CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125390Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

From: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
BLA: 125390
Agency receipt date: December 16, 2010
Drug: metreleptin
Sponsor: Amylin Pharmaceuticals, LLC (Bristol-Myers Squibb Company)

Indication: adjunct to diet as replacement therapy to treat the complications of leptin deficiency in patients with congenital or acquired generalized lipodystrophy

Reviewing Division: Division of Metabolism and Endocrinology Products

Introductory Comments: The pharm/tox reviewer and supervisor concluded that the nonclinical data support approval of metreleptin for the indication listed above.

The recommended pharmacologic class for metreleptin is leptin analog.

Fertility, embryofetal development and pre/postnatal development studies of metreleptin were conducted in mice. While no malformations were observed in fetuses, an increase in dystocia and prolonged gestation was noted at clinically relevant doses. The division recommended describing these findings in labeling. Biological plausibility of this effect is supported by published data showing leptin inhibition of uterine contractility in vitro.

Carcinogenicity studies of metreleptin were not conducted. The reviewer summarized literature that shows that leptin may play a role in tumor promotion or progression. The reviewer and supervisor suggest some wording for labeling that mentions this literature.

Conclusions:
I agree with the division pharm/tox conclusion that metreleptin can be approved from the pharm/tox perspective. Description in labeling of the dystocia induced by metreleptin in mice and of the potential role of leptin in tumor promotion and progression seems appropriate. I have provided other comments on labeling separately.

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/s/

PAUL C BROWN
02/21/2014

Memorandum

SUPERVISOR MEMO

Date:	05 Dec 2013
RE:	BLA 125390
Sponsors:	BMS (Amylin Pharmaceuticals)
Drug	Metreleptin

Amylin Pharmaceuticals is seeking marketing approval for metreleptin, proposed trade name Myalept, as a treatment of metabolic disorders associated with inherited or acquired lipodystrophy in the pediatric and adult populations. Metreleptin is a 147-amino acid analog of human leptin, differing from mature, circulating human leptin by the addition of a single amino terminal methionyl group. If approved, metreleptin would be a first-in-class treatment option for the inherited/acquired lipodystrophic orphan population.

Generalized lipodystrophy is characterized by a partial or severe loss of adipose tissue. Excessive deposition of lipid at alternate sites, particularly the liver and muscle, contributes significantly to the severe metabolic phenotype of lipodystrophy (e.g., insulin resistance, hypertriglyceridemia). Leptin levels are low in this condition, with the degree of leptin deficiency roughly scaling with the degree of adipose loss in the individual. Treatment with metreleptin is thus intended as replacement therapy, but post-treatment levels of metreleptin can exceed the normal range of leptin in many cases, due to a higher baseline level of leptin (e.g., in partial lipodystrophy) or to drug accumulation secondary to complexing with anti-drug antibodies.

Dr. Basso, the primary nonclinical reviewer, concludes that the pharmacology and toxicology data support approval of metreleptin. *I concur with Dr. Basso's assessment.*

Most of the toxicology studies were completed in the 1990s when the clinical program was focused on an obesity indication. In normal mice and dogs, administration of metreleptin for up to 6 months severely reduced food intake and gain of body weight, with higher doses resulting in poor body condition. All other adverse findings in these studies were considered secondary to emaciation. These studies are more relevant to the obese population than to the leptin-deficient clinical lipodystrophy population; nonetheless, the results did not identify any major risks when the clinical program expanded to include lipodystrophy. Of note, metreleptin monotherapy appears to be ineffective for weight loss in the obese clinical population.

The carcinogenic potential of metreleptin was based on the assessment of proliferation markers in the chronic toxicology studies, and was further informed by the published literature. Two-year rodent studies were not required to support the BLA for lipodystrophy. This decision was based

primarily on the intent of correcting the leptin-deficient state of the clinical population (i.e., hormone replacement), and also on the scope of available relevant literature and on potential confounding by anti-drug antibody responses in rodents. No neoplastic or pre-neoplastic lesions were observed in the chronic mouse and dog studies, and PCNA staining failed to show evidence of active proliferation in a panel of target tissues. Being a synthetic peptide, metreleptin tested negative in a battery of genotoxicity assays. Based on these findings in normal, healthy animals, metreleptin did not exhibit carcinogenic activity. There is, however, an extensive literature on leptin's role in cell growth, immunity, and cancer. There is no evidence indicating that metreleptin's pharmacodynamic activity differs in any meaningful way from endogenous leptin, and therefore the literature on endogenous leptin is considered entirely relevant. As discussed in more detail in Dr. Basso's review and in FDA's advisory committee briefing document, a fair amount of evidence demonstrates that leptin promotes proliferative and anti-apoptotic responses in human cancer cells *in vitro* and *in vivo*, and that leptin is permissive in support of lymphopoiesis and autoimmunity.

The sponsor recognizes that there is a theoretical possibility that exogenous leptin could act as a tumor promoter, but insists that no evidence supports such a conclusion. A theoretical risk remains a risk nonetheless, and the occurrence of several cases of lymphoma in the clinical program clouds the sponsor's latter proclamation of 'no evidence'. We recommend that Myalept's drug label include a statement recognizing the pertinent literature regarding leptin and cancer biology.

I support Dr. Basso's recommended wording for Section 13:

> *Two-year carcinogenicity studies in rodents have not been conducted with metreleptin. No proliferative or pre-neoplastic lesions were observed in mice or dogs following treatment up to six months. However, leptin is reported in the literature to promote cell proliferation in vitro and tumor progression in some mouse models of cancer.*
>
> *Metreleptin was not mutagenic with or without metabolic activation in the Ames bacterial mutagenicity assay or in an in vitro chromosomal aberration assay in human peripheral blood lymphocytes. Metreleptin was not mutagenic or clastogenic in an in vivo micronucleus assay in mice.*

The reproductive and developmental toxicology studies identified prolonged or difficult labor (dystocia) in pregnant mice administered sub-clinical doses of metreleptin. This led to deaths of some dams and low survival of pups within the first few days post-partum. Dr. Basso cited a publication by Moynihan et al[1] that leptin can inhibit human uterine contractility in vitro, providing at least a plausible basis for the dystocia observed in mice. Infertility can be part of the clinical sequelae of lipodystrophy, and it is feasible that fertility and pregnancies may be more common after improvement in metabolic status with metreleptin therapy. Whether a clinical risk of dystocia exists might depend on the prevailing serum level of metreleptin in the individual, with higher risk tracking with higher, supraphysiological serum levels of metreleptin. The clinical risk, if any, would need to be considered against the potential consequences of worsening metabolic status should metreleptin be discontinued during (late) pregnancy.

[1] Moynihan AT, et al. (2006) Am J Obstet Gynecol. 195, 504-509.

I agree with Dr. Basso's recommendation of Pregnancy Category C, stating in Section 8: '*Based on results from mouse studies, metreleptin may result in dystocia or prolonged gestation. Metreleptin should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus*.'

Lastly, it is worth recognizing that metreleptin has proven to be highly immunogenic in rats, dogs, and humans. Given its one-amino acid difference from endogenous leptin, such immunoreactivity is astonishing. Nearly all rats and dogs (and humans) develop metreleptin-binding antibodies after repeated exposure to metreleptin. Potential neutralizing activity in rats and dogs was not evaluated but, if present, did not appear to ablate pharmacological activity of metreleptin, evidenced by a consistent change in body weight over time. The plasma level of metreleptin, however, increased rather dramatically upon repeated exposure, a consequence of reduced clearance of ADA complexes with metreleptin. In general, the nature and impact of anti-drug antibody responses, including neutralizing activity, in the clinical population cannot be reliably predicted by immune responses observed in rats and dogs exposed to metreleptin. Substantial neutralization of metreleptin and residual endogenous leptin by a neutralizing ADA response in lipodystrophic patients could have potential clinical consequences, such as worsening of metabolic status and compromised immunologic capacity, in addition to a potential loss of efficacy.

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/s/

TODD M BOURCIER
12/06/2013
Pharm/tox supports approval

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

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	125390
Supporting document/s:	Original submission
Applicant's letter date:	December 16, 2010
CDER stamp date:	December 16, 2010
Product:	Metreleptin/Myalept
Indication:	Diabetes mellitus and/or hypertriglyceridemia in pediatric and adult patients with lipodystrophy
Applicant:	Bristol-Myers Squibb
Review Division:	Division of Metabolic and Endocrine Products
Reviewer:	Federica Basso
Supervisor/Team Leader:	Todd Bourcier
Division Director:	Jean-Marc Guettier
Project Manager:	Pat Madara

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1 Executive Summary

1.1 Introduction

Metreleptin is a recombinant analog of native human leptin, differing from the native molecule by a single methionyl group added to the amino-terminal end. Leptin is a naturally occurring hormone predominantly secreted by adipose tissue that plays a central role in the neurohormonal regulation of energy homeostasis and fat and glucose metabolism.

The sponsor proposes metreleptin for the treatment of metabolic disorders associated with lipodystrophy, including diabetes and/or hypertriglyceridemia in *pediatric* and *adult* patients with inherited or acquired lipodystrophy.

1.2 Brief Discussion of Nonclinical Findings

Pivotal non-clinical studies were conducted in mice and dogs using subcutaneous administration of metreleptin. Safety margins have been estimated based on body surface area (BSA) and on Day 1 AUC, given that the presence of anti-drug antibodies interfered with toxicokinetic analysis in the chronic animal studies. Clinical AUC was estimated from a clinical study conducted in healthy subjects, as pharmacokinetic parameters were not analyzed in lipodystrophy patients.

		BSA basis		**AUC basis**	
Species, study duration	NOAEL (mg/kg)	NOAEL (mg/m^2)	Safety margins*	AUC (ng.h/mL)	Safety margins**
Mice, 6-month	1	3	<<1x	563	<<1x
Dog, 6-month	1.5	30	2x	6457	4x

* Based on human dose of 10mg/day or $12.5mg/m^2$ in a 20Kg subject, using allometric factor of 25

** Based on Day 1 AUC of 1770ng.h/mL in healthy subjects (Study LEPT-950272)

Pharmacology

Pharmacology studies were conducted by the sponsor in normal and transgenic mouse models of obesity, including the leptin-deficient obese ob/ob mice. These mice share a similar metabolic profile and similar leptin deficiency (ob/ob mice) with the lipodystrophic mouse. However, none of these rodent models exhibit total or partial loss of adipose tissue, which is characteristic and causative of the phenotype in lipodystrophic disease, and which result in more severe metabolic imbalances compared to that observed in

ob/ob mice[1]. Therefore, results with the obese rodent models are of limited usefulness in understanding the effects of metreleptin in lipodystrophic metabolic disorders.

Several studies investigating the effect of recombinant leptin (but not metreleptin) in lipodystrophic mice have been published by independent groups. These studies show that administration of recombinant murine leptin corrects hyperphagia, decreases body weight and almost completely rescues insulin resistance and diabetes in several models of lipodystrophic mice. Higher leptin doses were required to achieve efficacy in the more severe lipodystrophic mice, characterized by total loss of adipose tissue, compared to lipodystrophic mice with some residual amount of fat. The mechanism by which recombinant leptin improves insulin sensitivity and plasma glucose may extend beyond simply reducing food intake and body weight. Indeed, food restriction alone surprisingly did not significantly lower plasma insulin and glucose in lipodystrophic mice.

Metreleptin did not adversely affect the cardiovascular, pulmonary, CNS, renal, and gastrointestinal systems following acute administration in a standard battery of safety pharmacology studies in mice and rats. In addition, no treatment related adverse effects in cardiovascular function were observed in the chronic dog study.

General toxicity
Pivotal toxicology studies were conducted in CD1 mice and Beagle dogs in studies up to 6 months duration. These studies administered metreleptin produced by Amgen manufacturing facilities in the 1990s.The predominant finding in both species was a marked, dose-related decrease in food intake and body weight consistent with the pharmacodynamic activity of metreleptin. Excessive body weight loss resulted in a few deaths, most notably in mice following a scheduled overnight fasting, due to poor nutritional status and body state. Changes in serum chemistry in mice and dogs (increased red cell mass, urea, cortisol, and decreased albumin and total protein) and histopathology findings of gastric erosions, lympohocytolysis, and injection site cellulitis in mice were also most likely secondary to the anorexigenic effect of metreleptin. Clinical condition improved upon dose cessation which correlated with a quick rebound in food intake. Multi-organ perivasculitis following the first month of dosing and chronic plasmacytic vasculitis thereafter was observed in dogs, likely related to antidrug antibody or to leptin-mediated modulation of the inflammatory/immune response[2].

Anti-drug antibody developed within two weeks of dosing in both species. The ADA did not result in a loss of pharmacological activity, but did increase plasma levels and exposure by decreasing metreleptin clearance.

Genotoxicity
Metreleptin (manufactured by Amgen and Sandoz) was not mutagenic in a battery of genetic toxicology studies including the Ames bacterial assay, a mammalian cell mutagenicity assay, and an *in vivo* mouse micronucleus study.

Carcinogenicity
Two year carcinogenicity studies in mice and rats were not required to support the lipodystrophy indication. No pre-neoplastic lesions were observed in the chronic mouse and dog studies, and immunostaining for the proliferation marker PCNA, conducted in several tissues in the mouse 28-day and the dog 6-month toxicity studies, did not show a proliferative response to metreleptin in any of the tissues examined. However, there is compelling evidence from the literature suggesting that leptin may promote tumor progression by stimulating cell proliferation and angiogenesis, and by inhibiting apoptosis [3-6]. In vitro studies demonstrate that leptin increases cell proliferation and decreases apoptosis in several cancer cell lines, including breast, colon, prostate, ovary, thyroid and hematopoietic cell lines[4]. In vivo studies show that leptin is associated with mammary tumor development and progression. Leptin deficient mice do not develop transgene-induced mammary tumors and leptin treatment increases tumor size by 100% in a mouse model of mammary neoplasia[7-9]. Knock-down of leptin receptor or administration of a leptin receptor antagonist decreased tumor growth in several mouse models of breast cancer[10-12]. Conversely, lower leptin levels in lipodystrophic mice are associated with an increased incidence of mammary tumors and decreased tumor latency[13], suggesting that a lipodystrophic background might influence leptin's pro-tumorigenic activity identified in other studies. The same lipodystrophic mouse model also showed increased susceptibility to chemically-induced skin tumors compared to both wild type and ob/ob mice[14], additionally suggesting that susceptibility to tumorigenesis is higher in lipodystrophic mice independent of leptin status.

Conflicting data were, however, reported in the public literature on the contribution of leptin to colon, lung, pancreas, and prostate cancers.

It is not known whether metreleptin modulates cancer risk in lipodystrophic patients, as metreleptin administration to lipodystrophic subjects raises leptin levels to just above physiological levels (25-30ng/ml). Furthermore, leptin-mediated cancer risk may differ among subjects with different type of lipodystrophies and their associated underlying metabolic state.

Reproductive and developmental toxicity
Reproductive and developmental toxicity was assessed in fertility, early embryonic development, and pre- and post-natal development studies in mice. Metreleptin did not adversely affect fertility and was not teratogenic in mice at doses up to 7-fold the clinical dose. Metreleptin caused dystocia or prolonged gestation at all doses, starting at below the clinical dose based on body surface area. Dystocia caused maternal death during labor, and resulted in litter loss and stillbirth. Lower pup survival was also observed between postnatal day 0 and 4, likely related to maternal stress during parturition. Although the mechanism of action for leptin-induced dystocia is not clear, there is in vitro evidence suggesting that leptin inhibits both frequency and amplitude of spontaneous and oxytocin-induced contractions in human myometrium[15].

Consistent with the pharmacodynamic activity of metreleptin, lower food intake resulted in reduced maternal weight from gestation throughout lactation and led to reduced pup

weight at birth and into adulthood. However, no behavioral, developmental or reproductive abnormalities were observed in the first or second generations.

Leptin plays a permissive role in pubertal developmental and reproduction[16]. Therefore, it is likely that lipodystrophic women may become pregnant during metreleptin treatment. The peri-natal study in rats suggests that metreleptin may result in prolonged or difficult labor (dystocia), possibly related to an inhibitory effect on myometrial contraction. Short-term discontinuation of metreleptin near the time of parturition could be considered; however, it is recognized that deterioration of metabolic status upon removal of metreleptin may also present its own clinical hazard during pregnancy.

Low metreleptin concentrations (less than 1% the maternal plasma concentration) were observed in the fetal serum and amniotic fluid, suggesting minimal placental transfer.

1.3 Recommendations

1.3.1 Approvability

AP (Approval)
Pharmacology/Toxicology recommends approval of BLA125390.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Pregnancy Category C

Risk summary
There are no adequate and well-controlled studies of metreleptin in pregnant women. Based on results from mouse studies, metreleptin may result in dystocia or prolonged gestation. Metreleptin should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Clinical considerations

Animal data
Metreleptin administered to pregnant mice during the period of organogenesis was not teratogenic at doses up to 7-fold the clinical dose, based on body surface area.

In a pre- and post-natal development study in mice, metreleptin administered at doses of 3, 10 and 30mg/kg from gestation day 6 to lactation day 21 caused prolonged gestation at all doses, starting at below the clinical dose. Prolonged gestation resulted in the death of some females during parturition and lower survival of offspring within the immediate post-natal period. Decreased maternal body weight was observed from gestation throughout lactation at all metreleptin doses, and resulted in reduced weight of offspring at birth which persisted into adulthood. However, no developmental

abnormalities were observed and reproductive performance of the first or second generations was not affected at any dose.

Placental transfer of metreleptin into the fetus was low (approximately 1%) following subcutaneous dosing from gestation days 11 to 17.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Two-year carcinogenicity studies in rodents have not been conducted with metreleptin. No proliferative or pre-neoplastic lesions were observed in mice or dogs following treatment up to six months. However, leptin is reported in the literature to promote cell proliferation in vitro and tumor progression in some mouse models of cancer.

Metreleptin was not mutagenic with or without metabolic activation in the Ames bacterial mutagenicity assay or in an *in vitro* chromosomal aberration assay in human peripheral blood lymphocytes. Metreleptin was not mutagenic or clastogenic in an *in vivo* micronucleus assay in mice.

In a fertility study in mice, metreleptin had no adverse effects on mating, fertility, or early embryonic development at doses up to 7 times the clinical dose based on body surface area.

2 Drug Information

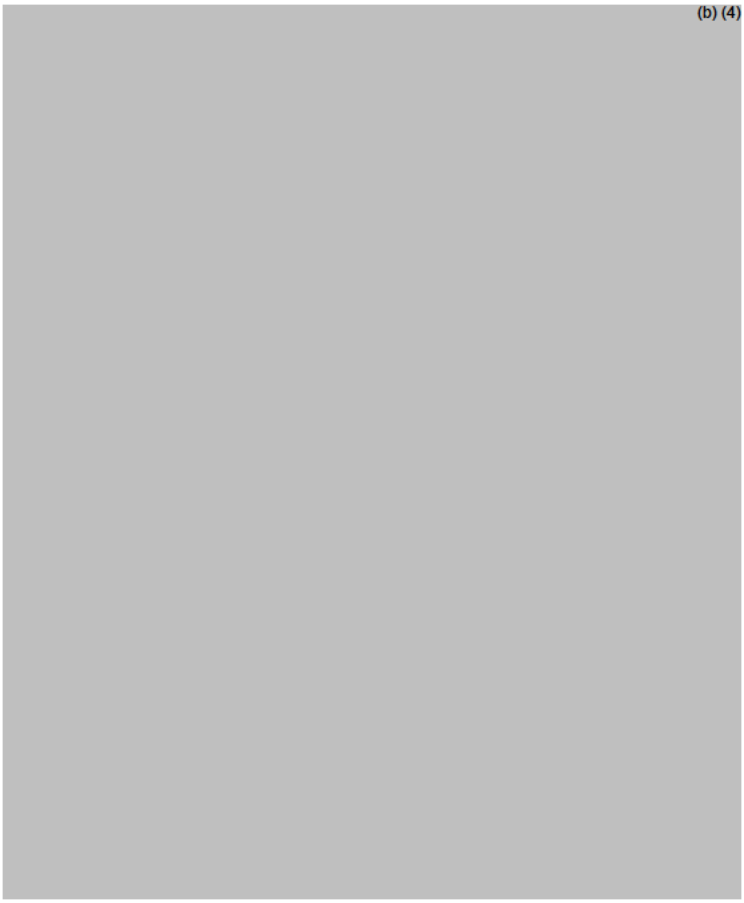
2.1 Drug

CAS Registry Number	186018-45-1
Generic Name	Metreleptin
Code Name	r-metHuLeptin, AC164594, AC100
Chemical Name	*N*-Methionyl leptin (human)
Molecular Formula/Molecular Weight	$C_{714}H_{1167}N_{191}O_{221}S_6$/16,156 Daltons

Structure or Biochemical Description

(b) (4)

Figure 1: Metreleptin Drug Substance Structure



Pharmacologic Class: Recombinant analog of human leptin

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 101824, 50259

2.3 Drug Formulation

Metreleptin is supplied as a sterile, white, solid, lyophilized cake containing 11.3mg of metreleptin and packaged in glass vials with (b) (4) stoppers. Upon reconstitution with 2.2mL of bacteriostatic water for injection (BWFI), the formulation is a sterile, clear, colorless solution for injection, at a concentration of 5mg/mL metreleptin, and with a withdraw-able volume of 2mL (10mg) for parenteral (i.e., subcutaneous) administration. The solution is buffered to pH 4.25 with glutamic acid and contains glycine, sucrose, and polysorbate 20.

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

Metreleptin is indicated for the treatment of diabetes mellitus and/or hypertriglyceridemia in pediatric and adult patients with inherited or acquired lipodystrophy. FDA granted metreleptin orphan status given the rarity of the clinical condition. The degree of rarity is unclear, but the sponsor estimated that genetic forms of lipodystrophy have a one-in-a-million prevalence in the general population. The sponsor did not provide an estimate for the prevalence of the acquired form of lipodystrophy, which may be more difficult to recognize and diagnose clinically.

Lipodystrophy is characterized by a partial or profound loss of adipose tissue mass. Consequently, serum triglycerides reach very high levels and are stored at ectopic sites, notably the skeletal and liver tissues, inducing a state of severe insulin resistance and other accompanying metabolic disturbances, often at an early age (e.g., diabetes, hepatic steatosis, risk of pancreatitis). Impairment of female fertility (polycystic ovary syndrome, amenorrhea) may also be present. Patients with congenital/acquired lipodystrophy generally survive to middle-age, often succumbing to gastrointestinal hemorrhage secondary to portal hypertension and liver injury.

Serum leptin, being produced by adipose tissue, is lower though not absent in lipodystrophic patients with low adipose mass. The sponsor explains that the leptin deficiency contributes to metabolic abnormalities in part by causing hyperphagia, which exacerbates the proximal event of hypertriglyceridemia. Metreleptin is intended to eliminate hyperphagia and thereby reduce the extent of hypertriglyceridemia and associated hyperinsulinemia. Impairment of fertility is also plausibly related to very low leptin levels, which may be improved by treatment with metreleptin. (b) (4) Whether metreleptin has other metabolic effects separate from suppressing appetite in this condition is not clear.

Therapeutic plasma drug levels of metreleptin were maintained in lipodystrophic patients at 20-35ng/ml over a period of several months (study NIH 991265/20010769). This plasma drug level exceeds the normal range of leptin in healthy men and is at the higher end of the normal range in healthy women (*Table 1, below, excerpted from sponsor's submission*). Thus, treatment with metreleptin provides a plasma drug level that borders on pharmacologic relative to the healthy, non-lipodystrophic population.

Table 1: Leptin Concentrations in a Representative Sample of Healthy Subjects in the US Population and in Patients With Different Types of Lipodystrophy

Fasting Serum Leptin Concentrations (ng/mL)					
Healthy Subjects Aged ≥20 y [1]		Patients With Lipodystrophy [2]			
		Generalized		Partial	
Men (n = 2937)	Women (n = 3366)	CGL (n = 18)	AGL (n = 11)	FPLD (n = 46)	APL (n = 18)
4.6 (1.4-15.6)	12.7 (3.3-40.4)	0.6 (0.05-3.7)	2.2 (0.05-11)	2.9 (0.23-9)	6.2 (1.2-10)

CGL = congenital generalized lipodystrophy; AGL = acquired generalized lipodystrophy; FPLD = familial partial lipodystrophy-Dunnigan variety; APL = acquired partial lipodystrophy.
[1] Values are Geometric Mean (range), in the 3rd NHANES adapted from (Ruhl CE, 2001, pg 295).[13]
[2] Values are Median (range), adapted from (Haque WA, 2002, pg 2395).[7]
Note: Both these studies used the LINCO RIA kit to measure leptin concentrations.[14]

The recommended dose is 0.06mg/kg for pediatric patients weighing 40 kg or less, 2.5mg/day in males above 40kg, and 5mg/day in females above 40kg. Maximum daily dose would not exceed 10mg/day or 12.5, 7.8, and 6.2 mg/m^2 in subjects with a body weight of 20, 40, and 60kg, respectively.

Based on single dose pharmacokinetics collected from healthy adult subjects, the maximum proposed dose of 10mg to a 60kg individual would provide approximate exposure of ~1770 ng*h/ml AUC and 178ng/ml Cmax.

Metreleptin Recommended Daily Dose by Weight and Gender

Baseline Weight	Daily Dose (Injection Volume)
≤40 kg (males and females)	0.06 mg/kg (0.012 mL/kg)
>40 kg	
Males	2.5 mg (0.5 mL)
Females	5.0 mg (1.0 mL)

2.7 Regulatory Background

Nearly all nonclinical studies of metreleptin were completed in the 1990s and early 2000s when the clinical program was focused on an obesity indication. An additional toxicology study in mice was conducted in 2010 to bridge changes in the API due to a change in the manufacturer of metreleptin.

4 Pharmacology

4.1 Primary Pharmacology

Early pharmacology studies were conducted in normal and transgenic mouse models of obesity, including the leptin-deficient obese ob/ob mice, when the development program was focused on treating general obesity. These models share some metabolic disturbances with a lipodystrophic phenotype, e.g., insulin resistance, hyperglycemia, hypertriglyceridemia, and fatty liver. Also, absent or low leptin is common between

leptin-deficient ob/ob mice and lipodystrophic mice, but the deficiency is genetic in the former and secondary to fat loss in the latter. Importantly, none of the rodent obesity models exhibit total or partial loss of fat tissue, which is characteristic and causative of the phenotype in lipodystrophic diseases. In addition to leptin, adipose tissue secretes several other adipokines and pro-inflammatory cytokines which are involved in endocrine regulation of insulin resistance. Thus, metabolic imbalances and metreleptin efficacy may differ between fatless lipodystrophic mice and obese non-lipodystrophic mice, including leptin deficient ob/ob mice. The studies in non-lipodystrophic animal models conducted by the sponsor are therefore of limited usefulness in understanding the effects of metreleptin in lipodystrophic metabolic disorders.

Several mouse models of lipodystrophy have been developed in the last two decades[17]. All are characterized by various degrees of fat ablation, leptin deficiency, insulin resistance, hyperglycemia, fatty liver, and hypertriglyceridemia. These models include the Ap2-nSREBP-1c mice, the A-ZIP/F1 mice, and conjugated linoleic acid (CLA) induced lipodystrophic mouse. Some residual fat characterizes the nSREBP-1c transgenic mouse and the CLA-induced lipodystrophic mouse, whereas essentially no white adipose tissue remains in the A-ZIP/F1 mice[18].

The following studies investigating the effect of leptin in mouse models of lipodystrophy were not conducted or supported by the sponsor, and none of the studies used metreleptin. The recombinant murine leptin used in these studies was purchased from (b) (4)

Administration of exogenous recombinant leptin to lipodystrophic mice generally decreased plasma glucose, insulin, hepatic triglycerides, and improved insulin sensitivity (Fig. 1, 2). Efficacy depended on the severity of adipose tissue ablation, with higher doses being required to achieve efficacy in the more severe lipodystrophic models (e.g., A-ZIP/F1 mice) [1,19,20]. Over-expression of leptin to high levels in the liver (50ng/ml) also reduced insulin resistance, diabetes, and hepatic steatosis in A-Zip/F-1 mice (Fig. 3)[21].

The mechanism by which recombinant leptin improves insulin sensitivity and plasma glucose may extend beyond simply reducing food intake and body weight. For example, food restriction did not lower plasma insulin and glucose in Ap2-nSREBP-1c and LepTg/A-Zip transgenic mice (Figure 4 and 5, compared to Figure 1), although liver triglycerides did decrease notably. Similarly, recombinant leptin decreased serum insulin and glucose concentration in CLA-lipodystrophy without significant changes in food intake, body weight, and fat mass (Fig. 6). Generally, food restricting obese rodents will reduce plasma glucose and triglycerides, and improve insulin sensitivity. It is unclear why a similar effect is apparently not seen in lipodystrophic models. It is not possible to quantify the contribution of primary and secondary effects of recombinant leptin to metabolic improvement from these studies.

Figure 1. Metabolic parameters in ap2-nSREBP-1c Tg mice following leptin administration (5ug/day)[19]

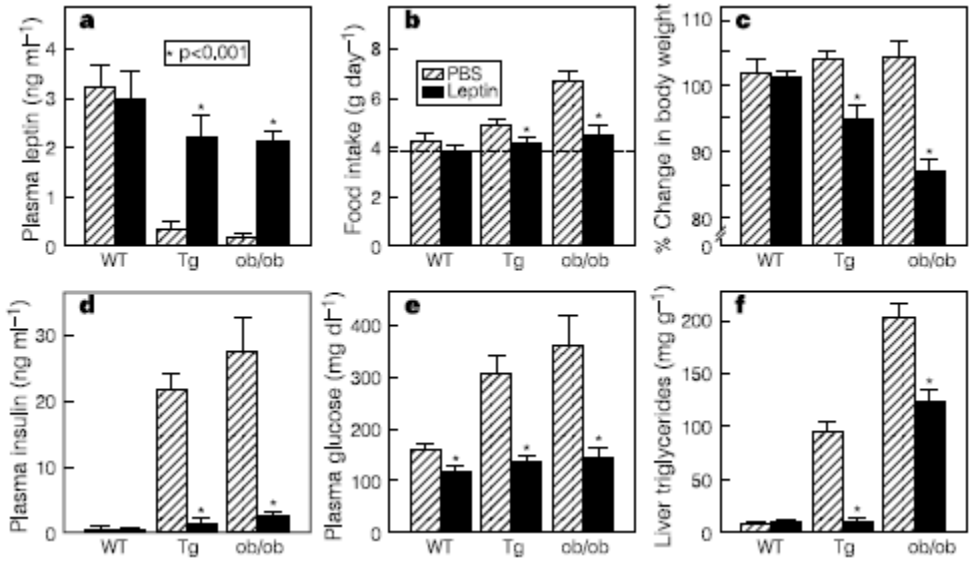


Figure 2. Metabolic parameters in A-ZIP/F1 mice following leptin administration (30ug/day)[1]

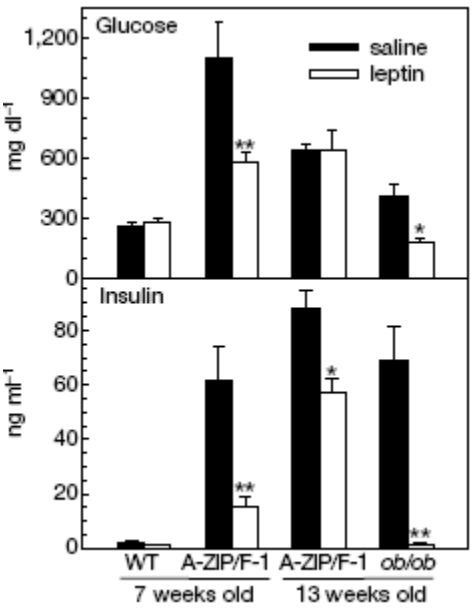
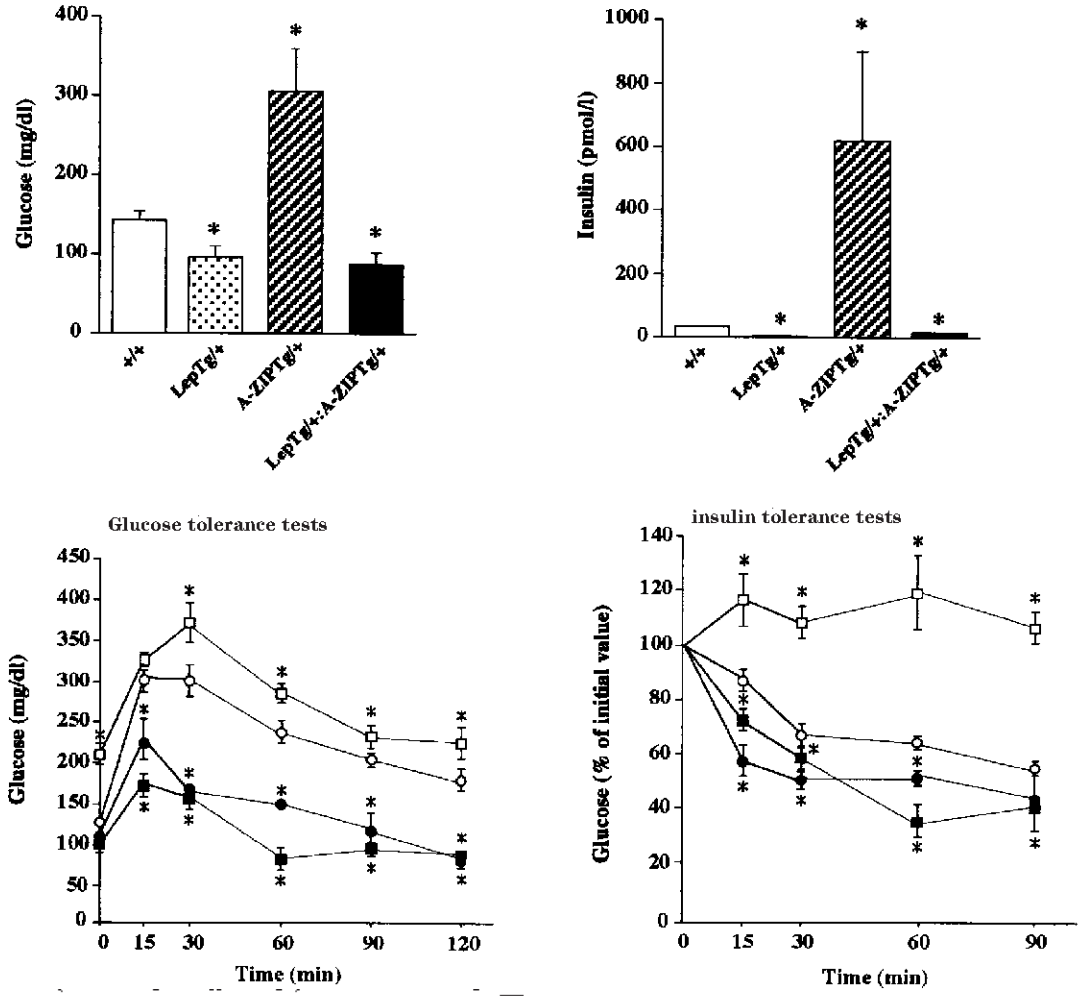


Figure 3. Metabolic parameter in A-ZIP mice crossed with LepTg mice[21]



+/+ (○), LepTg/+ (●), A-ZIPTg/+ (□), and LepTg/+:A-ZIPTg/+ (■) mice

Figure 4. Metabolic parameters in ap2-nSREBP-1c mice following food restriction[19]

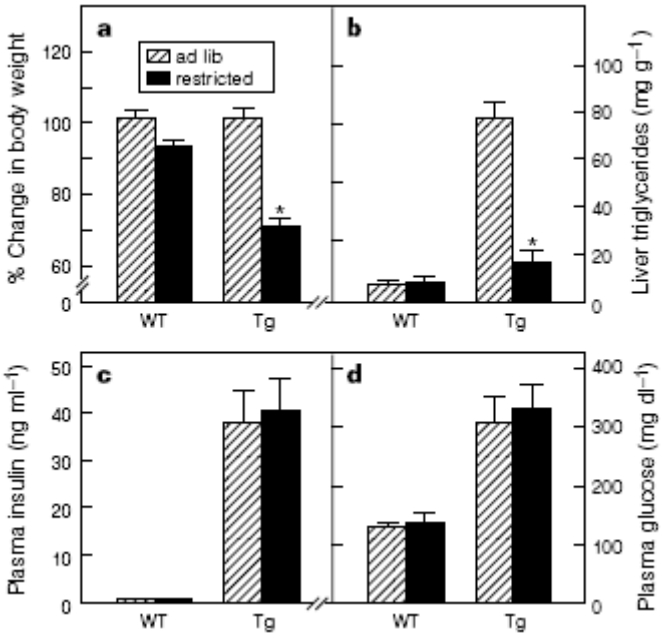
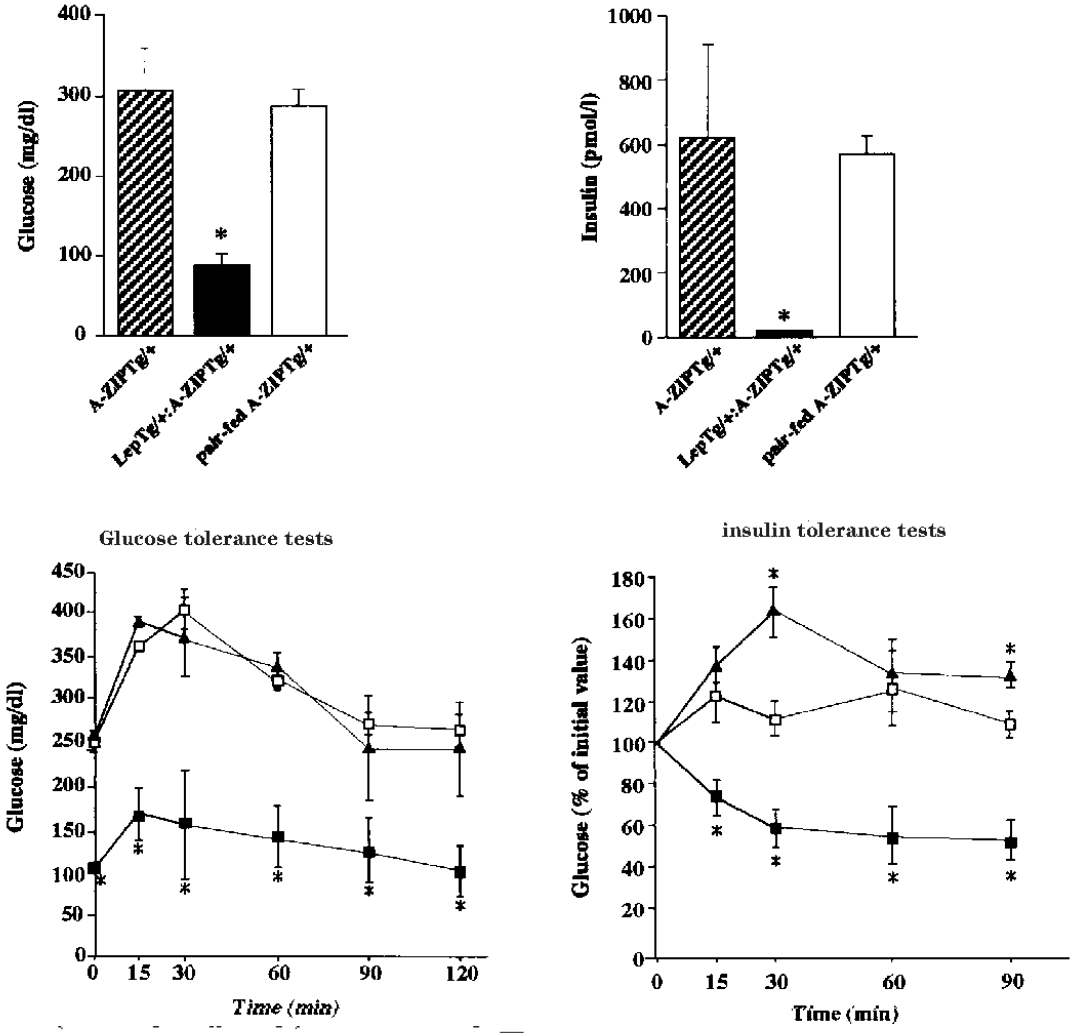


Figure 5. Metabolic parameter in pair-fed A-ZIP Tg mice[21]



+/+ (○), LepTg/+ (●), A-ZIPTg/+ (□), and LepTg/+:A-ZIPTg/+ (■) mice

Figure 6. Serum leptin, insulin, and ITT in CLA-induced lipodystrophy mice following leptin administration (5ug/day for 4 weeks)[20]

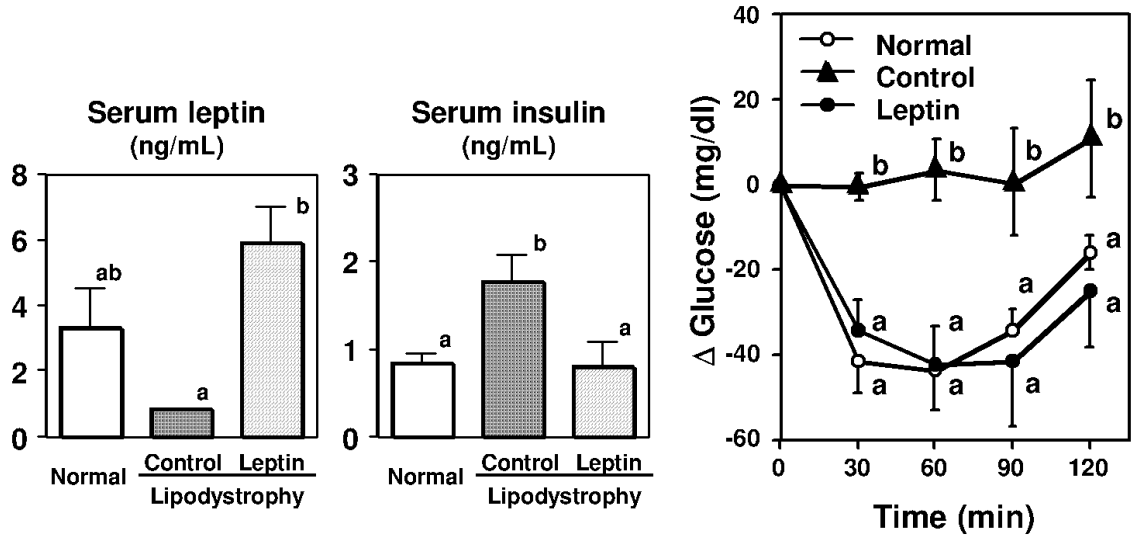


Table 1. Leptin effect on body weight, food intake, and fat mass in CLA mice[20]

		Lipodystrophy model	
	Normal	Control	Leptin
Final body weight (g)	21.7 ± 0.5	22.1 ± 0.3	21.3 ± 0.2
Food intake (g)	61.2 ± 1.3	58.4 ± 2.1	59.6 ± 1.2
Liver (g/100 g body weight)	4.11 ± 0.05 [a]	6.47 ± 0.77 [b]	5.65 ± 0.29 [b]
Whit adipose tissue (g/100 g body weight)			
Epididymal	1.87 ± 0.22 [a]	0.290 ± 0.029 [b]	0.228 ± 0.030 [b]
Perirenal	0.836 ± 0.104 [a]	0.199 ± 0.018 [b]	0.178 ± 0.018 [b]
Omental	1.13 ± 0.09 [a]	0.652 ± 0.022 [b]	0.740 ± 0.029 [b]
Subcutaneous	1.95 ± 0.16 [a]	0.329 ± 0.014 [b]	0.344 ± 0.017 [b]

[a, b] Different superscript letters show significant difference at $P < 0.05$.

Synopsis of the sponsor's pharmacology studies in normal, obese and diabetic rodents

Administering metreleptin to leptin deficient ob/ob mice decreased food intake, body weight gain, fat mass, serum levels of glucose and insulin, and hepatic levels of triglycerides, glycerol, and cholesterol compared to control mice. These effects were more notable in ob/ob mice compared to pair-fed mice (Fig.7, Table 2), but the effect of metreleptin and pair-feeding were qualitatively the same. Metreleptin did not significantly alter metabolic parameters in normal C57BL/6J mice or in obese Ay, NZO, and AKR/j mice likely because, contrary to ob/ob mice which have very low levels of endogenous leptin, obese mice have supra-physiological levels of leptin and become leptin resistant (data not shown).

The effect of metreleptin on insulin resistance at the whole body level and in liver, skeletal muscle, and adipose tissue was investigated in normal rats and in several models of diabetic-insulin resistant rats. Metreleptin improved insulin sensitivity, as indicated by the increased rate of tissue glucose uptake and glycogen synthesis, and by decreased production of hepatic glucose in both normal and diabetic STZ rats. In glucose-intolerant Wistar rats, metreleptin prevented plasma glucose increase following an intraperitoneal glucose load, and prevented the high fat diet induced decrease in skeletal muscle glucose uptake. Stimulation of glucose uptake in the muscle and brown adipose tissue, but not in the white adipose tissue, was observed in SD rats following an IV bolus of glucose (data not shown). Based on the literature, the sponsor also claims that leptin administration stimulates fatty acid oxidation in liver and muscle in normal rodents, by activating AMPK and PPAR alpha, thus activating carnitine palmitoyl tranferase-1, and acyl-CoA oxidase, and inhibiting lipogenic enzymes including acyl-CoA carboxilase and stearoyl-CoA desaturase-1 [22,23].

Figure 7. Body weight changes and food intake in ob/ob mice following metreleptin administration (10mg/kg for 21 days)

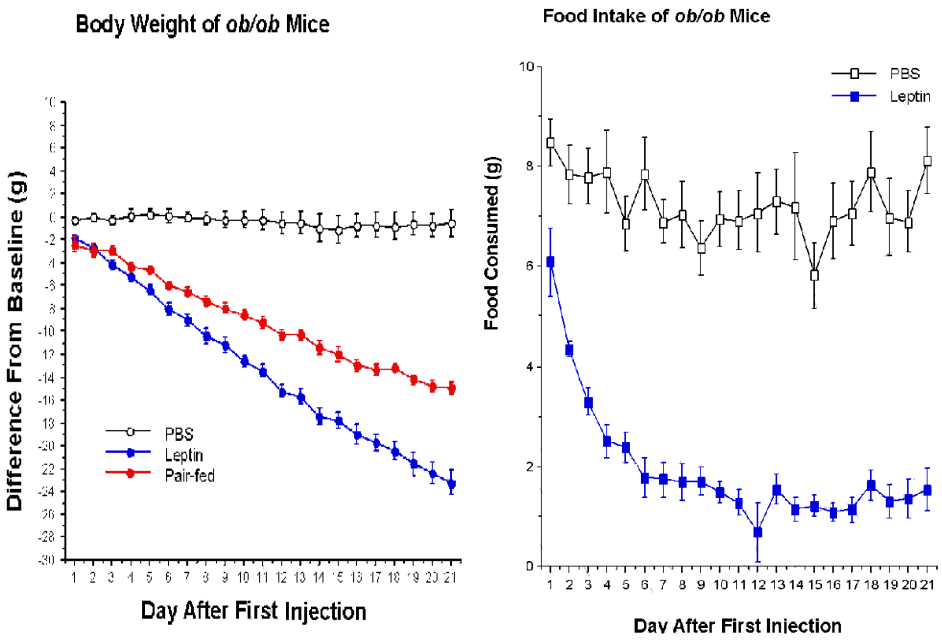


Table 2. Serum chemistry and hepatic lipids in ob/ob mice

Ob/ob mice (% change vs. control)				
	Metreleptin		Pair-fed	
	Wk 1	Wk 3	Wk 1	Wk 3
Serum chemistry				
Glucose	-60*	-70*	-25	-30*
Cholesterol	-46*	-60*	-21	-52*
Triglycerides	-46*	-60*	-38*	-44*
Glycerol	-23*	-38*	-	-
Insulin	-15X*	-21X*	-2X*	-
Liver lipids				
Cholesterol	-37*	-56*	-32*	-
Triglycerides	-28	-50	-39	+2X*
Glycerol	-44*	-44*	-64* #	-7X * #

* p<0.05-0.0001 vs. PBS
# p<0.05-0.0001 vs. Metreleptin

4.3 Safety Pharmacology

A comprehensive battery of safety pharmacology studies was conducted by the sponsor in normal mice, rats and dogs following a single subcutaneous dose of metreleptin. Safety assessment of the central nervous system in mice and rats showed no adverse effects with metreleptin at doses up to 30mg/kg (7X and 14X MRHD, BSA basis, respectively). The effect of metreleptin on the cardiovascular system was evaluated in conscious telemetered rats and dogs. No treatment related changes in hemodynamic or ECG parameters were observed in either species at doses up and above 14X MRHD (BSA basis). In addition, no treatment related changes in cardiovascular function were observed following 6-month of subcutaneous dosing in dogs at doses up to 1.5mg/kg (18-fold the clinical AUC, based on Day 1 AUC). The renal, respiratory, and gastrointestinal systems were not adversely affected by metreleptin at doses up to 30mg/kg (14X MRHD, BSA basis).

Table 3. Summary of safety pharmacology studies (sponsor's table)

Study Type	Species	Duration	NOAEL		Human Safety Margins**		
			(mg/kg/d)	(mg/m^2/d)	20 kg	40 kg	60 kg
Safety Pharmacology							
CNS	Mouse	Single Dose	30	90	7.2	12	14
	Rat	Single Dose	30	180	14	23	29
Cardiovascular	Rat	Single Dose	30	180	14	23	29
	Dog	Single Dose	25	500	40	64	81
Respiratory	Mouse	Single Dose	30	90	7.2	12	14
Renal	Rat	Single Dose	30	180	14	23	29
Gastrointestinal	Rat	Single Dose	30	180	14	23	29

** Calculated by dividing the appropriate HED by the proposed maximum daily dose of 10 mg/day (or 0.5, 0.25 and 0.167 mg/kg/day for patients with a body weight of 20 kg, 40 kg and 60 kg, respectively).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Pharmacokinetics was established for both intravenous and subcutaneous routes of administration in dogs, mice, and pregnant mice. Elimination of metreleptin was assessed in nephrectomized mice, which serves as a pivotal study that the sponsor relies upon in predicting a predominant renal clearance mode in human subjects.

Overall, the pharmacokinetics in mice and dogs is similar to humans in terms of volume of distribution (2-5x plasma volume) and absorption from subcutaneous administration. The plasma half-life differs moderately across species, from ~0.5h in mice, 1-2hrs in dogs, and 3-5hrs in humans. Anti-drug antibody responses were prominent in mice and dogs, but also occur in human subjects, with the primary consequence of increasing plasma drug levels.

Exposure in pregnant mice is higher than in non-pregnant mice. However, drug levels in fetal tissues and amniotic fluid is less than 1% of maternal drug levels.

Clinical Pharmacokinetics and Exposure

Therapeutic exposure in lipodystrophic patients was reportedly maintained at 20 to 35ng/ml over a period of several months (Clinical study NIH 991265/20010769). The sponsor's dosing recommendations propose that the dose can be escalated up to a maximum of 10mg/day, presumably for pediatrics as well as adults. Exposure at a dose of 10mg must be extrapolated from Day 1 exposure in healthy subjects, which is ~1770 ng*h/ml AUC and 178ng/ml Cmax.

Table: Day 1 Pharmacokinetics in Healthy Adult Volunteers

Study	**LEPT-950272 (SC)**	
Study Day	**Day 1**	
Dose Group (mg/kg)	**0.1**	**0.3**
N	31	26
C_{avg} (ng/mL)	—	—
T_{max} (hr)	4.3 (1.5)	4.0 (1.5)
C_{max} (ng/mL)	119 (32)	343 (104)
nC_{max} (ng/mL/mg/kg)	1190 (320)[a]	1143 (347)[a]
$t_{½}$ (hr)	4.3 (2.7)	3.8 (1.4)
$AUC_{0-\infty}$ (ng*hr/mL)	1180 (267)	3657 (781)
AUC/D (ng*hr/mL/mg/kg)	11800 (2675)	12190 (2603)
CL (mL/kg/hr)	89 (20)	86 (18)
Vz (mL/kg)	519 (276)	444(117)

Pharmacokinetics and Exposure in Mice
Following a single subcutaneous administration, metreleptin absorption was rapid with Tmax ranging from 10 to 30min. After reaching Cmax, serum levels declined in a biphasic manner with half-lives of approximately 24min and 6h. The relative bioavailability was high (82%). Exposure increased dose proportionally and slight accumulation was noted following 10 days of dosing.
Following IV administration in mice, clearance was similar to the glomerular filtration rate (~600mL/h/kg), suggesting that the main route of elimination is filtration through the kidneys. Volume of distribution at steady state was approximately 3 times the plasma volume in mice (~50mL/Kg), suggesting that metreleptin distributes, albeit not extensively, to extravascular sites.

Table 4. Single dose mouse TK summary

Male CD1 mice, single dose									
Route	**Dose, mg/kg**	**Tmax (h)**	**Cmax (ng/mL)**	**AUC (ng.h/mL)**	**T1/2 (h)**	**F**	**Vss (mL/Kg)**	**CL (mL/h/Kg)**	**MRT (h)**
SC	0.3	0.28	377	348	0.408	0.71			
	1	0.14	1520	1230	0.388	0.84			
	3	0.50	3810	3780	0.379	0.83			
	10	0.39	12600	14000	0.436	0.89			
IV	0.3			491	0.446		142	611	0.23
	1			1470	0.491		87.2	681	0.21
	3			4530	0.484		85.5	663	0.26
	10			15800	0.476		65.3	633	0.25

Table 5. Repeated dose mouse TK summary

Male CD1 mice, 10 days									
Dose, mg/kg	**Tmax (h)**			**Cmax (ng/mL)**			**AUC (ng.h/mL)**		
	D1	**D7**	**D10**	**D1**	**D7**	**D10**	**D1**	**D7**	**D10**
1	0.16	0.28	0.39	1440	1393	1534	1263	1453	1891
10	0.39	0.5	0.28	10700	12480	14700	10256	12644	17937

Pregnant mice
Following subcutaneous administration of metreleptin (10mg/kg) to pregnant and non pregnant mice, metreleptin exposure and elimination half life was significantly higher in pregnant mice compared to non pregnant mice (up to 3 and 5 fold, respectively). Low metreleptin concentrations (less than 1% the maternal plasma concentration) were observed in the fetal serum and amniotic fluid, suggesting minimal placental transfer. Following repeated dosing from GD11 to GD17, little accumulation was observed in the maternal and fetal serum (1.3 fold increased compared to single dose).

Table 6. Pregnant mice TK summary (sponsor's table)

Treatment	Parameter	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-t} (ng•hr/mL)	V_z/F (mL/kg)	CL/F (mL/hr/kg)	$MRT_{0-\infty}$ (hr)
Single Dose (GD 17)	**Maternal**	0.78	17850.3	41851.7	995.5	234.2	3.6
	Fetal	1.30	61.1	141.3	--	--	2.1
	Amniotic	3.50	75.9	517.6	--	--	7.5
Daily Dose (GD 11-17)	**Maternal**	0.76	19240.0	52725.8	963.7	182.2	3.8
	Fetal	1.29	87.7	194.3	--	--	1.8
	Amniotic	0.74	38.0	498.0	--	--	9.7
Single Dose	**Nonpregnant**	0.25	20044.7	19381.9	491.7	515.9	0.8

Pharmacokinetics and Exposure in Dogs

Following a single subcutaneous administration, metreleptin absorption was moderately rapid (Tmax: 3-4hrs). The terminal half-life, approximately 2 hrs, was longer than the half-life measured following IV administration, indicating that absorption appeared to be the rate-limiting step following SC administration. Bioavailability ranged between 0.72 and 0.91%. Exposure increased dose proportionally and slight accumulation was noted following 13 days of dosing.

Following IV administration, clearance of metreleptin averaged 205mL/h/kg, approximately 85% of the glomerular filtration rate in the dog (240mL/h/Kg), and volume of distribution at steady state was approximately 3-4 times the plasma volume in the dog (~50mL/Kg), suggesting slight distribution to extravascular sites.

Table 7. Single dose dog TK summary

Beagle dogs, males, single dose									
Route	**Dose, mg/kg**	**Tmax (h)**	**Cmax (ng/mL)**	**AUC (ng.h/mL)**	**T1/2 (h)**	**F**	**Vss (mL/Kg)**	**CL (mL/h/Kg)**	**MRT (h)**
SC	0.3	2.8	180	1320	2.1	0.9			
	3	4	1080	11700	2.7	0.7			
IV	0.3			1450	1.16		193	215	0.91
	3			16400	1.46		165	194	0.88

Table 8. Repeated dose dog TK summary

Beagle dogs, males + females, 13 days									
Dose, mg/kg	**Tmax (h)**			**Cmax (ng/mL)**			**AUC (ng.h/mL)**		
	D0	**D6**	**D13**	**D0**	**D6**	**D13**	**D0**	**D6**	**D13**
0.3	4	2.5	2	155	194	361	1220	1240	2520
3	4	2.5	2.3	1440	2180	2750	11500	13500	21600

Immunogenicity
Serum reactivity ratios, indicative of an antibody response against metreleptin, increased substantially after two weeks of dosing in mice and dogs. This was accompanied by an increase in plasma drug levels and exposure, but not by a diminution of pharmacodynamic activity. The sponsor suggests that reduced renal filtration and thus reduced clearance of antibody-metreleptin complexes explains the increase in drug levels, which is reasonable. Of note, ADA responses to metreleptin occurred with high frequency (>60%) in studies with lipodystrophic patients and obese subjects, and was also associated with increased plasma drug levels, mirroring the case in mice and dogs.

Metabolism
No metabolism studies were performed with metreleptin.

Elimination
Metreleptin appears to be cleared almost entirely by renal elimination.
Renal clearance of metreleptin was investigated in male CD1 mice following an I.V. dose of 10mg/kg administered to three groups of mice: control, sham-operated, and nephrectomized mice. Serum concentrations of metreleptin were determined at several time points within 24h of dosing using a validated enzyme immunoassay.

Results:

- Serum concentration of metreleptin was approximately 30 fold higher in nephrectomized mice compared to control and sham-operated animals.
- Clearance of metreleptin decreased 97% in nephrectomized mice compared to control and sham-operated mice, consistent with the significance of the kidneys in elimination of this protein
- Decreased Vss in the nephrectomized animals suggests that the kidneys also served as a distribution site for metreleptin.

Pharmacokinetic Parameters	Group 1 (Control)	Group 2 (Sharm-operated)	Group 3 (Nephrectomized)
AUC (ng•h/mL)	14400	15100	439000
CL (mL/h/kg)	697	662	22.8
AUMC (ng•h^2/mL)	4182	4444	2400000
V_c[a] (mL/kg)	91.9±5.5	80.2±16	45.4±6.0
V_{ss} (mL/kg)	203	195	125
MRT (h)	0.291	0.295	5.47

[a] Vc was calculated from each animal euthanized at the 1 minute time point in each group. The mean±SD is shown.

6 General Toxicology

6.2 Repeat-Dose Toxicity

Toxicology studies considered relevant for support of BLA 125390 include a 1-month and a combined 3/6 month study in mice, and a combined 1/3/6 month study in dogs. These studies administered metreleptin subcutaneously once daily and incorporated drug-free recovery periods. Also, a 1-month bridging toxicology study in mice was conducted to qualify the metreleptin drug substance following a change in manufacturer from Amgen to Sandoz.

Summary of Pivotal Toxicology Studies in Swiss Albino Mice

Two short-term, 28-day studies were conducted in healthy adult Swiss Albino mice using either intravenous or subcutaneous administration of drug at dosages of 1, 10, and 100mg/kg. Both studies yielded similar findings: excessive reductions in weight gain and food consumption, depletion of adipose fat mass, and inflammation at the site of injection (subcutaneous route). Some animals succumbed to the anorexic effect of leptin and were found dead, some with gastric erosions, and primarily at the 100mg/kg dose. Most deaths occurred following an overnight fast prior to scheduled termination, indicating greater susceptibility to stressful conditions due to poor body state (emaciation).

A combined 3/6 month toxicology study in Swiss Albino mice was conducted using doses of 0.3, 1, 3, 10, and 30 mg/kg, administered subcutaneously, followed by a 28 day recovery period. All doses resulted in reduced food consumption and lower weight gain compared to controls, with females being significantly more affected than males. Consistent with the 1-month study, several deaths were encountered at 3, 10, and 30mg/kg on Day 91 following a scheduled overnight fast, again reflecting susceptibility due to generally poor nutritional status and body state. Subsequent deaths over the next 3 month dosing period occurred in all dose groups, including controls, and were not considered clearly related to treatment. Gross and histopathological findings were reasonably related to the anorexigenic effect of metreleptin, with frequency and severity generally correlating with dose. Such findings included increased red cell mass, lower organ weight and emaciated carcasses, gastric erosions with dark discoloration, fat atrophy, lympohocytolysis, and injection site cellulitis. Mild centrilobular hepatocellular degeneration was observed at month 3 at doses ≥1mg/kg, but not at month 6. A rebound in weight gain and reversal of histological findings were evident in the recovery period. Due to the deaths following overnight fasting at ≥3mg/kg at month 3, the reviewer agrees with the sponsor's NOAEL of 1mg/kg, though some findings were evident at the 0.3 and 1mg/kg dosages.

Correcting for body mass, the NOAEL of 1mg/kg (3mg/m^2) in the 6 month mouse study provided no safety margin to the clinical dose in adults (6.2mg/m^2) or in pediatric patients (12.5mg/m^2). The anorexia-related adverse findings in mice were monitored in the course of clinical trials and were not evident in studies of lipodystrophy patients.

Therefore, at the proposed clinical dose, these anorexia-related findings in mice do not reflect a human risk.

A one-month bridging toxicology study was conducted in Swiss albino mice to determine comparability between the toxicity of metreleptin drug substance manufactured at Amgen and the metreleptin drug substance manufactured at Sandoz. The Amgen product has been used in the non-clinical and clinical studies conducted during the development of metreleptin, whereas the Sandoz drug substance will be used for the marketed drug product. The bridging study showed similar toxicological profile between the two metreleptin drug substances. Similar to Amgen-metreleptin, the main findings observed with the Sandoz-metreleptin were reductions in weight gain and food intake, depletion of adipose tissue mass, inflammation at the injection site, as well as mortality at the high dose.

Synopses of Pivotal Toxicology Studies in Mice

Swiss Albino Mice: 28-day intravenous toxicity study
Dose: 1, 10, 100mg/kg (3, 30, 300mg/m^2)
Safety margins (relative to clinical dose of 6.2-12.5mg/m^2):
<1, 2, 24 fold MRHD (BSA basis)

Key Study Findings

- Mortality at ≥10mg/kg
- Decrease in body weight gain at HD
- Increased in urea (48%) and AST (1.5X) in HD females
- Tubular degeneration, hydronephrosis, pyelitis and proteinaceous deposits in the kidney were noted in HD animals
- Depletion in adipose tissues in all treated groups, with increased severity at HD
- Increased severity of chronic inflammation at the injection sites at ≥10mg/kg
- Proteinaceous deposits and inflammation in the urinary bladder at HD.

Swiss Albino Mice: 28-day subcutaneous (SC) toxicity study
1, 10, 100mg/kg (3, 30, 300mg/m^2)
Day 1 Cmax: 0.4, 1.5, 37μg/mL
Day 1 AUC: 563, 6020, 85000 ng*h/ml
Safety margins (relative to clinical dose of 6.2-12.5mg/m^2):
0.5x, 5x, 73x, based on AUC
<1x, 2-5x, 24-48x based on BSA

Key findings

- Mortality was observed at ≥10mg/kg, due to food and water deprivation before scheduled necropsy.
- Dose related decrease in body weight gain was observed in all treated animals.
- Urea was increased at ≥10mg/kg, without microscopic changes in the kidney

- Increase in AST (2X) and ALT (4X) was observed in HD females, and correlated microscopically with hepatocellular degeneration. HD males also showed hepatocellular degeneration, without hepatic enzyme leakage
- Fat atrophy and increased severity of injection site inflammation was noted in treated animals
- Plasma drug levels were nearly absent by 8 hours post-dose due to the short half-life in mouse plasma
- Immunogenicity was observed at all doses, affecting drug exposure (increased) by Day 14.

Swiss Albino Mice: 3/6 month SC toxicity study followed by a 28-day recovery period

0.3, 1, 3, 10, 30 mg/kg, or
0.9, 3, 9, 30, 90 mg/m^2
Safety margins (relative to clinical dose of 6.2-12.5mg/m^2):
~0.1x, <1x, 1x, 2-5x, 7-15x, based on BSA

Study no.:	REST70254
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 1, 1995
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	r-metHuLeptin, lot # 1112285M5, 13005L5, 1101226B6 (Amgen sourced API)

Key Study Findings

- Mortality occurred at ≥3mg/kg at 3 months, due to food/water restriction one day prior to scheduled interim necropsy.
- Reduced weight gain was observed in all treated groups throughout dosing.
- Increased AST (2-fold) was observed in HD females.
- Fat atrophy and inflammation at the injection site was noted at all doses.
- NOAEL, based on mortality, was 1mg/kg (3mg/m^2), below the clinical exposure

Methods

Doses:	0.3, 1, 3, 10, 30 mg/kg
Frequency of dosing:	Once a day
Route of administration:	SC injection
Dose volume:	1.5, 5, 15, 2, 6 mL/kg
Formulation/Vehicle:	Histidine 11mM, sorbitol 5% (citrate buffer)
Species/Strain:	Swiss Albino mice, (b) (4)
Number/Sex/Group:	15/sex/group
Age:	6 weeks old
Weight:	Males: 26.3-34.3g. Females: 20.5-28.9g
Satellite groups:	15/sex/group 28 day recovery
Unique study design:	15/sex/group were euthanized at 3- and 6-mo of dosing. Toxicokinetic was not measured in this study.

Observations and Results
Mortality
3-month:
Four, two and four animals in the 3, 10, and 30mg/kg group, respectively, died on Day 91 following overnight food and water deprivation prior to scheduled necropsy, which likely was a contributing factor in the deaths. Body weight gain in most of these mice was below the group average. Six of these mice exhibited pallor, decreased activity, hunched posture and were cold to touch prior to death. The remaining animals showed no adverse clinical signs throughout the study.
One animal in the HD group died on Day 33 without showing any prior adverse effects.

3-6 month: Between Day 91 and the end of study, several animals were found dead or were sacrificed in poor condition in all dose groups, including control. There were no treatment-related clinical observations and none of the deaths were clearly attributable to treatment.

Table 9. Mouse mortality at 3 and 6 months

Mortality at 3-month				
Dose	**Animal ID**	**Day**	**BW gain (g)**	**Findings**
0.3	2043-M	71 S	4.5*	Urogenital region: swollen, skin lesion
1	-	-	-	-
3	4033-M	91 FD	3.6*	Decreased activity, cold to touch, hunched posture, moderate dehydration
	4034-M	91 FD	0.8*	Decreased activity, cold to touch, hunched posture, moderate dehydration.
	4543-F	91 FD	2.6	Decreased activity, cold to touch, hunched posture, moderate dehydration
	4521-F	91 FD	-0.1*	No adverse clinical signs
10	5514-F	91 FD	0.4*	No adverse clinical signs
	5521-F	91 FD	2	No adverse clinical signs
30	6032-M	91 FD	1.9*	Decreased activity, cold to touch, hunched posture, moderate dehydration
	6043-M	91 FD	-0.2*	Decreased activity, cold to touch, hunched posture, moderate dehydration
	6531-F	91 FD	5#	Decreased activity, cold to touch, hunched posture, moderate dehydration
	6523-F	33 FD	-2.3*	No adverse clinical signs

Mortality between 3-6 months				
Dose	**Animal ID**	**Day**	**BW gain (g)**	**Findings**
0	1621-F	165 S	9	Decreased activity, hunched posture, increased respiratory rate. Cause of death: amyloidosis
	1114-M	156 FD	14.2#	No adverse clinical signs
0.3	2071-M	139 S	10.3#	Decreased activity. Osteosarcoma
1	3073-M	176 FD	10.9	No adverse clinical signs
	3093-M	127 S	6.2*	Limited usage, severe, right-left forepaw
	3113-M	97 S	10.1#	No adverse clinical signs Cause of death: amyloidosis
	3574-F	179 FD	7.7	No adverse clinical signs. Cause of death: amyloidosis
3	4611-F	96 FD	7.5#	No adverse clinical signs. Cause of death: amyloidosis
10	5071-M	98 FD	-0.6*	No adverse clinical signs
	5093-M	151 FD	5.4*	No adverse clinical signs
	5592-F	152 S	5.1#	Decreased activity, cold to touch
	5601-F	148 FD	-2*	Hunched posture, moderate dehydration (D133-148). Lymphosarcoma
30	6553-F	177 FD	-1*	No adverse clinical signs

* Body weight gain < group average
# Body weight gain >group average
S: sacrificed. FD: found dead

Clinical Signs
3-month: Clinical observations included cold to touch, dehydration, hunched posture, pallor and/or decreased activity for most males at ≥ 1 mg/kg/day and females at ≥ 0.3 mg/kg/day. Since these findings only occurred on Day 91, they were considered related to an interaction between treatment with metreleptin and overnight food and water deprivation prior to necropsy.

6-month: There were no treatment-related clinical observations. Animals were not fasted overnight prior to necropsy.

Body temperature
No treatment-related effects were noted.

Body Weights
Dose related decrease in body weight gain and body weight relative to the control groups was observed in all treated males and in females at ≥1mg/kg during the first three months of dosing. Lower body weight was substantial in females at higher dose groups. Lower body weight compared to controls continued throughout 6-months but was less notable. At 6 months, body weight was significantly reduced only in females starting at ≥1mg/kg compared to control females. Body weight gain quickly rebounded in dosed males and females during the recovery period, indicating that metreleptin retained anorexic activity for the duration of the 6m dosing period.

Table 10. Mouse body weight gain and body weight

	BW gain (% of baseline)				BW (vs. controls)			
	3-mo		6-mo		3-mo		6-mo	
Dose	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
0	+24	+26	+34	+34				
0.3	+20	+21	+29	+28	-6**	-2	-6	-3
1	+17	+14	+27	+22	-6**	-11**	-5	-9**
3	+14	+6	+31	+13	-10**	-14**	-4	-15**
10	+10	+3	+28	+14	-12**	-18**	-6	-14**
30	+10	+1	+28	+10	-11**	-21**	-5	-17**

*p<0.05, **p<0.01 (Dunnett's test)

Figure 8. Male mice body weight percent change from baseline

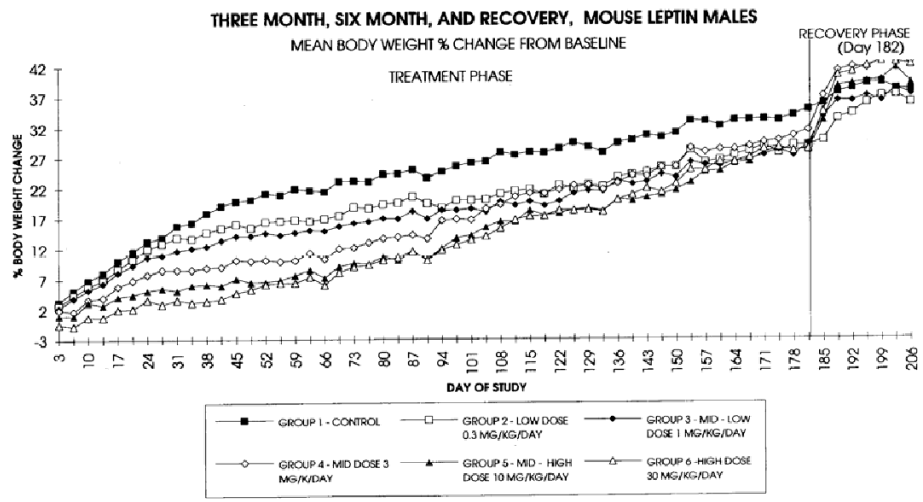
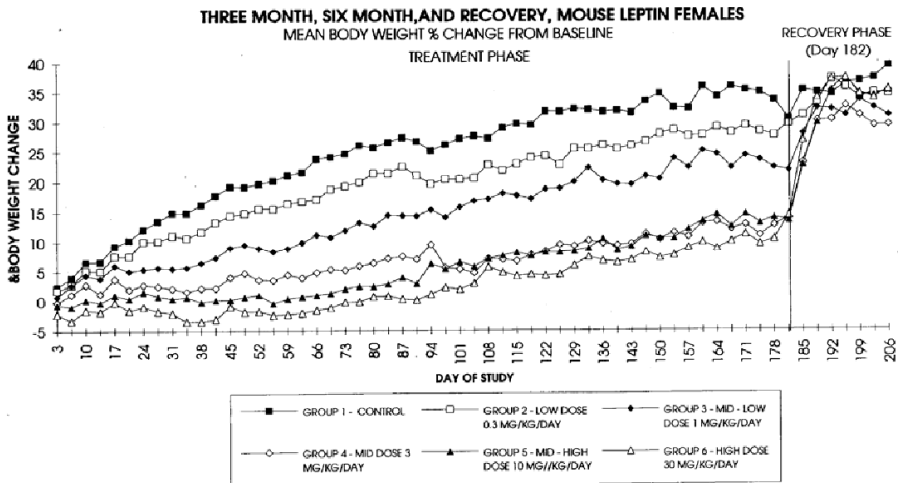


Figure 9. Female mice body weight percent change from baseline



Feed Consumption
Statistically reduced food intake was observed at ≥1mg/kg during the first 4 days of treatment. The reduced intake generally persisted at ≥ 3 mg/kg/day throughout approximately Day 71 in males and throughout Day 179 in females.

Ophthalmoscopy
Unremarkable

Hematology
Slight to moderate increase in red blood cells, hemoglobin and hematocrit was noted in males at ≥ 3 mg/kg/day and in females at 30 mg/kg/day. These changes were not dose related and not significant, except for RBC in females at 10mg/kg.

Table 11. Mouse hematology parameters at 6-month

Hematology-Males						
Dose	**0**	**0.3**	**1**	**3**	**10**	**30**
RBC ($x10^6$)	7.73	7.61	7.95	8.23 (6%)	8.70* (13%)	8.35 (8%)
HB (g/dL)	12.8	12.6	12.8	13.3 (4%)	13.7 (7%)	13.8 (8%)
HTC (%)	36.8	35	37	39.1 (6%)	41 (11%)	40.9 (11%)

* p<0.05 (Dunnett's)

Hematology-Females						
Dose	**0**	**0.3**	**1**	**3**	**10**	**30**
RBC ($x10^6$)	7.85	8.2	7.78	7.76	7.28	8.86 (13%)
HB (g/dL)	13.3	13.5	12.9	12.5	12.2	14.4 (8%)
HTC (%)	37.6	38.7	37.8	38.3	35.4	42.5 (13%)

Clinical Chemistry
- Increased AST (2-fold) was observed in HD females.
- Decreased in triglycerides was noted in females at ≥1mg/kg, without dose relationship.

Table 12. Mouse clinical chemistry at 6-month

Clinical chemistry-Females						
	0	**0.3**	**1**	**3**	**10**	**30**
AST (U/L)	68.6	111	135.6	107.6	91.6	129.2*
TG (mg/dL)	94.3	51.6	30.8**	18.6**	35.8*	30.0**

* p<0.05, ** p<0.01 (Dunnett's)

Urinalysis
Unremarkable

Gross Pathology
Increased incidence of dark discoloration of ingesta and/or digesta dark areas in the glandular portion of the stomach, carcass emaciation was observed in treated animals, and was more notable in females.

Table 13. Mouse gross pathology

Gross pathology-Males						
Dose	**0**	**0.3**	**1**	**3**	**10**	**30**
Carcass emaciation	1	1	1	2	3	2
Digesta discoloration	1	1	1	2	3	3
Stomach dark area	2	1	2	1	0	2

Gross pathology-Females						
Dose	**0**	**0.3**	**1**	**3**	**10**	**30**
Carcass emaciation	0	7	11	10	11	12
Digesta discoloration	0	2	3	4	4	2
Stomach dark area	0	4	5	4	4	2

Organ Weights
Treatment related changes were observed in females only, and are attributed to the greater decrement in body weight relative to control (-13% to -21%) at doses ≥ 1mg/kg. Dose related decrease in absolute liver weight (at ≥1mg/kg), spleen and heart weights (at ≥3mg/kg), and kidneys and pituitary weights (at ≥10mg/kg) was observed. The decrease in liver and spleen weight was greater than the decrease in body weight.

Body weight was ~5%-6% lower in dosed males, but did not result in substantial changes in organ weights relative to control males.

Table 14. Mouse absolute organ weights

Females						
Dose	**0**	**0.3**	**1**	**3**	**10**	**30**
BW	29.8	27.9	25.9** (-13%)	24.3** (-18%)	24.7** (-17%)	23.5** (-21%)
Brain	0.533	0.517	0.533	0.512	0.501** (-6%)	0.510* (-4%)
Liver	1.299	1.183	1.049* (-19%)	1.015** (-22%)	.0994** (-23%)	0.903** (-30%)
Spleen	0.124	0.108	0.103	0.088** (-29%)	0.086** (-31%)	0.081** (-35%)
Heart	0.178	0.165	0.158	0.145** (-19%)	0.141** (-21%)	0.137** (-23%)
Kidneys	0.449	0.428	0.414	0.410	0.396* (-12%)	0.371** (-17%)
Pituitary	0.03	0.03	0.03	0.03	0.02** (-33%)	0.02** (-33%)

* p<0.05, ** p<0.01 (Dunnett's)

Histopathology
Adequate Battery: Yes

Peer Review: No

Histological Findings:
Liver: Slight to mild centrilobular hepatocellular degeneration was observed in females at ≥ 1mg/kg at week 12, but not at week 24.

Fat: Increased incidence and severity of mesenteric and perirenal fat atrophy was observed in all treated animals at weeks 12 and 24. In the males, Incidence of fat atrophy was slightly decrease at week 24 compared to week 12.

Injection site: Cellulitis was noted in all groups at week 12 and 24, with increased incidence at higher doses.

Lymphocytolysis, characterized by multifocal to diffuse single cell necrosis, was observed in lymph nodes, thymus and spleen of all treated animals at week 12, and was considered to be secondary to poor body conditions.

Stomach: Gastric erosions, characterized by superficial to complete epithelium sloughing, was noted at ≥1 mg/kg, with higher incidence at week 12.

Pancreas: A slight decrease in the relative number of zymogen granules was also noted in treated groups, with higher incidence at week 12.

None of the observed histopathological findings were present at the end of the recovery period.

Table 15. Mouse histopathology

Histopathology-Week 12												
	Males						Females					
	0	0.3	1	3	10	30	0	0.3	1	3	10	30
Fat atrophy												
Mesenteric	2	7	7	10	9	12	7	8	15	14	14	12
Perirenal	2	4	6	10	10	11	1	6	13	14	15	15
Injection site												
Cellulitis	1	0	6	2	4	7	6	0	5	12	6	10
Liver												
Centril. hepat. degeneration						0			2	1	1	3
Lymphocytolysis												
LN mandibular			3	3	3	4			2	6	3	4
LN mesenteric			1	1	1	4		1	2	2	1	2
Spleen	0	2	5	6	7	9	1	7	10	11	11	13
Thymus	5	6	5	11	10	12	5	8	13	14	13	14
Stomach												
Erosion	0	0	2	8	5	8	1	4	9	11	13	13
Pancreas												
Decreased zymogen		1	1	5	4	6	1	4	9	9	7	6

Histopathology-Week 24												
	Males						Females					
	0	0.3	1	3	10	30	0	0.3	1	3	10	30
Fat atrophy												
Mesenteric	1	2	2	5	5	4	0	7	12	15	12	14
Perirenal	0	1	1	2	3	3	0	2	6	8	10	11
Injection site												
Cellulitis	1	7	8	5	5	9	6	6	10	9	11	12
Liver												
Centril. hepat. degeneration						0						0
Necrosis				1	2	1	1	1	0	1	0	0
Lymphocytolysis												
LN mandibular						0						0
LN mesenteric						0						1
Spleen						0						1
Thymus	2	1	2	0	0	0	0	2	3	2	3	3
Stomach												
Erosion	1	1	1	0	0	1	0	1	3	3	2	3
Pancreas												
Decreased zymogen	1	1	1	1	2	2	0	4	5	5	4	5

Toxicokinetics
TK was not included as an endpoint in this study.

Immunogenicity
Metreleptin was immunogenic at all doses, apparent in most animals in most dose groups by month 3 of dosing (earliest time point examined). Despite the ADA, metreleptin retained pharmacological activity for the duration of the 6m dosing period.

Table 16. Mouse immunogenicity (sponsor's table)

		% SEROCONVERSION		
DOSE (mg/kg/day)	GENDER	Month 3	Month 6	Month 7 (Recovery)
0	M	0	0	0
0.3	M	80	40	100
1	M	75	60	60
3	M	40	100	100
10	M	100	100	100
30	M	100	100	100
0	F	0	0	0
0.3	F	0	60	40
1	F	67	60	75
3	F	50	100	60
10	F	100	100	100
30	F	100	100	100

Dosing Solution Analysis
Dose concentration was below the nominal concentration for lot # 10305L5 (0.11 and 3.44mg/ml for 0.2 and 5mg/ml, respectively). Dose concentration for lot # 1101226B6 was within acceptable variations. Lot # 1112285M5 was not analyzed.

Summary of Pivotal Toxicology Studies in Dogs

The predominant finding from the 1 month and the combined 1/3/6 month toxicology studies in beagle dogs was the profound effect of metreleptin on food consumption and body weight. The dose range tested in these studies captured the pharmacological range from a relatively mild reduction in weight gain at the lowest dose to excessive body weight loss that exceeded tolerability at the highest dose. The highest dose compatible with tolerability in dogs was 1.5mg/kg, which defined the NOAEL for the chronic study. The safety margin at the 1.5mg/kg dose is 5-fold to the clinical dose, based on AUC.

Most other findings in the toxicology study were reasonably related to the excessive anorexic effect of metreleptin, particularly in serum chemistry (e.g., decreased protein, fatty acids, and increased BUN, hydroxybutyrate), gross/micro histology of the gastrointestinal tract (stomach/GI hemorrhage) and adipose tissue (atrophy). Upon cessation of drug during the various recovery periods, food intake rapidly improved, as did the resumption of weight gain and normalization of other endpoints.

Metreleptin provoked an ADA rapidly and in nearly all dosed dogs. The ADA did not result in a loss of pharmacological activity, but did increase plasma levels and exposure.

Metreleptin provoked a cellular and humoral response, indicated by the ADA, and including reduced thymic size, lymphoid hyperplasia, and acute & chronic perivasculitis. It is plausible, even likely, that the perivasculitis is related to the ADA. However, it is possible that the severe multi-organ perivasculitis observed at the 5mg/kg dose after a month of dosing, and the subsequent chronic plasmacytic vasculitis seen at all dose groups thereafter, may also reflect pro-inflammatory action of metreleptin[2], independent of the ADA response.

Synopses of Pivotal Toxicology Study in Dogs

Dog: 28-day SC bolus injection and infusion toxicity study

Dose: 0.5, 5mg/kg, administered as s.c. bolus or s.c. infusion (10, 100mg/m^2)
Safety margins: 1, 8 fold MRHD (BSA basis)

Key Study Findings

- Both doses substantially reduced body weight from baseline, ≥30% with bolus injection, ≥5-10% with infusion. Food consumption correlated with weight loss.
- Dosed dogs lost both fat and lean body mass, determined by DEXA
- Dosed dogs were dehydrated/emaciated, showed fat atrophy and reduced pancreatic zymogen granules
- Bone marrow hypocellularity and increased M:E ratio suggested suppressed erythropoiesis, likely secondary to stress of starvation
- Injection site inflammation/swelling at all doses
- Bolus dosing more effectively reduced body weight than infusion dosing.
- No treatment-related deaths or moribundity was observed.

Dogs: 1/3/6 month SC toxicity study followed by a 28-day recovery period

Study no.:	REST070248
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 15, 1995
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	r-metHuLeptin, #10235L5, 10305L5, 1112285M5 (Amgen sourced API)

Key Study Findings

- Metreleptin exerted pharmacological activity (e.g., weight loss/reduced weight gain) at all doses tested, from relatively minimal effect at the lowest dose (0.05mg/kg) to excessive effect that exceeded tolerability at the highest dose (5mg/kg).

- The most profound effect on food consumption, body weight, and associated endpoints were observed in the first month of dosing, which persisted at an attenuated level thereafter to the end of the study.

- Metreleptin provoked a cellular or humoral immune response, indicated by the presence of thymic atrophy, acute multi-organ perivasculitis, chronic plasmacytic perivasculitis, and possibly lymphoid hyperplasia. The relationship of these findings to the ADA response is plausible, even likely, but not certain.

- In general, observations related to metreleptin were reversible upon cessation of treatment; this was particularly evident with food consumption and body weight gain.

- The sponsor proposed a NOAEL of 1.5mg/kg, citing the highest dose considered most tolerable in the study. This reviewer agrees with the sponsor's assessment, but additionally cites severe multi-organ perivasculitis present at 5mg/kg as the potentially more important finding (if unrelated to the ADA response) that defines the 1.5mg/kg NOAEL. Exposure at the NOAEL provides ~5-fold margin to the clinical dose, based on AUC (day 1 dog AUC: ~6500ng*h/ml; day 1 human AUC, estimated for 0.15mg/kg: 1170ng*h/ml)

Additional Comment: Dogs were not exposed to steady-state levels at any dose of metreleptin tested in this study. Given a measured ~2hr half-life in dog plasma, metreleptin was absent in all blood samples collected 24h post-dose in this study.

Methods

Doses:	0.05, 0.15, 0.5, 1.5mg/kg for 6-months 5mg/kg for 3-months
Frequency of dosing:	Once daily
Route of administration:	SC injection, daily,
Dose volume:	1mL
Formulation/Vehicle:	10mM Histidine, 5% sorbitol.
Species/Strain:	Beagle Dogs
	27/sex from (b) (4)
	50/sex from (b) (4)
Number/Sex/Group:	12/9/9/14/14/14/sex
Age:	5-6 months old
Weight:	Males: 7.3-10.5kg. Females: 6.4-9.7kg
Satellite groups:	TK group: 1/sex/group
	Recovery group: 2 to 3 animals per sex entered the recovery phase at week 4, 8, 12, 24
Unique study design:	3/sex/group underwent necropsy at dosing week 4, 8, 12, 24

Observations and Results
Mortality
One female in the 0.5mg/kg group and one male in the 1.5mg/kg group were euthanized during weeks 6 and 8, respectively. Both animals were prostrated and the male had decreased respiration. Body weight loss in the female and male was 41% and 37% respectively. Both euthanizations appeared due to severe nutritional stress.

Clinical Signs
- Thinness was observed at ≥0.15mg/kg at month 3 and 6.
- Dermal atonia was observed at ≥0.5mg/kg at month 3, but was less frequent at month 6.
- Longer capillary refill time (CRT) was observed in dogs at ≥0.5mg/kg at month 3, with some improvement by month 6.

Table 17. Dog clinical signs

3-month (total occurrence/no. of animals)							
Dose		**0**	**0.05**	**0.15**	**0.5**	**1.5**	**5**
N		**3**	**3**	**3**	**3**	**4**	**9**
Thin	M	0	1/1	0	2/2	3/3	1/1
	F		0	1/1	3/3	3/3	2/2
Dermal atonia	M				0	2/2	1/1
	F			0	3/3	2/2	1/1
CRT >2sec	M	1/1	0	0	1/1	2/2	4/4
	F			0	2/2	8/6	4/4

6-month (total occurrence/no. of animals)						
Dose		**0**	**0.05**	**0.15**	**0.5**	**1.5**
N		**3**	**3**	**3**	**3**	**3**
Thin	M			2/2	0	2/2
	F		0	2/2	2/2	2/2
Dermal atonia	M			1/1	0	0
	F			1/1	1/1	0
CRT 1-2sec	M				0	5/5
	F				0	6/6

Body temperature
Unremarkable

Body Weights
Metreleptin very robustly and dose-dependently reduced body weight within the first few weeks of dosing. The reduction in BW is relative to baseline, not to control, indicating actual loss of BW. Its anorexic effect waned but did not disappear with continued dosing over the 6 month period. A sustained effect on body weight (either less weight gain or actual weight loss vs. baseline) was observed in males and females at doses ≥ 0.15mg/kg.

A rebound in weight gain was observed during the recovery period following the 6 month dosing period, at a rate that exceeded weight gain in the control dogs.

Excessive weight loss exceeded tolerability in males and females within the first 3 months of dosing at 5mg/kg. This dose was discontinued at week 12 and the dogs remained on-study as an unplanned recovery group. Weight gain resumed and the clinical condition normalized after dosing was halted.

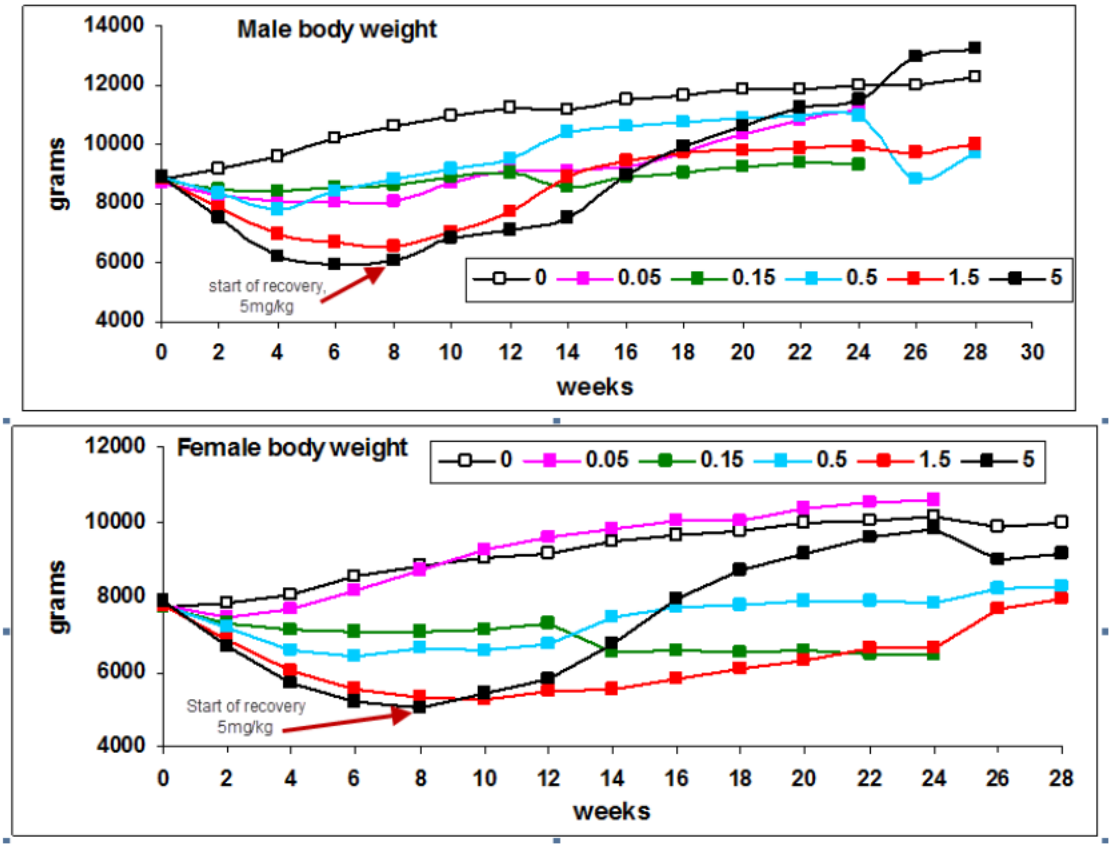
The sponsor noted that the diet of some animals in the dosed groups was supplemented with Alpo and/or boluses of dextrose/electrolytes when nutritional status was considered at-risk.

Table 18. Body Weight.

Male Body Weight: Percent change from Baseline				
Dose	1-mo	3-mo	6-mo	Recovery (6-mo cohort)
0	+9	+27	+36	+4
0.05	-7	+5	+27	*nd*
0.15	-5	+2	+6	*nd*
0.5	-11	+7	+23	+20
1.5	-22	-13	+13	+15
5*	-31	-20		+90*

Female Body Weight: Percent change from Baseline				
Dose	1-mo	3-mo	6-mo	Recovery (6-mo cohort)
0	+5	+18	+31	+2
0.05	-1	+23	+36	*nd*
0.15	-10	-6	-17	*nd*
0.5	-18	-14	+1	+6
1.5	-24	-30	-15	+11
5*	-29	-27		+49*

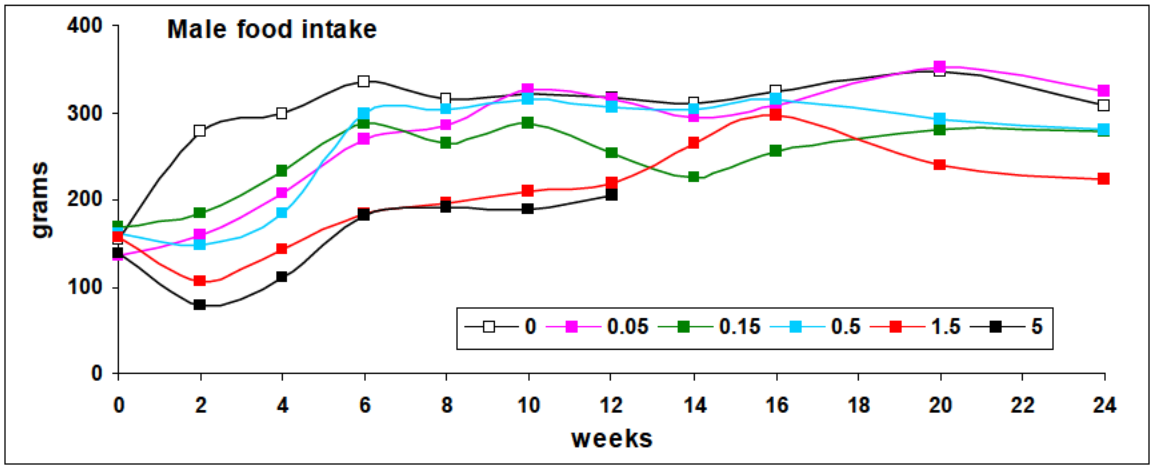
***Dosing of entire 5mg/kg cohort was stopped at week 12, but continued in non-dosing recovery to the end of the study. 'Recovery' of the 5mg/kg group denotes weight gained from month 3 after dosing was discontinued.*

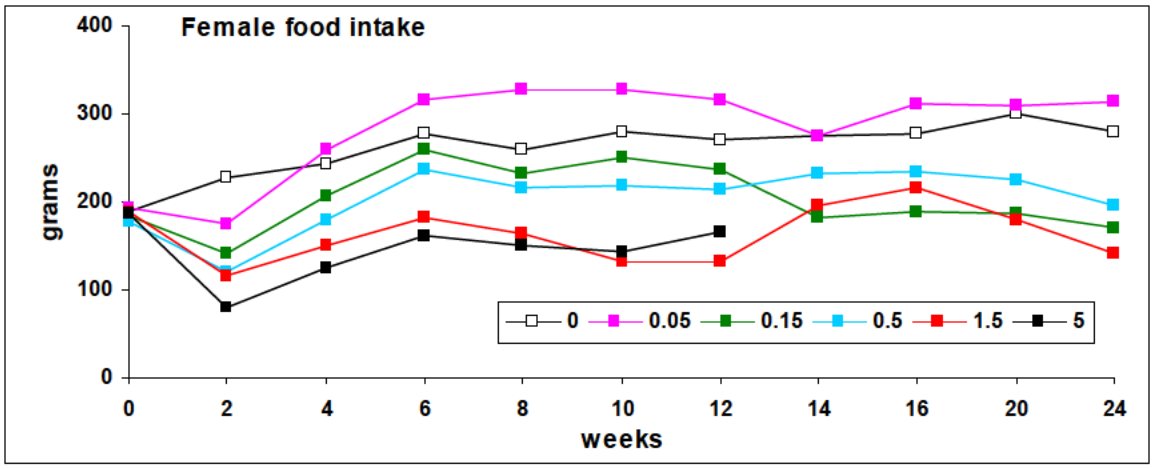


Feed Consumption

In correlation with its effect on body weight, metreleptin very robustly and dose-dependently suppressed food intake during the first few weeks of dosing. As the suppressive effect on food intake waned, the dogs tended to gain more body weight. Given the absence of other suspect toxicities, it is presumed that the changes in body weight were primarily due to metreleptin's suppressive effect on appetite and food intake.

Table 19. Dog food consumption





Ophthalmoscopy
Unremarkable

ECG
ECG was recorded prior to initiation of dosing and during weeks 3, 7, 11, 23, and 27. No treatment related changes were observed.

Hematology
Decrease in red blood cells, hemoglobin and hematocrit was observed at ≥0.5mg/kg at the end of weeks 8 and 12 of dosing, but recovered by the end of week 24 of dosing.

Bone marrow smears were prepared, but microscopic examination was not deemed to be necessary by the sponsor, pathologist or study director.

Table 20. Dog hematology parameters

Hematology-Males							
		0	**0.05**	**0.15**	**0.5**	**1.5**	**5**
RBC mil/uL	week 8	6.02	5.89	6.19	5.74	5.35	4.42**
	week 12	6.55	6.13	6.28	5.63**	5.34**	5.02**
	week 24	6.75	6.99	6.33	6.37	7.32	na
HB g/dL	week 8	14.0	13.7	14.5	13.6	12.2*	10.1**
	week 12	15.0	14.0	14.4	13.3	12.0**	11.4**
	week 24	15.6	15.9	14.2	14.9	16.2	na
HTC %	week 8	40.1	38.5	41.0	38.6	33.9**	28.2**
	week 12	45.9	42.6	43.7	39.4*	35.4**	34.0**
	week 24	45.7	46.3	41.3	42.9	47.2	na

* p<0.05, ** p<0.01

Hematology-Females							
		0	**0.05**	**0.15**	**0.5**	**1.5**	**5**
RBC mil/uL	week 8	6.14	5.74	5.99	5.67	5.11**	4.92**
	week 12	6.61	6.45	5.93	5.38**	4.82**	5.33**
	week 24	6.55	6.52	6.35	6.16	6.28	na
HB g/dL	week 8	14.4	13.6	13.9	13.5	11.7**	11.5**
	week 12	15.0	15.0	13.7	12.6*	10.9**	12.2*
	week 24	15.1	14.4	14.2	14.1	14.0	na
HTC %	week 8	40.6	38.3	39.6	37.5	32.8**	31.9**
	week 12	45.9	45.4	41.1	37.5*	32.6**	36.6**
	week 24	44.0	42.6	41.7	40.9	40.5	na

* $p<0.05$, ** $p<0.01$

Clinical Chemistry
Metreleptin changed several parameters within the first 1- to 3-months of dosing, with only minor changes being observed by 6 months. Some of these early changes include decreased albumin, total protein, free fatty acids and vitamin A. Increases in ALT, urea nitrogen, butyl hyrdoxybutyrate, and cortisol were also observed. This profile of changes is suggestive of nutritional stress, which is further suggested by the temporal association between the change in these parameters and suppressed food intake and loss of body weight.

Urinalysis
Reduced urine volume was noted early in the dosing phase, consistent with the extreme anorexic effect of metreleptin during this time. Parameters were not clearly affected by metreleptin later in the study (e.g., pH, specific gravity).

Gross Pathology
Adverse gross findings were absent at the 6 month time point. At the earlier 1 and 3 month time points, gastrointestinal findings included dark red contents and evidence of hemorrhage, primarily at the 5mg/kg dose, but also in single animals at lower dose groups. The reviewer agrees with the sponsor that the adverse gastrointestinal observations were secondary to a state of starvation early in the study. Also, a complete absence of adipose tissue was reported for some single animals across the dose range, consistent with excessive pharmacological activity of metreleptin.

Organ Weights
Thymus weight (and occasionally visible size) was lower in most dose groups when examined at months 1 and 3, with an apparent return to control levels by month 6. Statistical significance was reached only once, at the 1 month time point in the 5mg/kg dose, but non-significant decreases were observed in most other dose groups through month 3, nonetheless. Normalization to brain weight or body yielded a similar finding. The cause for a smaller thymus is unclear. Other decreases were observed in liver, kidney, heart, and pancreas, most notably at week 4, with an apparent return to control levels by month 6.

Table 21. Thymus Weight in Dogs

Thymus Weight in Males (normalized to brain weight)						
Month	***0***	***0.05***	***0.15***	***0.5***	***1.5***	***5***
1m	10	6.35	10.5	5.3	3.7	1.4*
3m	12.3	12.1	11.9	5.9	4.4	4.4
6m	6.6	7.7	8.2	13.1	13.4	

Thymus Weight in Females (normalized to brain weight)						
Month	***0***	***0.05***	***0.15***	***0.5***	***1.5***	***5***
1m	11.2	6.5	7.9	4.9	3.5	2.3
3m	13.5	(20.9)	9.6	3.9	4.5	6.4
6m	10.3	11.5	4.8	5.1	11.9	

Shaded area indicate numerical or statistical* dose-related decrease in thymus weight.
*p<0.05, ** p<0.01

Histopathology
Adequate Battery: Yes

Peer Review: Thyroid tissue was peer reviewed by two external pathologists, at the request of the sponsor. Other tissues were not peer reviewed. The sponsor contracted with (b) (4) to conduct the primary histopathology reading.

Histological Findings

Perivasculitis:
An unanticipated finding was the occurrence of perivasculitis in response to metreleptin. It appears that metreleptin provoked a cellular/humoral immune reaction that affected multiple organs early after initiation of treatment (evident at 1 month), resolving into a chronic plasmacytic infiltratrate only at the injection sites at later time points (months 3 and 6).

Two of three males and females in the 5mg/kg dose group exhibited multi-organ perivasculitis after the first month of dosing. Affected organs included the injection site, adipose, liver, lungs, heart, kidneys, muscle, skin, stomach, and urinary bladder. The lesions were moderate to severe and were described as involving 'significant' infiltrates of lymphocytes, plasma cells, and neutrophils. The incidence in lower dose groups at one month was low and sporadic, with one control female showing vasculitis at the placebo injection site. However, the metreleptin-dosed cases showed vasculitis in the adipose tissue and bladder. The recovery animals from the 5mg/kg dose group did not show perivasculitis, suggesting that the effect is reversible once dosing is stopped.

The acute multi-organ perivasculitis at one month evolved to a chronic plasmacytic infiltrate at three and six months that was confined to the injection site, but was now seen in all dose groups. Severity was minimal to moderate, but severe at the 5mg/kg dose (3m time point). Of note, there were cases of control dogs having this lesion, which confounds interpretation. However, given the clarity of the response at 5mg/kg

and the rather consistently increased incidence across the dose groups, this finding is considered related to metreleptin treatment at all doses, with the most severe effects occurring at 5mg/kg.

Histopathology: Perivasculitis in Males						
Dose	0	0.05	0.15	0.5	1.5	5
Month 1						
Perivasculitis	1	0	1	0	0	2
Comment:	injection site		adipose			multi-organ, severe
Month 3						
Chronic plasmactyic perivasculitis, injection site	0	2	3	3	3	2 severe
Month 6						
Chronic plasmactyic perivasculitis, injection site	1	3	1	2	3	nd

Histopathology: Perivasculitis in Females						
Dose	0	0.05	0.15	0.5	1.5	5
Month 1						
Perivasculitis	1	1	0	1	0	2
Comment:	injection site	adipose		bladder		multi-organ, severe
Month 3						
Chronic plasmactyic perivasculitis, injection site	2	3	2	2	2	3 severe
Month 6						
Chronic plasmactyic perivasculitis, injection site	0	2	2	1	3	nd

Thymus Gland
Thymic atrophy was reported in dosed groups at each of the examined time points. The clearest dose-dependence appears to be at months 1 and 3. Of note, this histological finding generally correlates with smaller thymic size and weight in the dosed groups. Dogs from the recovery groups also variably showed persistence of thymic atrophy, so reversibility of this effect is either slow or absent.

Histopathology: Thymus in Males						
Dose	**0**	**0.05**	**0.15**	**0.5**	**1.5**	**5**
Month 1						
Atrophy	0	1	0	0	2	3
Month 3						
Atrophy	0	0	0	2	2	1
Month 6						
Atrophy	0	1	1	1	0	nd

Histopathology: Thymus in Females						
Dose	**0**	**0.05**	**0.15**	**0.5**	**1.5**	**5**
Month 1						
Atrophy	0	1	1	0	1	3
Month 3						
Atrophy	0	0	1	2	2	1
Month 6						
Atrophy	0	0	2	2	0	nd

Adipose Tissue
Consistent with metreleptin's anorexic effect in this study, atrophy of the adipose tissue was observed in most animals at doses ≥ 0.15mg/kg, being most prominent at the 0.5, 1.5, and 5mg/kg. Evidence of recovery was seen in concert with resumption of weight gain after cessation of dosing.

Histopathology: Adipose in Males						
Dose	**0**	**0.05**	**0.15**	**0.5**	**1.5**	**5**
Month 1						
Atrophy	0	1	2	3	3	3
Month 3						
Atrophy	0	1	1	1	2	2
Month 6						
Atrophy	0	0	2	0	3	nd

Histopathology: Adipose in Females						
Dose	**0**	**0.05**	**0.15**	**0.5**	**1.5**	**5**
Month 1						
	0	1	1	2	3	2
Month 3						
	0	0	1	2	3	2
Month 6						
	0	0	2	2	3	nd

Gastrointestinal Tract
The gross findings of stomach/intestinal injury at 1 and 3 months dosing at 5mg/kg was accompanied by histological evidence of supportive inflammation and submucosal hemorrhage. These findings were absent by 6 months.

Lymphoid tissue
The pathologist made note of prominent lymphoid hyperplasia in two high dose dogs at 1 month (male 3778 and female 3810). Similar lymphoid hyperplasia was reported sporadically in other dose groups, including controls, at later time points. The response at 5mg/kg is reasonably associated with treatment, but it is difficult to ascribe the isolated cases at lower doses to metreleptin. However, given the apparent immunostimulative response to metreleptin, a relationship to treatment cannot be entirely dismissed.

Thyroid Gland
There appeared to be some difficulty among the pathologists in describing some minimal to mild histological changes in the thyroid gland. At issue is the presence of small thyroid lobes but with focal 'hyperplastic follicles'. One interpretation was that the change resulted from a chronic elevation in TSH, but such evidence for an increase is

absent. The other interpretation is that the change is consistent with thyroid histology in beagles. Slightly greater incidence and severity of follicular hyperplasia was seen in high dose dogs at the 5mg/kg dose. Given the relatively minor histological change and the transitory nature of the finding, the review does not consider the thyroid findings of any obvious toxicological consequence.

PCNA analysis
PCNA analysis was conducted in controls and 5mg/kg groups at month 1, 3 and 6. Tissue examined included adrenals, brain, colon, duodenum, kidney, liver (hepatocytes and endothelial cells), lung, mammary gland, pancreas (islet and exocrine), pituitary, spleen (red pulp and white pulp), stomach, testes and thyroid. Tissue sections were incubated with a monoclonal antibody to PCNA and reagents required for the avidin-biotin peroxidase method for the detection of the antigen-antibody complex. PCNA expression in cells in all phases of the cell cycle was localized by the chromagen 3,3'diaminobenzidine.

Metreleptin induced a slight increase in cell proliferation in the lung of HD males at 6-month. However, the proliferating indices were similar or below that of control groups at 1 and 3 months, so this increase was not considered biologically significant.

In female dogs there was insufficient mammary gland tissue that precluded evaluation of a proliferating index at 1- and 3-month.

Table 22. Dog PCNA analysis (sponsor's table)

Males

Dose	Time (mo.)	Adrenal[b]	Brain (hypothalamus)[d]	Colon[f]	Stomach[f]	Duodenum[f]	Kidney[d]	Liver[d]	Endothelium Liver[g]	Lung[e]
Control	1	2.0	6.7	9.3	0.7	22.7	6.3	4.3	1.7	30.0
5 mg/kg/day	1	1.7	3.3	10.0	0.7	26.7	3.0	2.0	0.3	20.7
Control	3	1.7	4.3	11.0	1.7	17.7	3.3	5.0	0.3	37.7
5 mg/kg/day	3	1.3	4.7	8.7	0.3	18.3	3.3	1.7	0.7	26.0
Control	6	2.0	4.0	8.7	0.7	21.0	3.0	1.7	0.7	17.3
5 mg/kg/day	6	1.0	7.7	8.0	0.0	24.3	3.0	3.7	0.0	29.0

Dose	Time (mo.)	Mammary Gland[d]	Pancreas Islets[b]	Pancreas Exocrine[b]	Pituitary[b]	Testes Leydig Cells[a]	Thyroid[b]	Spleen Red Pulp[b]	Spleen White Pulp[c]
Control	1	ND	0.3	2.0	1.0	1.7%	1.0	2.3	331.3
5 mg/kg/day	1	ND	0.0	0.3	0.7	0.7%	0.0	3.3	338.0
Control	3	ND	0.3	1.7	1.3	2.7%	0.3	1.7	285.7
5 mg/kg/day	3	ND	0.0	2.0	1.0	3.0%	0.3	2.0	419.0
Control	6	ND	0.0	1.3	0.7	0.7%	0.0	1.7	234.0
5 mg/kg/day	6	ND	0.0	1.0	0.7	2.0%	0.7	1.7	271.0

Females

Dose	Time (mo.)	Adrenal[b]	Brain (hypothalamus)[d]	Colon[f]	Stomach[f]	Duodenum[f]	Kidney[d]	Liver[d]	Endothelium Liver[g]	Lung[e]
Control	1	0.7	8.7	7.7	0.3	15.3	2.3	2.7	0.0	29.0
5 mg/kg/day	1	1.0	2.7	15.7	0.3	21.3	3.0	3.3	0.3	27.7
Control	3	1.0	5.7	5.7	0.7	23.7	1.3	1.7	0.0	27.7
5 mg/kg/day	3	1.3	4.3	11.0	1.0	21.3	2.7	4.7	0.0	23.3
Control	6	1.0	2.7	10.3	1.0	29.7	2.3	1.3	0.0	29.3
5 mg/kg/day	6	1.3	2.3	10.7	0.0	27.0	4.0	0.7	0.0	30.0

Dose	Time (mo.)	Mammary Gland[d]	Pancreas Islets[b]	Pancreas Exocrine[b]	Pituitary[b]	Testes Leydig Cells[a]	Thyroid[b]	Spleen Red Pulp[b]	Spleen White Pulp[c]
Control	1	ND	0.0	0.7	0.7	ND	0.0	2.0	364.0
5 mg/kg/day	1	ND	0.0	0.3	1.0	ND	0.0	2.3	355.3
Control	3	ND	0.0	1.3	0.3	ND	0.0	1.0	297.0
5 mg/kg/day	3	50.0	0.0	1.0	0.7	ND	0.3	2.0	302.3
Control	6	155.3	0.0	1.7	1.0	ND	0.0	1.7	306.3
5 mg/kg/day	6	87.7	0.3	1.3	1.0	ND	0.0	1.7	297.3

Toxicokinetics

Blood samples were collected from 1 male/female per dose group on days 1, 7, 14, and 28 of the study. Dogs were serially bled on each sampling day at 0, 1, 2, 4, 8, and 24h following dosing. Exposure expressed as AUC was similar on Days 1 and 7, but the assay became confounded by antibody development by Day 14 (marked increase in exposure).

Of special note, metreleptin was absent in serum samples collected 24h post-dose, indicating that dogs were not subjected to steady-state exposure in this study. The measured half-life of metreleptin in dogs is ~2 hours.

Table 23. Dog toxicokinetics

Male Toxicokinetics									
	AUC0-24 (ng.h/mL)			Cmax (ng/ml)			Tmax (h)		
	D1	D7	D14*	D1	D7	D14*	D1	D7	D14
0.05	214.3	250.8	602.3	30.9	47.7	101.0	2	2	2
0.15	596.6	675.5	1766.9	70.1	91.1	288.2	2	2	2
0.5	3065.4	2488.6	18422.1	291.1	302.1	1680.1	2	2	2
1.5	6457.0	9981.8	64901.9	629.4	1026.9	6700	2	4	2
5	32334.8	32980.6	NC	3268.9	3962.3	NC	4	2	NC

Female Toxicokinetics									
	AUC0-24 (ng.h/mL)			Cmax (ng/ml)			Tmax (h)		
	D1	D7	D14*	D1	D7	D14*	D1	D7	D14
0.05	238.1	268.4	NC	23.7	31.9	NC	2	2	NC
0.15	932.8	771.9	2347.7	88.3	107.2	358	2	2	2
0.5	3406.8	2640.4	27482.1	470.5	443.6	2649.4	2	2	2
1.5	6881.8	7863.9	37241.8	938.5	1312.7	3979.8	2	2	2
5	21475.3	19076.4	64817.3	2380.9	4058.7	7205.1	4	2	4

**D14 data confounded by presence of ADA*

Immunogenicity
Metreleptin was immunogenic at all doses. Neutralizing antibody assay was not done. However, whether neutralizing or not, sustained body weight loss observed throughout the study indicates that pharmacological activity persisted despite the ADA.

Table 24. Dog immunogenicity (sponsor's table)

	Study Month					
	1	1-month recovery	2	3	6	1-month recovery
DOSE (mg/kg/day)	% SEROCONVERSION					
0.00	4	-	6	0	36	33
0.05	88	-	100	100	100	-
0.15	100	-	100	91	100	-
0.50	94	100	90	95	36	40
1.50	100	100	100	94	91	25
5.00	100	100	100	89	25	0

Dosing Solution Analy**sis**
Leptin concentration in the 0.05mg/kg group was approximately half of the nominal concentration at weeks 1, 4, 13 and 26.

One-Month Bridging Toxicology Study in Mice

One-month bridging toxicology study was conducted in CD1 mice to determine comparability between the toxicological profile of metreleptin drug substance manufactured at Amgen and the metreleptin drug substance manufactured at Sandoz. The Amgen product has been used in the non-clinical and clinical studies conducted to support the development of metreleptin, whereas the Sandoz drug substance will be used for the marketed metreleptin product.

Design:
The bridging study with the Sandoz drug substance was conducted at metreleptin doses of 1, 10, and 100mg/kg to replicate a previous study conducted with the Amgen drug substance. Metreleptin was administered subcutaneously for 28 days, followed by a recovery period of 4 weeks.

	Amgen DS	Sandoz DS
Metreleptin DS batch #	10235L5, 10305L5	48201701, 4821072
Year conducted	1995	2010
Strain	Swiss albino	CD1 albino (swiss)
CRO name and location	(b) (4)	
Duration	28-day + 4-week recovery	
Doses	1, 10, 100mg/kg	
Route	SC injection	
Vehicle	20mg/mL Glycine, 10mg/mL Sucrose, 1.471mg/mL L-Glutamic Acid, 0.1mg/mL Polysorbate 20 in Sterile Water	

Key Study Findings

- Tolerability was exceeded at 100mg/kg, as 5 mice were found dead or euthanized on days 10-13 of dosing. While a cause of death is not clear, excessive weight loss was probably a contributing factor.

- The primary drug-related finding was a reduction in weight gain. Mice lost body weight relative to baseline at the MD and HD. Mice at the LD gained body weight but the gain was substantially less than in the controls. The body weight rapidly returned in all dose groups during recovery.

- Changes in body weight generally tracked with changes in food intake.

- Histologically, fat atrophy/depletion was seen in the subcutis and hilar fat deposits of the kidney. Liver rarefaction was less common with dosing, indicating depletion of glycogen stores.

- Injection sites and other skin sites showed mixed cell infiltrates with a NOAEL of 1mg/kg (LD). This was not observed in recovery animals.

- Exposure accumulated substantially after 28 days of dosing, likely secondary to an anti-drug antibody response (immunogenicity data were not submitted)

Conclusion:
The toxicological profile of metreleptin drug substance from Amgen and Sandoz are comparable.

7 Genetic Toxicology

The in vitro studies were conducted in 2011 with the Sandoz drug substance manufactured at 1000L scale (lot # B097141), whereas the in vivo study was conducted in 1996 with the Amgen drug substance (lot # 1108136H6). The drug substance from Amgen and Sandoz showed a similar toxicological profile in a 1-month bridging study

conducted in CD1 mice, in the Ames test and in the in vitro mammalian chromosome aberration test.

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

Study no.:	REST110186
Study report location:	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	September 19, 2011
GLP compliance:	Yes
QA statement:	No
Drug, lot #, and % purity:	Yes AC164594 (Metreleptin), B097141 (Sandoz 1000L scale), 95.2%

Key Study Findings

Metreleptin was not mutagenic in any bacterial strain tested.

Methods

Strains:	TA1535, TA1537, TA98, TA100 and WP2 *uvrA*
Concentrations in definitive study:	160, 320, 640, 1280, 2560, 5000μg/plate
Basis of concentration selection:	Limit dose recommended by regulatory guidelines
Negative control:	20mg/mL glycine, 10mg/mL sucrose, 1.4mg/mL L-glutamic acid and 0.1mg/mL polysorbate 20 in Sterile Water for Injection.
Positive control:	- S9: NaAz, 9AC, 2NF, NQO + S9: 2AA, BaP
Metabolic activation system	Phenobarbital/5,6-benzoflavone induced rat liver S9
Formulation/Vehicle:	Same as negative control
Incubation & sampling time:	The plate incorporation method was used without S9 and the "treat and wash modification" of the method (Thompson *et al* 2005) was used in the presence of S9 to avoid feeding effects resulting from breakdown of the test article due to the proteolytic nature of S9. Incubation was at 37C for ~65hrs

Study Validity

The mean revertant colony count of the vehicle control was within the current historical control range of the laboratory. All positive controls increased the number of revertant colony at least twice (1.5 fold for strain TA100) the concurrent vehicle control levels. Therefore the study is considered valid.

Results

No substantial increases in the revertant colony counts were obtained with any bacterial strain following exposure to metreleptin in either the plate incorporation or treat and wash assay, in the absence or presence of S9 mix.

Table 25. Ames: Plate incorporation assay (sponsor's table)

Table 5 **AC164594 - Plate Incorporation Assay - Confirmatory Test**

Strain	Conc. (µg/plate)	S9	Number of revertants					Plate observations *			Fold response †
			x_1	x_2	x_3	mean	SD	x_1	x_2	x_3	
TA1535	Vehicle A	0	61	52	57	**57**	5				1.0
	320	0	43	59	68	**57**	13				1.0
	640	0	46	40	49	**45**	5				0.8
	1280	0	53	53	58	**55**	3				1.0
	2560	0	57	60	50	**56**	5				1.0
	5000	0	51	48	72	**57**	13				1.0
TA1537	Vehicle A	0	12	27	14	**18**	8				1.0
	320	0	15	14	18	**16**	2				0.9
	640	0	25	11	11	**16**	8				0.9
	1280	0	17	12	17	**15**	3				0.9
	2560	0	15	16	20	**17**	3				1.0
	5000	0	12	23	14	**16**	6				0.9
TA98	Vehicle A	0	46	29	32	**36**	9				1.0
	320	0	20	23	24	**22**	2				0.6
	640	0	27	28	40	**32**	7				0.9
	1280	0	23	32	32	**29**	5				0.8
	2560	0	26	31	28	**28**	3				0.8
	5000	0	32	32	32	**32**	0				0.9
TA100	Vehicle A	0	151	132	127	**137**	13				1.0
	320	0	138	131	136	**135**	4				1.0
	640	0	121	123	119	**121**	2				0.9
	1280	0	122	141	120	**128**	12				0.9
	2560	0	128	114	121	**121**	7				0.9
	5000	0	140	134	137	**137**	3				1.0
WP2 uvrA	Vehicle A	0	46	72	35	**51**	19				1.0
	320	0	43	52	43	**46**	5				0.9
	640	0	36	67	58	**54**	16				1.1
	1280	0	46	50	34	**43**	8				0.8
	2560	0	45	44	52	**47**	4				0.9
	5000	0	54	57	37	**49**	11				1.0

* No comments on the plate or background lawn

† Fold response in mean revertants compared to concurrent vehicle control

SD Sample standard deviation

Table 26. Ames: Treat and wash assay (sponsor's table)

Table 6 **AC164594 - Treat and Wash Assay - Confirmatory Test**

Strain	Conc. (µg/plate)	S9	Number of revertants					Plate observations *			Fold response †
			x_1	x_2	x_3	**mean**	SD	x_1	x_2	x_3	
TA1535	Vehicle A	+	71	80	60	**70**	10				1.0
	320	+	74	88	88	**83**	8				1.2
	640	+	72	81	85	**79**	7				1.1
	1280	+	75	70	68	**71**	4				1.0
	2560	+	91	69	77	**79**	11				1.1
	5000	+	77	83	58	**73**	13				1.0
TA1537	Vehicle A	+	14	25	22	**20**	6				1.0
	320	+	18	17	13	**16**	3				0.8
	640	+	29	18	10	**19**	10				0.9
	1280	+	12	22	22	**19**	6				0.9
	2560	+	18	27	20	**22**	5				1.1
	5000	+	24	14	20	**19**	5				1.0
TA98	Vehicle A	+	34	38	36	**36**	2				1.0
	320	+	30	34	30	**31**	2				0.9
	640	+	31	31	39	**34**	5				0.9
	1280	+	34	41	36	**37**	4				1.0
	2560	+	38	42	40	**40**	2				1.1
	5000	+	32	25	33	**30**	4				0.8
TA100	Vehicle A	+	113	115	119	**116**	3				1.0
	320	+	134	138	134	**135**	2				1.2
	640	+	132	117	108	**119**	12				1.0
	1280	+	152	130	153	**145**	13				1.3
	2560	+	106	123	138	**122**	16				1.1
	5000	+	105	129	119	**118**	12				1.0
WP2 *uvrA*	Vehicle A	+	49	59	54	**54**	5				1.0
	320	+	75	65	59	**66**	8				1.2
	640	+	63	60	58	**60**	3				1.1
	1280	+	42	48	57	**49**	8				0.9
	2560	+	66	55	58	**60**	6				1.1
	5000	+	50	55	50	**52**	3				1.0

* No comments on the plate or background lawn

† Fold response in mean revertants compared to concurrent vehicle control

SD Sample standard deviation

7.2 *In Vitro* Assays in Mammalian Cells

Study title: In Vitro Mammalian Chromosome Aberration Test in Human Peripheral Blood Lymphocytes

Study no.:	REST110185
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 19, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AC164594 (Metreleptin), B097141 (Sandoz 1000L scale), 95.2%

Key Study Findings

Metreleptin did not induce chromosome aberrations in the presence or absence of metabolic activation.

Methods

Cell line:	Human peripheral blood lymphocytes
Concentrations in definitive study:	10 to 5000µg/mL 4 hr treatment period +/- S9 10 to 4000µg/mL in the 21 hr treatment period
Basis of concentration selection:	Short term: Maximum dose level by the OECD. Long term: Toxicity
Negative control:	20mg/mL glycine, 10mg/mL sucrose, 1.4mg/mL L-glutamic acid and 0.1mg/mL polysorbate 20 in Sterile Water for Injection.
Positive control:	MMC without S9; CP with S9
Metabolic activation system	Phenobarbital/5,6-benzoflavone induced rat liver S9
Formulation/Vehicle:	Same as negative control
Incubation & sampling time:	Short term: 4 hrs + 17 hrs recovery at 37C Long term: 21hr at 37C After colcemid addition, all cultures were incubated at 37C for ~ two hours prior to harvesting.

Study Validity

The negative control results were within the historical control range, while the positive control resulted in increased incidence of aberrant cells (at least twice) compared with the concurrent control. Therefore, the study is considered valid.

Results

Metreleptin did not cause any increases in the proportion of aberrant metaphases, or in the incidence of chromatid or chromosome gaps or polyploidy.

Table 27. Chromosomal aberration assay in HPBL (sponsor's table)

Treatment	Conc. (µg/mL)	MI	RMI (%)	Number cells examined	% Aberrant	Number of aberrations					Incidental observations †			
						b	e	B	E	other	(g	G	P	C)
4 hours treatment in the absence of S9 (0S9)														
Vehicle‡	-	6.9	100	200	0.0	0	0	0	0	0	1	0	0	1
AC164594	1280	8.1	118	200	0.5	1	0	0	0	0	2	0	0	0
	2560 D	7.4	108	200	0.0	0	0	0	0	0	2	0	0	0
	5000 D	8.0	117	200	0.5	1	0	0	0	0	1	0	0	1
MMC	0.10	10.1	147	200	11.0*	16	9	1	0	0	3	1	0	0
4 hours treatment in the presence of S9 (+S9)														
Vehicle‡	-	8.9	100	200	0.0	0	0	0	0	0	0	0	0	0
AC164594	1280	6.5	73	200	1.0	2	0	0	0	0	0	0	1	0
	2560ppt	9.4	106	200	0.0	0	0	0	0	0	0	0	0	0
	5000ppt	8.9	100	200	0.5	1	0	0	0	0	0	0	0	0
CP	6.0	7.1	80	200	8.0*	17	1	0	0	0	2	0	0	1
21 hours treatment in the absence of S9 (0S9)														
Vehicle‡	- A	4.2	100	200	0.5	0	1	0	0	0	1	0	0	0
	-	3.7	100	200	0.0	0	0	0	0	0	1	0	0	0
AC164594	1280	4.2	114	200	1.0	2	0	0	0	0	1	0	0	1
	2560ppt	3.4	92	200	0.0	0	0	0	0	0	1	0	0	0
	4000 A,ppt	2.5	59	200	1.5	4	0	0	0	0	0	0	0	0
MMC	0.05	3.7	102	200	9.0*	18	0	2	0	0	3	0	0	0

MI, RMI	Mitotic Index, Relative Mitotic Index (vehicle = 100%)
b, e, g	Chromatid break, exchange, gap
B, E, G	Chromosome break, exchange, gap
other	Includes pulverized chromosomes and cells with > 8 aberrations
P	Polyploidy and endoreduplication
C	Centromeric disruption
†	g, G, P and C are excluded from the calculation of % aberrant cells
‡	A vehicle dose volume of 1250 µL was used for both 4 hour treatment regimes and vehicle dose volumes of 640 µL and 1000 µL were used in the 21 hour treatment regime
A	Vehicle and experimental culture used a high dose volume of 1000µL and were therefore compared when determining the RMI
D	Cloudy media at the end of treatment
ppt	Precipitate visible in the culture medium at the end of treatment
*	Substantial increase in the number of aberrant metaphases

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

Study title: Mammalian erythrocyte micronucleus test

Study no:	REST20285
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 23, 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	r-metHuLeptin, lot # 1108136H6 (Amgen)

Key Study Findings
Metreleptin was not clastogenic in mice at doses up to 100mg/kg (24X MRHD).

Methods

Doses in definitive study:	10, 30, 100mg/kg
Frequency of dosing:	Single dose
Route of administration:	IV injection
Dose volume:	5ml/kg
Formulation/Vehicle:	Acetate formulation (Acetate 10mM, sorbitol 5%)
Species/Strain:	CD1 male mice, from (b) (4)
Number/Sex/Group:	5 males/group
Satellite groups:	NA
Basis of dose selection:	HD was the lethal dose in a 28-day mouse study
Negative control:	Acetate formulation (Acetate 10mM, sorbitol 5%)
Positive control:	CP, 50mg/kg

Study Validity
The mean incidence of micronucleated polychromatic erythrocytes did not exceed 5/1000 polychromatic erythrocytes (0.5%) in the negative control, and the incidence of micronucleated polychromatic erythrocytes increased significantly in the positive control group. Therefore, the study was considered valid.

Results
- No mortality or adverse clinical signs were noted during the course of the study.
- No increases in micronucleated polychromatic erythrocytes were observed in male mice at 24 and 48 hours after dosing.

Table 28. Mouse bone marrow micronucleus assay (sponsor's table)

Treatment	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean +/- sd)	Change From Control (%)	Micronucleated Polychromatic Erythrocytes Number per 1000 PCEs (Mean +/- sd)	Number per PCEs Scored
r-metHuLeptin Vehicle [acetate formulation]							
5 ml/kg	M	24	5	0.53 ± 0.02	---	0.7 ± 0.27	7 / 10000
Recombinant Methionyl Human Leptin(r-metHuLeptin) [acetate formulation]							
10 mg/kg	M	24	5	0.51 ± 0.05	-4	0.5 ± 0.35	5 / 10000
30 mg/kg	M	24	5	0.52 ± 0.03	-2	0.8 ± 0.76	8 / 10000
100 mg/kg	M	24	5	0.47 ± 0.08	-11	0.5 ± 0.35	5 / 10000
CP, 60 mg/kg	M	24	5	0.42 ± 0.04	-21	47.7 ±15.14	*477 / 10000
r-metHuLeptin Vehicle [acetate formulation]							
5 ml/kg	M	48	5	0.57 ± 0.02	---	0.6 ± 0.65	6 / 10000
Recombinant Methionyl Human Leptin(r-metHuLeptin) [acetate formulation]							
10 mg/kg	M	48	5	0.55 ± 0.08	-4	0.5 ± 0.50	5 / 10000
30 mg/kg	M	48	5	0.53 ± 0.03	-7	0.7 ± 0.27	7 / 10000
100 mg/kg	M	48	5	0.56 ± 0.04	-2	0.7 ± 0.45	7 / 10000
CP, 60 mg/kg	M	48	5	0.31 ± 0.07	-46	19.3 ± 4.59	*193 / 10000

[i] *, p≤0.05 (Kastenbaum-Bowman Tables)

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8 Carcinogenicity

A two year carcinogenicity study in mice and rats was not conducted. The assessment of carcinogenic potential of metreleptin relies on chronic toxicity studies and published literature. No pre-neoplastic lesions were observed in the chronic mouse and dog studies, and immunostaining for the proliferation marker PCNA did not show an increase in cell proliferation in a broad variety of cells, including adrenal, brain, colon, duodenum, kidney, liver, endothelial cells in the liver, lung, mammary gland, pancreas, pituitary, spleen, stomach, and thyroid.

However, there is growing evidence from the published literature that leptin may promote tumor progression by stimulating cell proliferation, migration, invasion through activation of several oncogenic pathways such as the Janus kinase-signal transducer and activator of transcription (JAK-STAT), the mitogen-activated protein kinase (MAPK), and the phosphatidylinositol 3-kinase (PI3K) [4,5]. Leptin also contributes to tumorigenesis by inhibiting apoptosis and promoting angiogenesis[3].

Following the Division's request, the sponsor conducted a literature review to assess any potential relationship between leptin and cancer. The sponsor's review identified several potential associations, primarily from in vitro studies, between leptin signaling and various cancer types (breast cancer, lymphoma, leukemia, papillary thyroid cancer, prostate cancer, and renal cell carcinoma), but often with conflicting data. The sponsor concluded that it is possible that leptin signaling may influence cancer progression; however, no clear association has been established.

This reviewer agrees with the sponsor that the majority of the in vitro studies suggest an association between leptin and cancer cell proliferation. The sponsor's assessment relies mostly on in vitro evidence and few in vivo models. This reviewer found several additional data in mouse models of cancer which support a role of leptin in tumorigenesis. Based on our review, the link between leptin and tumor promotion appears particularly remarkable in breast cancer, whereas conflicting data were observed in animal models of colon, lung, prostate, and pancreatic cancer. These studies are briefly summarized below.

Leptin signaling-deficient ob/ob and db/db mice do not develop transgene-induced mammary tumors[7,8]. Administration of recombinant leptin increased tumor size by 100% in nude mice bearing MCF-7 cell tumor xenografts[9], whereas inhibition of leptin signaling by knock down or antagonism of leptin receptor significantly delays the onset and reduces the growth of syngeneic, xenograft and chemically-induced mammary tumors in mice[10-12]. However, the lipodystrophic A-ZIP-F1 mouse, characterized by total loss of white adipose tissue and undetectable levels of circulating leptin, showed increased incidence and decreased latency of transgene-induced mammary tumors, which was attributed to a systemic pro-inflammatory and pro-mitogenic environment[13]. A-ZIP/F1 mice also are more prone to chemically induced skin papillomas compared to either wild-type animals and ob/ob mice[14].

The role of leptin in colon cancer progression is more controversial. Obese KK-Ay mice, with elevated leptin levels, are more prone to chemical induced colon cancer compared to lean controls[24], whereas the absence of leptin signaling inhibited chemically-induced colorectal tumor growth in ob/ob and db/db mice[25,26]. This inhibition, however, was not confirmed by another group[27]. Administration of recombinant murine leptin during the late-stage of colorectal cancer increased tumor sizes in ob/ob mice[26], but did not promote the growth of colon cancer xenografts in nude mice or intestinal tumorigenesis in ApcMin/+ mice[28]. Finally, murine leptin inhibited initial stages of colon cancer in the Fischer rat[29].

In contrast with the above data, ob/ob and db/db mice showed increased tumor size and faster tumor growth in xenograft mouse models of lung[30], prostate[31], and pancreatic tumors[32].

Human data

Both leptin and its receptor are expressed in human breast[33], colorectal[34,35], esophageal[36], brain[37], papillary thyroid[38,39], endometrial[40], ovarian[41], prostate[42], diffuse large B-cell lymphoma (DLBCL)[43], acute/chronic myeloid leukemia[44], and acute lymphoblastic leukemia[45]. Leptin serum concentration was associated with cancer in some, but not all studies. Therefore, a definitive positive correlation between elevated leptin levels and cancer has not been established in humans.

The sponsor also reported of several malignancies (peripheral T cell lymphoma, leukemia, and cystadenocarcinoma) which occurred in lipodystrophy patients never treated with metreleptin and stated that “these findings suggest that some lipodystrophy patients, notably those with autoimmune diseases, may be at increased risk for malignancies, especially hematologic malignancies. The mechanism(s) are unknown but it has been suggested that normal function and/or normal quantity of adipocytes in the bone marrow are important factors supporting normal hematopoiesis[46-48]. Although it has not been studied, it is possible that such regulation may be impaired in lipodystrophy. In addition, autoimmune conditions are associated with increased risk of malignant lymphoproliferative disorders”.

Conclusion

There is a fair amount of evidence indicating that leptin promotes proliferative, mitogenic, pro-angiogenic and anti-apoptotic responses in human cancer cells *in vitro* and in animal models of breast cancer. However, the association between circulating leptin and human cancer risk or progression is still unclear. If a link between leptin and cancer is established in humans, it will remain to be determined 1) whether leptin modulates cancer risk in lipodystrophy patients, in which metreleptin administration raises leptin levels to just above physiological levels (25-30ng/ml) and 2) whether leptin-mediated cancer risk differs among subjects with different type of lipodystrophies and their associated underlying diseases.

The sponsor has concluded that “although there is a theoretical possibility that exogenous treatment with leptin could contribute to or stimulate growth of pre-existing

malignancies, neither the Sponsor's clinical studies, nor review of the literature support that conclusion".

9 Reproductive and Developmental Toxicology

Reproductive and developmental toxicity of metreleptin was assessed by the sponsor in fertility, early embryonic development, and pre- and post-natal development studies in normal mice administered metreleptin by subcutaneous injection.
These studies did not reveal any adverse effects on fertility, embryo/fetal development, and on offspring growth and development. Metreleptin caused dystocia or prolonged gestation at all doses, so that a NOAEL for dystocia could not be established. A phase exposure study, in which metreleptin was administered at different intervals during gestation, revealed that metreleptin-induced dystocia occurs predominantly during the late gestation period (>GD15).

9.1 Fertility and Early Embryonic Development

Study title: Fertility and Early Embryonic Development to Implantation of r-metHuLeptin Administered Subcutaneously in Mice

Study no.:	REST 70257
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 19, 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	r-metHuLeptin, lot # 1112285M5

Key Study Findings
Metreleptin did not affect reproductive performance or early embryonic development in mice at doses up to 30mg/kg (7X MRHD).

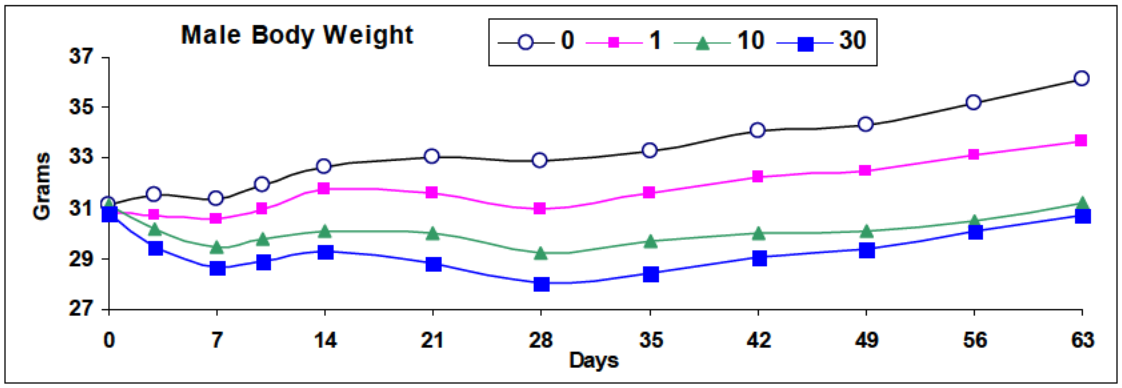
Methods

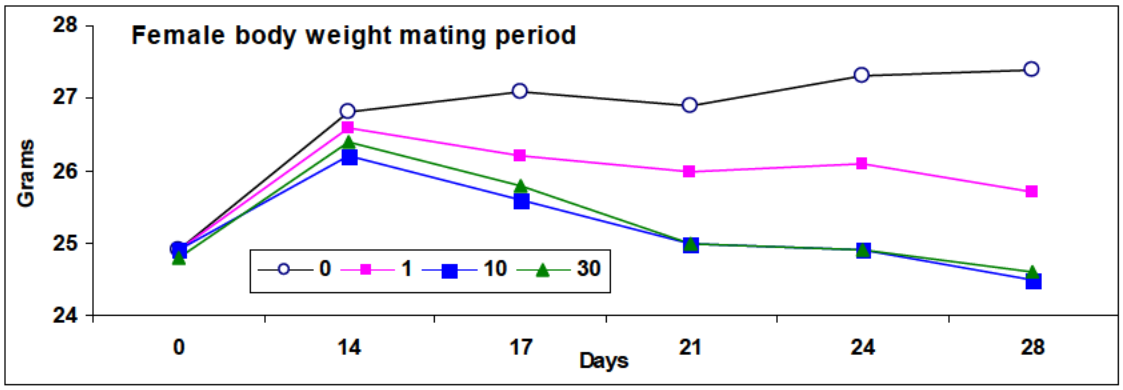
Doses:	1, 10, 30mg/kg
Frequency of dosing:	Once a day
Dose volume:	10ml/kg
Route of administration:	SC injection
Formulation/Vehicle:	Histidine formulation (Histidine 10mM, sorbitol 5%)
Species/Strain:	CD1 mice, (b) (4)
Number/Sex/Group:	25/sex/group
Satellite groups:	NA
Study design:	Males were dosed for 28 days prior to pairing and until the day prior to necropsy. Females were dosed for 14 days prior to pairing through GD 6. A laparotomy was performed on each F0 female with evidence of mating on GD 15. All males were euthanized following the last laparotomy.

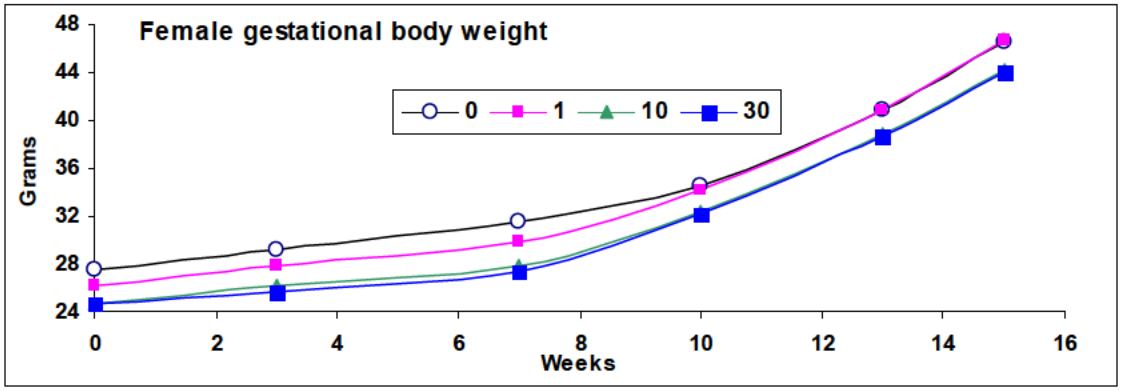
Observations and Results
Mortality
None

Clinical Signs
Unremarkable

Body Weight
Decrease in body weight gain was observed in all treated animals during mating. Gestational body weight gain was significantly reduced in the HD group between days 0 and 10, and in all treated females between days 7-10. After day 10, body weight gain was similar across all groups.







Feed Consumption
Decreased food intake was observed between gestation days 3-7 at ≥10mg/kg.

Toxicokinetics
NA

Dosing Solution Analysis
Metreleptin concentration was within 10% of nominal concentration.

Necropsy
No treatment related changes were observed.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)
There were no treatment related changes in reproductive performance or early embryonic development.

Table 29. Female reproductive performance (sponsor's table)

Daily Dose (mg/kg/day)	Control	1	10	30
Mean Number Estrous Cycles/14 days	-	-	-	-
Mean Cycle Length (days)	-	-	-	-
Mean Pre-Coitus Interval (days)	2.1	2.5	2.8	2.3
Female Mating Index (%)	100	92	96	100
Number of Pregnant Females	25	25	25	22
Female Fertility Index (%)‡	100	100	100	88
Mean Number Corpora Lutea	15.2	14.4	13.9	14.2
Mean Number Implantations	12.8	13.2	12.0	12.4
Mean % Preimplantation Loss	14.2	7.8	14.2	12.0
Mean Percent Viable Embryos	91.8	94.5	85.9	90.7
Mean Percent Early Resorptions	7.4	5.2	13.7	9.0
Mean Percent Late Resorptions	0.9	0.3	0.3	0.3
Percent of Dead Embryos	0	0	0	0
Mean % Postimplantation Loss	8.2	5.5	14.1	9.3

9.2 Embryonic Fetal Development

Embryofetal development study in mice

Study no.:	REST70258
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 13, 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	r-metHuLeptin, lot # 1112285M5

Key Study Findings

- Dose related decrease in gestational body weight gain (7-10%)
- No treatment related changes in Intrauterine growth and survival
- No treatment related malformations were observed.
- Maternal and developmental NOAEL: 30mg/kg, or 7X MRHD

Methods

Doses:	1, 10, 30mg/kg
Frequency of dosing:	Once a day
Dose volume:	10ml/kg
Route of administration:	SC injection
Formulation/Vehicle:	Histidine formulation (Histidine 10mM, sorbitol 5%)
Species/Strain:	CD1 mice, from (b) (4)
Number/Sex/Group:	25 mated females/group
Satellite groups:	NA
Study design:	Metreleptin was administered to female mice once daily from GD 6 through 15. A laparohysterectomy was performed on all surviving animals on GD 18.

Observations and Results

Mortality

None treatment related.

One female in the 10 mg/kg/day group aborted on GD 16. This female was internally normal and had 12 fetuses with no apparent malformations *in utero*. No abortions were seen in the 30 mg/kg/day group; therefore, this single abortion in the mid dose group was considered to be unrelated to treatment.

Clinical Signs

Unremarkable

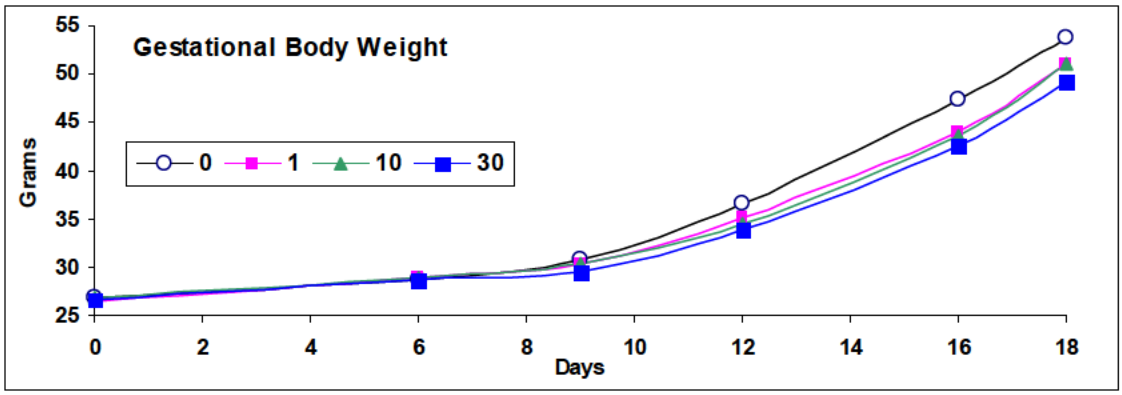
Body Weight

Dose related decrease in gestational body weight gain was observed in all treated females. Body weight was dose dependently reduced in all treated groups on GD 16, and in the HD group only on GD18. Gravid uterine weight was unaffected by treatment.

Table 30. Mouse body weights

Dose, mg/kg	BW gain (% of baseline)		Decrement (% vs. controls)	
	GD6-16	GD0-18	GD16	GD18
0	65	100		
1	52	93	-7*	-5
10	50	90	-8**	-5
30	48	85	-10**	-8**

*p<0.05, ** p<0.01



Feed Consumption
Food consumption was significantly decreased in treated groups during GD12-16.

Toxicokinetics
NA

Dosing Solution Analysis
Test article concentrations were within ±10% of the nominal values.

Necropsy
Unremarkable

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)
No treatment related changes in corpora lutea, resorptions, pre- and postimplantation losses, viable fetuses, and fetal weight were observed.

Offspring (Malformations, Variations, etc.)
No treatment related malformations or variations were observed.

Embryofetal development study in rabbits: Dose-ranging study
Dose: 0.3, 1, 10, 30mg/kg
Maternal and fetal NOAEL: 30mg/kg (360mg/m^2)
Safety margins: 72 and 30 fold, relative to clinical dose of 5-12mg/m^2, respectively

Study no.:	REST70267
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 12, 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	r-metHuLeptin, lot # 1108136H6

Key Study Findings

- No treatment related changes in gestational body weight gain
- No treatment related changes in Intrauterine growth and survival
- No treatment related malformations were observed.
- Maternal and developmental NOAEL: 30mg/kg, or 7X MRHD (BSA basis)

Study Design:

Metreleptin was administered by subcutaneous injection to artificially inseminated NZW rabbits (5/group) from GD6 through GD20 at doses of 0.3, 1, 10, and 30mg/kg once daily. A laparohysterectomy was performed on all surviving animals on GD29.
The objective of this study was to select dosage levels for a definitive developmental toxicity study. However, a pivotal study was never conducted.

Results

Mortality

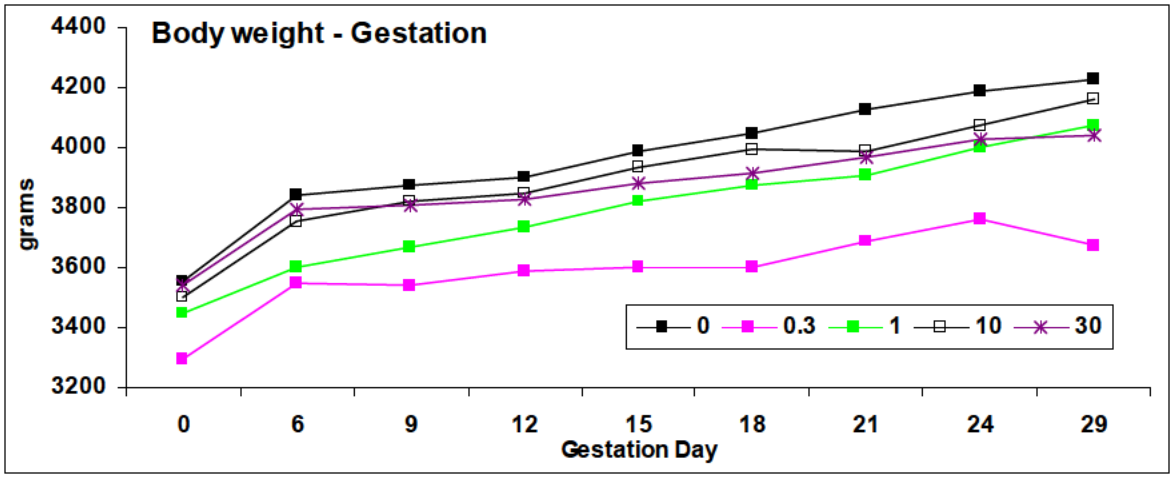
None

Clinical Signs

Increased incidence of very slight edema and very slight erythema was observed at the injection site in the 10 and 30mg/kg groups, most often between gestation days 10 and 17.

Body Weight

No significant changes in body weight gain were observed throughout gestation



Feed Consumption

No treatment related changes were observed in any dose groups compared to controls

Toxicokinetics

TK was not analyzed in this study

Dosing Solution Analysis
Concentration in the 0.3mg/kg group was -42% and -32% the nominal concentration during the first and last week of dosing, respectively.

Necropsy
There were no treatment related macroscopic changes in the dams.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)
No treatment related changes in viable fetuses, corpora lutea, resorptions, pre- and postimplantation losses and fetal weight were observed.

Offspring (Malformations, Variations, etc.)
External malformations were noted in one fetus in the 30mg/kg group (1 out of 27, corresponding to an incidence of 3.7%) and included thoracogastroschisis, spina bifida, acephaly, carpal flexure, micromelia, adactyly, and brachydactyly. Because of the low incidence, the reviewer agrees with the sponsor that this finding is likely not treatment related.

9.3 Pre and Postnatal Development

A study of the effects of metreleptin on pre- and postnatal development in mice

Study no.:	REST70259
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 16, 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	r-metHuLeptin, lot # 1111056L6

Key Study Findings
F0 dams
- All doses reduced gestational weight gain 10-16%
- Dystocia (difficult birth) affected a small number of dams from all dose groups, but was not seen in controls: Prolonged gestation, death during parturition, litter loss, and increased stillbirths were observed.

F1 generation
- Early survival PND0-4 was lower in all dose groups, but similar to controls thereafter
- Reduced pup weight was noted from pre-weaning into adulthood
- Delay in the occurrence of vaginal patency was observed in all treated females
- No adverse effects on F1 reproductive performance were observed.

F2 generation
- Metreleptin did not affect survival or intrauterine growth of the F2 fetuses.

Reviewer comment: Metreleptin resulted in dystocia in every dose group. This was apparent in those few individuals found dead or in extremis, but likely affected many others despite no clear evidence of distress. Of note, leptin reportedly inhibits the contractile apparatus of myocytes in vitro[49]. No NOAEL was established for the apparent dystocia in pregnant mice.

It is feasible that poor viability from PND0-4 results from stress during birth, though this is not certain. No NOAEL was established for the F1 due to this finding. Survival thereafter, however, is similar to control. While F1 animals gained less body weight with a likely small delay in maturation, overall survival and fertility were not adversely effected.

There is no data on the presence of metreleptin in milk or on lactational transfer of metreleptin. However, systemic exposure would not be expected from oral ingestion of metreleptin. The lower body weight of pups which persisted to adulthood may be due to the initial lower birth weight.

One female clinical trial participant with congenital generalized lipodystrophy became pregnant at age 23 while on metreleptin treatment for approximately 7 years. Metreleptin treatment was continued throughout pregnancy and breast feeding (approximately 5 months). The patient delivered vaginally after inducing labor. The baby was born as a still birth but was successfully resuscitated. Later, the baby was diagnosed with shoulder dystocia and Erb's palsy. At the time of the last follow-up information from the investigator, the infant was a healthy and well-developing 5-month-old with improving Erb's palsy. The still birth and shoulder dystocia were considered by the sponsor not related to leptin but rather to the fact that the infant was delivered vaginally, was large for gestational age (from maternal gestational diabetes), and had become caught in the birth canal with resultant traumatic birth injury (Module 2, summary clinical safety). Nevertheless, the dystocia observed in pregnant mice at all doses of metreleptin suggests that a relationship to drug treatment should not be dismissed.

	Adverse effects	**NOAEL**	**Safety Margin to MRHD (mg/m^2)***		
			12.5	7.8	6.2
F0	Dystocia ↓ Weight gain	<3mg/kg	<1X	<1X	<1X
F1	↓ Survival <PND4 ↓ Weight gain	<3mg/kg, 9mg/m^2	<1X	<1X	<1X
F2	None	30mg/kg, 90mg/m^2	7X	12X	15X

*10mg/day or 12.5, 7.8, and 6.2 mg/m^2 in subjects with a body weight of 20, 40, and 60kg, respectively.

Based on 2-fold higher AUC but similar Cmax in pregnant versus non-pregnant CD1 mice, estimated exposure is as follows:

	AUC, ng*h/ml	Cmax, ug/ml
Dose		
3mg/kg	~7000	3.5
10mg/kg	~12,000	12
30mg/kg	~36,000	36

Estimated AUC and Cmax at the 3mg/kg dose in pregnant mice is ~7-fold and ~20-fold higher, respectively, compared to clinical AUC and Cmax at 10mg/day.

Methods

Doses:	3, 10, 30mg/kg
Frequency of dosing:	Once a day
Dose volume:	10ml/kg
Route of administration:	SC injection
Formulation/Vehicle:	Acetate formulation
Species/Strain:	CD1 mice, from (b) (4)
Number/Sex/Group:	25 females/group
Satellite groups:	NA
Study design:	Metreleptin was administered to female mice from GD 6 through LD20. All females were allowed to deliver and rear their offspring to LD 21. Twenty-five males and females per group were randomly selected to obtain a minimum of one male and one female per litter for the F1 parental generation. From these selected pups, ten males and females per group were selected for neurobehavioral testing. The F1 animals in each group (25/sex) were mated, avoiding sibling pairings. On GD18, F1 females were necropsied, and fetuses were examined externally for malformations and variations. The F1 males were necropsied following the last laparohysterectomy in the F1 females.

Observations and Results
F_0 generation

Mortality
Two, one, and three F0 females in the 3, 10, and 30 mg/kg/day groups, respectively, were found dead or euthanized in moribund condition.

In the 3mg/kg group:
#1007 and #1104 were found death on GD17 and LD1, respectively. No adverse macroscopic findings were noted. Female #1104 died during parturition.

In the 10mg/kg group:
#1102 death occurred on post-mating day 20 following prolonged gestation.

In the 30mg/kg group:

#1011 died during parturition with a pup lodged in the vaginal opening.
#1021 was found dead on GD7, following 2 days of dosing, non treatment related.
#1098 was gravid and euthanized on post-mating day 19 following prolonged gestation.

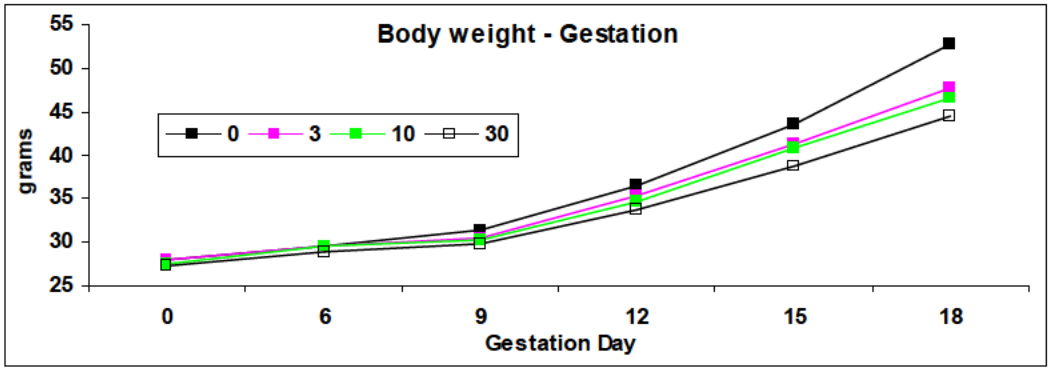
Clinical Signs
Unremarkable

Body Weight
Gestation
Dose related decrease in body weight gain and body weight was observed throughout gestation

Table 31. Mouse body weights during gestation

Dose, mg/kg	BW gain (grams)		Decrement (%)	BW (% control)
	GD6-18	GD0-18	GD18	
0	23.2	25.0	-	
3	18.2**	19.7**	-21	-10**
10	17.0**	19.0**	-24	-12**
30	15.4**	16.9**	-32	-16**

** p<0.01

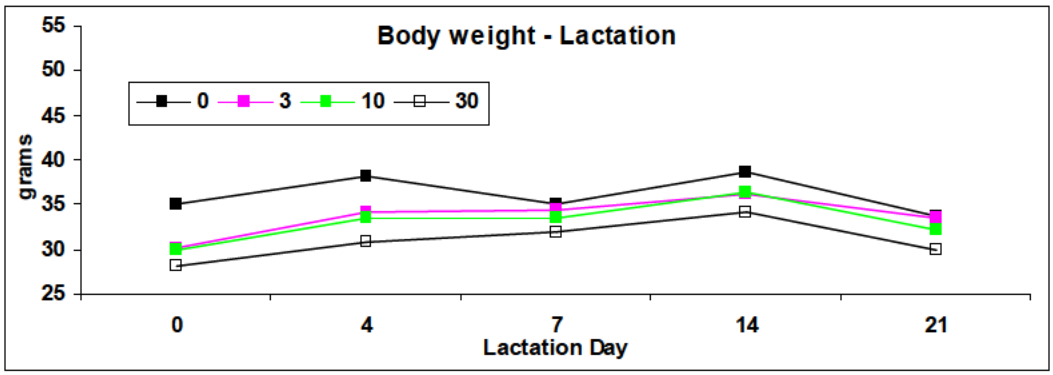


Lactation
Body weight gain was similar across all groups. The statistical significance was due to a weight gain drop in the control group between LD14-21.
Body weight was significantly decreased on LD4 in all treated groups (dose-related). By the end of lactation, body weight was similar to controls in the LD and MD groups, and still lower in the HD group, although a partial recovery was noted (-12% vs. -20%, on LD21 and LD4, respectively).

Table 32. Mouse body weights during lactation

Dose, mg/kg	BW gain (grams)	BW (% control)	
	LD0-21	LD4	LD21
0	-1.2	-	-
3	3.2**	-11**	-1
10	2.2**	-13**	-5
30	1.8**	-20**	-12*

*p<0.05, ** p<0.01



Feed Consumption
Food consumption was reduced in all treated groups on GD12-18, and throughout lactation

Toxicokinetics
Not done. However, the toxicokinetic study in pregnant versus non-pregnant CD1 mice showed that AUC is approximately 2-fold higher in pregnancy, whereas Cmax remains similar to non-pregnant mice. This suggests lower clearance of drug in pregnant animals.

Gestation, Parturition Performance
Failure to Deliver
The number of females that delivered litters was imbalanced across the dose groups: 0, 1, 2, and 5 for control, 3, 10, and 30mg/kg, respectively. The imbalance reflected a lower pregnancy rate in the dosed groups (100%, 96%, 96%, 88%) and more resorptions in the dosed groups versus controls (0, 0, 1, 2). This imbalance is not related to treatment, as both fertility and pre/post-implantation endpoints were not affected by metreleptin in the fertility and embryofetal development studies.

Gestation and Parturition
Metreleptin appeared to result in prolonged gestation or difficult parturition (dystocia) in a small number of females at all dose groups. Given the day Females #1102 and 1098 were found dead or euthanized, the reviewer assumes that difficult parturition was

encountered. Gestation length (19-20 days) and parturition was reportedly similar to control for the remaining individual females in the dosed groups.

Individual cases of prolonged gestation or evidence of dystocia	
Control	None reported
3mg/kg	#1104, Died during parturition, found dead LD1,
10mg/kg	#1102, Prolonged gestation, found dead post-mating day 20
30mg/kg	#1011, Prolonged gestation, died during parturition
	#1098, Prolonged gestation, euthanized post-mating day 19

Litter Loss
Three, two, and five females in the 3, 10, and 30mg/kg groups, respectively, had total litter loss between lactation day 0 and 14.

Table 33. F_0 uterine content

Daily Dose (mg/kg/day)	Control	3	10	30
F_0 Females				
Number Pregnant	25	25	25	25
Number Died or Sacrificed Moribund	0	2	1	3
Number with Total Litter Loss	0	3	2	5
Clinical Observations	-	-	-	-

Necropsy
Unscheduled deaths
Two, one and three females in the 3, 10 and 30mg/kg groups, respectively, were found dead or euthanized in extremis. All of these females were gravid. No macroscopic findings were observed except dark red stomach content in the female at 10mg/kg).

Females with total litter loss: No macroscopic findings.

Scheduled necropsy
Post-mating Day 25: Unremarkable
Lactation Day 21: Treatment-related dark contents or areas in the gastrointestinal tract were observed in one female per treated group (out of 19, 20, 12 examined females).

F_1 weaning period
Survival (note, excludes dams with total litter loss)
Dosed females gave birth to similar numbers of pups, but all doses of metreleptin resulted in a higher incidence of stillborns. Also, all doses of metreleptin resulted in lower pup survival from birth to post-natal day 4. Survival from PND4 to weaning

(PND21) was similar to control, with a numerical lowering at the 30mg/kg dose ('postnatal survival to weaning' in Table 43).

The stillborns and found-dead pups did not exhibit any pattern of malformations indicative of a teratogenic effect of metreleptin. However, many were cannabilized or autolyzed which precluded examination.

Table 34. F_1 generation litter data (sponsor's table)

Daily Dose (mg/kg/day)	Control	3	10	30
F_1 Litters (preweaning)				
Number Litters Evaluated	25	22	22	17
Mean Number Pups/Litter	12.2	11.3	11.9	11.5
Mean Number Liveborn Pups/Litter	12.2	9.8*	10.0	8.1**
Mean Number of Stillborn Pups/Litter	0.0	1.5	1.9	3.4
Postnatal Survival to Day 4 (%)	97.5	78.8**	78.1**	61. 7**

Daily Dose (mg/kg/day)	Control	3	10	30
Postnatal Survival to Weaning (%)	93.0	93.5	96.3	89.4
Mean Pup Body Weights – Day 21 (g)	9.43	7.79**	7.71**	6.34**
Pup Sex Ratios (% Males)	48.1	48.5	56.3	49.8
Pup Clinical Signs	-	-	-	-
Pup Necropsy Observations	-	-	-	-

Clinical signs
Unremarkable

Body weight
Birth weight was lower in all dose groups compared to control. Weight gain thereafter was similar to control at 3 and 10mg/kg but slightly lower at 30mg/kg. Lower body weight persisted to adulthood in all dosed groups, which appears to be largely due to the initial lower birth weight.

Table: Birth weight on Day 1, and Pre- weaning body weight gain

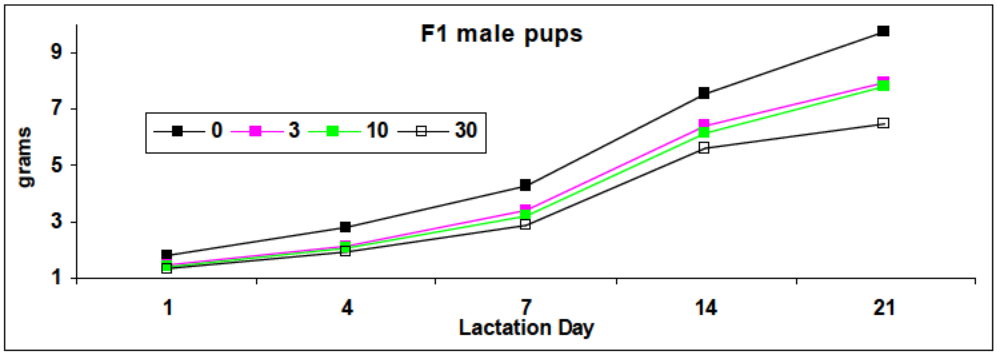
Birth weight

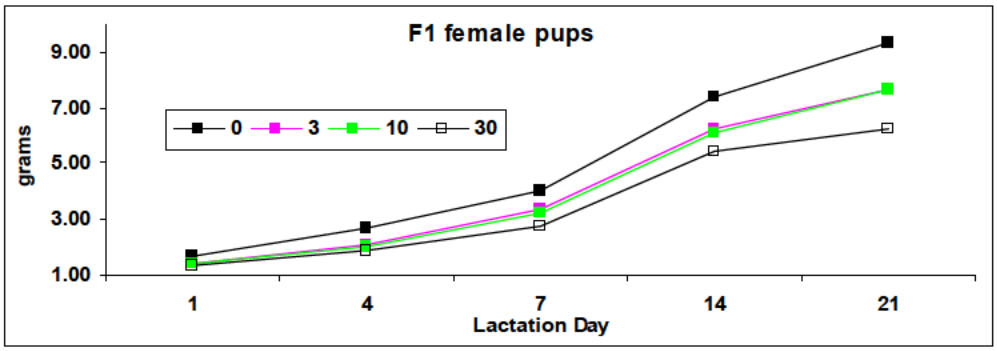
PRE-WEANING

DOSE GROUP:		0 MG/KG/DAY	3 MG/KG/DAY	10 MG/KG/DAY	30 MG/KG/DAY
DAY 1	MEAN	1.75	1.42**	1.41**	1.33**
	S.D.	0.176	0.122	0.135	0.094
	N	25	20	20	13

Dose, mg/kg	BW gain (grams)		Decrement (% vs. controls)			
	LD1-LD21		LD1		LD 21	
	Males	Females	Males	Females	Males	Females
0	443	449				
1	449	445	-20**	-18**	-19**	-18**
10	444	449	-20**	-18**	-20**	-18**
30	378	376	-25**	-23**	-34**	-33**

*p<0.05, ** p<0.01





Necropsy
No treatment related abnormalities were reported at the scheduled necropsy of pups at LD/PND 21.

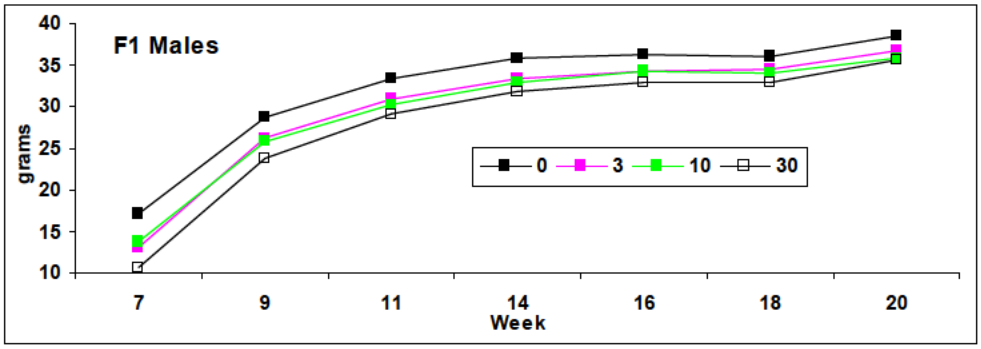
F1 post-weaning period
Mortality
During Week 6 (PND 23-25), one F_1 male each in the 10 and 30 mg/kg/day groups and one F_1 female in the 30 mg/kg/day group were found dead or euthanized pre-terminally. No significant clinical findings or macroscopic changes were observed in these animals.

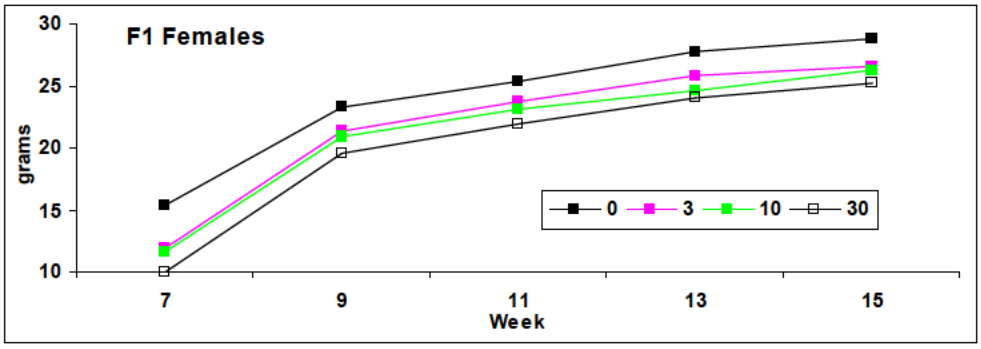
Body weight
Body weight at the end of week 18 in males and week 15 in females was still dose dependently reduced. Body weight gain was similar across all groups.

Table 35. F_1 post-weaning body weights

Dose, mg/kg	BW gain (wk 7-15/18) (grams)		Decrement (% vs. controls)	
	Males	Females	Males	Females
0	21	14		
3	24	15	-5	-8**
10	22	15	-7**	-9**
30	25	15	-8**	-13**

*p<0.05, ** p<0.01





Physical development

Delay in the occurrence of vaginal patency was observed in all F1 females. A slight delay, not significant, in balanopreputial separation was noted in HD males

Table 36. F_1 physical development (sponsor's table)

Daily Dose (mg/kg/day)	Control	3	10	30
F_1 Females (Postweaning)				
Number Evaluated Postweaning	25	25	25	25
Number Died or Sacrificed Moribund	0	0	0	1
Mean Age of Vaginal Patency (days)	32.1	35.2*	35.1*	39.7**
F_1 Males (Postweaning)				
Number Evaluated Postweaning	25	25	25	25
Number Died or Sacrificed Moribund	0	0	1	1
Preputial Separation (average days)	33.8	33.9	34.0	35.6

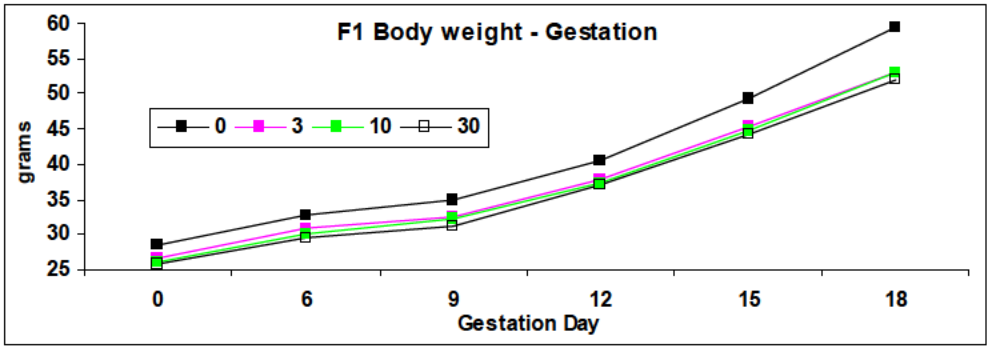
Neurological assessment
No treatment related changes in auditory startle test, motor activity, or Biel maze swimming trials were observed.

F1 mating to sacrifice period
Body weight
Body weight gain was similar across all groups. Body weight on GD18 was still reduced in all treated females compare to controls.

Table 37. F_1 gestational body weights

Dose, mg/kg	BW gain (grams)	Decrement (% vs. controls)
	GD0-18	GD18
0	31	
1	27	-11**
10	27	-11**
30	26	-12**

*p<0.05, ** p<0.01



Reproduction:
No adverse effects on F_1 reproductive performance (mating and fertility indices) were observed. Gravid uterine weight was unaffected by treatment. The number of corpora lutea, implantations and pre-implantation loss were not affected by metreleptin treatment.

Table 38. F_1 reproductive performance and uterine content (sponsor's table)

F_1 Females (Postweaning)				
Daily Dose (mg/kg/day)	Control	3	10	30
Mean Number of Days Prior to Mating	3.3	2.1	2.5	3.1
Number of Females Sperm Positive	23	23	24	22
Number of Pregnant Females	25	25	25	23
Mean Number Corpora Lutea	16.3	15.0	14.3	15.3
Mean Number Implantations	13.4	12.1	10.8	12.3
Mean % Preimplantation Loss	16.3	18.7	24.7	18.6

Necropsy:
No treatment related changes were observed in the F_1 males or females that were found dead, euthanized pre-terminally in moribund condition, or at the scheduled necropsies.

F_2 Generation

- Intrauterine growth and survival of the F_2 fetuses were not affected by F_0 maternal treatment.
- No changes in fetal body weight were observed.
- No treatment related external malformations or variations were observed.

Table 39. F_2 litter data (sponsor's table)

Daily Dose (mg/kg/day)	Control	3	10	30
F_2 Litters				
Mean Number Live Conceptuses/Litter	12.5	10.6	10.2*	11.3
Mean Number Resorptions	6.5	13.2	6.3	7.6
Number of Dead Conceptuses	0	0	0	0
Mean % Postimplantation Loss	6.8	13.2	6.3	8.0
Fetal Body Weights (g)	1.34	1.37	1.41	1.36
Fetal Sex Ratios (% males)	53.4	52.4	49.1	50.5
Fetal Anomalies	2	0	1	0

* Significantly different from the control group at 0.05 using Dunnett's test

A phase exposure pre- and postnatal development study in mice

Study no.:	REST70256
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 15, 1999
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	r-metHuLeptin, lot # 31033F7A

Key Study Findings
F_0 dams

- Prolonged gestation and dystocia was observed in females treated on GD6-18.
- Total litter loss was noted in females treated on GD15-18 and GD6-18

F_1 pups

- Decrease live litter size and postnatal survival was observed in females treated on GD6-18.

Reviewer Comments: Metreleptin caused dystocia when administered to pregnant CD1 mice during the late gestation period (>GD15). Dystocia was indicated by numerous deaths of dams near the time of parturition, difficulty during parturition, and a higher stillborn and mortality rate among pups on PND0-4.

This is consistent with the pre/post-natal study conducted in CD1 mice at the same 10mg/kg dose of metreleptin. Note that dystocia-related deaths were also observed at 3mg/kg in the pivotal pre/post-natal study, but this dose was not evaluated in the phased-administration study.

Methods

Doses:	10mg/kg
Frequency of dosing:	Once a day
Dose volume:	10ml/kg
Route of administration:	SC injection
Formulation/Vehicle:	Glutamic acid 9mM, glycine 2.4%, Tween 0.01%, sucrose 1%
Species/Strain:	CD1 mice, (b) (4)
Number/Sex/Group:	50/group
Satellite groups:	NA
Study design:	Metreleptin was administered by sc injection to three groups of female mice on GD6-15, GD15-18, and GD6-18. Females that were dosed on GD 15-18 also received placebo on GD6-14. 25/group were allowed to deliver and rear their offspring to LD21. 25/group had a laparohysterectomy was performed on GD18. Fetuses were examined externally for malformations and variations.

Observations and Results

F_0 Dams

Survival

Prolonged gestation and/or dystocia was observed in one female each of Group 1 (control), Group 2 (GD6-15) and Group 3 (GD 15-18), and in five females in Group 4 (GD 6-18). Most of these females died or were euthanized *in extremis* on GD 19, except for one female each in Group 2 (GD6-15) and Group 4 (GD 6-18). The surviving female in group 4 had total litter loss.

All females in the laparohysterectomy group survived to the scheduled necropsy on GD 18.

Table 40. Prolonged gestation and distocya

Group	Treatment	ID		Clinical signs
Control		9161	Euthanized	Hypoactive, cool to touch, pale in color
10mg/kg	GD6-15	9296		Pale in color
	GD15-18	9294	Euthanized	
	GD6-18	9261		Pale in color, total litter loss
		9345	Euthanized	Hypoactive, cool to touch, pale in color, red material on ventral abdominal area.
		9290	Found dead	Hypoactive, cool to touch, pale in color
		9332	Found dead	Hypoactive, cool to touch, pale in color
		9322	Found dead	

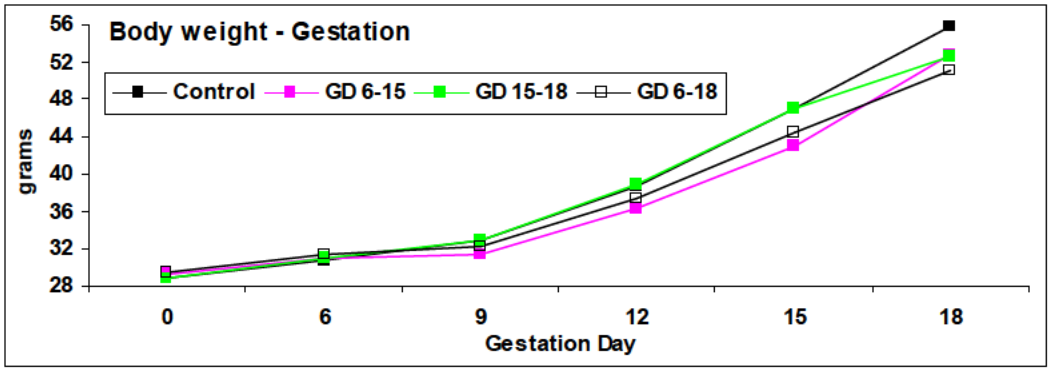
Clinical signs
Unremarkable

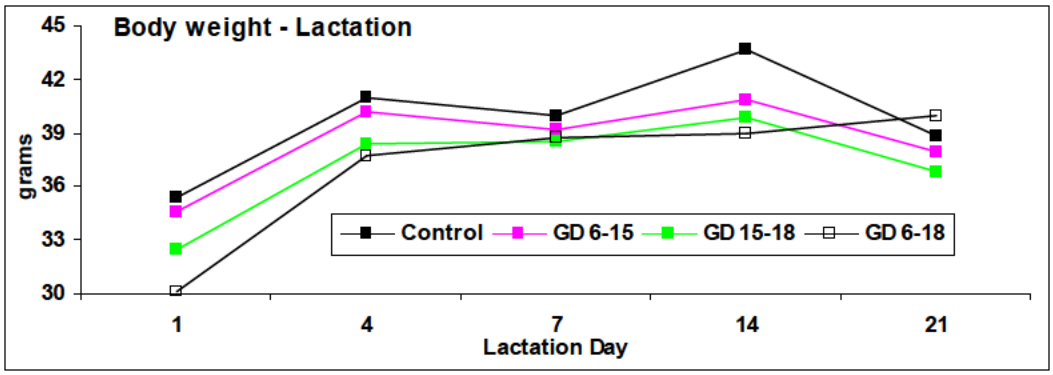
Body weight
Body weight gain was decreased in Group 2 (GD6-15) and Group 4 (GD6-18). Dose related decrease in body weight was observed on GDs 15 and 18. Body weight was still decreased in Group 3 (GD 15-18) and Group 4 (GD6-18) on LDs 1 and 4, but recovered by LD7.

Table 41. Gestation and lactation body weight

Dose, mg/kg	BW gain (grams)		Decrement (% vs. controls)			
	GD6-15	GD6-18	GD15	GD18	LD1	LD21
Control	53	81				
GD 6-15	39	71	-9**	-5*	-2	-2
GD 15-18	52	69	0	-6**	-8**	-5
GD 6-18	42	62	-5**	-8**	-15**	3

*p<0.05, **p<0.01





Feed consumption

Food consumption was reduced in Group 3 (GD 15-18) and Group 4 (GD 6-18) during GD15-18, and in Group 4 (GD 6-18) during lactation days 1-4.

Serum chemistry

- Decrease in glucose, cholesterol, triglycerides, and FFA was observed in Group 3 (GD 15-18) and Group 4 (GD 6-18).
- Increase in phosphorus was observed in all treated groups.

Table 42. Serum chemistry

	Control	GD 6-15	GD 15-18	GD 6-18
BUN	23.5	22	27.8	29.7*
Glucose	143	144	120	104**
Cholesterol	54	61	32**	30**
Triglycerides	167	263**	74**	83**
FFA	536	404	281*	290*
Phosphorus	7.8	9.5*	9.3*	10**

All: mg/dL. *p<0.05, **p<0.01

Uterine content

- Longer duration of gestation was observed in Group 4 (GD 6-18)
- Increased incidence of females with total litter loss was observed in Group 3 (GD15-18) and Group 4 (GD6-18)

Figure 10. F_0 uterine content

Daily Dose (mg/kg/day)	Control (Dosed GD 6-18)	10 (Dosed GD 6-15)	10 (Dosed GD 15-18)	10 (Dosed GD 6-18)
F_0 Females				
Laparohysterectomy Phase				
Number Pregnant	25	24	25	25
Number Died or Sacrificed Moribund	0	0	0	0
Clinical Observations	-	-	-	-
Necropsy Observations	-	-	-	-
Natural Delivery Phase				
Number Pregnant -	23	22	25	24
Number Died or Sacrificed Moribund	2	0	1	4
Females with Total Litter Loss	0	0	1	9
Clinical Observations	-	-	-	-
Necropsy Observations	-	-	-	-
Mean Duration of Gestation (days)	18.3	18.5	18.6	18.8*

Necropsy observation
Unremarkable

Toxicokinetics
NA

Dosing Solution Analysis
Test article concentrations were within 10% of the target dose concentrations.

F_1 litter data
Survival
Decrease in live litter size and in post natal survival (birth to PND 4) was observed in Group 3 (GD15-18) and Group 4 (GD6-18).

Figure 11. F_1 litter data

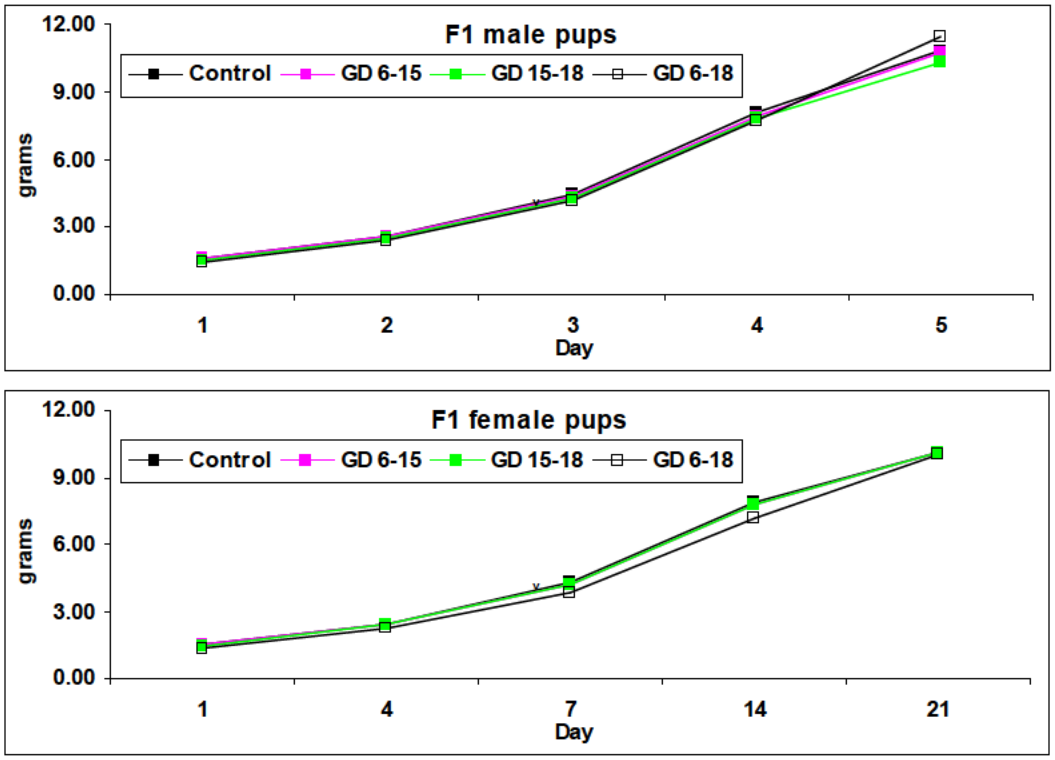
Daily Dose (mg/kg/day)	Control (Dosed GD 6-18)	10 (Dosed GD 6-15)	10 (Dosed GD 15-18)	10 (Dosed GD 6-18)
F_1 Litters (Preweaning)				
Number Litters Evaluated	22	22	24	22
Mean Number Pups/Litter	12.9	12.8	11.4	11.0*
Mean Number Liveborn Pups/Litter	12.3	12.6	10.5	6.0**
Mean Number Stillborn Pups/Litter	0.6	0.2	0.9	5.0
Postnatal Survival to Day 4 (%)	92.9	96.1	87.4	32.9**
Postnatal Survival Day 4 – Weaning (%)	94.3	94.3	97.3	100.0
Mean Pup Body Weights – Day 21 (g)	10.56	10.42	10.20	10.39
Pup Sex Ratios (% males)	52.8	47.1	47.4	48. 2
Pup Clinical Signs	-	-	-	-
Pup Necropsy Observations	-	-	-	

GD = gestation day; PND = post natal day.
- = No noteworthy findings.
* Significantly different from the control group at 0.05 using Dunnett's test

Clinical signs
Unremarkable

Body weight
Body weight was slightly decreased in male and female pups (-13% vs. controls) in Group 4 (GD6-18) on PND 1. By day 4, no differences were observed.

Figure 12. F_1 pups body weigh



Offspring (Malformations, Variations, etc.)
No treatment related external malformations were observed, either in the live or stillborn pups.

10 Special Toxicology Studies

Severity of injection site reactions was assessed in Sprague-Dawley rats and in New Zealand rabbits. Subcutaneous administration of metreleptin provoked local inflammation at the injection site, characterized by perivascular cuffing, mixed cell infiltrates, and macrophage giant cells. Severity of the response appeared dose-dependent. Injection site reactions were also monitored in mice and dogs in the context of the pivotal toxicology studies, and in the clinical trials. Overall, metreleptin provokes a minimal to moderate injection site reaction marked by a chronic cellular infiltrate upon repeated subcutaneous administration.
In addition, the sponsor conducted an antigenicity study in guinea pigs induced subcutaneously and then challenged intravenously with metreleptin. Metreleptin tested positive for antigenicity in this now-outdated assay. Metreleptin provokes an anti-drug antibody response in mice, dogs, and human subjects, though the response does not appear to reduce pharmacological activity. Case reports of anti-metreleptin anti-bodies cross-reacting and neutralizing endogenous leptin have been identified in the obesity development program for metreleptin.

Metreleptin did not induce hemolysis as determined by assays conducted with rat and human blood samples

11 Integrated Summary and Safety Evaluation

Pharmacology
Metreleptin is a recombinant analog of native human leptin, differing from the native molecule by a single methionyl group added to the amino-terminal end. Leptin is a naturally occurring hormone predominantly secreted by adipose tissue that plays a central role in the neuro-hormonal regulation of energy homeostasis and fat and glucose metabolism.

Pharmacology studies were conducted by the sponsor in normal and transgenic mouse models of obesity, including the leptin-deficient obese ob/ob mice. These mice share a similar metabolic abnormalities (insulin resistance, hyperglycemia, hypertriglyceridemia, and fatty liver) and similar leptin deficiency (ob/ob mice) with the lipodystrophic mouse. However, none of these rodent models exhibit total or partial loss of adipose tissue, which is characteristic and causative of the phenotype in lipodystrophic disease. It was shown that fatless lipodystrophic mice are more diabetics than ob/ob mice, and that patients with generalized lipoatrophy are more prone to diabetes than those who lack leptin[1]. Also, it is the loss of adipose tissue that causes leptin deficiency in the lipodystrophic mouse, in contrast to a genetic mutation in the ob/ob mouse. These differences between obese and fatless leptin deficient mice suggest that obese leptin deficient mice used by the sponsor are of limited usefulness in proof of concept studies of metreleptin in lipodystrophy.

Several mouse models of lipodystrophy have been developed in the last two decades[17]. All are characterized by various degrees of fat ablation, leptin deficiency, insulin resistance, hyperglycemia, fatty liver, and hypertriglyceridemia. The effect of commercially available recombinant murine leptin has been evaluated by independent groups in the Ap2-nSREBP-1c mice, the A-ZIP/F1 mice, and conjugated linoleic acid (CLA) induced lipodystrophic mouse. The A-ZIP/F1 mouse has the most severe lipodystrophic phenotype, with almost complete lack of white adipose tissue, in contrast to the nSREBP-1c transgenic mouse and the CLA-induced lipodystrophic mouse which have some residual fat[18].
Administration of exogenous recombinant murine leptin to lipodystrophic mice generally decreased plasma glucose and insulin, hepatic triglycerides, and improved insulin sensitivity. Higher doses of leptin were required to achieve efficacy in the more severe lipodystrophic models (e.g., A-ZIP/F1 mice). The mechanism by which recombinant leptin improves insulin sensitivity and plasma glucose may extend beyond simply reducing food intake and body weight. For example, food restriction did not lower plasma insulin and glucose in Ap2-nSREBP-1c and LepTg/A-Zip transgenic mice, although liver triglycerides did decrease notably. Similarly, recombinant-leptin decreased serum insulin and glucose concentration in CLA-lipodystrophy without significant reductions in food intake and body weight.

Safety pharmacology
Safety pharmacology assessment of cardiovascular, gastrointestinal, neurological and pulmonary effects of metreleptin was performed in mice, rats and dogs. None of these studies identify significant liabilities of acute metreleptin exposure.

ADME
Metreleptin is rapidly absorbed and highly bioavailable in mice and dogs following subcutaneous administration. Elimination half life is short in both species (<2h), so that steady-state exposure was not achieved in the chronic dog study (and likely in mice as well, although TK analysis was not conducted in mice). Pharmacokinetics data from healthy subjects also show a short drug half-life in humans (3-5hrs), suggesting that metreleptin accumulation following repeated doses is unlikely. Metreleptin is almost entirely cleared by renal elimination, based on a mouse study showing a 97% decrease in metreleptin clearance in nephrectomized mice compared to control and sham-operated mice

General toxicity
General toxicity was assessed in CD1 mice and in Beagle dogs in studies up to 6-month duration. The predominant finding observed in both species was a profound, dose related decrease in food intake and body weight, which is consistent with the pharmacodynamic activity of metreleptin. Excessive body weight loss resulted in few deaths, most notably in mice following a schedule overnight fasting, due to poor nutritional status and body state. Changes in serum chemistry in mice and dogs (increased red cell mass, urea, cortisol, and decreased in albumin and total protein) and histopathology findings of gastric erosions, lympohocytolysis, and injection site cellulitis in mice were also secondary to the anorexigenic effect of metreleptin, and improved at dose cessation following a quick rebound in food intake. Although these findings were observed at the clinical dose, they do not likely reflect a risk for humans, given that severe body weight loss does not occur in lipodystrophic subjects treated with metreleptin. In addition, rapid resumption of food intake at dose cessation should quickly alleviate any metreleptin related anorexigenic effects in humans.

Anti drug antibodies were detected in mice and dogs starting at two weeks of dosing and were generally present throughout recovery. ADAs were not neutralizing based on the sustained weight loss observed in mice and dogs, but lead to increased metreleptin exposure overtime, most likely by decreasing metreleptin clearance.
Anti drug antibodies also developed in lipodystrophic patients and obese subjects following repeated dosing of metreleptin, and were also associated with increased plasma drug levels.

Genotoxicity
Metreleptin had no mutagenic or clastogenic potential in an *in vitro* bacterial mutation assay, an *in vitro* mammalian cell mutagenicity assay, and an *in vivo* mouse micronucleus study.

Reproductive and developmental toxicity
Reproductive and developmental toxicity was assessed in fertility, early embryonic development, and pre- and post-natal development studies in mice.
Metreleptin did not adversely affect fertility and was not teratogenic in mice at doses up to 7-15 fold the clinical dose based on BSA. A decrease in maternal body weight during gestation and lactation resulted in reduced pup weight at birth, which persisted into

adulthood. However, no adverse effects on behavioral, developmental, or reproductive performances were observed in the F1 and F2 generations. Metreleptin caused dystocia or prolonged gestation at all doses, starting at below the clinical dose based on BSA. Dystocia resulted in the death of some females during parturition, and consequent litter loss and stillbirth in metreleptin treated groups. Reduced pup viability was also observed from postnatal day 0 to 4, and it is likely related to maternal stress during labor. A phase exposure study, in which metreleptin was administered at different intervals during gestation, showed that metreleptin induced dystocia occurs predominantly during the late gestation period (>GD15). It appears that leptin impairs the contractile apparatus of the myocyte: a potent and cumulative inhibitory effect on both frequency and amplitude of spontaneous and oxytocin-induced contractions was observed in strips of human myometrium incubated with leptin[15].
Based on these data, discontinuation of metreleptin near the time of parturition could be considered to avoid the possible risk of dystocia.

12 Appendix/Attachments

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/s/

FEDERICA BASSO
11/05/2013

TODD M BOURCIER
11/06/2013
pharm/tox supports AP

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

BLA Number: 125390 **Applicant: Amylin** **Stamp Date: March 27, 2013**

Drug Name: Metreleptin **BLA Type: Priority**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		DS for the to-be-marketed formulation is manufactured from Sandoz, whereas the DS for non clinical studies was manufactured by Amgen. A 28-day bridging toxicology study showed comparability of the two DS sources.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?			NA No additional pre-clinical studies were requested by the Division at the pre-NDA meeting (September 2007)

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Impurities in the metreleptin drug substance lots manufactured at Sandoz in 2011 are similar to the impurities contained in the Sandoz DS lots manufactured in 2007, used in the 28-day bridging study in mice.
11	Has the applicant addressed any abuse potential issues in the submission?		X	
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		X	

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ____Yes____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

There is large *in vitro* and *in vivo* evidence linking leptin to cancer development and progression in several tissues/organs. Please address the potential of metreleptin in modulating cancer risk in the lipodystrophy population.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

FEDERICA BASSO
05/15/2013

TODD M BOURCIER
05/15/2013
I concur