Dose, mg/14-d	0		5		10		15	
	M	F	M	F	M	F	M	F
Wt changes %	-	-	↓22%	↓16%	↓20%	↓14%	↓26%	↓15%

Food consumption: Mean FC was statistically slightly lower (approximately ↓ 10%, irrespective of dose-level and sex) in treated M throughout the study, and during the first 11 weeks of treatment in females.

Ophthalmoscopy: Unremarkable

Hematology: There were no treatment-related differences between the C and treated groups at the end of the dosing period.

Clinical chemistry: The main changes in serum chemistry parameters at the end of the study are shown in the scanned table below.

Sex	Male				Female			
	0	5	10	15	0	5	10	15
Dose-level (mg)								
Calcium (mmol/L)	2.72	2.58**	2.57**	2.55**	2.70	2.56**	2.55**	2.54**
I. Phosphorus (mmol/L)	1.83	1.76	1.62**	1.60**	1.28	1.45	1.31	1.44
Glucose (mmol/L)	6.94	6.73	6.68	7.35	7.86	6.92**	6.75**	6.86**
Protein (g/L)	73	68**	69**	67**	76	68**	69**	70**
Albumin (g/L)	37	34	36	34*	43	36**	35**	36**
Albumin/Globulin	1.05	1.04	1.08	1.03	1.28	1.13*	1.06**	1.09**
Triglycerides (mmol/L)	0.73	0.40*	0.39*	0.39**	0.41	0.27	0.28	0.28
ALP (IU/L)	199	216	195	179	80	117*	121**	137**

statistically significant when compared to controls: * p<0.05. **: p<0.01

Best Possible Copy

Urinalysis: Unremarkable

Gross pathology: Subcutaneous nodules (generally shown as yellowish or grey/white) at the injection sites were seen with a high frequency in treated groups, notably at 10 and 15 mg. Scabs and thickened subcutaneous tissue were occasionally seen in treated animals at the injection sites. White color of the pancreas was noted in all treated groups.

Organ weights: Liver weights (mean abs. and rel.) in all treated groups were lower compared to the C, and were considered drug-related.

Abs. wt: ↓ 29, 29, and 36% M and ↓ 20, 26, 31% F at 5, 10, and 15 mg, respectively;
Rel. wt: ↓ 10, 10, and 12% M and ↓ 7, 14, and 21% F at 5, 10, and 15 mg, respectively.

Histopathology: Major histopath findings were seen at injection sites with granulomatous inflammation and vasculitis.

The most prominent finding consisted of the presence of slight to marked subcutaneous granulomas or subcutaneous granulomatous inflammation in all treated groups, with a low incidence at 5 mg, and a high incidence at 10 and 15 mg. These findings were sometimes accompanied by inflammation, dermal fibrosis and/or subcutaneous fibrosis, and correlated with the subcutaneous nodules and thickened subcutaneous tissue seen grossly.

At 10 and 15 mg, granulomas had sometimes formed abscesses and/or ruptured, correlating with the scabs occasionally noted at macroscopic examination.

These findings were summarized in the scanned table below.

GROUP:		1M	2M	3M	4M
NUMBER OF ANIMALS:		(1)	(2)	(3)	(4)
		20	19	20	20
Injection Site 1 x 2	# EX	20	2	16	20
Focal/diffuse hyperkeratosis		20	2	16	20
Focal/diffuse acanthosis		20	2	16	20
Subcutaneous inflammatory cell infiltration		0	0	1	0
Subcutaneous fibrosis		0	0	1	1
Subcutaneous granuloma		0	2	15	15
Focal chronic dermal inflammation		0	0	0	3
Focal folliculitis		0	0	0	1
Focal subcutaneous granulomatous inflammation		0	1	2	3
Focal dermal fibrosis with adnexal atrophy		0	0	2	1
Intrgranulomal acute inflammatory cells		0	1	0	0
Injection Site 2 x 2	# EX	20	5	19	20
Focal/diffuse hyperkeratosis		20	5	19	19
Focal/diffuse acanthosis		20	4	19	18
Subcutaneous fibrosis		0	1	1	1
Subcutaneous granuloma		0	2	16	18
Focal dermal fibrosis with adnexal atrophy		0	2	10	13
Dermal granuloma		0	0	1	2
Focal folliculitis		0	0	0	1
Focal subcutaneous granulomatous inflammation		0	0	3	4
Intrgranulomal acute inflammatory cells		0	0	2	0
Injection Site 3 x 2	# EX	20	6	17	20
Scab formation		0	0	1	0
Focal/diffuse hyperkeratosis		20	6	17	20
Focal/diffuse acanthosis		18	5	17	19
Subcutaneous granuloma		0	5	16	13
Focal dermal fibrosis with adnexal atrophy		0	2	9	13
Focal subcutaneous granulomatous inflammation		0	0	1	6
Focal folliculitis		0	0	1	0
Injection Site 4 x 2	# EX	20	7	20	20
Scab formation		0	1	0	0
Focal/diffuse hyperkeratosis		20	7	20	20
Focal/diffuse acanthosis		20	7	20	20
Subcutaneous fibrosis		0	1	2	1
Subcutaneous inflammatory cell infiltration		0	1	1	2
Subcutaneous granuloma		0	5	18	19
Focal dermal fibrosis with adnexal atrophy		0	1	3	5
Focal folliculitis		1	0	0	3
Focal subcutaneous granulomatous inflammation		0	0	4	1
Injection Site 5 x 2	# EX	20	15	20	20
Scab formation		0	0	0	1
Focal/diffuse hyperkeratosis		20	15	20	20
Focal/diffuse acanthosis		20	15	20	20
Subcutaneous inflammatory cell infiltration		0	4	9	2
Subcutaneous fibrosis		0	3	1	3
Subcutaneous granuloma		0	12	20	19
Focal dermal fibrosis with adnexal atrophy		0	5	10	16
Focal dermatitis		0	0	0	1
Dermal granuloma		0	0	0	1
Focal subcutaneous granulomatous inflammation		0	0	2	2
Focal epithelial erosion		0	0	0	1

GROUP:		1F	2F	3F	4F
NUMBER OF ANIMALS:		(1)	(2)	(3)	(4)
		20	20	20	20
Injection Site 1 x 2	# EX	20	3	11	20
Scab formation		1	0	1	0
Focal/diffuse hyperkeratosis		20	3	11	20
Focal/diffuse acanthosis		15	3	11	18
Subcutaneous inflammatory cell infiltration		0	0	0	1
Subcutaneous fibrosis		0	0	0	1
Subcutaneous granuloma		0	3	10	13
Focal folliculitis		0	0	0	1
Focal subcutaneous granulomatous inflammation		0	0	1	7
Focal dermal fibrosis with adnexal atrophy		0	1	7	10
Injection Site 2 x 2	# EX	20	5	13	20
Scab formation		1	1	0	1
Focal/diffuse hyperkeratosis		16	4	13	20
Focal/diffuse acanthosis		11	4	13	20
Subcutaneous fibrosis		0	0	0	1
Subcutaneous granuloma		0	2	13	18
Focal dermal fibrosis with adnexal atrophy		0	3	11	16
Focal subcutaneous granulomatous inflammation		0	1	1	1
Focal ulceration		0	1	0	1
Injection Site 3 x 2	# EX	20	11	14	20
Scab formation		0	4	1	0
Focal/diffuse hyperkeratosis		18	11	14	20
Focal/diffuse acanthosis		13	11	13	20
Subcutaneous inflammatory cell infiltrate		0	0	0	3
Subcutaneous granuloma		0	9	13	9
Focal dermal fibrosis with adnexal atrophy		0	6	7	11
Focal subcutaneous granulomatous inflammation		0	1	2	7
Focal ulceration		0	4	2	4
Intrgranulomal acute inflammatory cells		0	0	1	0
Injection Site 4 x 2	# EX	20	11	19	20
Scab formation		1	0	0	0
Focal/diffuse hyperkeratosis		16	10	19	20
Focal/diffuse acanthosis		11	8	19	19
Subcutaneous fibrosis		0	0	1	0
Subcutaneous inflammatory cell infiltration		0	0	1	1
Subcutaneous granuloma		1	8	19	19
Focal dermal fibrosis with adnexal atrophy		0	4	13	13
Focal subcutaneous granulomatous inflammation		0	1	0	3
Dermal granuloma		0	0	0	1
Intrgranulomal acute inflammatory cells		0	0	1	1
Injection Site 5 x 2	# EX	20	17	16	20
Scab formation		2	2	1	5
Focal/diffuse hyperkeratosis		17	16	16	20
Focal/diffuse acanthosis		9	16	16	20
Subcutaneous inflammatory cell infiltration		0	3	2	0
Subcutaneous fibrosis		0	1	1	2
Subcutaneous granuloma		0	17	16	16
Focal dermal fibrosis with adnexal atrophy		0	11	12	17
Focal subcutaneous granulomatous inflammation		0	0	0	4
Focal epithelial erosion		0	0	0	1
Focal ulceration		0	0	0	3
Intrgranulomal acute inflammatory cells		0	0	0	1
Fistula		0	0	0	1

GROUP:	1M	2M	3M	4M
NUMBER OF ANIMALS:	(1)	(2)	(3)	(4)
Injection Site 6 x 2 # EX	20	16	19	20
Scab formation	0	3	4	7
Focal/diffuse hyperkeratosis	20	16	19	18
Focal/diffuse acanthosis	19	16	19	20
Subcutaneous fibrosis	0	2	3	5
Subcutaneous inflammatory cell infiltration	0	7	14	10
Intramuscular granuloma	0	0	0	1
Focal dermal fibrosis with adnexal atrophy	0	8	10	13
Focal dermal inflammation	0	4	1	6
Subcutaneous granuloma	0	12	18	19
Focal ulceration/necrosis	0	0	1	3
Focal subcutaneous granulomatous inflammation	0	1	2	1
Focal ulceration	0	3	3	3
Focal dermal arteritis	0	1	0	0
Focal epithelial erosion	0	2	1	0
Dermal granuloma	0	1	0	0
Focal folliculitis	0	0	1	0
Intracutaneous acute inflammatory cells	0	0	1	1

GROUP:	1M	2M	3M	4M
NUMBER OF ANIMALS:	(1)	(2)	(3)	(4)
Injection Site 7 x 2 # EX	20	3	8	20
Focal/diffuse acanthosis	20	3	7	20
Focal/diffuse hyperkeratosis	20	3	8	20
Focal scab formation	0	0	1	2
Subcutaneous granuloma	0	2	8	7
Focal subcutaneous granulomatous inflammation	0	1	0	4
Dermal chronic inflammation	0	0	0	2
Focal dermal fibrosis with adnexal atrophy	1	1	0	6
Focal epithelial erosion	0	0	0	2
Subcutaneous inflammatory cell infiltration	0	0	1	0

GROUP:	1F	2F	3F	4F
NUMBER OF ANIMALS:	(1)	(2)	(3)	(4)
Injection Site 6 x 2 # EX	20	17	20	20
Scab formation	0	7	8	7
Focal/diffuse hyperkeratosis	14	17	20	19
Focal/diffuse acanthosis	8	17	20	19
Subcutaneous fibrosis	0	1	3	0
Subcutaneous inflammatory cell infiltration	0	6	8	4
Intramuscular granuloma	0	0	0	1
Focal dermal fibrosis with adnexal atrophy	0	9	16	16
Focal dermal inflammation	0	9	7	8
Subcutaneous granuloma	0	12	19	11
Focal ulceration/necrosis	0	0	0	5
Focal subcutaneous granulomatous inflammation	0	4	4	7
Focal ulceration	0	3	6	5
Focal epithelial erosion	0	2	1	1
Intracutaneous acute inflammatory cells	0	0	1	2
Fistula	0	0	1	1
Haemorrhage	0	0	0	1

GROUP:	1F	2F	3F	4F
NUMBER OF ANIMALS:	(1)	(2)	(3)	(4)
Injection Site 7 x 2 # EX	20	0	1	20
Focal/diffuse acanthosis	15	0	1	17
Focal/diffuse hyperkeratosis	16	0	1	20
Focal scab formation	3	0	1	1
Subcutaneous granuloma	0	0	0	1
Focal subcutaneous granulomatous inflammation	0	0	0	9
Dermal chronic inflammation	0	0	1	0
Focal dermal fibrosis with adnexal atrophy	0	0	0	5
Subcutaneous inflammatory cell infiltration	0	0	0	1
Focal ulceration	0	0	0	1

Best Possible Copy

Acanthosis and hyperkeratosis of the skin at the injection sites were noted in all groups, including the C. Both findings were minimal or mild, with a low incidence at 5 mg, and a high incidence at 10 and 15 mg. The severity of the findings was occasionally moderate or marked in treated groups, and they were often associated with the ulceration and rupture of granulomas.

Minimal to moderate acute vasculitis was noted at the injection sites at 5, 10, or 15 mg. Males were more affected than females. This finding was not dose-dependent. Incidence was comparable in males treated at 5 or 10 mg (11/17 or 12/19 for site 6) and was much lower at 15 mg (4/20) as shown in the scanned table below. In females, incidence was higher at 5 mg (9/17) than 10 mg (3/20) and no lesions were seen at 15 mg. In the most severe lesions that were seen in male rats treated at 5 mg, moderate vasculitis (small and mid-size arteries of most recent injection sites 6, 5 and 4) was characterized by a fibrinoid medial necrosis, focal endothelial destruction and/or thickening, disruption of the internal lamina elastica, and by perivascular inflammation. RBC accumulation was also occasionally seen in the tunica media of arteries.

Incidence and mean severity of vasculitis microscopic finding

Sex Group	Male				Female			
	1	2	3	4	1	2	3	4
Dose-level (mg)	0	5	10	15	0	5	10	15
Finding: VASCULITIS								
Injection site 4	0	1/8 (1.0)	0	0	0	0	1/19 (1.0)	0
Injection site 5	0	3/16 (1.0)	2/20 (1.0)	0	0	1/17 (1.0)	1/16 (1.0)	0
Injection site 6	0	11/17 (1.6)	12/19 (1.6)	4/20 (1.3)	0	9/17 (1.4)	3/20 (1.0)	0

(): mean severity; 0: not found; site No. 4, (thoracic right), site No. 5, (dorsal right), site No. 6, (flank right).

Intimal thickening was noted in vessels, mostly in small and mid-size arteries, from all injection sites in all groups including control rats. The most severe "intimal thickening" changes were seen equally at injection sites from rats treated at 10 or 15 mg, while female rats treated at 10 mg were slightly less affected compared to 15 mg except at the most recent injection site #6. At 5 mg, changes of lower severity were seen and fewer animals were affected. In all treated groups, the most severe changes were observed at the most recent injection sites (#6 and #5), while the oldest sites (#7 and #1) had the less pronounced changes. In control rats, intimal thickening remained generally marginal and restricted to isolated vessels (see the scanned table below).

Incidence and mean severity of intimal thickening microscopic finding

Sex Group	Male				Female			
	1	2	3	4	1	2	3	4
Dose-level (mg)	0	5	10	15	0	5	10	15
Finding: INTIMAL THICKENING								
Injection site 7	1/20 (1.0)	3/4 (1.3)	5/8 (1.2)	13/20 (1.6)	2/20 (1.0)	0	1/1 (1.0)	13/20 (1.7)
Injection site 1	2/20 (1.0)	2/3 (1.5)	11/16 (1.6)	13/20 (1.9)	1/20 (1.0)	1/3 (1.0)	8/11 (1.9)	15/20 (2.0)
Injection site 2	1/20 (1.0)	1/6 (2.0)	17/19 (1.7)	18/20 (1.9)	0	3/4 (2.0)	13/13 (1.8)	19/20 (2.4)
Injection site 3	4/20 (1.3)	7/7 (1.6)	15/17 (1.9)	16/20 (2.4)	1/20 (1.0)	10/11 (2.0)	11/14 (1.9)	19/20 (2.3)
Injection site 4	5/20 (1.4)	5/8 (1.6)	16/20 (1.4)	16/20 (2.0)	2/20 (1.5)	6/11 (1.0)	12/19 (1.6)	20/20 (1.7)
Injection site 5	2/20 (1.0)	13/16 (1.3)	19/20 (2.0)	19/20 (2.2)	0	10/17 (2.3)	14/16 (2.4)	20/20 (2.1)
Injection site 6	3/20 (1.0)	14/17 (1.4)	19/19 (2.1)	17/20 (2.4)	1/20 (1.0)	15/17 (1.6)	19/20 (2.4)	20/20 (2.0)

(): mean severity; 0: not found; site No. 1, (thoracic left), site No. 2, (dorsal left), site No. 3, (flank left), site No. 4, (thoracic right), site No. 5, (dorsal right), site No. 6, (flank right), site No. 7 (scapular).

Pancreas

A minimal to mild increased quantity of zymogen granules was noted in all treated males and females with a lower incidence in the control animals. A dose-relationship for the

Best Possible Copy

incidence and severity was evident in females but not in males. This finding generally correlated with the white color of this organ at necropsy.

Dose, mg/14-d	0		5		10		15	
Sex	M	F	M	F	M	F	M	F
# Examined	20	20	19	20	20	20	20	20
Increased zymogen	5	1	14	6	19	16	17	17

Eyes

Minimal or mild retrobulbar inflammation/hemorrhage, noted in all groups, was more frequently observed in the treated males and females.

Harderian glands

Minimal to mild chronic-active inflammation was noted in all groups, with a greater incidence in the treated males and females without a clear dose-relationship.

According to the sponsor, the inflammation, as seen in both eyes and Harderian glands, was considered to be a result of orbital bleeding (sample collection). Thus it was considered to be of no toxicological importance.

Toxicokinetics:

Serum drug concentration was analyzed with a validated radioimmunoassay method at IPSEN Pharma SA. Because the number of rats showing non-specific binding (NSB) values over 10% was so high on weeks 13 and 25 (246 out of 288 samples), only these animals were considered on each week to perform TK analysis.

Following the first dosing, mean values of TK parameters were very similar for male and female rats at the three doses. AUC_t values were of 284.3, 597.5 and 809.2 ng.mL/day (males) and 244.5, 557.7 and 814.4 ng.mL/day (females), respectively, at the three doses.

Following the 7th and 13th dosing, lanreotide serum levels in female rats were substantially higher than those obtained in males at the three dose levels.

Lanreotide serum levels of rats with NSB<10% were usually lower than those of rats showing an immunogenic response against lanreotide. So, the exposure values determined following the 7th and 13th dosing might be overestimated, in which higher than proportional increase of the exposure level in females between 5 and 10 mg was noted, and apparently drug accumulation occurred in the mid- and end-term of dosing compared to the initial exposure levels.

A total of 18 male rats and 30 female rats presented a specific antibody response to lanreotide with titers ranging from 4 to 128 and from 4 to 512, respectively. Female rats seemed to be more sensitive to lanreotide immunogenicity and no marked differences between doses indicated.

Sex	Male			Female		
Group	2	3	4	2	3	4
Dose-level (mg)	5	10	15	5	10	15
<i>First administration (D1 to D14)</i>						
C _{max} (ng/mL)	106	150	165	169	140	169
AUC _τ (ng/mL).day	284	598	809	245	558	814
<i>Seventh administration (D85 to D99)</i>						
C _{max} (ng/mL)	279	1635	2256	1278	2482	2572
AUC _τ (ng/mL).day	1460	9412	11881	5744	19637	17338
<i>Thirteenth administration (D169 to D183)</i>						
C _{max} (ng/mL)	755	1373	2055	1620	4294	3699
AUC _τ (ng/mL).day	4713	9853	14220	9460	32918	32917

AUC_τ: area under the serum concentration-time curve from 0 to τ within the dosing interval (14 days) at steady state. D = Day.

Study title: Twenty-six Week Toxicity Study by Repeated Subcutaneous Injection Every 14 Days in Beagle Dogs (Lanreotide Autogel)

Key study findings: Lanreotide Autogel was s.c. dosed once every 14 days for 26 weeks (total of 13 dosing) to beagle dogs at dose levels of 60, 120 or 360 mg/animal/injection at 26 defined injection sites (2 sites for each dosing occasion, i.e. a half of each full dosage was given into 1 site) in the dorsum and flanks. No reversibility observation was performed.

- No mortality.
- Local reaction at the injection sites (yellowish nodules) in all lanreotide treated animals and diarrhea in most lanreotide treated animals were the major clinical signs.
- Body wt in males decreased during the first 8 weeks of treatment (↓ 7.4, 9.6 and 10% at LD, MD, and HD, respectively, vs. C); by the end of treatment, males' body weights were still slightly lower compared to C (↓3, 12, and 4%, respectively). No significant wt changes were seen in treated females.
- No marked findings in EKG, hematology and serum chemistry parameters.
- Injection site tissue reactions were the major histopathologic findings at all dose levels tested, including the presence of infiltration of the subcutaneous tissue by macrophages and/or granulomatous inflammation. Overall, severity of granulomatous inflammation increased with the dose from ≥ 120 mg and decreased with the age of lesion (indicative of reversibility). Minimal to slight changes in the kidney (cortical tubular vacuolation) and lung at ≥ 120 mg.
- The systemic NOAEL is 120 mg/14-d/animal (AUC = 721 ng/ml/d sex combined).

Study no.: 28224 TCC / —

Best Possible Copy

Volume #, and page #: Vol A3.45, and pages 1 to 962

Conducting laboratory and location: _____

Date of study initiation: 10/12/2004

GLP compliance: yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Lanreotide Autogel, batch # 04K2205, and purity not specified (mean peptide content of — in each syringe)

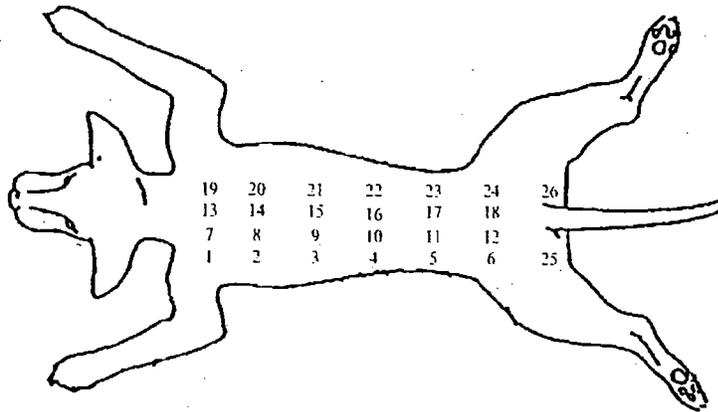
Methods

Doses: 0, 60, 120, 360 mg/animal/14 days

Species/strain: Dog/Beagle

Number/sex/group or time point (main study): 3/s/g

Route, formulation, volume, and infusion rate: s.c. injection in the dorsum and flanks using pre-filled syringes fitted with a single use needle. Each animal received 13 injections every 14 days over the treatment period. Two injection sites were used on each treatment occasion, and each site was used only once (26 dosing sites are presented in the figure below). Control animals received the vehicle only (0.9% sterile isotonic saline solution), batch # FVI08A and FVG29A.



Satellite groups used for toxicokinetics or recovery: TK samples were taken from the animals in main study without satellite groups.

Age: 6 months old at the beginning of treatment

Weight: 8.2 to 10.2 kg for males and 5.8 to 9.4 kg for females

Sampling times: Animals were sacrificed after 26 bi-weekly dosing

Unique study design or methodology: none

Observations and times:

Mortality: twice a day during treatment period

Clinical signs: once a day

Body weights: once pretest, once prior to first dosing, and once a week till the end of treatment

Food consumption: once daily throughout the treatment duration

Ophthalmoscopy: pretest and in Weeks 13 and 25 in all animals

EKG: pretest and in Weeks 13 and 25 at 2 hrs after dosing

Hematology: collected once pretest and in Weeks 13 and 25

Clinical chemistry: collected once pretest and in Weeks 13 and 25

Urinalysis: collected once pretest and in Weeks 13 and 25

Gross pathology: at necropsy

Organ weights: see histopath table

Histopathology: see histopath table

Results

Mortality: No deaths

Clinical signs: Limited to local reaction at the injection sites, including nodules in all lanreotide autogel treated animals and transient scabs and abscesses in some animals at some injection sites. All these findings were dose-related (scanned Table 1). Liquid feces were episodically observed in most lanreotide-treated animals.

Table 1: Local reactions at the injection sites (cumulated for all 78 injection sites/sex/group and over the full study duration)

Group	1		2		3		4	
Dose-level (mg/injection site)	0		30		60		180	
Dose-level (mg/animal/14 days)	0		60		120		360	
Sex	M	F	M	F	M	F	M	F
Nodules	0	0	78	78	78	78	78	78
Wounds	0	0	1	5	6	3	1	3
Abscesses	0	0	9	10	25	20	5	32
Scabs	0	0	11	12	21	17	4	23
Erythema	0	1	0	0	0	0	0	1

Body weights: Body wt in males decreased during the first 8 weeks of treatment compared to C for 7.4, 9.6 and 10% at LD, MD, and HD, respectively; by the end of treatment, males' body weights were still slightly lower compared to C (↓3, 12, and 4%, respectively). No significant wt changes were seen in treated females.

Food consumption: Unremarkable

Ophthalmoscopy: No drug-related findings

EKG: Unremarkable

Hematology: Unremarkable

Clinical chemistry: Unremarkable

Urinalysis: No marked differences between treated and C animals

Gross pathology:

Subcutaneous injection sites

Yellowish nodules most often with yellowish contents and/or thickened subcutaneous tissue were observed at some injection sites in all treated animals, and was not observed in any of the controls.

Nodules/thickened subcutaneous tissue were observed at all sites injected in all low-dose males. However, the nodules clinically observed following the injection on Days 1, 15, 29, 43, 57, 71 and 85 totally disappeared in 2/3 or 3/3 males within 14 to 26 weeks prior to the termination.

Nodules/thickened subcutaneous tissue were observed at all sites injected in all MD males. However, the nodules clinically observed following the injection on Days 1, 15, 43, 57, 71, 85, 99 and 141 totally disappeared in 2/3 or 3/3 males within 6 to 26 weeks prior to the termination.

Liver

Liver enlargement in 1/3 females at 360 mg correlated with higher liver weight and glycogen content.

Gallbladder

Dilatation of the gallbladder was noted in 1/3 males at 60 mg, 2/3 males and females at 120 mg and 1/3 males and 2/3 females at 360 mg. In 1/3 females at 360 mg, this correlated with adhesion of intraluminal substance to epithelium; probably this excessive mucus could explain the gallbladder dilatation. However a relationship to treatment with the drug can not be excluded.

Organ weights: Slightly increased liver wt was noted in all treated males and HD females, which may be associated with increases in glycogen content in hepatocytes at HD, and no other histopath correlation was found in the liver; while the lower testis wt at HD males was not associated with histopath findings.

Difference in mean organ wt (% from control):

Sex Group	Male			Female		
	2	3	4	2	3	4
Dose-level (mg/animal every 14 days)	60	120	360	60	120	360
<i>- Liver</i>						
. absolute	+2	-1	+16	-10	+6	+16
. relative	+5	+12	+18	-20	-4	+20
<i>- Testes</i>						
. absolute	+4	-12	-20			
. relative	+7	0	-18			

Histopathology:

- Subcutaneous infiltration at injection sites containing macrophages and/or granulomatous inflammation as shown in the scanned tables below were the major histopath findings related to treatment at all dose levels.

Male					Female						
SEX	DOSE GROUP:	1	2	3	4	SEX	DOSE GROUP:	1	2	3	4
NO. ANIMALS:		3	3	3	3	NO. ANIMALS:		3	3	3	3
-----					-----						
INJECTION SITE 1		3	3	3	3	INJECTION SITE 1		3	3	3	3
- Derm. Black Pig. La. M.:		1	-	2	2	- Derm. Black Pig. La. M.:		1	1	-	-
- Epider. Deg./Necrosis:		1	1	-	-	- Dermal Granuloma		-	1	-	-
- Collag. Degr. Up. Derm.:		2	-	-	-	- Folliculitis		1	-	-	1
- Dermal Granuloma		-	-	-	1	- *Derm. Macro. Infiltr.:		-	-	-	1
- *Subc. Macro. Infiltr.:		-	1	1	-	- *Hypoder. Granul. In.:		-	2	-	-
- *Subcut. Granul. Infl.:		-	2	2	2	- *Subcut. Granul. Infl.:		-	1	2	1
- *Prom. Mono. Cel. Infi.:		-	-	1	1	- *Prom. Mono. Cel. Infi.:		-	1	1	1
- *Subcuta. Fibrosis		-	-	1	-	- *Dermal Fibrosis		-	-	1	1
						- *Subcuta. Fibrosis		-	-	-	1
-----					-----						
INJECTION SITE 2		3	3	3	3	INJECTION SITE 2		3	3	3	3
- Derm. Black Pig. La. M.:		2	1	2	-	- Derm. Black Pig. La. M.:		2	1	3	1
- Acanthosis		1	-	-	-	- Hyperkeratosis		1	-	-	-
- Epid. Deg./Necrosis		1	-	-	-	- Acanthosis		1	-	-	-
- Infl. Cel. Inf. Dermis		-	-	-	1	- Infl. Cel. Inf. Dermis		-	1	-	-
- *Der./Subc. M. Mac. In.:		-	1	-	-	- *Der./Subc. M. Mac. In.:		-	-	-	1
- *Subc. Macro. Infiltr.:		-	-	1	1	- *Hypoder. Granul. Inf.:		-	1	3	-
- *Subcut. Granul. Infl.:		-	-	2	1	- *Subcut. Granul. Infl.:		-	2	-	1
- *Prom. Mono. Cel. Infi.:		-	-	2	1	- *Prom. Mono. Cel. Infi.:		-	1	2	-
- *Granulation Tissue		-	-	-	1	- *Der./subc. M. Fibros.:		-	-	-	1
- *Subcuta. Fibrosis		-	-	-	1	- *Subcuta. Fibrosis		-	-	1	-
- *Multinu./giant Cel.:		-	-	2	1	- *Subcutane. Necrosis		-	-	1	-
-----					-----						
INJECTION SITE 3		3	3	3	3	INJECTION SITE 3		3	3	3	3
- Derm. Black Pig. La. M.:		1	1	-	1	- Derm. Black Pig. La. M.:		1	1	3	1
- Folliculitis		1	-	-	-	- *Der./Subc. M. Gra. Ti.:		-	-	-	1
- *Derm. Macro. Infiltr.:		-	1	-	-	- *Subc. Macro. Infiltr.:		-	1	2	1
- *Subc. Macro. Infiltr.:		-	3	-	2	- *Subcut. Granul. Infl.:		-	2	-	-
- *Subcuta. Fibrosis		-	1	-	-	- *Prom. Mono. Cel. Infi.:		-	1	-	-
- *Subcut. Granul. Infl.:		-	-	1	-	- *Multinu./Giant Cells:		-	2	1	-
- *Prom. Mono. Cel. Infi.:		-	-	-	2	-----					
-----					-----						
INJECTION SITE 4		3	3	3	3	INJECTION SITE 4		3	3	3	3
- Derm. Black Pig. La. M.:		1	-	1	1	- Derm. Black Pig. La. M.:		2	1	1	2
- Dermal Inflamm. Cells		-	-	1	-	- Hyperkeratosis		1	-	-	-
- *Subcut. Macr. Infiltr.:		-	3	1	1	- Acanthosis		1	-	-	-
- *Subcut. Granul. Infl.:		-	-	1	1	- Dermal Granuloma		-	1	-	-
- *Prom. Mono. Cel. Infi.:		-	-	1	1	- Folliculitis		1	-	-	-
- *Multinu./Giant Cel.:		-	-	1	-	- *Der./subc. M. Fibros.:		-	-	2	1
-----					-----						
INJECTION SITE 5		3	3	3	3	INJECTION SITE 5		3	3	3	3
- Derm. Black Pig. La. M.:		1	1	1	2	- Derm. Black Pig. La. M.:		-	2	-	2
- Folliculitis		-	1	-	1	- Folliculitis		1	-	-	-
- Collag. Degr. Up. Derm.:		1	1	-	-	- Subcutane. Hemorrhage:		1	-	-	-
- Subcutane. Hemorrhage:		-	-	1	-	- Acanthosis		-	-	-	1
- Subcutaneous Edema		-	-	-	1	- Superf. Leuko. Exocyt.:		-	-	-	1
- *Subcut. Macr. Infiltr.:		-	1	1	-	- *Der./Hyp. Macro. Inf.:		-	-	2	-
- *Subcut. Granul. Infl.:		-	2	2	3	- *Subcut. Macr. Infiltr.:		-	-	1	1
- *Prom. Mono. Cel. Infi.:		-	2	1	3	- *Derm./Hypo. Gran. In.:		-	-	-	1
- Collagen Fibr. Basop.:		-	-	1	-	- *Subcut. Granul. Infl.:		-	2	1	1
-----					-----						
						- *Prom. Mono. Cel. Infi.:		-	1	-	-
						- *Gran. In. Ar. Eosin. M.:		-	-	-	1
						- *Granulation tissue		-	-	-	1
						- *Derm./Hypode. Fibros.:		-	-	-	1
						- *Der./subc. M. Fibros.:		-	-	1	-
						- *Derm./Subcut. Fibros.:		-	-	1	-
						- *Subcutane. Fibrosis		-	1	-	-
						- *Subcutane. Necrosis		-	1	-	-
						- Collagen Fibr. Basop.:		1	1	-	1
						- *Multinu./giant Cells:		-	1	-	-

Best Possible Copy

Male					
SEX	DOSE GROUP	1	2	3	4
NO. ANIMALS:		3	3	3	3
INJECTION SITE 6					
- Derm. Black Pig. La. M.:		3	3	3	3
- Folliculitis		-	1	-	1
- Dermal Inflamm. Cells		1	-	-	-
- Dermal Granuloma		-	-	1	-
- subcutane. Hemorrhage:		-	-	-	1
- *Subc. Macro. Infiltr.:		-	1	-	1
- *Subcut. Granul. Infl.:		-	1	1	3
- *Granulation Tissue		-	1	-	-
- *Prom. Mono. Cel. Infi.:		-	1	1	3
- *Subcuta. Fibrosis		-	-	-	1
- *Multinu./Giant Cel.:		-	1	1	1
- Collag. Fibr. Basoph.:		1	1	-	1
INJECTION SITE 7					
- Derm. Black Pig. La. M.:		3	3	3	3
- Subcutane. Hemorrhage:		2	1	-	-
- Folliculitis		-	-	-	1
- *Subc. Macro. Infiltr.:		-	-	2	-
- *Hypod. Granulo. Infl.:		-	1	1	-
- *Subcut. Granul. Infl.:		-	1	1	3
- *Prom. Mono. Cel. Infi.:		-	1	1	2
- *Hypodermal Fibrosis:		-	-	1	-
- *Subcuta. Fibrosis		-	-	-	1
- *Multinu./Giant Cel.:		-	1	1	1
INJECTION SITE 8					
- Derm. Black Pig. La. M.:		3	3	3	3
- *Der. Macro. Infiltr.:		1	-	-	-
- *Der./Subc. Mu. Mac. I.:		-	-	1	-
- *Der./Subc. Mu. Gra. I.:		-	1	-	-
- *Der./Subc. Mu. Fibro.:		-	-	1	-
- Subcutane. Hemorrhage:		-	-	1	-
- *Subc. Macro. Infiltr.:		-	-	1	-
- *Subcut. Granul. Infl.:		-	2	1	3
- *Prom. Mono. Cel. Infi.:		-	1	2	2
- *Subcuta. Fibrosis		-	1	-	-
- *Multinu./Giant Cel.:		-	1	1	-
INJECTION SITE 9					
- Derm. Black Pig. La. M.:		3	3	3	3
- Derm. Black Pig. La. M.:		1	-	1	-
- *Subc. Macro. Infiltr.:		-	2	-	2
- *Subcut. Granul. Infl.:		-	1	-	2
- *Prom. Mono. Cel. Infi.:		-	-	-	2
- *Multinu./Giant Cel.:		-	1	-	1
INJECTION SITE 10					
- Derm. Black Pig. La. M.:		3	3	3	3
- Derm. Black Pig. La. M.:		2	1	-	-
- Acanthosis		1	-	-	-
- Derm. Collag. Degrada.:		1	-	-	-
- Derm. Infl. Cel. Inf.		-	-	1	-
- Subcuta. Hemorrhage:		-	1	-	-
- *Der./Subc. Mu. Mac. I.:		-	-	1	-
- *Subc. Macro. Infiltr.:		-	1	2	-
- *Subcut. Granul. Infl.:		-	3	-	3
- *Der./Subc. Mu. Fibro.:		-	-	1	-
- *Prom. Mono. Cel. Infi.:		-	2	-	2
- *Subcuta. Fibrosis		-	2	1	1
- *Multinu./Giant Cel.:		-	1	2	1

Female					
SEX	DOSE GROUP	1	2	3	4
NO. ANIMALS:		3	3	3	3
INJECTION SITE 6					
- Derm. Black Pig. La. M.:		3	3	3	3
- Acanthosis		1	-	1	2
- Folliculitis		1	-	-	-
- *Hypod. Granul. Infl.:		-	2	-	1
- *Subcut. Granul. Infl.:		-	1	3	1
- *Prom. Mono. Cel. Infi.:		-	1	2	-
- *Gran. In. Ar. Eosin. M.:		-	1	-	-
- *Subcuta. Fibrosis		-	1	-	1
- *Multinu./Giant Cel.:		-	2	2	-
- *Subcutane. Necrosis		-	1	-	-
- Collag. Fibr. Basoph.:		1	-	-	1
INJECTION SITE 7					
- Derm. Black Pig. La. M.:		3	3	3	3
- Hyperkeratosis		1	-	-	2
- *Der./Hyp. Macro. Inf.:		-	-	1	-
- *Hypod. Granulo. Infl.:		-	2	-	1
- *Subcut. Granul. Infl.:		-	1	2	2
- *Prom. Mono. Cel. Infi.:		-	1	1	-
- *Subcuta. Fibrosis		-	1	-	1
- *Multinu./Giant Cel.:		-	2	-	-
INJECTION SITE 8					
- Derm. Black Pig. La. M.:		3	3	3	3
- Derm. Black Pig. La. M.:		1	-	-	-
- Folliculitis		1	-	-	-
- *Der./Subc. Mu. Mac. I.:		-	-	-	1
- *Hypoder. Macro. Infi.:		-	-	-	1
- *Hypoder. Granul. Inf.:		-	-	2	1
- *Subcut. Granul. Infl.:		-	-	1	1
- *Prom. Mono. Cel. Infi.:		-	-	2	1
- *Multinu./Giant Cel.:		-	-	1	-
INJECTION SITE 9					
- Derm. Black Pig. La. M.:		3	3	3	3
- Derm. Black Pig. La. M.:		2	2	1	1
- Subcut. Hemorrhage		-	-	1	-
- Folliculitis		1	-	-	-
- Derm. Infl. Cel. Infil.:		-	1	-	-
- *Der./Hypod. Macr. I.:		-	-	1	-
- *Subc. Macro. Infiltr.:		-	-	1	-
- *Derm./Hypod. Gran. I.:		-	-	-	1
- *Hypod./Subc. Gran. I.:		-	1	-	-
- *Derm./Hypod. Fibros.:		-	-	1	1
- *Subcut. Granul. Infl.:		-	1	2	2
- *Prom. Mono. Cel. Infi.:		-	-	2	2
- *Subcutane. Fibrosis		-	1	-	1
- *Multinu./Giant Cel.:		-	3	-	-
INJECTION SITE 10					
- Derm. Black Pig. La. M.:		3	3	3	3
- Derm. Black Pig. La. M.:		1	1	-	-
- Dermal Granuloma		1	-	-	-
- Subcuta. Hemorrhage		-	1	-	-
- *Dermal Macro. Infil.:		-	1	-	-
- *Der./Subc. Mu. Mac. I.:		-	-	-	1
- *Subc. Macro. Infiltr.:		-	1	-	-
- *Der./Subc. Mu. Gra. I.:		-	-	1	-
- *Subcut. Granul. Infl.:		-	1	2	2
- *Der./Subc. Mu. Fibro.:		-	-	-	1
- *Prom. Mono. Cel. Infi.:		-	-	3	-
- *Subcuta. Fibrosis		-	2	-	-
- *Multinu./Giant Cel.:		-	-	2	-

Best Possible Copy

Male					
SEX	DOSE GROUP	1	2	3	4
NO. ANIMALS:		3	3	3	3
INJECTION SITE 11		3	3	3	3
- Derm.Black Pig.La.M.:		-	-	-	1
- Folliculitis		-	1	-	-
- Myof.Deg./Nec.S.Mus.:		-	-	1	-
- *Derm.Infl.Cel.Infi.:		-	-	1	-
- *Subc.Macro.Infiltr.:		-	1	1	-
- *Subcut.Granul.Infl.:		-	1	2	3
- *Granulation Tissue		-	-	-	2
- *Prom.Mono.Cel.Infi.:		-	1	2	2
- *Subcuta. Fibrosis		-	-	2	1
- *Multinu./giant Cel.:		-	1	-	1
INJECTION SITE 12		3	3	3	3
- Subcutane.Hemorrhage:		-	-	1	1
- Derm.Black Pig.La.M.:		-	1	-	-
- Acanthosis		1	-	-	-
- Derm.Collag.Degrada.:		1	-	-	-
- *Subc.Macro.Infiltr.:		-	1	1	-
- *Subcut.Granul.Infl.:		-	1	2	3
- *Subcuta. Fibrosis		-	1	2	1
- *Prom.Mono.Cel.Infi.:		-	1	2	2
- *Multinu./Giant Cel.:		-	1	1	-
- Collag.Fibre Basoph.:		-	-	-	1
INJECTION SITE 13		3	3	3	3
- Derm.Black.Pig.La.M.:		1	2	1	-
- Dermal Granuloma		-	-	1	-
- Folliculitis		-	-	-	1
- *Subc.Macro.Infiltr.:		-	1	1	-
- *Subcut.Granul.Infl.:		-	-	-	3
- *Subcuta. Fibrosis		-	-	-	1
- *Prom.Mono.Cel.Infi.:		-	-	-	3
- *Multinu./Giant Cel.:		-	-	1	1
INJECTION SITE 14		3	3	3	3
- Derm.Black.Pig.La.M.:		1	-	-	-
- Subcuta. Hemorrhage		-	1	-	1
- Hyperkeratosis		1	-	-	-
- Acanthosis		1	-	-	-
- Folliculitis		-	-	1	-
- *Subcut.Granul.Infl.:		-	2	1	3
- *Prom.Mono.Cel.Infi.:		-	1	-	3
- *Multinu./Giant Cel.:		-	-	1	-
- *Der./Hypod.Macr.In.:		-	1	-	-
- *Dermal Fibrosis		-	-	1	-
- *Subcuta. Fibrosis		-	1	-	1

Female					
SEX	DOSE GROUP	1	2	3	4
NO. ANIMALS:		3	3	3	3
INJECTION SITE 11		3	3	3	3
- Derm.Black Pig.La.M.:		1	1	-	-
- *Der./Subc.Mu.Mac.I.:		-	-	-	1
- *Subcut.Granul.Infl.:		-	2	2	2
- *Granulation Tissue		-	-	-	1
- *Prom.Mono.Cel.Infi.:		-	1	2	1
- *Gran.In.Ar.Eosin.M.:		-	-	-	1
- *Der./Subc.Mu.Fibro.:		-	-	-	1
- *Subcuta. Fibrosis		-	-	1	1
- *Multinu./giant Cel.:		-	2	1	-
- *Subcutaneous Necro.:		-	-	-	2
INJECTION SITE 12		3	3	3	3
- Derm.Black Pig.La.M.:		1	2	-	-
- Hyperkeratosis		1	-	-	-
- Acanthosis		1	-	-	-
- *Der./Subc.Mu.Mac.I.:		-	-	-	1
- *Subc.Macro.Infiltr.:		-	-	1	-
- *Der./Sub.M.Gra.Inf.:		-	1	-	1
- *Subcut.Granul.Infl.:		-	-	3	2
- *Granulation Tissue		-	-	-	1
- *Der./Subc.Mu.Fibro.:		-	-	-	1
- *Subcuta. Fibrosis		-	1	-	1
- *Prom.Mono.Cel.Infi.:		-	1	1	1
- *Gran.In.Ar.Eosin.M.:		-	-	1	1
- *Multinu./Giant Cel.:		-	1	2	-
- Collag.Fibre Basoph.:		-	1	1	-
- *Subcutane.Necrosis		-	-	2	2
INJECTION SITE 13		3	3	3	3
- Derm.Black.Pig.La.M.:		3	-	-	-
- Folliculitis		1	-	-	-
- *Der./Subc.Mu.Mac.I.:		-	-	-	1
- *Derm.Macro.Infiltr.:		-	-	-	1
- *Dermal fibrosis		-	-	-	1
- *Subc.Macro.Infiltr.:		-	2	-	-
- *Der./Sub.M.Gra.Inf.:		-	-	-	1
- *Subcut.Granul.Infl.:		-	-	3	1
- *Subcut.Fibroplasia		-	1	-	-
- *Subcuta. Fibrosis		-	2	-	-
- *Prom.Mono.Cel.Infi.:		-	-	1	1
- *Multinu./Giant Cel.:		-	-	3	-
INJECTION SITE 14		3	3	3	3
- Derm.Black.Pig.La.M.:		1	-	-	-
- Subcuta. Hemorrhage		-	-	-	1
- Folliculitis		1	-	1	-
- *Subcut.Macro.Infil.:		-	1	-	-
- *Subcut.Granul.Infl.:		-	1	3	3
- *Granulation tissue		-	-	1	1
- *Prom.Mono.Cel.Infi.:		-	1	2	2
- *Gran.In.Ar.Eosin.M.:		-	-	-	3
- *Multinu./Giant Cel.:		-	-	2	-
- *Subcuta. Fibrosis		-	1	-	2
- *Subcutane.Necrosis		-	-	-	1

Best Possible Copy

Male				
SEX	1	2	3	4
DOSE GROUP:	1	2	3	4
NO. ANIMALS:	3	3	3	3
INJECTION SITE 15	3	3	3	3
- Derm.Black.Pig.La.M.	1	1	-	-
- subcut.Hemorrhage	-	-	-	1
- *Dermal Fibrosis	-	-	2	-
- *Der./Sub.Mus.M.Infl.	-	1	1	-
- *Derm.Macro.Infiltr.	-	-	1	-
- *Subc.Macro.Infiltr.	-	1	-	-
- *Subcut.Granul.Infl.	-	1	-	3
- *Prom.Mono.Cel.Infi.	-	-	-	3
- *Multinu./Giant Cells	-	-	-	1
INJECTION SITE 16	3	3	3	3
- Derm.Black.Pig.La.M.	1	1	1	-
- Collag.Degr.Up.Derm.	2	-	-	-
- subcut.Hemorrhage	-	-	-	1
- *Subc.Macro.Infiltr.	-	1	1	-
- *Subcut.Granul.Infl.	-	2	-	3
- *Prom.Mono.Cel.Infi.	-	1	-	3
- *Multinu./Giant Cel.	-	1	-	-
- *Subcuta.Fibrosis	-	-	1	3
INJECTION SITE 17	3	3	3	3
- Derm.Black.Pig.La.M.	-	1	1	-
- Folliculitis	-	-	-	1
- Subcut.Hemorrhage	-	-	1	1
- *Der./Sub.Mu.Gran.T.	-	-	1	-
- *Der./Subc.Mu.Mac.I.	-	-	1	-
- *Subcut.Granul.Infl.	-	2	1	3
- *Subcuta.Fibrosis	-	1	1	2
- *Prom.Mono.Cel.Infi.	-	-	1	3
INJECTION SITE 18	3	3	3	3
- Derm.Black.Pig.La.M.	1	2	-	-
- Dermal Hemorrhage	-	-	1	-
- Subcutane.Hemorrhage	-	-	1	-
- *Subc.Macro.Infiltr.	-	1	-	1
- *Derm.Granulo.Infil.	-	-	1	-
- *Subcut.Granul.Infl.	-	1	1	3
- *Prom.Mono.Cel.Infi.	-	1	1	3
- *Derm.Granulat.Tiss.	-	-	1	-
- *Gran.In.Ar.Eosin.M.	-	-	1	-
- *Subcuta.Fibrosis	-	-	1	2
- *Subcutane.Necrosis	-	-	-	1
- *Multinu./Giant Cel.	-	-	1	-

Female				
SEX	1	2	3	4
DOSE GROUP:	1	2	3	4
NO. ANIMALS:	3	3	3	3
INJECTION SITE 15	3	3	3	3
- Derm.Black.Pig.La.M.	1	-	1	1
- subcut.Hemorrhage	-	1	-	1
- Hyperkeratosis	1	-	-	-
- Acanthosis	1	-	-	-
- Folliculitis	1	-	-	-
- *Der./Sub.Mus.M.Infl.	-	-	1	-
- *Subc.Macro.Infiltr.	-	2	-	1
- *Subcut.Granul.Infl.	-	1	1	2
- *Prom.Mono.Cel.Infi.	-	-	-	2
- *Granulation Tissue	-	2	-	-
- *Gran.In.Ar.Eosin.M.	-	-	-	2
- *Multinu./Giant Cells	-	-	1	-
- *Subcut.Collag.Degr.	-	-	-	1
- *Subcut.Fibroplasia	-	-	-	1
- *Der./Subc.Mu.Fibro.	-	-	1	-
- *Subcutaneous Fibro.	-	2	-	2
- *Subcutane.Necrosis	-	-	-	2
INJECTION SITE 16	3	3	3	3
- Derm.Black.Pig.La.M.	2	1	1	-
- *Derm.Macro.Infiltr.	-	-	-	1
- *Subc.Macro.Infiltr.	-	2	-	1
- *Subcut.Granul.Infl.	-	-	2	2
- *Prom.Mono.Cel.Infi.	-	-	1	2
- *Gran.In.Ar.Eosin.M.	-	-	-	1
- *Multinu./Giant Cel.	-	1	2	-
- *Dermal Fibrosis	-	-	-	1
- *Subcuta.Fibrosis	-	2	-	1
- *Subcutane.Necrosis	-	-	-	1
INJECTION SITE 17	3	3	3	3
- Derm.Black.Pig.La.M.	-	1	1	1
- Der./Subc.Mu.Hemorr.	-	-	1	-
- Subcut.Hemorrhage	-	1	-	-
- Acanthosis	-	-	1	-
- Epider.Deg./Necrosis	-	-	1	-
- Skin Ulceration	-	-	1	-
- *Dermal Macrophages	-	1	-	-
- *Dermal Granul.Tiss.	-	1	1	-
- *Der./Sub.Mu.Gran.T.	-	-	1	-
- *Der./Subc.Mu.Mac.I.	-	1	-	-
- *Subcut.Macro.Infil.	-	3	-	1
- *Der./Subc.Mu.Gra.I.	-	-	1	-
- *Subcut.Granul.Infl.	-	2	2	2
- *Der./Subc.Mu.Fibro.	-	-	1	-
- *Subcuta.Fibrosis	-	2	-	1
- *Prom.Mono.Cel.Infi.	-	1	-	1
- *Subcutane.Necrosis	-	-	-	1
- *Gran.In.Ar.Eosin.M.	-	-	2	1
- *Multinu./Giant Cel.	-	3	2	-
- Der./Subc.Mu.Necros.	-	-	1	-
INJECTION SITE 18	3	3	3	3
- Derm.Black.Pig.La.M.	-	-	-	1
- Subcutane.Hemorrhage	-	1	-	-
- Hyperkeratosis	1	-	-	-
- Acanthosis	1	-	1	-
- *Der./Sub.Mus.M.Infl.	-	-	1	1
- *Subc.Macro.Infiltr.	-	-	1	-
- *Subcut.Granul.Infl.	-	2	2	2
- *Granulation Tissue	-	-	-	1
- *Prom.Mono.Cel.Infi.	-	2	1	1
- *Gran.In.Ar.Eosin.M.	-	2	1	2
- *Der./Subc.Mu.Fibro.	-	-	1	1
- *Subcuta.Fibrosis	-	2	3	2
- *Subcutane.Fibropla.	-	1	-	-
- *Subcutane.Necrosis	-	-	1	2
- *Multinu./Giant Cel.	-	1	1	-

Best Possible Copy

Male					Female						
SEX	DOSE GROUP	1	2	3	4	SEX	DOSE GROUP	1	2	3	4
NO. ANIMALS:		3	3	3	3	NO. ANIMALS:		3	3	3	3
INJECTION SITE 19					INJECTION SITE 19						
- Derm. Black Pig. La. M.:		3	3	3	3	- Derm. Black Pig. La. M.:		3	3	3	3
- Epider. Dege./Necros.:		2	-	1	-	- Acanthosis		3	1	-	-
- Folliculitis		-	1	-	-	- Folliculitis		-	-	-	1
- Derm. Inflamm. Cell Ag.:		-	1	-	1	- Derm. Inflamm. Cell Ag.:		1	-	-	-
- *Hypod. Granul. Infl.:		-	-	1	-	- *Der./Hypod. Macr. In.:		-	1	1	-
- *Subcut. Granul. Infl.:		-	3	1	3	- *Der./Subc. M. Gra. In.:		-	-	-	1
- *Granulation Tissue.:		-	2	1	-	- *Subcut. Granul. Infl.:		-	1	2	2
- *Prom. Mono. Cel. Infi.:		-	3	1	3	- *Granulation Tissue.:		-	-	-	1
- *Dermal fibrosis		-	-	1	-	- *Prom. Mono. Cel. Infi.:		-	1	1	1
- *Subcuta. Fibrosis		-	2	-	1	- *Dermal fibrosis		-	1	1	-
- *Gran. In. Ar. Eos. Mat.:		-	2	-	-	- *Subcuta. Fibrosis		-	1	2	2
- *Multinu./Giant Cel.:		-	-	-	1	- *Gran. In. Ar. Eos. Mat.:		-	-	1	2
- *Subcutane. Necrosis		-	1	-	-	- *Multinu./Giant Cel.:		-	1	2	-
INJECTION SITE 20					INJECTION SITE 20						
- Derm. Black Pig. La. M.:		3	3	3	3	- Derm. Black Pig. La. M.:		3	3	3	3
- Subcutane. Hemorrhage:		1	1	1	-	- *Derm. Macro. Infiltr.:		-	1	-	2
- Folliculitis		-	1	-	-	- *Dermal fibrosis		-	-	1	-
- *Dermal/subc. Fibros.:		-	-	1	-	- *Derm. Granul. Inflamm.:		-	1	1	2
- *Dermal fibrosis		-	-	1	-	- *Subcut. Granul. Infl.:		-	1	2	1
- *Derm. Granul. Inflamm.:		-	1	2	-	- *Prom. Mono. Cel. Infi.:		-	2	2	1
- *Subcut. Granul. Infl.:		-	2	-	3	- *Subcuta. Fibrosis		-	-	-	1
- *Prom. Mono. Cel. Infi.:		-	2	1	3	- *Gran. In. Ar. Eosin. M.:		-	-	1	2
- *Subcuta. Fibrosis		-	1	-	2	- *Subcut. Macro. Infil.:		-	-	-	1
- *Granulation Tissue		-	1	-	1	- *Multinu./giant Cel.:		-	3	2	-
- *Gran. In. Ar. Eosin. M.:		-	1	-	-	- *Subcutane. Necrosis		-	-	1	2
- *Multinu./giant Cel.:		-	2	-	1	INJECTION SITE 21					
INJECTION SITE 21					INJECTION SITE 21						
- Derm. Black Pig. La. M.:		3	3	3	3	- Derm. Black Pig. La. M.:		3	3	3	3
- Subcutis Hemorrhage		1	1	-	-	- Acanthosis		-	2	-	1
- *Subcut. Granul. Infl.:		-	1	-	-	- Hyperkeratosis		-	-	1	1
- *Prom. Mono. Cel. Infi.:		-	3	2	3	- Skin Ulceration		-	-	-	1
- *Granulation Tissue		-	2	2	3	- *Subcutane. Macro. In.:		-	3	-	-
- *Subcutane. Fibropla.:		-	1	-	-	- *Der./Subc. M. Gra. In.:		-	-	-	1
- *Subcuta. Fibrosis		-	3	1	3	- *Subcut. Granul. Infl.:		-	1	3	2
- *Gran. In. Ar. Eosin. M.:		-	2	-	1	- *Prom. Mono. Cel. Infi.:		-	1	2	2
- *Multinu./giant Cel.:		-	-	1	1	- *Granulation Tissue		-	-	1	1
- *Subcutane. Necrosis		-	1	-	-	- *Subcuta. Fibrosis		-	1	2	2
INJECTION SITE 22					INJECTION SITE 22						
- Derm. Black Pig. La. M.:		3	3	3	3	- Derm. Black Pig. La. M.:		3	3	3	3
- Subcutis Hemorrhage		1	-	-	1	- Folliculitis		3	-	-	1
- Folliculitis		1	1	-	-	- Acanthosis		2	-	-	-
- Hyperkeratosis		-	1	-	-	- Skin Ulceration		1	-	-	-
- Acanthosis		-	1	-	-	- *Der./Sub. Mu. Mac. In.:		-	-	1	-
- *Derm. Granul. Tissue		-	1	-	-	- *Subcut. Macro. Infil.:		-	-	1	-
- *Derm. Macro. Infiltr.:		-	1	1	-	- *Subcut. Granul. Infl.:		-	2	2	3
- *Subcut. Macro. Infil.:		-	-	1	-	- *Granulation Tissue		-	-	2	1
- *Subcut. Granul. Infl.:		-	1	2	3	- *Prom. Mono. Cel. Infi.:		-	2	-	2
- *Granulation Tissue		-	1	1	-	- *Gran. In. Ar. Eosin. M.:		-	-	1	3
- *Prom. Mono. Cel. Infi.:		-	1	2	3	- *Subcutane. Fibropla.:		-	1	-	-
- *Gran. In. Ar. Eosin. M.:		-	1	-	1	- *Subcuta. Fibrosis		-	1	1	2
- *Derm./Subc. Fibropl.:		-	1	-	-	- *Multinu./giant Cel.:		-	2	1	1
- *Subcuta. Fibrosis		-	-	-	3	- *Subcutane. Necrosis		-	-	2	1
- *Multinu./giant Cel.:		-	-	3	1	INJECTION SITE 22					
INJECTION SITE 22					INJECTION SITE 22						
- Derm. Black Pig. La. M.:		3	3	3	3	- Derm. Black Pig. La. M.:		3	3	3	3
- Folliculitis		1	-	-	-	- Folliculitis		3	-	-	1
- Acanthosis		-	1	-	-	- Acanthosis		1	-	-	-
- *Derm. Granul. Tissue		-	1	-	-	- Skin Ulceration		-	-	1	-
- *Derm. Macro. Infiltr.:		-	1	1	-	- *Der./Sub. Mu. Mac. In.:		-	-	1	-
- *Subcut. Macro. Infil.:		-	-	1	-	- *Subcut. Macro. Infil.:		-	-	1	-
- *Subcut. Granul. Infl.:		-	1	2	3	- *Subcut. Granul. Infl.:		-	2	2	3
- *Granulation Tissue		-	1	1	-	- *Granulation Tissue		-	-	2	1
- *Prom. Mono. Cel. Infi.:		-	1	2	3	- *Prom. Mono. Cel. Infi.:		-	2	-	2
- *Gran. In. Ar. Eosin. M.:		-	1	-	1	- *Gran. In. Ar. Eosin. M.:		-	-	1	3
- *Derm./Subc. Fibropl.:		-	1	-	-	- *Subcutane. Fibropla.:		-	1	-	-
- *Subcuta. Fibrosis		-	-	-	3	- *Subcuta. Fibrosis		-	1	1	2
- *Multinu./giant Cel.:		-	-	3	1	- *Multinu./giant Cel.:		-	2	1	1
INJECTION SITE 22					INJECTION SITE 22						
- Derm. Black Pig. La. M.:		3	3	3	3	- Derm. Black Pig. La. M.:		3	3	3	3
- Folliculitis		1	-	-	-	- Folliculitis		1	-	-	-
- Acanthosis		-	1	-	-	- Acanthosis		-	-	1	-
- *Derm. Granul. Tissue		-	1	-	-	- Skin Ulceration		-	-	1	-
- *Derm. Macro. Infiltr.:		-	1	1	-	- *Der./Sub. Mu. Mac. In.:		-	-	1	-
- *Subcut. Macro. Infil.:		-	-	1	-	- *Subcut. Macro. Infil.:		-	-	1	-
- *Subcut. Granul. Infl.:		-	1	2	3	- *Subcut. Granul. Infl.:		-	2	2	3
- *Granulation Tissue		-	1	1	-	- *Granulation Tissue		-	-	2	1
- *Prom. Mono. Cel. Infi.:		-	1	2	3	- *Prom. Mono. Cel. Infi.:		-	2	-	2
- *Gran. In. Ar. Eosin. M.:		-	1	-	1	- *Gran. In. Ar. Eosin. M.:		-	-	1	3
- *Derm./Subc. Fibropl.:		-	1	-	-	- *Subcutane. Fibropla.:		-	1	-	-
- *Subcuta. Fibrosis		-	-	-	3	- *Subcuta. Fibrosis		-	1	1	2
- *Multinu./giant Cel.:		-	-	3	1	- *Multinu./giant Cel.:		-	2	1	1
INJECTION SITE 22					INJECTION SITE 22						
- Derm. Black Pig. La. M.:		3	3	3	3	- Derm. Black Pig. La. M.:		3	3	3	3
- Folliculitis		1	-	-	-	- Folliculitis		1	-	-	-
- Acanthosis		-	1	-	-	- Acanthosis		-	-	1	-
- *Derm. Granul. Tissue		-	1	-	-	- Skin Ulceration		-	-	1	-
- *Derm. Macro. Infiltr.:		-	1	1	-	- *Der./Sub. Mu. Mac. In.:		-	-	1	-
- *Subcut. Macro. Infil.:		-	-	1	-	- *Subcut. Macro. Infil.:		-	-	1	-
- *Subcut. Granul. Infl.:		-	1	2	3	- *Subcut. Granul. Infl.:		-	2	2	3
- *Granulation Tissue		-	1	1	-	- *Granulation Tissue		-	-	2	1
- *Prom. Mono. Cel. Infi.:		-	1	2	3	- *Prom. Mono. Cel. Infi.:		-	2	-	2
- *Gran. In. Ar. Eosin. M.:		-	1	-	1	- *Gran. In. Ar. Eosin. M.:		-	-	1	3
- *Derm./Subc. Fibropl.:		-	1	-	-	- *Subcutane. Fibropla.:		-	1	-	-
- *Subcuta. Fibrosis		-	-	-	3	- *Subcuta. Fibrosis		-	1	1	2
- *Multinu./giant Cel.:		-	-	3	1	- *Multinu./giant Cel.:		-	2	1	1
INJECTION SITE 22					INJECTION SITE 22						
- Derm. Black Pig. La. M.:		3	3	3	3	- Derm. Black Pig. La. M.:		3	3	3	3
- Folliculitis		1	-	-	-	- Folliculitis		1	-	-	-
- Acanthosis		-	1	-	-	- Acanthosis		-	-	1	-
- *Derm. Granul. Tissue		-	1	-	-	- Skin Ulceration		-	-	1	-
- *Derm. Macro. Infiltr.:		-	1	1	-	- *Der./Sub. Mu. Mac. In.:		-	-	1	-
- *Subcut. Macro. Infil.:		-	-	1	-	- *Subcut. Macro. Infil.:		-	-	1	-
- *Subcut. Granul. Infl.:		-	1	2	3	- *Subcut. Granul. Infl.:		-	2	2	3
- *Granulation Tissue		-	1	1	-	- *Granulation Tissue		-	-	2	1
- *Prom. Mono. Cel. Infi.:		-	1	2	3	- *Prom. Mono. Cel. Infi.:		-	2	-	2
- *Gran. In. Ar. Eosin. M.:		-	1	-	1	- *Gran. In. Ar. Eosin. M.:		-	-	1	3
- *Derm./Subc. Fibropl.:		-	1	-	-	- *Subcutane. Fibropla.:		-	1	-	-
- *Subcuta. Fibrosis		-	-	-	3	- *Subcuta. Fibrosis		-	1	1	2
- *Multinu./giant Cel.:		-	-	3	1	- *Multinu./giant Cel.:		-	2	1	1
INJECTION SITE 22					INJECTION SITE 22						
- Derm. Black Pig. La. M.:		3	3	3	3	- Derm. Black Pig. La. M.:		3	3	3	3
- Folliculitis		1	-	-	-	- Folliculitis		1	-	-	-
- Acanthosis		-	1	-	-	- Acanthosis		-	-	1	-
- *Derm. Granul. Tissue		-	1	-	-	- Skin Ulceration		-	-	1	-
- *Derm. Macro. Infiltr.:		-	1	1	-	- *Der./Sub. Mu. Mac. In.:		-	-	1	-
- *Subcut. Macro. Infil.:		-	-	1	-	- *Subcut. Macro. Infil.:		-	-	1	-
- *Subcut. Granul. Infl.:		-	1	2	3	- *Subcut. Granul. Infl.:		-	2	2	3
- *Granulation Tissue		-	1	1	-	- *Granulation Tissue		-	-	2	1
- *Prom. Mono. Cel. Infi.:		-	1	2	3	- *Prom. Mono. Cel. Infi.:		-	2	-	2
- *Gran. In. Ar. Eosin. M.:		-	1	-	1	- *Gran. In. Ar. Eosin. M.:		-	-	1	3
- *Derm./Subc. Fibropl.:		-	1	-	-	- *Subcutane. Fibropla.:		-	1	-	-
- *Subcuta. Fibrosis		-	-	-	3	- *Subcuta. Fibrosis		-	1	1	2
- *Multinu./giant Cel.:		-	-	3	1	- *Multinu./giant Cel.:		-	2	1	1
INJECTION SITE 22					INJECTION SITE 22						
- Derm. Black Pig. La. M.:		3	3	3	3	- Derm. Black Pig. La. M.:		3	3	3	3
- Folliculitis		1	-	-	-	- Folliculitis		1	-	-	-
- Acanthosis		-	1	-	-	- Acanthosis		-	-	1	-
- *Derm. Granul. Tissue		-	1	-	-	- Skin Ulceration		-	-	1	-
- *Derm. Macro. Infiltr.:		-	1	1	-	- *Der./Sub. Mu. Mac. In.:		-	-	1	-
- *Subcut. Macro. Infil.:		-									

Male				
SEX	1	2	3	4
DOSE GROUP:	1	2	3	4
NO. ANIMALS:	3	3	3	3
INJECTION SITE 23	3	3	3	3
- Epider. Degene./Necr.:	1	-	-	-
- Derm. Granulo. Infiltr.:	1	-	-	-
- Collag. Degr. Up. Derm.:	2	-	-	-
- Acanthosis	1	-	-	-
- Subcutane. Hemorrhage:	-	1	2	-
- *Subcut. Granul. Infil.:	-	3	2	3
- *Prom. Mono. Cel. Infil.:	-	2	1	2
- *Granulation Tissue	-	1	3	-
- *Subcuta. Fibrosis	-	2	3	3
- *Subcutane. Fibropla.:	-	1	-	-
- *Subc. Collag. Degrad.:	-	1	-	-
- *Subcutaneous Edema	-	-	1	-
- *Gran. In. Ar. Eosin. M.:	-	2	1	2
- *Subcutane. Necrosis	-	1	1	2
- *Multinu./Giant Cel.:	-	-	-	1
INJECTION SITE 24	3	3	3	3
- Derm. Black Pig. La. M.:	1	-	-	-
- Skin Ulceration	-	-	1	-
- Dermal Granul. Tissue:	-	-	1	-
- *Subc. Macro. Infiltr.:	-	1	-	-
- *Subcut. Granul. Infil.:	-	1	3	3
- *Prom. Mono. Cel. Infil.:	-	1	-	2
- *Gran. In. Ar. Eosin. M.:	-	1	1	-
- *Granulation Tissue	-	-	3	-
- *Multinu./Giant Cel.:	-	-	-	1
- *Subcutane. Necrosis	-	-	2	1
- *Subcuta. Fibrosis	-	1	-	2
- Collagen Fibre Baso.:	1	-	-	-
INJECTION SITE 25	3	3	3	3
- Derm. Black Pig. La. M.:	1	1	-	1
- Subcut. Hemorrhage	-	1	-	-
- subcutaneous Edema	-	-	-	1
- Collag. Degr. Up. Derm.:	1	-	-	-
- *Hypod. Granul. Infil.:	-	-	2	-
- *Subcut. Granul. Infil.:	-	3	1	3
- *Prom. Mono. Cel. Infil.:	-	3	-	1
- *Granulation Tissue	-	-	3	2
- *Gran. In. Ar. Eosin. M.:	-	2	2	-
- *Hypodermal Fibrosis:	-	-	1	-
- *Subcutane. Fibropla.:	-	1	-	-
- *Subcuta. Fibrosis	-	2	1	1
- *Hypoderm. Necrosis	-	-	2	-
- *Subcutane. Necrosis	-	2	1	2
- *Multinu./giant Cel.:	-	1	-	-
- *Subcut. Edema/Fibro.:	-	1	-	-
INJECTION SITE 26	3	3	3	3
- Derm. Black Pig. La. M.:	-	2	-	-
- Superf. Skin Ulcerat.:	-	1	-	-
- Hyperkeratosis	1	-	-	-
- Acanthosis	1	1	-	-
- Collag. Degr. Up. Derm.:	1	-	-	-
- *Hypod. Granul. Infil.:	-	-	1	-
- *Subcut. Granul. Infil.:	-	3	2	3
- *Prom. Mono. Cel. Infil.:	-	3	-	1
- *Granulation Tissue	-	2	3	3
- *Gran. In. Ar. Eosin. M.:	-	2	1	-
- *Multinu./giant Cel.:	-	1	-	-
- *Hypodermal Fibrosis:	-	-	1	-
- *Subcutane. Fibropla.:	-	1	-	-
- *Subcuta. Fibrosis	-	2	1	1
- *Hypoderm. Necrosis	-	-	1	-
- *Subcutane. Necrosis	-	2	2	2
- *Collag. Degr./Necro.:	-	-	-	1
- Collag. Fibre Basoph.:	-	1	-	-

Female				
SEX	1	2	3	4
DOSE GROUP:	1	2	3	4
NO. ANIMALS:	3	3	3	3
INJECTION SITE 23	3	3	3	3
- Derm. Black Pig. La. M.:	1	3	1	1
- Folliculitis	1	-	-	-
- Superf. Leuko. Exudate:	-	1	1	-
- Acanthosis	1	1	1	1
- Skin Ulceration	-	1	-	-
- Hyperkeratosis	-	1	-	-
- Derm. Collag. Degrad.:	-	1	-	-
- Subcutane. Hemorrhage:	-	3	-	-
- *Subcut. Granul. Infil.:	-	3	2	3
- *Prom. Mono. Cel. Infil.:	-	2	3	1
- *Granulation Tissue	-	1	1	2
- *Subcuta. Fibrosis	-	2	3	1
- *Subcutane. Fibropla.:	-	2	-	-
- *Subc. Collag. Degrad.:	-	1	-	-
- *Gran. In. Ar. Eosin. M.:	-	3	1	2
- *Subcutane. Necrosis	-	3	1	3
- *Multinu./Giant Cel.:	-	1	3	-
INJECTION SITE 24	3	3	3	3
- Derm. Black Pig. La. M.:	1	1	-	1
- Subcutane. Hemorrhage:	-	2	-	1
- Derm. Infl. Cell. Infil.:	-	1	-	-
- Acanthosis	-	-	-	1
- Dermal Edema	-	1	-	-
- *Subcut. Granul. Infil.:	-	2	3	3
- *Prom. Mono. Cel. Infil.:	-	2	2	1
- *Gran. In. Ar. Eosin. M.:	-	2	1	1
- *Granulation Tissue	-	1	1	2
- *Subcutane. Necrosis	-	2	3	3
- *Subcuta. Fibrosis	-	2	-	1
- *Subcutane. Fibropla.:	-	3	-	1
- Subc. Collag. Degrad.:	-	1	-	-
- Collagen Fibre Baso.:	2	1	-	1
INJECTION SITE 25	3	3	3	3
- Derm. Black Pig. La. M.:	1	1	2	1
- Subcut. Hemorrhage	-	1	-	-
- Folliculitis	1	-	-	-
- Hyperkeratosis	-	1	-	-
- Acanthosis	-	2	-	-
- *Hypod. Granul. Infil.:	-	-	-	2
- *Subcut. Granul. Infil.:	-	2	3	1
- *Granulation Tissue	-	2	3	1
- *Gran. In. Ar. Eosin. M.:	-	2	3	2
- *Hypodermal Fibrosis:	-	-	-	1
- *Subcutane. Fibropla.:	-	1	-	-
- *Hypoderm. Necrosis	-	-	-	1
- *Subcutane. Necrosis	-	2	3	1
- *Multinu./giant Cel.:	-	-	-	2
- *Subcut. Collag. Degr.:	-	1	-	-
INJECTION SITE 26	3	3	3	3
- Derm. Black Pig. La. M.:	-	-	-	2
- Hyperkeratosis	2	-	-	-
- Acanthosis	1	1	-	-
- Subcutane. Hemorrhage:	1	1	-	-
- Collag. Degr. Up. Derm.:	-	1	-	-
- Derm. Inflam. Ce. Infil.:	-	1	-	-
- *Hypod. Granul. Infil.:	-	1	-	2
- *Subcut. Granul. Infil.:	-	2	3	1
- *Prom. Mono. Cel. Infil.:	-	3	-	-
- *Granulation Tissue	-	3	3	2
- *Gran. In. Ar. Eosin. M.:	-	3	3	2
- *Multinu./giant Cel.:	-	1	-	1
- *Hypodermal Fibropl.:	-	-	-	1
- *Subcutane. Fibropla.:	-	1	-	-
- *Subcuta. Fibrosis	-	1	-	-
- *Hypoderm. Necrosis	-	1	-	1
- *Subcutane. Necrosis	-	2	3	1
- Collag. Fibre Basoph.:	1	-	-	-

Best Possible Copy

- Some changes noted in kidneys and lungs are summarized in the following table with a severity from minimal to slight, which may or may not be directly related to the treatment:

Dose, mg/animal/inj.	Sex	0		60		120		360	
		M	F	M	F	M	F	M	F
Kidney	No. Examd	3	3	3	3	3	3	3	3
	Eosino. Tubul.dropl.	0	0	0	0	0	0	2	0
	Cortical vacuo. tubu. Cells	0	0	0	0	0	0	3	0
Lungs	No. Examd	3	3	3	3	3	3	3	3
	Bronchiolitis	0	0	0	0	0	0	1	3
	Perivascular lymph cell aggregates	0	1	1	1	2	2	2	1

Toxicokinetics:

AUC values were 313, 694 and 1711 ng/ml.day in males and 389, 748 and 1236 ng/ml.day in females (week 25).

The non-specific binding (NSB) was evaluated during the study to control analytical interferences. At 60 mg/animal, 1 male and 1 female showed a NSB greater than 30% and a possible start of putative specific antibody response. Furthermore, a total of 5 dogs (1 male and 1 female at LD, 1 female at MD and 2 females at HD) showed NSB values between 10% and 30%. Unexpectedly high serum levels of lanreotide were determined in these dogs probably because of the interaction of lanreotide with circulating antibodies. The dogs which showed NSB greater than 30% and those with NSB values between 10 to 30% were not taken into account in the mean TK parameters calculations because this could artificially alter the mean concentration and TK results. In male dogs, AUC seemed to increase proportionally to the increasing dose level. In female dogs, dose proportionality was lower at high dose levels. As expected (due to the long half life), repeated administration at 14 day intervals seemed to produce a moderate accumulation of lanreotide in male and female dogs. A moderate accumulation ranged from 1.76-3.15 in males and 1.60- 2.55 in females was observed.

Table 2.6.4-19. Pharmacokinetic Values of Repeat Dose s.c. Lanreotide Autogel in Male and Female Dogs (Mean ± SD) (13 Doses at 14 Day Intervals)

Parameter	60 mg/animal		120 mg/animal		360 mg/animal	
	M	F	M	F	M	F
T _{max} , day*	0.167	0.167	0.167	0.334	0.167	0.500
C _{max} , ng/ml	63.244 ± 18.5	79.374	142.439 ± 15.1	138.577 ± 38.502	316.435 ± 59.718	210.366 ± 14.8
C _{max} /D	1.05 ± 0.308	1.323	1.187 ± 0.1260	1.155 ± 0.321	0.879 ± 0.166	0.584 ± 0.0412
AUC _t , ng/ml/day	313 ± 86.8	389	694 ± 206	748 ± 173	1711 ± 162.9	1236 ± 319
AUC _t /D	5.21 ± 1.45	6.49	5.78 ± 1.720	6.23 ± 1.44	4.75 ± 0.453	3.43 ± 0.887
AUC _{t, NSB}	1.00	1.00	2.22	1.92	5.47	3.18
Rac _{t, AUC}	1.76	2.51	3.15	2.55	3.04	1.60

*Median

Best Possible Copy

Histopathology inventory

Study	28223 TCR	28224 TCC
Species	Rat	Dog
Adrenals	x*	x*
Aorta	x	x
Bone Marrow smear	x	x
Bone (femur)	x	x
Brain	x*	x*
Cecum	x	x
Cervix		
Colon	x	x
Duodenum	x	x
Epididymis	x*	x*
Esophagus	x	x
Eye	x	x
Fallopian tube		
Gall bladder	x	x
Gross lesions	x	x
Harderian gland		
Heart	x*	x*
Ileum	x	x
Injection site	x	x
Jejunum	x	x
Kidneys	x*	x*
Lachrymal gland		
Larynx		
Liver	x*	x*
Lungs	x	x
Lymph nodes, cervical		
Lymph nodes mandibular	x	x
Lymph nodes, mesenteric	x	x
Mammary Gland	x	x
Nasal cavity		
Optic nerves	x	x
Ovaries	x*	x*
Pancreas	x	x
Parathyroid	x	x
Peripheral nerve	x	x
Pharynx		
Pituitary	x	x
Prostate	x	x
Rectum	x	x
Salivary gland	x	x
Sciatic nerve	x	x
Seminal vesicles		
Skeletal muscle	x	x
Skin	x	x
Spinal cord	x	x
Spleen	x*	x*
Sternum	x	x
Stomach	x	x
Testes	x*	x*
Thymus	x*	x*
Thyroid	x*	x*
Tongue	x	x
Trachea	x	x
Urinary bladder	x	x
Uterus	x	x
Vagina	x	x
Zymbal gland		

x, histopathology performed
 *, organ weight obtained

2.6.6.4 Genetic toxicology

All the resubmitted genotox study reports with BIM 23014 (lanreotide acetate) listed below have been reviewed previously under _____

In vitro:

- Study to determine the ability of SAB 2335 (BIM 23014) to induce mutation in four histidine-requiring strains of *Salmonella typhimurium* (IPS 4/S S2)
- Mutagenicity test on bacteria (*Salmonella typhimurium* his- and *E. coli* trp-) using B:N. Ames' techniques with BIM 23014 (IPL-R 930514)
- Study to determine the ability of SAB 2335 to induce mutation to 6-thioguanine resistance in mouse lymphoma L5178Y cells using a fluctuation assay (IPS 4/ML)
- Study to evaluate the chromosome damaging potential of SAB 2335 by its effects on cultured human lymphocytes using an in vitro cytogenetics assay (IPS 4/HLC)
- Test for chromosome aberrations by in vitro human lymphocyte metaphase analysis on the compound BIM 23014 (IPL-R 930406)

In vivo:

- Investigations on the influence of BIM 23014 on the testicular DNA synthesis and repair in the mouse (23014 Mu 6R)
- Study to evaluate the potential of SAB 2335 to induce micronuclei in the polychromatic erythrocytes of CD-1 mice (IPS 4/MNT)

The following five in vitro and in vivo genotox studies reviewed are "new studies" with lanreotide acetate (the new manufacturer/formulation) submitted under current NDA.

Study title: Lanreotide acetate: reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and one tryptophan-requiring strain of *Escherichia coli*

Key findings: Lanreotide acetate did not induce mutation in four histidine-requiring strains of *Salmonella typhimurium* and one tryptophan-requiring strain of *E. coli* when tested under the conditions of this assay at concentrations up to 5000 µg/plate (a precipitating dose) in the absence and in the presence of S-9 activation.

Study no.: 434/85

Volume #, and page #: Vol A3.53, and pages 1-60

Conducting laboratory and location: _____

Date of study initiation: 02/04/2002

GLP compliance: yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: Lanreotide acetate, batch #KO12 (Ipsen Pharma Biotech)/M11286 (Dreux), and purity —

Methods

Strains/species/cell line: Four strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and one strain of *E. coli* (WP2 uvrA).

Doses used in definitive study:

Experiment	S-9	Concentration of treatment solution (mg/mL)	Final concentration (µg/plate)
Range-finder Experiment and Mutation Experiment 1	- and +	0.016	1.6
		0.08	8
		0.40	40
		2.00	200
		10.00	1000
		50.00	5000
Mutation Experiment 2	and +	0.2048*	20.48
		0.512*	51.2
		1.28*	128
		3.20*	320
		8.00*	800
		20.00*	2000
		50.00*	5000

* Concentration of treatments solutions used for the Experiment 2 pre-incubation treatments were twice that stated above, in order to permit treatments at the final concentration stated, whilst volume additions were reduced to 0.05 mL.

Basis of dose selection: In an initial toxicity range-finding study in strain TA100 using concentration of 1.6, 8, 40, 200, 1000 and 5000 µg/plate, plus solvent and positive controls, evidence of toxicity manifested by a slight thinning of the background lawn and marked decrease in revertant numbers was observed at the top dose 5000 µg/plate with and without S9 activation, and precipitation of the test compound was also observed at this dose level. In both experiments 1 and 2, HD 5000 µg/plate was retained.

Negative controls: The solvent DMSO

Positive controls:

**APPEARS THIS WAY
ON ORIGINAL**

Chemical	Source	Stock * concentration (µg/mL)	Final concentration (µg/plate)	Use Strain(s)	S-9
2-nitrofluorene (2NF)		50	5.0	TA98	-
Sodium azide (NaN ₃)		20	2.0	TA100, TA1535	-
9-aminoacridine (AAC)		500	50.0	TA1537	-
4-nitroquinoline 1-oxide (NQO)		20	2.0	WP2 uvrA	-
Benzo[a]pyrene (B[a]P)		100**	10.0	TA98	+
2-aminoanthracene (AAN)		50**	5.0	TA100, TA1535,	+
		100**	10.0	TA1537 WP2 uvrA	+

* With the exception of NaN₃, which was prepared in water, all stock solutions were prepared in sterile anhydrous analytical grade dimethyl sulphoxide (DMSO). All stock solutions were stored in aliquots at 1-10°C in the dark, with the exception of B[a]P and NQO which were stored in aliquots at -80°C in the dark.

** For Experiment 2 pre-incubation treatments, stock solutions of these positive control compounds were twice the concentration stated. This enabled the volume additions to be reduced to 0.05 mL (thus avoiding solvent-induced toxicity) and achievement of the final concentrations per plate as detailed above.

Incubation and sampling times: Pre-incubation for 1 hr at 37°C for the treatment in the presence of S9 was included. Then the normal plate-incorporation procedure was conducted and 2-day incubation at 37±1°C was carried out.

Results

Study validity:

The assay was considered valid if the following criteria were met:

- the mean negative control counts fell within the normal ranges as provided with the study report
- the positive control chemicals included clear increases in revertant numbers confirming discrimination between different strains
- no more than 5% of the plates were lost through contamination or some other unforeseen event

The test compound was considered to be mutagenic if:

- the assay was valid (see above)
- Dunnetts test gave a significant response ($p \leq 0.01$) and the data set(s) showed a significant dose correlation
- the positive responses were reproducible

Colonies were counted electronically using a  Colony Counter or manually where confounding factors such as split agar affected the accuracy of the automatic counter.

Best Possible Copy

All criteria mentioned above are met and the study is considered valid.

Study outcome:

No lanreotide acetate treatment of any of the test strains in any of the experiments including the range finding study resulted in a statistically significant increase in revertant numbers. The mean colony counts from both experiments 1 and 2 in the condition with and without S9 activation are summarized in the following scanned tables.

Lanreotide Acetate: Summary of mean revertant colonies (-S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	WP2 uvrA
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 µl	38 ± 8	107 ± 7	18 ± 7	6 ± 1	10 ± 3
Lanreotide Acetate	1 G	42 ± 1	106 ± 8	18 ± 3	7 ± 1	10 ± 3
	8	32 ± 7	110 ± 11	17 ± 2	9 ± 1	7 ± 4
	40	34 ± 4	111 ± 14	15 ± 3	6 ± 3	10 ± 3
	200	41 ± 7	112 ± 7	17 ± 7	6 ± 3	9 ± 3
	1000	43 ± 1	103 ± 7	19 ± 4	11 ± 3	12 ± 9
	5000	24 ± 4 (S, Ppt)	25 ± 2 (S+ Ppt)	22 ± 1 (Ppt)	(1+ Ppt)	12 ± 5 (Ppt)
Positive controls	Compound	2NF	NaN ₃	NaN ₃	AAC	NQO
	Dose Level	5 µg	2 µg	2 µg	50 µg	2 µg
	Mean ± SD	494 ± 25	555 ± 15	424 ± 30	109 ± 9	346 ± 26

- SD Standard deviation
- 2NF 2 Nitrofluorene
- NaN₃ Sodium azide
- AAC 2 Acetylaminofluorene
- NQO 4 Nitroquinoline 1-oxide
- S Slight thinning of background lawn
- Ppt Precipitation of test article observed
- 1 Toxic no revertant colonies

Best Possible Copy

Lanreotide Acetate: Summary of mean revertant colonies (+S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	WP2 uvrA
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 µl	46 ± 3	108 ± 8	21 ± 4	9 ± 5	9 ± 1
Lanreotide Acetate	1.6	40 ± 6	105 ± 8	23 ± 1	8 ± 1	7 ± 3
	8	43 ± 6	105 ± 7	24 ± 6	12 ± 4	13 ± 2
	40	42 ± 1	100 ± 6	15 ± 5	11 ± 3	8 ± 3
	200	45 ± 12	99 ± 11	18 ± 6	8 ± 4	10 ± 4
	1000	38 ± 4	103 ± 3	15 ± 2	11 ± 2	10 ± 1
	5000	30 ± 4 (S- Ppn)	45 ± 5 (S+ Ppn)	11 ± 1 (Ppn)	11 ± 1 (1- Ppn)	12 ± 5 (Ppn)
Positive controls	Compound	B[a]P	AAN	AAN	AAN	AAN
	Dose Level	10 µg	5 µg	5 µg	5 µg	10 µg
	Mean ± SD	311 ± 14	1472 ± 62	224 ± 11	139 ± 17	181 ± 36

SD Standard deviation
 B[a]P Benzo[a]pyrene
 AAN 2-Aminoanthracene
 S Slight thinning of background lawn
 Ppn Precipitation of test article observed
 T Toxic, no revertant colonies

Best Possible Copy

**APPEARS THIS WAY
ON ORIGINAL**

Lanreotide Acetate: Summary of mean revertant colonies (-S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	WP2 uvrA
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 µl	31 ± 10	145 ± 8	23 ± 5	8 ± 4	15 ± 2
Lanreotide Acetate	20.48	35 ± 6	124 ± 16	21 ± 2	9 ± 1	12 ± 4
	51.2	27 ± 6	110 ± 16	20 ± 2	8 ± 3	12 ± 3
	128	33 ± 2	102 ± 9	17 ± 1	12 ± 1	15 ± 4
	320	35 ± 3	128 ± 12	18 ± 12	11 ± 6	13 ± 3
	800	27 ± 9	135 ± 25	20 ± 3	11 ± 4	11 ± 6
	2000	36 ± 7	114 ± 7	15 ± 8	7 ± 1 (S)	11 ± 5
	5000	14 ± 2 (S + Ppn)	- (T + Ppn)	17 ± 4 (Ppn)	- (T + Ppn)	9 ± 1 (Ppn)
Positive controls	Compound	ZNF	NaN ₃	NaN ₃	AAC	NQO
	Dose Level	5 µg	2 µg	2 µg	50 µg	2 µg
	Mean ± SD	608 ± 41	765 ± 16	511 ± 29	212 ± 17	539 ± 25

Best Possible Copy

- SD Standard deviation
- ZNF 2 Nitrofluorene
- NaN₃ Sodium azide
- AAC 9 Aminoacridine
- NQO 4 Nitroquinoline 1 oxide
- S Slight thinning of background lawn
- Ppn Precipitation of test article observed
- T Toxic, no revertant colonies

**APPEARS THIS WAY
ON ORIGINAL**

Lanreotide Acetate: Summary of mean revertant colonies (+S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	WPZ <i>uvrA</i>
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	50 µl	37 ± 4	124 ± 10	22 ± 6	12 ± 1	14 ± 6
Lanreotide Acetate	20.48	35 ± 5	120 ± 17	20 ± 1	14 ± 7	12 ± 7
	51.2	39 ± 10	114 ± 15	16 ± 3	15 ± 3	11 ± 2
	128	40 ± 13	122 ± 9	13 ± 5	15 ± 5	8 ± 3
	320	29 ± 3	117 ± 19	21 ± 2	13 ± 3	12 ± 4
	800	23 ± 4	102 ± 9	23 ± 6	9 ± 3	11 ± 3
	2000	28 ± 11 (S+ Ppa)	87 ± 12 (S+ Ppa)	15 ± 1 (Ppa)	(T- Ppa)	9 ± 1 (Ppa)
	5000	(T+ Ppa)	(T- Ppa)	13 ± 1 (S+ Ppa)	(T+ Ppa)	12 ± 2 (S+ Ppa)
Positive controls	Compound	B[a]P	AAN	AAN	AAN	AAN
	Dose Level	10 µg	5 µg	5 µg	5 µg	10 µg
	Mean ± SD	328 ± 64	1069 ± 69	132 ± 10	223 ± 18	33 ± 2

Best Possible Copy

SD Standard deviation

B[a]P Benz[a]pyrene
AAN 2-Aminoanthracene

S Slight thinning of background lawn
Ppa Precipitation of test article observed
? Toxic, no revertant colonies

Study title: Lanreotide acetate: mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells using the microtiter fluctuation technique

Key findings: When tested up to toxic and precipitating concentrations, lanreotide acetate did not induce mutation at the tk locus of L5178Y mouse lymphoma cells in two independent experiments, in the absence or presence of S-9. It is concluded that, under the conditions conducted in this study, lanreotide acetate is not mutagenic in the absence or presence of S-9 activation.

Study no.: — 434/87

Volume #, and page #: Vol A3.53, and pages 1 to 59

Conducting laboratory and location: _____

Date of study initiation: 01/25/2002

GLP compliance: yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: Lanreotide acetate, batch # M11286, and purity

Methods

Strains/species/cell line: L5178Y TK^{+/+} mouse lymphoma cells

Doses used in definitive study: Doses used in range finding, mutation experiments 1 and 2 are given in the following scanned table:

Experiment	S-9	Treatment period (hrs)	Concentration of treatment solution (mg/mL)	Final concentration (µg/mL)
Range-finder	- and +	3	5.469	54.69
			10.94	109.4
			21.88	218.8
			43.75	437.5
			87.50	875
			175.0	1750
			0.6836	6.836
	-	24	1.367	13.67
			2.734	27.34
			5.469	54.69
			10.94	109.4
			21.88	218.8
			43.75	437.5
			87.50	875
1	- and +	3	10.0	100
			20.0	200
			40.0	400
			80.0	800
			120.0	1200
			140.0	1400
			160.0	1600
	-	24	10.0	100
			20.0	200
			40.0	400
			60.0	600
			80.0	800
			90.0	900
			100.0	1000
2	-	24	120.0	1200
			150.0	1500
			20.0	200
			40.0	400
			80.0	800
			100.0	1000
			120.0	1200
	-	3	140.0	1400
			160.0	1600
			20.0	200
			40.0	400
			80.0	800
			100.0	1000
			120.0	1200

Best Possible Copy

Basis of dose selection: Cytotoxicity range finding studies were performed in the absence and presence of S9 activation under 3 hr or 24 hr treatment.

For 3 hr treatment, six dose levels were tested from 54.69 to 1750 µg/ml (limited by solubility). Precipitate was observed at 1750 µg/ml and 875 µg/ml. Extreme toxicity (<10% relative survival) was observed at the highest dose tested (1750 µg/ml) in the absence and presence of S9. The highest dose yielded >10% relative survival was 875 µg/ml, which yielded 52% and 77% relative survival in the absence and presence of S9, respectively. The raw plate counts and relative survival values are shown in the scanned Table 1 below.

Table 1
Lanreotide Acetate: raw plate counts and adjusted relative survival
in the cytotoxicity range-finder (3 hour treatment)

Treatment (µg/mL)	In the absence of S-9					In the presence of S-9				
	Day 0 cells x 10 ⁵	Survival% (Day 0)	%CE (Day 0)	%S	%RS	Day 0 cells x 10 ⁵	Survival% (Day 0)	%CE (Day 0)	%S	%RS
0	5.15	73	89.30	89.30	100.00	5.17	72	86.64	86.64	100.00
54.69	5.32	62	64.87	67.02	75.04	5.15	75	94.99	94.62	109.21
109.4	4.55	77	101.24	89.45	100.16	4.94	68	77.01	73.58	84.93
218.8	4.88	80	111.98	106.11	118.82	5.13	75	94.99	94.25	108.78
437.5	4.42	71	84.09	72.17	80.82	5.12	70	81.64	80.85	93.31
875A	4.92	52	48.76	46.58	52.16	5.05	64	68.66	66.80	77.10
1750PA	3.20	0	0.00	0.00	0.00	4.77	5	3.34	3.08	3.56

%CE % Day 0 cloning efficiency
 %S % Day 0 survival adjusted to take account of loss of cells during treatment
 § Positive wells per plate. 96 wells scored unless otherwise stated e.g. 52/95
 § 1.6 cells/well plated for survival.
 %RS Percent relative survival adjusted by post treatment cell counts
 P Precipitate observed at the time of treatment
 A Precipitate observed at the end of the treatment incubation period (observed in the presence of S-9 only at 875 µg/ml.)

For 24 hr treatment range finding study, nine dose levels were tested in the absence of S9 from 6.836 to 1750 µg/ml (limited by solubility). Following the 24 hr treatment incubation, precipitate was observed at 1750 µg/ml. Complete toxicity was observed at the highest dose tested (1750 µg/ml). The highest dose yielded 10% relative survival was 875 µg/ml. The raw plate counts and relative survival values are shown in the scanned Table 2 below.

Table 2

Lanreotide Acetate: raw plate counts and adjusted relative survival in the cytotoxicity range-finder (24 hour treatment)

Treatment (µg/mL)	In the absence of S-9				
	Day 0 cells x 10 ³	Survival * (Day 0)	%CE (Day 0)	%S	%RS
0	5.59	73	89.30	89.30	100.00
6.836	5.14	73	89.30	82.11	91.95
13.67	5.88	66	72.70	76.47	85.63
27.34	5.73	70	81.64	83.69	93.71
54.69	4.75	77	101.24	86.03	96.34
109.4	4.83	71	84.09	72.66	81.36
218.8	4.47	73	89.30	71.41	79.96
437.5	3.52	73	89.30	56.23	62.97
875	2.10	31	24.37	9.16	10.25
1750PA	1.56	0	0.00	0.00	0.00

Best Possible Copy

§ Positive wells per plate. 96 wells scored unless otherwise stated. eq. 52.95
1.6 cells/well plated for survival.
%RS Percent relative survival adjusted by post treatment cell counts
P Precipitate observed at the time of treatment
A Precipitate observed at the end of the treatment incubation period

Based on these data, top dose 1800 or 1500 µg/ml was used in definitive studies 1 or 2, respectively.

Negative controls: The solvent DMSO

Positive controls:

Chemical	Source	Stock* concentration (µg/mL)	Final concentration (µg/mL)	S-9
4-nitroquinoline 1-oxide (NQO)	/ /	15#	0.15 #	-
		20#	0.20 #	-
benzo(a)pyrene (BP)	/ /	200	2.00	+
		300	3.00	+

* All solutions were prepared in anhydrous analytical grade dimethyl sulphoxide (DMSO). BP and NQO stock solutions were stored as frozen aliquots at -80°C in the dark.
For Experiment 2 -S-9 (24 hour treatment), stock solutions of NQO were 2 and 4 µg/ml. to give final concentrations of 0.02 and 0.04 µg/mL, respectively.

Incubation and sampling times: 3 hr treatment with and without S9 for Experiment 1; 24 hr treatment without S9 and 3 hr treatment with S9 for Experiment 2.

Results

Study validity:

The assay would be considered valid if all the following criteria were met:

- the mutant frequencies in the negative control cultures fell within the normal range (above 60 mutants per 10^6 viable cells but not more than three times the historical mean value)
- at least one concentration of each of the positive controls induced a clear increase in mutant frequency (the difference between the positive and negative control mutant frequencies was greater than half the historical mean value)
- the plating efficiencies of the negative controls from the mutation experiments were between the range of 60% to 140% on Day 0 and 70% to 130% on Day 2.

The test article would be considered to be mutagenic if all the following criteria were met:

1. the assay was valid according to the above mentioned validity criteria
2. the mutant frequency at one or more doses was significantly greater than that of the negative control ($p < 0.05$)
3. there was a significant dose-relationship as indicated by the linear trend analysis ($P < 0.05$).

Study outcome:

Based on the validity criteria and study results, the assay is deemed valid.

A summary of the results for Experiments 1 and 2 is shown in the scanned Table 3 below. No statistically significant increases in mutant frequency were observed following treatment with lanreotide acetate at any dose level tested in the absence or presence of S9 in the two definitive studies.

It is noted that in Experiments 1 and 2, more than one precipitating dose was tested in the absence and presence of S9. Some of these precipitating doses were extremely toxic (i.e. ↓ survival) and were not plated out, but in a number of cultures in both experiments (particularly in the presence of S9), precipitate was observed only at the end of the treatment incubation period, according to the report. However, for all treatment regimens in both experiments, a minimum of four doses (including the lowest precipitating dose) were analyzed in accordance with the requirements of the protocol, hence the validity of the study was not prejudiced.

Table 3
Lanreotide Acetate: summary of results

Experiment 1 (3 hour treatment +/- S-9)

Treatment (µg/mL)	-S-9				Treatment (µg/mL)	-S-9			
	%RS	RTG	MF§			%RS	RTG	MF§	
0	100.00	1.00	85.47		0	100.00	1.00	90.53	
100	82.36	1.06	79.37	NS	100	125.39	1.04	78.39	NS
200	89.05	1.11	72.82	NS	200	114.50	0.98	77.10	NS
400	84.04	1.15	71.15	NS	400	114.38	1.02	87.03	NS
800	60.39	0.89	85.71	NS	800A	106.36	0.89	75.64	NS
1200A	2.09	0.08	106.15	NS	1200A	20.64	(0.29)	(87.45)	
1400PA §	0.87				1400PA	10.48	0.07	115.59	NS
1600PA §	0.08				1600PA §	2.18			
1800PA §	0.13				1800PA §	0.00			
Linear trend				NS	Linear trend				NS
NQO					BP				
0.15	69.43	0.86	395.82		2	87.84	0.50	588.94	
0.2	53.70	0.86	397.00		3	43.91	0.45	828.93	

Experiment 2 (24 hour treatment - S-9, 3 hour treatment + S-9)

Treatment (µg/mL)	-S-9				Treatment (µg/mL)	-S-9			
	%RS	RTG	MF§			%RS	RTG	MF§	
0	100.00	1.00	109.84		0	100.00	1.00	125.37	
100	80.95	1.22	72.67	NS	200	88.23	0.96	101.87	NS
200	76.39	1.31	91.37	NS	400	88.43	0.98	99.72	NS
400	66.35	1.15	87.95	NS	800	79.23	0.84	125.14	NS
600	42.21	0.74	91.89	NS	1000A	38.60	0.52	117.96	NS
800	18.14	0.27	103.40	NS	1200A	17.91	0.21	118.08	NS
900 X	0.89	0.13	#		1400PA X	5.78	0.03	#	
1000 X	1.53	0.04	#		1600PA X	3.50	0.01	#	
1200A §	0.10								
1500PA §	0.00								
Linear trend				NS	Linear trend				NS
NQO					BP				
0.02	55.20	1.12	282.47		2	64.25	0.72	674.70	
0.04	84.83	1.00	401.03		3	74.74	0.46	651.21	

- § 5-TFT resistant mutants/10⁶ viable cells 2 days after treatment
- %RS Percent relative survival adjusted by post treatment cell counts
- § Not plated for viability - 5-TFT resistance
- §§ Treatment excluded from analysis due to excessive heterogeneity
- ! Data in parentheses indicates marked heterogeneity observed
- ! Based on one replicate only
- X Treatment excluded from final test statistics due to excessive toxicity
- # Mutant frequency not reported; cultures yielded < 10% RS, but 10-20% RS achieved at a lower dose
- NS Not significant
- P Precipitate observed at the time of treatment
- A Precipitate observed at the end of the treatment incubation period

Best Possible Copy