CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

211150Orig1s000

NON-CLINICAL REVIEW(S)

Tertiary Pharmacology/Toxicology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 211150
Submission date: December 14, 2018
Drug: pitolisant
Applicant: Bioprojet Pharma (Harmony Biosciences)
Indication: Treatment of excessive daytime sleepiness and cataplexy in adult patients with narcolepsy
Reviewing Division: Division of Psychiatry Products

Discussion:
The pharmacology/toxicology reviewer and supervisor recommended that pitolisant could be approved from the pharmacology/toxicology perspective for the indication listed above.

The pharmacology/toxicology reviewer identified CNS-related clinical signs including convulsions as the most prominent toxic effect of pitolisant. Such effects were observed in multiple species at exposures that were higher than clinical exposures although NOAELs were at exposures that were equal to or lower than the clinical exposure.

A two-year carcinogenicity study of pitolisant was conducted in rats and a 6-month carcinogenicity study was conducted in tgRasH2 mice. The executive carcinogenicity assessment committee concluded that these studies were adequate and that there were no drug-related neoplasms in either study.

Pitolisant possess two pharmacologic activities that may warrant established pharmacologic class terms. Pitolisant acts as an inverse agonist and antagonist at the histamine-3 (H3) receptor. Histamine-3 receptor antagonist and histamine-3 receptor inverse agonist would be appropriate established pharmacologic classes for pitolisant.

Conclusions:
The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. I agree that this NDA may be approved for the above indication. I note that CNS effects have been evaluated clinically. I have provided comments on labeling separately.

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/s/

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	211150
Supporting document/s:	SDN 2, eCTD 0002
Applicant's letter date:	12/14/2018
CDER stamp date:	12/14/2018
Product:	Pitolisant
Indication:	Treatment of excessive daytime sleepiness and cataplexy in adult patients with narcolepsy
Applicant:	Bioprojet Pharma (Harmony Biosciences)
Review Division:	Division of Psychiatry Products
Reviewer:	James Miller, PhD
Supervisor/Team Leader:	Aisar Atrakchi, PhD
Division Director:	Tiffany Farchione, MD, Acting
Project Manager:	Brendan Muoio, PharmD

Disclaimer

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Any information or data necessary for approval of NDA 211150 that Bioprojet does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 211150.

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1 Executive Summary

1.1 Introduction

Pitolisant is a new molecular entity under development by Bioprojet Pharma for the treatment of excessive daytime sleepiness and cataplexy in adult patients with narcolepsy. The mechanism(s) of action of pitolisant is unclear. However, its efficacy could be mediated through its activity as an antagonist/inverse agonist at the histamine H3 receptors. Pitolisant is approved outside the U.S.

1.2 Brief Discussion of Nonclinical Findings

Pitolisant binds with relatively high affinity and selectivity to histamine-3 (H3) receptors (Ki = 1 nM) compared to other histamine receptors (H1, H2, or H4; Ki ≥ 10 µM). Pitolisant acts as an inverse agonist and antagonist at the H3 receptor and upon binding causes disinhibition of histaminergic neurons resulting in increased synthesis and release of histamine. In *in vivo* animal models, pitolisant administration caused increased duration of wakefulness with an associated decrease in slow wave and paradoxical (REM) sleep. In addition to enhanced wakefulness, pitolisant decreased the number of narcoleptic attacks and a reduced the total duration of narcolepsy when administered in the narcolepsy mouse model (Orexin$^{-/-}$). Pitolisant effects on additional neurotransmitter systems was demonstrated by *in vivo* microdialysis studies in rodents where pitolisant increased acetylcholine, noradrenaline and dopamine levels in the prefrontal cortex. Dopamine levels, however, were not affected in the striatum including the nucleus accumbens, a region known to be associated with abuse potential. In safety pharmacology studies, pitolisant was a moderate hERG channel inhibitor with an IC_{50} of 1.32 µM. However, in *in vivo* studies in rat, rabbit and dog, pitolisant had minimal to no effect on QTc intervals and no pro-arrhythmic potential. In telemetered dogs, no QTc effects were observed up to 14 times the maximum recommended human dose of 35.6 mg, based on C_{max}. In the CNS, pitolisant produced slight sedation with pronounced core muscle hypotony. Pitolisant was also proconvulsant after pentylenetetrazol (PTZ) challenge in mice with increased spasms and tremors at 4 times MRHD and convulsions at 7 times the MRHD, based on mg/m^2.

Pitolisant is rapidly and effectively absorbed, however, oral bioavailability is low (~2% in rat and 27% in monkey) due to extensive first-pass metabolism. After oral administration, pitolisant and its metabolites were widely distributed to tissues including the brain. The highest concentrations were measured in liver, kidney, adrenal glands and pancreas in addition to GI tract organs/tissues. The metabolic profile of pitolisant is complex and species-dependent with humans and monkeys being the most similar. Pitolisant is extensively metabolized primarily by CY2D6 with potential auxiliary metabolism by CYP3A4; only 2% excreted unchanged. The inactive metabolite BP1.3484 is the major circulating metabolite in both monkey and human. Multiple other major circulating metabolites are present as well as prominent metabolites were recovered in urine. In rats, the major metabolite is BP1.2526, which has low activity at the H3 receptor and has been demonstrated to cause convulsions in rats when administered alone, i.v. This metabolite is found at relatively low levels in both humans and monkeys.

Single and Repeat dose toxicity studies were conducted in mice, rats, and monkeys up to 1-month, 6-month and 9-month in duration, respectively. The primary target organ of toxicity across all species was the CNS, with clinical signs that included hypoactivity, salivation, staggering gait, tremors and convulsions. In rats and monkeys, repeated oral administration of pitolisant at 13 times and 3 times the MRHD, respectively, based on C_{max}, produced convulsions in both males and females. Convulsions were first observed near T_{max} and usually resolved within 2 to 3 hours after administration. Convulsions were not observed after discontinuation of dosing and were not associated with microscopic findings in the brain. Additional target organs of toxicity after oral administration of pitolisant included liver and testes in mice; and lung, kidney and adrenals in rats. In general, all adverse findings resolved during recovery except for pale lung foci related to focal alveolar macrophage infiltration, which was still present in rats at the end of the recovery phase. No apparent adverse effects on other organ systems were observed in monkeys.

Pitolisant was non-genotoxic in an adequate battery of genotoxicity assays. Pitolisant was not carcinogenic and did not induce tumors in rats or mice at doses up to 4- and 10- times the MRHD of 40 mg, respectively, based on mg/m^2.

Pitolisant did not significantly affect mating or fertility indices at doses up to 22 times the MRHD, based on mg/m^2. Pitolisant caused abnormalities in sperm morphology and decreased motility without any significant effect on fertility at doses ≥ 13 times the MRHD, based on mg/m^2.

In pregnant rabbits, intramuscular administration of pitolisant during organogenesis caused maternal toxicity including significant body weight loss and decreased food consumption at doses ≥ 1 time the MRHD and instances of convulsions at 4 times the MRHD, based on AUC, respectively. At the maternally toxic doses, increased incidence of pre-implantation loss and abortion occurred with a consequent decrease in both the number of implantations and live fetuses. Pitolisant was not teratogenic, in rabbits, at doses up to 4 times the MRHD based on AUC, however, delayed skeletal development (incomplete ossification and supernumerary ribs) was observed. The no-observed-adverse-effect level for maternal and embryofetal development are 0.7 and 1.4 times the MRHD, based on AUC.

In pregnant rats, oral administration of pitolisant during organogenesis caused maternal toxicity including decreased body weight gain and food consumption at 22 times the MRHD, based on mg/m^2. No increase in fetal mortality, resorption or abortions occurred and no apparent fetal toxicity was observed. The no-observed-adverse-effect level for maternal and embryofetal development are 22 and 27 times the MRHD, based on mg/m^2.

In the pre- and post-natal developmental toxicity study in the rat, oral administration of pitolisant caused maternal toxicity including mortality, severe CNS clinical signs including convulsions and a significant decrease in body weight gain at 22 times the

MRHD based on mg/m^2. At the maternally toxic doses, fetal toxicity included stillbirths, postnatal pup mortality (due to lack of milk or failure to nurse) and decreased pup length and weight. Pitolisant was teratogenic at 22 times the MRHD based on mg/m^2, causing major malformations including cleft palate and abnormal limb flexure. A delay in postnatal development (decrease weight and length and delay in incisor eruption and testes descent) occurred at ≥ 13 times the MRHD. The no-observed-adverse-effect level for maternal and developmental toxicity is approximately 7 times the MRHD, based on mg/m^2.

After oral administration of ^{14}C-pitolisant, radioactivity is measurable in fetal tissue with peak levels occurring at 30 min post-dose. No retention in fetal tissue was observed. Radioactivity was measurable in the milk of lactating rats at concentrations 1 to 3 times higher than measured in plasma.

Due to low systemic exposure to the major human metabolite **BP1.8054** after oral administration of pitolisant in toxicological species, separate nonclinical studies with direct dosing of this metabolite were conducted in rat and nonclinical safety of this metabolite was adequately assessed. Significant systemic exposure was attained with all other human metabolites after oral administration of pitolisant resulting in adequate assessment of these metabolites in the nonclinical studies.

1.3 Recommendations

1.3.1 Approvability

Based on the review and evaluation of the results of pitolisant testing in animals, this application is recommended for approval from a Pharmacology/Toxicology perspective for the treatment of excessive daytime sleepiness and cataplexy in adult patients with narcolepsy.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Sections of the labeling supported by nonclinical data were being negotiated with the Applicant at the time of completion of this review.

2 Drug Information

2.1 Drug

CAS Registry Number: 903576-44-3

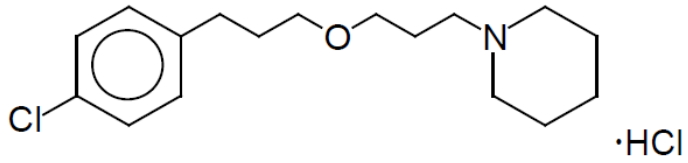
Generic Name: Pitolisant hydrochloride

Code Name: BF2.649

Chemical Name: IUPAC 1-{3-[3-(4-Chlorophenyl)propoxy]propyl}piperidine, hydrochloride

Molecular Formula/Molecular Weight: $C_{17}H_{26}ClNO$/296 (free base); 332 (HCl salt)

Structure or Biochemical Description



Pharmacologic Class: Histamine-3 (H3) receptor antagonist / inverse agonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

INDs (b) (4) and 111842

2.3 Drug Formulation

Immediate release, film-coated tablets in two strengths of 4.45 mg and 17.8 mg of pitolisant free base. The tablets are debossed with either an “S” or “H” on one side to indicate strength. The components and quantitative composition of pitolisant tablets are listed in the table below (Table 1).

Table 1. Composition of Pitolisant Drug Product

Ingredient	Function	Quality Standard	mg/Tablet per Strength		% (w/w) for
			4.45 mg	17.8 mg	
(b) (4)					
Pitolisant hydrochloride	API	3.2.S.4.1	5.00 [a]	20.0 [b]	
Microcrystalline cellulose	(b) (4)	USP/NF			(b) (4)
Crospovidone		USP/NF			
Talc		USP/NF			
Magnesium stearate		USP/NF			
Colloidal Silicon Dioxide		USP/NF			
					(b) (4)
Polyvinyl Alcohol –					(b) (4)
Titanium Dioxide					
					(b) (4)

a. Corresponding to 4.45 mg active substance, (b) (4)
b. Corresponding to 17.8 mg active substance,
c. (b) (4)
d.

[Excerpted from NDA211150, Description and Composition of the Drug Product; page 1]

2.4 Comments on Novel Excipients

There are no novel excipients in the drug product. All excipients are of compendial grade except for (b) (4), however, all components of this coating are compendial grade and therefore are considered to be acceptable.

2.5 Comments on Impurities/Degradants of Concern

The applicant conducted quantitative structure-activity relationship (Q)SAR assessment of 12 potential process impurities and degradants. An independent (Q)SAR for bacterial gene mutation was conducted by the FDA computational toxicology group using Derek Nexus 6.0.1 (DX0), Leadscope Model Applier 2.3.7-1, and CASE Ultra 1.7.0.5 GT1_BMUT model (CU). All 12 impurities were determined to be non-mutagenic, which

is consistent with the Applicant's conclusions and are therefore controlled as regular impurities.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed indication is for the treatment of excessive daytime sleepiness (EDS) and cataplexy in adult patients with narcolepsy. The maximum recommended human dose (MRHD) is 35.6 mg of pitolisant (free base)/day with a 3-week dose titration starting at 8.9 mg for the 1st week, increasing to 17.8 mg in the 2nd week and reaching the recommend dosage of 35.6 mg in the 3rd week.

2.7 Regulatory Background

- Pitolisant was approved by the European Medicines Agency (EMA), March of 2016, for the treatment of adults with narcolepsy with or without cataplexy.
- Pre-NDA meeting held on September 7, 2016
- Breakthrough therapy designation granted April 27, 2018 for the treatment of narcolepsy with cataplexy

3 Studies Submitted

3.1 Studies Reviewed

All nonclinical study reports relevant to the development of pitolisant for the treatment of excessive daytime sleepiness and cataplexy in adult narcolepsy patients have been reviewed.

3.2 Studies Not Reviewed

Due to approval being sought for an adult indication, juvenile animal studies were not reviewed.

3.3 Previous Reviews Referenced

Dr. Jerry Cott's reviews for the Carcinogenicity special protocol assessments (PSA) and corresponding ECAC meeting minutes (b) (4).

Dr. Melissa Banks-Muckenfuss' review for nonclinical information submitted (SDN10) including retrospective quantification of metabolite BP1.8054 in the Tgras.H2 Mouse carcinogenicity study under IND111842.

4 Pharmacology

4.1 Primary Pharmacology

***In Vitro* and *Ex Vivo* Pharmacodynamic Studies**

Binding affinity and functional potency of pitolisant and its metabolites for histamine-3 (H3) receptors were evaluated in several *In vitro* assays (Study nos. R-BF2.649-XL-001;

R-BF2-649-SK-001; R-BF2.649-XL-003). Pitolisant demonstrated high binding affinity for both native and recombinant human H3 receptors (Ki = 2.4 nM) and native mouse brain H3 receptor (Ki = 14 nM). The functional potency of pitolisant and its main metabolites at both the rat and monkey H3 receptors was evaluated using a CRE-gene reporter assay (Table 2 and Table 3). The parent compound demonstrated potent functional binding activity at H3 receptors in both species (K_B = 7.3 and 1.6 nM, respectively). A number of metabolites also showed moderate to high potency and similar to the parent, potency was greater at the monkey H3 receptor compared to the rat.

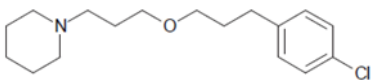
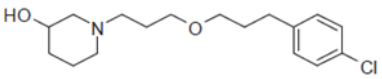
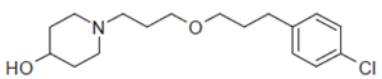
Table 2. Pitolisant Affinities to the Histamine-3 (H3) Receptor

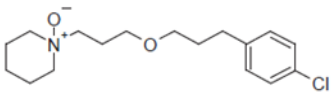
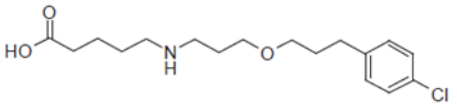
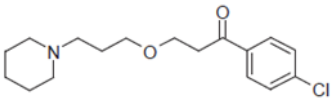
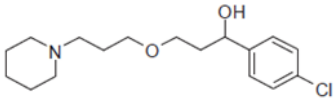
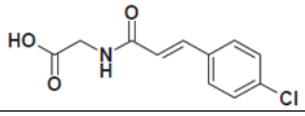
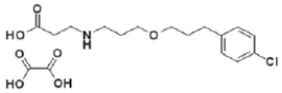
	Mouse	**Rat**	**Monkey**	**Human**
	Brain Native H3R	**Recombinant H3R ****	**Recombinant H3R ****	**Recombinant H3R ***
Test	Ki (nM)	K_B (nM)	K_B (nM)	Ki (nM)
[^{3}H](R)α-MeHA	5.7 ± 0.2			ND
[^{3}H]N$^\alpha$ methylhistamine	ND			1.0 ± 0.1
[^{125}I]iodoproxyfan	14 ± 1			2.4 ± 0.5
CRE gene reporter assay		7.3	1.6	

[^{3}H](R)α-MeHA: [^{3}H](R)α-methylhistamine. ND: not determined.
*: expression in rat glioma C6 cells.
**: expression in CHO cells.

[Excerpted from NDA211150, Pharmacology Written Summary; page 13]

Table 3. Summary of Binding Affinities of Pitolisant (BP2.649) and Metabolites at the Rat and Monkey Histamine-3 (H3) receptor

Compound #	Structure	rat H3	macaque H3
		gene reporter reversion HA Kb (nM)	gene reporter reversion HA Kb(nM)
BP2.649		7.3 ± 2.5	1.6
BP2.927		620 ± 151	43
BP2.928		311 ± 27	76

BP2.941		1000 ± 96	105
BP2.951		> 1000	> 1000
BP1.2525A		135 ± 46	11
BP1.2526A		25 ± 9	4.8
BP1.8054		> 1000	> 1000
BP1.8186		> 1000	> 1000

[Modified from NDA211150, Study Report R-BF2-649-SK-001; pages 7, 10]

Pitolisant effects on H3 receptor-mediated responses were evaluated in several in vitro assays (Study no. BF2.649-XL-003). The inverse agonist activity of pitolisant was demonstrated by the A23187-evoke [^{3}H]arachidonic acid release assay and [^{35}S]-GTPγS functional binding assay (EC_{50} = 1.5 nM) in human H3 receptor expressing CHO cells. The competitive antagonist activity of pitolisant at the H3 receptor was confirmed by inhibition of the imetit (H3 agonist)-induced [^{35}S]-GTPγS binding (K_B = 0.31 nM). Pitolisant also reversed the histamine-induced inhibition of [3H]-histamine release from rat cerebral cortex synaptosomes. Lastly, pitolisant demonstrated a positive functional response in the electrically-evoked twitches in isolated guinea pig ileum with a Ki = 5 nM.

Using the [^{35}S]-GTPγS functional assay (Study nos. R-BF2.649-IBB-007; R-BF2.649-IBB-009), the main human metabolites, BP1.8054 and BP1.9733 were inactive as antagonists at the human H3 receptor (K_B > 10 µM); whereas, BP2.928, demonstrated low potency antagonist activity (K_B = 874 nM).

At non-H3 histamine receptors, pitolisant demonstrated relatively low binding affinity with Ki or IC_{50} in the 1-10 micromolar range for H1 and H2 receptor to greater than 100 µM for the H4 receptor (Table 4; see secondary pharmacology for full list of off target binding).

Table 4. Binding Affinities of Pitolisant at Other Histamine Receptors

Receptors	Human H1R		Human H2R	Human H4R
Test System	[^{3}H]mepyr.	[^{35}S]GTPγS	[^{35}S]GTPγS	[^{3}H]histamine binding assay
Ki or IC_{50}	1.14 µM	> 10 µM	> 10 µM	>> 100 µM

[^{35}S]GTPγS: Histamine-Induced increase in [^{35}S]GTPγS binding. [^{3}H]mepyr.: Specific [^{3}H]mepyramine binding at recombinant human H_1 receptors expressed in HEK293 cells.

[Excerpted from NDA 211150, Pharmacology Written Summary; page 16]

***In Vivo* Pharmacodynamic Studies**

Pitolisant Effects on Histaminergic Activity

The ability of Pitolisant to increase histaminergic neurotransmission in vivo was examined in Swiss mice, C57BL/6 mice and Wistar rats (Study no. R-BF2.649-XL-004, non-GLP). Histaminergic activity was estimated through quantification of brain levels of tele-methylhistamine (t-MeHA), the main neuronal histamine metabolite. A single oral administration of pitolisant (3 mg/kg) significantly increased t-MeHA levels with a maximum effect at 90 min and a return to control levels by 6 h post-dose. The potency of pitolisant was similar in all rodent species and strains tested with no significant sex difference (See Table 5). The effect of pitolisant on histaminergic activity was maintained after repeated dosing for 4, 10 or 17 days with no significant reduction in potency. A significant rebound effect was observed when dosing was discontinued after repeated dosing for 4 days; however, this effect diminished as dosing duration increased and was non-significant compared to controls by 10 days of repeated dosing.

Table 5. Summary of Pitolisant potency on histaminergic activity in rodents

Male Swiss **OF1** mouse brain t-MeHA level	ED50 = 1.6 ± 0.6 mg/kg, p.o.
Male C57BL/**6J** mouse brain t-MeHA level	ED50 = **2.6** ± 0.5 mg/kg, p.o.
Female Swiss **CD-1** mouse brain t-MeHA level	ED50 = 2.1 ± 0.3 mg/kg, p.o.
Male Wistar rat brain t-MeHA level	ED50 # 3 mg/kg, p.o.
Male Swiss OF1 mouse brain t-MeHA level:	
4-day chronic treatment (10 mg/kg, p.o., b.i.d.)	At trough -34 ± 4 % At 10 mg/kg, p.o. chronic response # acute response
10-day chronic treatment (10 mg/kg, p.o., b.i.d.)	At trough -15 ± 3 % At 10 mg/kg, p.o. chronic response # acute response
17-day chronic treatment (10 mg/kg, p.o., o.d.)	At trough -5 ± 6 % At 10 mg/kg, p.o. chronic response # acute response

[Excerpted from NDA211150, Study Report R-BF2.649-XL-004-Amendment1; page 3]

Pitolisant Effects on t-MeHA, Acetylcholine, Dopamine and Noradrenaline Levels
A series of experiments were conducted to investigate the effects of pitolisant on other key neurotransmitter systems in both rats and mice using microdialysis and brain tissue sampling (Study no. R-BF2.649-XL-013).

In male Wistar rats, a single administration of pitolisant (10 mg/kg, i.p.) significantly increased extracellular dopamine (+219%), noradrenaline (+208%) and acetylcholine (+293%) levels in the prefrontal cortex as measured through microdialysis. Significant increases in extracellular acetylcholine (+269%) and t-MeHA levels (+55%) were measured in the hippocampus. **Reviewer Comment:** It is unclear whether the absence of data on dopamine and noradrenaline in the hippocampus and t-MeHA in the prefrontal cortex is due to a lack of an effect by pitolisant or a lack of quantification of these neurotransmitters in the respective regions.

In male OF1 mice, a single oral dose of pitolisant (10 mg/kg) significantly increased the DOPAC/DA ratio (+55%) in the prefrontal cortex alone at 90 min post-dose, indicating activation of dopaminergic activity in this region. Serotonin turnover was unaffected by pitolisant in all brain regions tested. As expected, t-MeHA levels were significantly elevated by pitolisant in all regions tested with the striatum and cortex showing the largest effect.

In male C57BL/6 mice, a single oral dose of pitolisant (10 mg/kg) slightly increased levels of the metabolite of norepinephrine MHPG (3-methoxy-4-hydroxyphenylglycol) in cortex (+13%), hippocampus (+10%), and hypothalamus (+15%) indicating minimal

noradrenergic activation in these regions. Dopamine and serotonin turnover was unaffected by pitolisant in all regions tested.

In Male wild type (WT) and orexin$^{-/-}$ mice, single oral administration of pitolisant (20 mg/kg) did not significantly affect dopamine and serotonin turnover when dosed alone or with modafinil (64 mg/kg, p.o.). In contrast, noradrenaline turnover significantly increased (+43% WT; +22% orexin$^{-/-}$) in the cortex after administration of pitolisant alone. Modafinil also increased noradrenaline turnover although this effect was not significant. An apparent synergistic effect on noradrenaline turnover (+86% WT; +121% orexin$^{-/-}$) was observed after co-administration of pitolisant with modafinil. Increased noradrenergic activity has been associated with an anti-cataplectic effect.

In male Wistar rats (Study no. R-BF2.649-XL-020), the effects of pitolisant on dopamine and serotonin levels and turnover were measured within the nucleus accumbens brain region. Pitolisant (10 mg/kg, p.o.) had no effect on extracellular levels of either dopamine or serotonin and their respective metabolites. In contrast, the reference article D-amphetamine demonstrated significant effects on dopamine levels in the nucleus accumbens brain region.

Pitolisant Effects on Wakefulness and Sleep Architecture

The wake promoting activity of pitolisant was evaluated in both mice (n=7) and cats (n=4) chronically implanted with intra-cortical and intra-muscular electrodes for continuous EEG/EMG recording (Study nos. R-BF2.64-Lin-001; R-BF2.64-Lin-002). In both species, single oral doses (up to 10 mg/kg in cats and 20 mg/kg in mice) increased the duration of wakefulness with an associated decrease in slow wave sleep and paradoxical sleep (REM). Additionally, significant increases in fast rhythms (frequency bands β and γ), which are associated with enhanced vigilance and attention, suggests possible pro-cognitive effects of pitolisant; though this effect was not assessed.

In the Orexin knock-out narcolepsy mouse model, a single oral administration of pitolisant (20 mg/kg) increased overall wakefulness with an associated decrease in both slow wave sleep and REM sleep (Study no. R-BF2.649-Lin-003). The effects on wake and sleep parameters were comparable to a single oral dose of modafinil (64 mg/kg). In contrast to modafinil, pitolisant suppressed both the number of narcoleptic attacks and the total duration of narcolepsy whereas modafinil had no effect on these parameters. Co-administration of pitolisant and modafinil had a significant synergistic effect on both increasing wakefulness and decreasing the number of narcoleptic episodes.

In the MPTP cat model of parkinsonism, animals demonstrate significant hypersomnia with marked behavioral somnolence and disturbances of paradoxical sleep. Single oral administration of pitolisant (10 mg/kg) during each phase (acute, transitory, and chronic) of the MPTP model, demonstrated significant increases in wakefulness with restored cortical activation and suppression of slow wave sleep (Study no. R-BF2.649-Lin-004). Additionally, in the chronic phase, administration of pitolisant substantially suppressed the increased paradoxical sleep. The wake promoting effects lasted for 5-8 h post-dose. Co-administration of pitolisant with anti-parkinsonian drugs, L-DOPA and ropinirole,

resulted in synergistic improvement in both wakefulness and locomotor activity. PK analysis demonstrated significant exposure to pitolisant after single oral dose of 10 mg/kg with a C_{max} of 1731 ng/mL and AUC of 3417 ng*h/mL. The major metabolite was BP2.951, the same major metabolite found in humans.

4.2 Secondary Pharmacology

Off-target binding affinity of pitolisant for a panel of 110 receptors, transporters and ion channels was evaluated using radioligand binding assays (Study no. 1009464P1). A summary of targets that were bound by pitolisant with a K_i < 1 µM are listed in the table below (Table 6).

Table 6. Main Findings in the General *In Vitro* Pharmacology Screen on Pitolisant

Receptors	Species	Binding assays Ki (µM)	Functional assays (µM)
Sigma-1 (σ1)	human	0.01*$ / 0.0005**	EC_{50} = 0.40**
Sigma-2 (σ2)	rat / human	0.05* / 0.007*	IC_{50} = 10**
Dopamine D3	human	0.38*	
5-HT_{2A}	human	0.544* / > 5**	Ki = 0.373**

* (b) (4), ** Bioprojet, $ IC_{50}

[Modified from NDA211150, Pharmacology Written Summary; page 17]

Pitolisant had high binding affinity for Both Sigma-1 and -2 receptors with a K_i in the low nanomolar range; similar to pitolisant's affinity for the H3 receptor. The effect of pitolisant on the function of sigma receptors was measured using cellular calcium flux (Study no. R-BF2.649-IBB-008). Pitolisant behaves as an agonist of Sigma-1 (EC_{50} = 402 nM) and as an antagonist of sigma-2 (IC_{50} ≈ 10 µM). Although pitolisant demonstrates relatively high affinity for these receptors, its functional potency at these receptors compared to the primary target is relatively low at pharmacologic concentrations.

Pitolisant demonstrated moderate binding affinity for both the serotonin 5-HT_{2A} and dopamine D3 receptors (Table 6). Under the [^{35}S]-GTPγS functional binding assay, pitolisant had low to moderate antagonistic activity at both receptors (Ki = 379 nM, and 1537 nM, respectively). Pitolisant was inactive as an antagonist at the 5-HT_{2A} in the calcium mobilization assay (Ki > 10 µM) and the [3H]-Spiperone displacement (Ki > 5 µM) (Study nos. R-BF2.649-TC-001; R-BF2.649-IBB-005).

Metabolites

Several human metabolites of pitolisant (BP2.951, BP1.8054, BP1.9733, BP1.3484, BP1.3473, BP1.8186, BP1.10749 and BP1.10556) were screened for off-target binding affinity in a comprehensive panel of over 170 receptors, channels and transporters. All but one of the metabolites tested showed little to no binding activity when tested at

concentrations of 3 and 10 µM. BP1.8186 was the only metabolite with a significant response of 60% inhibition at 3 µM for the sigma 1 receptor; Ki was not determined.

In [^{35}S]-GTPγS functional binding assays, the main human metabolites, BP2.951, BP1.8054 and BP1.9733 were inactive as agonists or antagonists up to 100 µM at the human D3 receptor. At the human 5-HT_{2A} receptor, only BP2.951 presented low potency antagonistic activity (Ki = 271 nM). BP2.951 does not penetrate the brain very effectively as demonstrated by only negligible levels being detected in the brain of rats after single oral administration of pitolisant. The likelihood of significant off-target activity at 5-HT_{2a} receptors in the brain is minimal.

4.3 Safety Pharmacology

Overall summary of safety pharmacology studies:
The safety pharmacology of pitolisant was evaluated in the cardiovascular, respiratory, CNS and gastrointestinal systems. In *in vitro* studies, pitolisant demonstrated moderate hERG channel inhibitory activity. However, *in vivo* studies in rat, rabbit and dog demonstrated minimal to no effect on QTc intervals and no pro-arrhythmic potential of pitolisant. At high IV doses of pitolisant (14 time the MRHD, based on C_{max}), heart rate and blood pressure increased significantly with a corresponding decrease in QTc interval (up to -14%) in beagle dogs. Respiratory function in anesthetized rats was minimally affected after IV administration to pitolisant, with a significant increase in inspiratory rate, a temporary decrease in expiratory rate, and a minimal increase in tidal volume. In the Irwin assessment in mice, slight sedation and pronounced core muscular hypotony were the major findings. Central excitation with the presence of straub tail and slight hypothermia occurred at the two highest doses. Pitolisant was determined to be pro-convulsant in mice under a sub-convulsant challenge by pentylenetetrazole (PTZ). Pitolisant increased the incidence and decreased the latency of tremors and spasms at the mid dose and convulsions at the high dose after PTZ challenge. Finally, no effect on motor coordination was observed in the rotarod test up to 60 mg/kg pitolisant in mice. In the gastrointestinal system, pitolisant slightly increased the ulcer score in fasted rats; however, pitolisant did not potentiate ulcer lesions after increased acid production by rivastigmine and indomethacin treatment.

Cardiovascular and Respiratory

In vitro Studies:

Effects of Pitolisant on hERG and other cardiac ion channel currents.
The inhibitory effects of Pitolisant on hERG channel tail currents was evaluated in hERG expressing HEK 293 cells (Study no. DHHL1001, GLP). Significant dose-dependent inhibition of hERG currents occurred at concentrations ≥ 0.3 µM with an IC_{50} of 1.32 µM. A second study (Study no. R-BF2.649-IBB-001, non-GLP) investigated the long-term effects (24 h) of Pitolisant on the surface expression of hERG channel protein. After the initial short-term exposure, hERG channel inhibition by Pitolisant was confirmed (-55.4% at 1 µM); however, no effect on the trafficking of hERG channel protein to the cell surface was detected during long-term incubation.

Pitolisant activity on a panel of cardiac-related ion channels was assessed through whole-cell patch-clamp in stably expressing cells (Study no. R-BF2.649-BSys-001, Non-GLP). Significant reduction in current amplitude occurred at 10 µM pitolisant for $Na_v1.5$ (slow inactivated state), $K_v4.3$, $K_v1.5$, $Ca_v1.2$, and $Ca_v3.2$; however, residual amplitude was greater than 50% for all ion channels listed. An IC_{50} value was only generated for $Ca_v1.2$ which was 9.46 µM.

Effects of Pitolisant on action potential parameters in human ventricular cardiomyocytes
Two non-GLP studies were conducted using conventional patch-clamp techniques with iPSC derived human cardiomyocytes to assess the effects of pitolisant on cardiac action potentials (Study nos. PS14G595, Non-GLP; PS14K611, Non-GLP). In the first study, pitolisant non-significantly increased action potential duration at 90% repolarization (APD90) by 16.5, 17.5 and 10.6% at 1, 3, and 10 µM, respectively. Significant decreases in APD20 of 26.2 and 38.1% occurred at 3 and 10 µM, respectively. In the second study using the same experimental system, the ability of pitolisant to alter the effects of a reference hERG channel blocker, dofetilide, on cardiac action potentials was determined. Perfusion of dofetilide (100 nM) caused a significant increase in APD90 which was fully reversed by perfusion of pitolisant (10 µM). Additionally, the perfusion of both resulted in a decrease in APD20. The results of the two studies are consistent with the inhibitory action of pitolisant on hERG channels as well as its calcium blocking effects.

Effects of Pitolisant on electrophysiological parameters in isolated rabbit Purkinje fibers
The pro-arrhythmic potential of pitolisant was assessed in two studies using isolated rabbit Purkinje fibers with a standard microelectrode technique (Study nos. R-B009-2.649, GLP; R-BF2.649-JFF-002, Non-GLP). Perfusion of pitolisant (0.1, 1, and 10 µM) induced a dose-dependent reduction in ADP50 (-27 ± 4 % at 10 µM), resting membrane potential, action potential amplitude and maximal upstroke velocity with all parameters reaching significance only at the high dose. The effects were mostly reversed upon wash-out of pitolisant. Pitolisant also caused a modest dose-dependent increase in APD90 (+9 ± 5 % at 10 µM). Although not significant during perfusion, the increase in APD90 continued to rise during the washout period and the effect was statistically significant compared to controls. Perfusion of the reference hERG channel blocker, dofetilide, extended action potential duration (APD) and resulted in early after depolarization (EAD) to occur, which is known to promote the occurrence of ventricular arrythmias. Co-perfusion of pitolisant at 10 µM shortened the APD and suppressed EAD induction. The results of these studies are consistent with the inhibitory action of pitolisant on potassium channels but also suggests effects on both calcium and sodium currents.

The binding affinities of several human metabolites of pitolisant for the hERG channel were evaluated by radioligand binding assays (Table 7). All metabolites, except for BP2.928, showed no significant binding with a Ki > 10 µM. At the MRHD of 40 mg, the serum levels of the major metabolites, BP1.8054 and BP1.9733, were 168 and 20.5 nM, respectively, corresponding to safety margins of 60 time and 487 times. BP2.928

demonstrated low binding affinity with a Ki of 4.2 µM and no binding at 1 µM. BP2.928 is not considered a significant human metabolite. The effects of the minor metabolite BP2.951 on hERG tail currents were evaluated. No significant inhibition was detected up to 17 µM. Serum levels of BP2.951 at the MRHD of 40 mg correspond to a safety margin of 201 times.

Table 7. Summary of *In Vitro* Effects of Pitolisant Metabolites on hERG Channel Currents

System Evaluated	Cell Type	Doses per Group	Number per Group	Noteworthy Findings	GLP	Study Number
In vitro effects of BP2.951, the main metabolite of pitolisant on Ikr current	HEK293	01 to 10 µM	N=3-4	**No inhibition of hERG channels up to 10 µM**	no	R-BP2.951-JFF-001
In vitro effects of BP2.951, the main metabolite of pitolisant on Ikr current	HEK293	0.7, 1.4, 4.4 and 17 µM	N=6	**No inhibition of hERG channels up to 17 µM**	yes	(b) (4) 10-0170-3
In vitro effects of BP2.982 and BP1.8054 metabolites of pitolisant on human hERG channel	HEK293	BP2.928 0.001 to 100 µM BP1.8054 1 to 100 µM	N=3	**BP2.928 Ki = 4.2 µM BP1.8054 Ki > 10 µM**	no	R-BF2.649-IBB-003 (b) (4) AB63150-1203678
In vitro effects of BP1.9733, a main metabolite of pitolisant on human hERG channel	HEK293	0.001 to 100 µM	N=3	**BP1.9733 Ki > 10 µM**	no	R-BF2.649-IBB-010 (b) (4) AB63150-1203679
In vitro effects of BP1.3473, 3484, BP1.8186, BP1.10749 and BP1.10556, metabolites of pitolisant on human hERG channel	HEK293	Up to 100 µM	N=3	**BP1.3473 Ki > 10 µM BP1.3484 Ki > 10 µM BP1.8186 Ki > 10 µM BP1.10556 Ki > 10 µM BP1.10749 Ki > 10 µM**	no	R-BF2.649-IBB-013 AB76488-1208135 AB76488-1208136 AB76488-1208133 AB76488-1208134 AB80437-1210427

[Modified from NDA211150, Pharmacology Tabulated Summary; page 27]

In vivo Studies:

Effects of Pitolisant on cardiovascular function in conscious telemetered dogs

Adverse cardiovascular effects of pitolisant were assessed in 3 separate studies using telemetered beagle dogs. In the first study (Study no. (b) (4) 08-136-4, GLP), single oral doses ranging from 5 to 15 mg/kg were administered to 3 male and 3 female dogs with a 48 h washout period between drug administrations. In two follow-up studies a single dose of pitolisant was administered by IV at 1.5 mg/kg (Study no. (b) (4) 08-282-2, GLP) and 4.5 mg/kg (Study no. (b) (4) 09-917-2, GLP). Cardiovascular parameters were monitored continuously over a 24 h period in all three studies.

Pitolisant administered orally to dogs up to 15 mg/kg and by IV at 1.5 mg/kg demonstrated no drug-related significant effects on measured cardiovascular

parameters including QT and QTc intervals. No arrhythmias or abnormal morphology of the electrocardiogram were noted. Oral administration resulted in relatively low exposure (C_{max} = 24.64 ng/mL at 15 mg/kg); however, IV administration did result in significant exposure levels (C_{max} = 294 ng/mL at 1.5 mg/kg).

Pitolisant administered IV at 4.5 mg/kg resulted in significant drug-related increases in arterial blood pressure (+50%) and heart rate (+89%) compared to pre-dose values. Maximum increases occurred within 10 min of administration and returned to pre-dose levels by 4 h post-administration. Because of the rapid increase in heart rate by pitolisant, a number of ECG intervals (PR, QRS, and QT) were shortened. The QTc interval was also shortened by 12% to 14% at 5 min post-administration and returned to predose values within 1 h. No arrhythmias or morphology changes in the electrocardiograms were noted. IV administration of 4.5 mg/kg pitolisant resulted in significant exposure with a C_{max} of 1044 ng/mL which corresponds to 14X the serum levels of pitolisant at the MRHD of 40 mg.

Effects of pitolisant on cardiovascular and respiratory function in rats

Anesthetized Sprague-Dawley rats were administered a continuous ascending dose of pitolisant (0 to 6 mg/kg) over a 30-minute period by IV infusion (Study no. FS-264903, GLP). Cardiovascular and respiratory parameters were measured at pre-infusion, during the 30 min dosing and at 5- and 10-minutes post-dosing. A dose-dependent reduction in heart rate compared to basal values occurred at ≥ 1 mg/kg of pitolisant and was statistically significant compared to the control group at ≥ 4 mg/kg. A maximum reduction of 95 bpm occurred at the highest dose of 6 mg/kg. Inspiratory flow rate increased significantly compared to controls at the highest dose and remained significantly elevated at the two post-infusion time points. Expiratory flow rate was significantly reduced compared to basal levels; however, only the flow rate at the 1st post-infusion timepoint was significantly different than control values. Minimal but statically significant increases in tidal volume compared to the control group occurred at 4 and 6 mg/kg.

Primary hemodynamic parameters including heart rate and blood pressures were continuously monitored for 24 h in conscious telemetered Wistar rats (N=7) after a single oral dose of pitolisant at 5 and 20 mg/kg (Study no. R-B042-2.649, GLP). A 3-day washout period occurred between the various treatments. Minimal yet significant increases in heart rate and RPP, compared to controls, occurred in the 5 mg/kg dose group at 20 min post-dose and returned to control levels by 1 h. At the high dose of 20 mg/kg these parameters were not significantly different than control values. The high dose resulted in significant increases in all blood pressure parameters; however, the increases were within physiological range. The elevated pressures occurred at 20 min post-dose and normalized by 1 h. In conscious telemetered Wistar rats, a single oral dose of 5 and 15 mg/kg pitolisant did not significantly affect any ECG parameter tested. A slight but non-significant reduction in heart rate was noted at 15 mg/kg and the effect persisted for up to 6 h post-dose (Study no. R-B008-2.649, Non-GLP).

Effects of pitolisant on cardiovascular function in rabbits

Cardiovascular effects of pitolisant were assessed in anesthetized New Zealand white rabbits (predominantly female, 81%) by standard surface ECG (Study no. R-B011-2.649, GLP). Pitolisant was administered by IV either as a slow infusion over 60 min with a single dose (1 mg/kg) or as an ascending dose up to 10 mg/kg with a 20 min duration for each dose step. A wash-out protocol was also used consisting of infusion of 3 mg/kg over 30 min and a 30 min wash-out period. Cardiovascular parameters were monitored continuously starting at 10 min pre-infusion and ending 60 min from the start of infusion. Slow infusion of 1 mg/kg resulted in no significant change in ECG parameters compared to baseline. During the ascending dose phase, decreased heart rate (-17%) and prolongation of the QT interval (+23%) was noted and reached significance during the last 10 min of infusion. However, the QT interval was not significantly different from controls after correction for heart rate (QTc). Additionally, mean blood pressure and RPP were significantly reduced during the last 10 min of infusion in the ascending dose phase. Similar effects to the ascending dose protocol were noted during the 30 min loading period of 3 mg/kg pitolisant with the wash-out protocol. These effects included decreased heart rate and increased QT with no effect on QTc. During the wash-out phase all parameters trended toward normalization with no exaggerated rebound effect noted. Similarly, mean blood pressure and RPP were reduced during loading phase and normalized during the wash-out phase with no exaggerated rebound effect noted.

Although a dose-related increase in the incidence of U-waves occurred in pitolisant treated animals, there was no increase in the U- to T-wave amplitude ratio (UTA) indicating a low potential for pro-arrhythmic activity of pitolisant.

The pro-arrhythmic potential of pitolisant was further assessed using Carlsson's anesthetized rabbit model for Torsades de pointes (TdP) (Study no. R-BF2.649-LL-009, Non-GLP). This model consists of animal sensitization by co-infusion of methoxamine, an α-adrenergic receptor agonist. Pitolisant was administered by slow IV infusion over 60 min in New Zealand white rabbits (n=3/dose) at 3 and 9 mg/kg. Changes in hemodynamic parameters were similar to those observed in non-sensitized animals as summarized in the study above. ECG parameters were also similarly affected as described in the above study with increases in cardiac intervals including QT but not QTc and the presence of U waves with only minimal effects on UTA ratio. There was also no indication of ventricular tachycardia or TdP events at any dose of pitolisant tested. In contrast, infusion of the positive control, clofilium tosylate, resulted in prolonged QTc interval, increased UTA and ventricular tachycardia and TdP events. The plasma level of pitolisant at the end of infusion of the high dose was approximately 3000 ng/mL which is > 40X the C_{max} at MRHD.

CNS

The effects of pitolisant on the central nervous system were assessed by the Irwin test following a single oral administration to male Swiss mice (8/group) at doses of 3, 10, 30, 60 and 100 mg/kg. All parameters were evaluated at 30, 60, 120 and 180 minutes post-dose. The most apparent effects were pronounced core muscular hypotony and slight

sedation. Pitolisant inhibited spontaneous motor activity, impaired touch responses and inhibited righting reflex. Increased vocalization and irritability were observed but these parameters were only significant at the HD. Clear indications of central excitation were noted. Pitolisant at 10 mg/kg and above significantly inhibited the provoked biting response. Core temperature was also decreased dose-dependently by pitolisant beginning at 30 min post-dose and returned to control levels by 180 min. Instances of catalepsy and straub tail were recorded in a few animals primarily at the highest dose tested.

The proconvulsant potential of pitolisant was assessed by pentylenetetrazol (PTZ) challenge in Swiss mice (Study no. FS-264902, GLP). Oral administration of pitolisant increased central excitation as demonstrated by increased incidence of tremors and spasms at 30 mg/kg as well as straub tail and convulsions at 60 mg/kg after challenge with sub-convulsant exposure to PTZ (30 mg/kg, i.p.). Although, both tremors and spasms occurred in control animals after PTZ challenge, the latency time was significantly reduced at the 2 highest doses of pitolisant. Pitolisant was determined to be proconvulsant at 30 and 60 mg/kg.

The effects of pitolisant on motor coordination was assessed by rotarod in male Swiss mice. A single oral gavage dose of pitolisant at 3, 10, 30 and 60 mg/kg were administered and non-accelerated rotarod was performed at 60, 90 and 120 min post-dose. No significant effects on motor coordination were observed at any dose of pitolisant.

Gastrointestinal

The effects of pitolisant on gastrointestinal acid production and ulcer formation was assessed in two single oral dose studies in rats (Study nos. R-BF2-649-XL-011; R-BF2-649-XL-025). Oral administration of pitolisant at 30 mg/kg caused a slight but significant increase in the gastric ulcer score at 6 h post-dose in fasted male Wistar rats. Co-administration of pitolisant did not potentiate formation of ulcers by indomethacin or rivastigmine, two compounds known to increase acid production and the formation of gastric ulcers.

4.4 Other Pharmacology Studies

Pitolisant demonstrated positive effects on learning and memory in the scopolamine-induced learning deficit mouse model and a model of natural forgetting in mice.

Pitolisant was evaluated for potential effects in psychotic disorders such as schizophrenia using several experimental models. Pitolisant significantly reduced methamphetamine-, dizocilpine- and MK-801-induced hyperactivity. Pitolisant also demonstrated positive effects on apomorphine-induced climbing behavior and disruption of pre-pulse inhibition.

In the DAT (-/-) KO mouse model of ADHD, pitolisant significantly reduced spontaneous locomotor activity and improved performance in the water-maze test.

The antiepileptic activity of pitolisant was evaluated in 3 models of epilepsy. Pitolisant failed to suppress epileptic seizures in the maximal electroshock seizure model. Pitolisant, however, was effective at suppressing spike and wave discharges in the genetic model of absence epilepsy (GAERS) and hippocampal seizures in the kainate temporal lobe epilepsy model. The induction of clonic seizures at the high dose of pitolisant in the kainate model suggests possible proconvulsant activity at higher doses.

Although these data demonstrate potential positive effects for a number of neurological and psychiatric disorders, these studies have limited relevance to the current indication of pitolisant.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

In vitro characterization of pitolisant demonstrated high aqueous solubility at both pH 1.0 and 6.8, high permeability with limited efflux in a Caco-2 cell model, and stability in both simulated gastric and intestinal fluid (Study nos. 15BIOPP1 non-GLP; 15BIOPP1GLPS317, GLP). These characteristics indicate the potential for high absorption of pitolisant.

In vivo pharmacokinetic parameters of pitolisant were evaluated after single and repeated oral dosing in multiple nonclinical species through limited dedicated PK studies or more extensively as part of general toxicology studies (See reviews for individual studies under the general toxicology section). In general, pitolisant is rapidly and effectively absorbed with peak plasma concentrations occurring between 0.25 to 1.5 h after administration in all species tested. Pitolisant systemic exposures tended to increase either dose proportionally or slightly greater than dose proportionally, particularly between lower doses. In general, pitolisant plasma levels tended to decrease after repeated dosing with an apparent increase in the formation of some metabolites; indicating induced metabolic activity after repeated dosing. No apparent sex differences in pitolisant exposure were observed in any tested species. Pitolisant was eliminated with a terminal half-life ($T_{½}$) ranging from 1 to 7 h.

Oral bioavailability of pitolisant was evaluated in male Swiss mice and Wistar rats following single oral and i.v. administrations of pitolisant at 10 mg/kg (free base) (Study no R-BF2.649-XL-005, non-GLP). Pitolisant was rapidly absorbed with maximal concentrations occurring at 15 min and 1 h after administration in rats and mice, respectively. Orally administered pitolisant exhibited relatively high bioavailability in mice (84%), whereas in rats a lower bioavailability was measured (37%; Table 8).

Table 8. Serum Concentrations and Bioavailability of Oral and IV Pitolisant in Mice and Rats

Species	Units	Oral		Intravenous			Bioavailability Ratio AUC_{PO}/AUC_{IV}
		C_{max}	AUC	AUC	$T_{½}$ dist (min)	$T_{½}$ elim (min)	
Mouse	nM or nM.h	1994 ± 222	7916	9449	13	126	0.84
	ng/mL or h.ng/mL	590 ± 66	2343	2797			
Rat	nM or nM.h	310 ± 73	764	2082	3	49	0.37
	ng/mL or h.ng/mL	92 ± 22	226	616			

Data are presented as mean ± standard error.
Values obtained from 6 animals per group except for oral treatment in rat (n=12). Mouse C_{max} was measured at 1 h; rat C_{max} was measured at 15 min.

[Excerpted from NDA211150, Pharmacokinetics Written Summary; page 19]

Elimination of pitolisant was faster in rats compared to mice with a $T_{½}$ of <1 h in rats versus 2 h in mice.

Reviewer Comment: Pitolisant concentrations were obtained using a radioreceptor assay which does not discriminate between the parent compound and any metabolites with H3 receptor activity (e.g. BP1.2525 and BP1.2526). Pitolisant plasma levels are therefore likely overestimated in this study.

Oral bioavailability was evaluated in male Sprague-Dawley rats and male Cynomolgus monkeys after oral and i.v. administration of ^{14}C-pitolisant (Study nos. (b) (4) DHHL1003, GLP; (b) (4) DHHL1004, GLP). In both rats and monkeys, near complete absorption of radioactivity occurred following oral administration, however, oral bioavailability of pitolisant was only 1.5% and 27%, respectively (Table 9; Table 10). The low bioavailability of the parent in both species is most likely related to extensive first pass metabolism with the formation of the major metabolites identified in these studies which included BP1.2526 and BP1.2525 in rat and BP2.951 in monkeys, in addition to other major metabolites not individually measured here.

Table 9. Pharmacokinetic Parameters of Total Radioactivity, Pitolisant, and Metabolites BP1.2525 and BP1.2526 in the Rat Following Oral Administration of Pitolisant at 30 mg/kg

Parameter	TRA	Analyte		
		Pitolisant	BP1.2525	BP1.2526
C_{max} (ng/mL)	5464	33.6	54.1	191
T_{max} (h)	1.00	0.250	0.250	0.250
$AUC_{0-\infty}$ (ng.h/mL)	76268	55.8	218@	579
$T_{1/2}$ (h)	17.2*	3.10	2.69*#	2.05
Absorption† (%)	143	NC	NC	NC
F (%)	*121*†	1.50	NC	NC

*: Only 3 data points used in regression analysis.
#: Period over which λ_z was determined is less than twice the resultant $T_{1/2}$
@: Extrapolated area is greater than 20% of total AUC.
†: Likely to be overestimated; calculated using $AUC_{0-\infty}$ from IV phase that is likely underestimated
F= bioavailability; NC = not calculated; TRA = Total radioactivity

[Excerpted from NDA211150, Pharmacokinetics Written Summary; page 23]

Table 10. Pharmacokinetic Parameters of Total Radioactivity, Pitolisant, and Metabolite BP2.951 in the Monkey Following Oral Administration of Pitolisant at 12 mg/kg

Parameter	Oral administration of 12 mg/kg (free base)		
	TRA	Pitolisant	BP2.951
C_{max} (ng/mL)	13724 (36.5)	314 (53.0)	2546 (59.5)
T_{max} (h)	1.02 (0.520-2.00)	1.01 (0.530-1.02)	1.01 (0.530-2.00)
AUC_{0-t} (ng.h/mL)	152216 (12.4)	1044 (42.7)	7639 (23.5)
$AUC_{0-\infty}$ (ng.h/mL)	161715 (10.4)	1050 (42.6)	7654 (23.6)
$T_{1/2}$ (h)	18.9 (16.4)	7.23 (39.7)	11.1 (29.8)
Absorption [a] (%)	90.1 (16.4)	NC	NC
F (%)	*96.4*	26.6 (50.5)	NC

Data are presented as geometric mean (%CV) except T_{max}, which is presented as geometric mean (range).
N = 4 animals
[a] Likely to be overestimated; calculated using $AUC_{0-\infty}$ from IV phase that is likely underestimated.
NC = not calculated; TRA = total radioactivity

[Excerpted from NDA211150, Pharmacokinetics Written Summary; page 23]

Distribution

Pitolisant is highly bound to plasma protein in all tested animal species (mouse, rat, dog, monkey) and humans (ranging from 87 to 97%; Study no. R-B113-2.649). In a second *in vitro* binding study, pitolisant was 96% plasma protein bound in both the mouse and human with serum albumin and alpha1-glycoprotein acid being the predominantly bound proteins (Study no. R-BF2.649-PhR-001). The plasma protein binding of significant human metabolites (BP1.8054, BP1.3473, BP1.3484, BP1.8186, BP1.10556, and BP1.10749) was also evaluated in human and animal serum (mouse, rat, dog, monkey, and rabbit; Study nos. R-B457-2.649; R-B393-1.9733). Plasma protein binding for BP1.3484, BP1.8186, and BP1.9733 were similar between animal species and humans; however lower plasma protein binding occurred with BP1.10556 and BP1.10749 in rodents compared to humans. BP1.8054 also showed lower protein binding in rodents compared to humans but only at higher metabolite concentrations. Evaluation of blood:plasma ratio of pitolisant and its main metabolites demonstrated no preferential distribution to the cellular components of whole blood (Study nos. R-B364-2.649; R-B392-1.9733; R-B458-2.659).

Three independent mass balance GLP studies, in rat, were conducted to evaluate distribution and excretion of pitolisant and its metabolites. Single oral doses of ^{14}C-pitolisant at 30 mg/kg (free base) were administered to male Sprague-Dawley and partially pigmented Lister-Hooded rats (Study nos. (b) (4) DHHL1003; (b) (4) BIP/02; (b) (4) BIP/07). After oral administration, radioactivity was rapidly distributed to systemic tissues with a T_{max} of 1 hour in roughly half of the tissues sampled. The T_{max} for radioactivity in the remaining tissues was approximately 6 h. The apparent volume of distribution (V_{ss}) was approximately 10-fold greater than total body water indicating extensive tissue distribution of pitolisant. As expected, the majority of radioactivity was observed in the GI tract. In addition to GI tract tissues, the highest concentration of radioactivity was measured in the liver, kidney, adrenal glands and pancreas. High concentrations of radioactivity were measured in the pigmented portion of the eye (Uveal tract) with radioactivity levels remaining elevated for an extended period ($T_{1/2}$ = 11.4 days; Only tissue at day 35 with quantifiable radioactivity). Levels of radioactivity were also greater in pigmented skin/fur compared to non-pigmented tissue; indicating some affinity for melanin. At 4 h post-dose, radioactivity levels were greater than plasma levels in two-thirds of sampled tissues. At 24 h post-dose radioactivity was below the limit of quantification in several tissues including the brain and spinal cord. A majority of radioactivity (92.1%) had been recovered by 24 h post dose, with roughly equal amounts being eliminated in urine and feces.

Similar to results in the rat studies, extensive and rapid distribution of radioactivity to systemic tissues was observed after oral administration of ^{14}C-pitolisant in male Cynomolgus monkeys with maximum concentrations occurring at 1 h post-dose in the majority of tissues and an apparent volume of distribution approximately 10-fold greater than total body water (Study no. (b) (4) DHHL1004, GLP). In addition to GI tract tissues, high levels of radioactivity were measured in liver, kidneys, prostate and seminal vesicles. A majority of radioactivity was recovered in the urine (~70%) with less than 5% recovered in the feces.

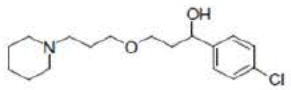
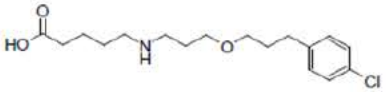
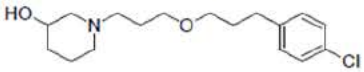
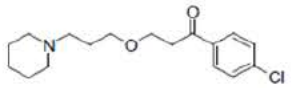
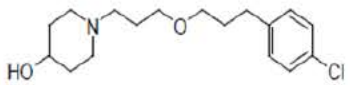
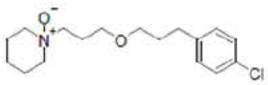
Following a single oral administration of ^{14}C-pitolisant at 30 mg/kg (free base) in pregnant SD rats (Study no QBR117544 BIP/06, GLP) radioactivity levels in fetal tissue peaked at 30 min post-dose and declined thereafter with a terminal half-life similar to or below maternal blood levels; indicating no significant retention in fetal tissue. The highest radioactivity levels in fetus associated tissues were measured in the amniotic sac, placenta, fetal liver and fetal brain. By 72 h post-dose, the majority of tissues were near or below the limit of quantification.

Metabolism

In Vitro Metabolism

The metabolic fate of pitolisant was evaluated in rat, dog, monkey and human hepatic microsomal preparations and primary hepatocyte cultures (Study no. (b) (4) CT-97, non-GLP). BP2.951 was the major metabolite formed in both monkey and human preparations, whereas BP1.2526 is the major metabolite formed in rat and dog (Table 11). The main enzymes involved in metabolizing pitolisant to the major metabolites, BP2.951 and BP1.2526 were identified using recombinant CYP isoforms and specific CYP inhibitors in human liver microsome preparations. Both CYP2D6 and CYP3A4 formed the main metabolites with CYP2D6 displaying higher affinity for pitolisant, while CYP3A4 had higher catalytic activity.

Table 11. The Structure and Relative Abundance of Main Metabolites from *In Vitro* Metabolism Across Various Species

Metabolite	Chemical Name/Structure	Rat		Dog		Monkey		Human	
		hep	mic	hep	mic	hep	mic	hep	mic
BP1.2526	benzylic alcohol 	+++	+++	+++	+++	+	+++	+	++
BP2.951	5-aminovaleric acid 	++	-	++	+/-	+++	+++	+++	+++
BP2.927	3-hydroxy piperidine 	-	+/-	+/-	-	+/-	-	+	++
BP1.2525	benzylic ketone 	+	++	+	+	+/-	-	+	+
BP2.928	4-hydroxy piperidine 	+/-	-	+++	++	+/-	+++	+	+
BP2.941	piperidine N-oxide 	-	-	+++	+	++	+++	++	++

hep = hepatocytes; mic = microsomes
+++ most abundant
++ moderate abundance
+ low abundance
+/- minor metabolite
- not detected
Source: (b) (4)CT-97

[Excerpted from NDA211150,Pharmacokinetics Written Summary; pages 51, 52]

In Vivo Metabolism and Excretion

After oral administration, pitolisant is rapidly and extensively metabolized across species with a complex metabolic profile (Table 12) resulting in less than 2% recovery of unchanged pitolisant after 24 h in most species including humans. The overall proposed biotransformation pathway is depicted in Figure 1. The primary metabolic route is oxidation followed by glucuronidation and glycine conjugation. Pitolisant is primarily metabolized by CYP2D6 with higher exposure levels occurring in CYP2D6 poor metabolizers. CYP3A4, identified as a prominent metabolism pathway *in vitro*, did

not appear to play a role *in vivo* as no change in pitolisant levels occurred with co-administration of CYP3A4 inhibitors grapefruit juice and itraconazole. However, co-administration with CYP3A4 inducer, rifampicin, reduced pitolisant exposure by approximately 50% indicating potential auxiliary metabolism by CYP3A4.

The majority of metabolites are inactive at the histamine H3 receptor. Complete loss of activity at the H3 receptor occurs with opening of the piperidine ring (e.g. BP1.3484, BP2.951 and BP1.8054) as well as with glucuronidation (e.g. BP1.9733). Oxidation alone at multiple different sites within the molecule partially reduces activity at the H3 receptor (e.g. BP1.2526 and BP1.2525; Table 12).

Metabolite Comparison Across Species

In humans, two mass balance studies were conducted using ^{14}C-pitolisant (radio-labeled at two different sites; Study nos. P11-01; P15-02). The major circulating metabolites identified in the first study were BP1.8054 and BP1.9733. In the second study BP1.3484 is the only major circulating metabolite that was prominent across all tested individuals. Metabolites BP1.3473, BP1.10749, and BP1.8054 were also major metabolites (>10%) but only in some individual subjects. Additionally, metabolites BP1.9733 and BP2.951 were prominent metabolites in urine in the second study.

Reviewer comment: The discrepancy in metabolite profiles between the two human mass balance studies is likely due to the incomplete tracking of radioactivity resulting from placement of the radio-label at a labile position of pitolisant in the first study.

In general, metabolite profiles were most similar between human, monkey and mice (Table 12). In monkeys and mice, metabolite BP1.3484 is the most prominent circulating metabolite with moderate levels of BP1.3473, BP1.9733 and BP2.951 also being present. Metabolism in the rat differed from monkeys and humans with the most abundant circulating metabolite being the oxidation product, BP1.2526, with BP1.2525 and BP2.951 also being present. In humans, BP1.2526 is found at relatively low concentrations.

Reviewer Comment: Except for BP1.8054, all identified major human metabolites were appropriately evaluated in nonclinical studies with adequate metabolite exposure levels being reached in the pivotal general toxicology, reproductive toxicology and carcinogenicity studies (Table 12; Table 13). Serum levels of metabolite BP1.9733 were directly quantified in the 9-month monkey study and retrospectively in the mouse carcinogenicity study. Due to low systemic exposure to metabolite BP1.8054 after oral administration of pitolisant in toxicological species, separate nonclinical studies with direct dosing of this metabolite were conducted in rat, which included a 13-week repeat dose toxicology study and embryo-fetal development study. Retrospective quantification of BP1.8054 in 26-week carcinogenicity study in TgrasH2 mice demonstrated adequate systemic exposure to this metabolite.

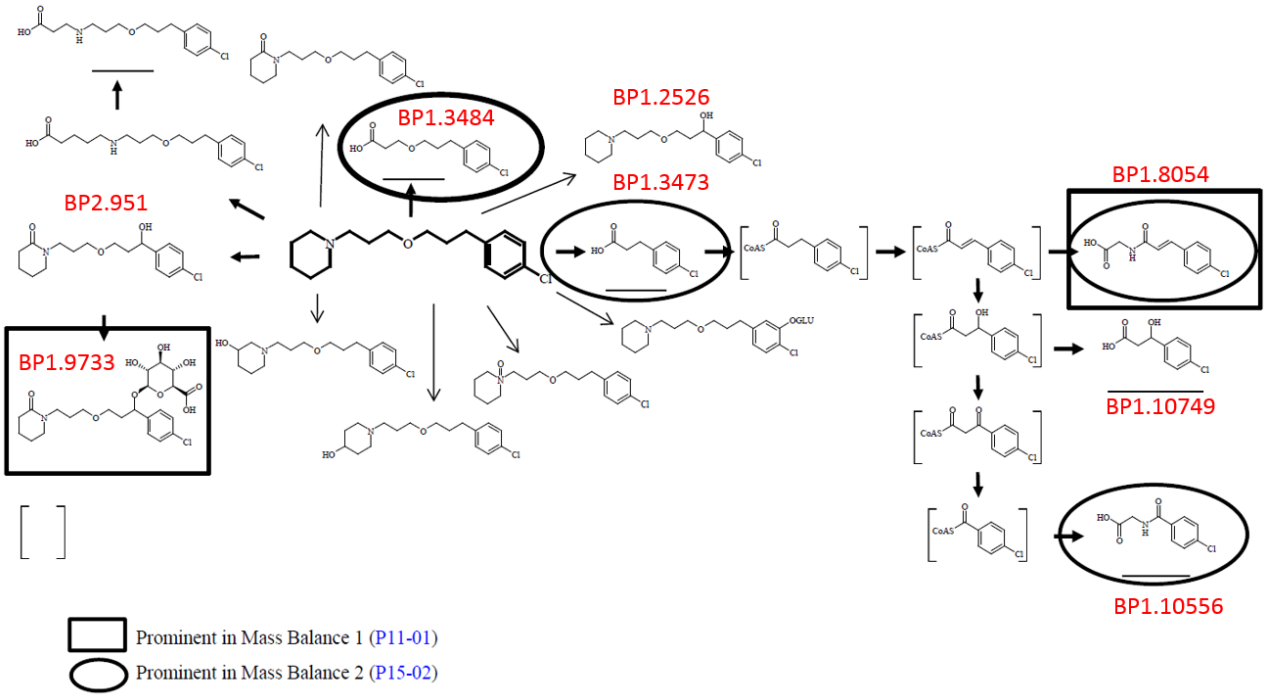
Table 12. Significant *In Vivo* Metabolites in Rats, Monkeys and Humans, and their Relative Abundance (in plasma/serum)

Component	**Mouse** BIP/12 B428 B449	**Rat** BIP/03 BIP/07	**Monkey** B322 B389 B452	**Human** P11-01 BIP/05	**Human** P15-02 BIP/09	**Human H3R Affinity** Ki (nM)
M200: BP1.10749	++	n.d.	n.d.	n.d.	++	> 10000
M213: BP1.10556	++	n.d.	++	n.d.	++	> 10000
M184: BP1.3473	+++	n.d.	+++	n.d.	+++	> 10000
M239: BP1.8054	++	n.d.	n.d.	+++	++	>10000
Glucoronide of mono-hydroxy pitolisant	n.d.	n.d.	+++	++	n.d.	n.d.
M501: BP1.9733	++	++	+++	+++	n.d.	> 10000
M242: BP1.3484	+++	n.d.	+++	n.d.	+++	> 10000
M311: BP1.2526	++	+++	++	+/-	+/-	10.1 ± 1.2
M299: BP1.8186	++	n.d.	++	++	++	> 10000
M327: BP2.951	++	n.d.	++	+++	+	> 1000
M311: BP2.941	n.d.	+	+	+	+/-	552 ± 38
M311: BP2.927	n.d.	+	+	+	+/-	114 ± 17
M311: BP2.928	n.d.	n.d.	n.d.	+/-	+/-	714 ± 91
M309: BP1.2525	+/-	++	n.d.	n.d.	n.d.	38 ± 8

n.d. = not determined
+++ Major
++ Moderate
+ Minor

[Modified from NDA211150, Pharmacokinetic Written Summary; pages 89, 90]

Figure 1. Possible Biotransformation Pathway in Humans



[Modified from NDA211150, Pharmacokinetic Written Summary; page 70]

Table 13. Serum Levels and Safety Margins for Prominent Human Metabolites in Monkey, Rat and Rabbit

Metabolite	Human Adult 40 mg		Monkey NOAEL 12 mg/kg			Rat NOAEL 30 mg/kg			Rabbit 16 mg/kg		
	Cmax (ng/mL)	AUC (ng*h/ml)	Cmax (ng/mL)	AUC (ng*h/ml)	Margin[$] (mk/hu)	Cmax (ng/mL)	AUC (ng*h/ml)	Margin[$] (rt/hu)	Cmax (ng/mL)	AUC (ng*h/ml)	Margin[$] (rab/hu)
BP1.3473	40.5	195	953	4219	**21.6**	137	847	**4.3**	541.5	7310	**37.5**
BP1.3484	42.9	739	8188	100357	**136**	156.7	872	**1.2**	1990	29838	**40.2**
BP1.9733	11.9	76	733.3	2851	**38**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[$] Safety margins based on AUC exposures
Source: Study nos. R-B452-2-649 and R-B389-9733

[Modified from NDA211150, Study Reports R-B452-2649; pages 28-30 and R-B389-9733; page 38-39]

Excretion

In both the human and monkey mass balance studies, the main route of excretion for pitolisant and its metabolites after oral administration was through urine with less than 2.5 and 5% of radioactivity being recovered in feces, respectively. In contrast, both urinary and biliary elimination occurred with roughly equal amounts or radioactivity being recovery in urine (49.1) and feces (44.9).

Drug-Drug Interaction

Effects of Pitolisant on Transporters

The substrate and inhibitor potential of pitolisant for drug transporters was evaluated, *in vitro,* with cell lines (MDR1-MDCK,BCRP-MDCK, and HEK293) expressing the following human transporters: P-gp, BCRP, OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, MATE1 and MATE2K (Study no. 14BIOPP1, non-GLP). Pitolisant was not a substrate of P-gp, BCRP, OATP1B1, OATP1B3, or OCT1. Pitolisant was not a significant inhibitor of P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 or MATE2K at the tested concentrations. Pitolisant was an inhibitor of OCT1 (IC_{50} of 0.795 µM), suggesting a potential Drug-Drug interaction with OCT1 substrates.

Effects of Pitolisant on CYP Enzymes

Multiple *in vitro* drug-drug interaction studies were conducted to evaluate the potential for pitolisant and its major metabolites to induce or inhibit CYP450 (CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2E1 and CYP3A4) and uridyl glucuronosyl transferase (UGT 1A1, 1A4, 1A6, 1A9, and 2B7) enzymes. Overall, pitolisant and its major human metabolites at the lowest concentrations tested, which corresponded to 50-fold the unbound plasma C_{max} at the MRHD of 40 mg pitolisant, were devoid of significant induction of catalytic activity of CYP450 and UGT enzymes tested in isolated human hepatocytes. However, using mRNA analysis, CYP3A4 showed weak induction by pitolisant and BP2.951 at 50-fold C_{max} at MRHD. In a clinical study, repeated administration of pitolisant up to 28 days did not increase levels of 4-β-hydroxycholesterol (Biomarker of CYP3A4 metabolism) indicating no induction of CYP3A4 activity in patients by pitolisant.

In recombinant CYP450 enzymes and isolated human liver microsomes, pitolisant did not significantly inhibit any of the tested CYP450 enzymes except for CYP2D6 (IC_{50} of 2.6 µM). Inhibition of CYP2D6 by pitolisant was not mechanism-based and appeared reversible indicating little too no potential for CYP2D6 inactivation after repeated dosing.

5.2 Toxicokinetics

Study title: 2-Week Toxicokinetic Study by the Oral Route (Gavage) in CB6F1-nonTgrasH2 Wild Type Mice (Study no. (b) (4) 44821 TSS, GLP)

A dedicate toxicokinetic study was conducted in CB6F1-nonTgrasH2 Wild Type Mice to evaluate the serum profile of pitolisant and several of its metabolites (BP2.951, BP1.2525, BP1.2526, BP1.3473, BP1.3484, BP1.8186, and BP10556) after oral administration of pitolisant. CB6F1-nonTgrasH2 Wild Type Mice (4/sex/timepoint for each day) were administered pitolisant once daily by oral gavage for 2-weeks at 75 mg/kg/day. Serum samples were collected for TK analysis at 0.25, 0.5, 1.5, 4, and 24 h on days 1 and 14. No mortality or clinical signs were noted, besides prostration in a single male.

Summary of toxicokinetic parameters for pitolisant its metabolites are listed in the table below (Table 14). Pitolisant was rapidly absorbed with a T_{max} of 0.25 h. Time to maximum exposure to the metabolites ranged from 0.25 to 1.5 h. No accumulation occurred for pitolisant and metabolites BP2.951, BP1.2525 and BP1.3484. Slight

accumulation was noted for metabolites, BP1.3473, BP1.8186, and BP1.10556. No apparent significant sex difference in drug exposure to pitolisant on day 1, however, exposure tended to be higher in females than males on day 14. The major circulating metabolite was BP1.3484, which represented 5.4-fold the parent AUC.

Table 14. Toxicokinetic Parameters for Pitolisant and its Metabolites after Daily Oral administration of Pitolisant for 2-weeks in CB6F1-nonTgrasH2 Wild Type Mice

	Day 1			Day 14			Day 14/Day 1 ratio	
	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng/mL*h)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng/mL*h)	C_{max}	AUC_{0-24h}
BF2.649	**0.25**	**1488**	**6073**	**038**	**1331**	**5980**	**0.89**	**0.98**
BP2.951	0.88	151	645	0.5	237	915	1.57	1.42
BP1.2525	0.5	24	220	0.5	6	9.7	0.24	0.04
BP1.2526	0.5	652	6828	0.5	313	2679	0.48	0.39
BP1.3473	0.25	187	1365	0.38	464	3440	2.48	2.52
BP1.3484	1.5	2875	19878	0.38	5481	32568	1.91	1.64
BP1.8186	0.38	46	192	0.5	144	737	3.20	3.82
BP1.10556	0.25	17	172	2.25	36	388	2.07	2.25

Study title: 6-Month Oral (Gavage) Repeat Dose Toxicokinetic Study in the Rat (Study no. (b) (4) HHL5000, GLP)

The toxicokinetic portion of the pivotal 6-month rat toxicity study was repeated under GLP conditions after an internal audit of the bioanalytical data by the conducting laboratory determined the toxicokinetic component was not GLP compliant. Sprague-Dawley rats (9/sex/group) were administered pitolisant once daily for 6 months at 5, 30 and 60 mg/kg/day. Blood samples for TK analysis were collected on day 1 and during weeks 13 and 26. Similar to the original study, sporadic convulsions were observed in both male and female rats in the HD 60 mg/kg/day group (4 animals of each sex). Additional clinical signs observed in a majority of HD animals included tremors, abnormal gait, agitation, subdued behavior and excessive salivation. Besides a slight increase in salivation in the MD group, no adverse clinical signs were noted in the LD and MD groups. Pitolisant did not affect body weight gain in any dose group.

Reviewer Comment: In contrast to the original study where convulsions were not observed until day 89 of dosing, no delay was observed in the current study with the majority of convulsions occurring prior to day 83. The reason for the delay in the original study was undetermined, however, an expanded brain histopathology did not show any indication of brain lesions being produced by pitolisant exposure.

A summary of toxicokinetic parameters for pitolisant and the major rat metabolite BP1.2526 are listed in the table below (Table 15). Pitolisant and metabolite BP1.2526 were absorbed rapidly with time to maximum serum concentrations (T_{max}) ranging between 0.17 to 0.5 h and 0.33 to 2 h, respectively. Serum levels of pitolisant and BP1.2526 declined with a terminal half-life $T_{1/2}$ ranging from ~2 to 7 h and ~1 to 10 h, respectively. Systemic exposure for both the parent and metabolite tended to increase

greater than dose-proportionally, particularly between the low and mid doses. Following repeated dosing, slight accumulation was noted with serum levels of pitolisant and its metabolites generally increasing over the 6-month duration. There was no apparent significant sex difference in exposure for pitolisant, however, metabolite BP2.951 levels were up to 13-fold higher in female rats compared to males. Although maximum exposure (C_{max}) to the main metabolite BP1.2526 was lower than that of pitolisant, the total exposure (AUC) to the metabolite was generally greater than that of the parent.

Table 15. Toxicokinetic parameters of Pitolisant and Metabolite BP1.2526 following repeated oral administration of Pitolisant in 6-month Rat Toxicokinetic Study

BF2.649 (parent)

Sex	Dose	C_{max} (ng/mL)			AUC_{0-t} (h*ng/mL)		
	(mg/kg/day)	Day 1	Week 13	Week 26	Day 1	Week 13	Week 26
Male	5	48	57	11	29	28	12
	30	428	729	558	236	758	681
	60	547	1140	877	355	2500	2070
Female	5	50	56	33	31	29	21
	30	431	1030	745	339	1100	996
	60	692	961	982	883	2280	3380

BP1.2526 (metabolite)

Sex	Dose	C_{max} (ng/mL)			AUC_{0-t} (h*ng/mL)		
	(mg/kg/day)	Day 1	Week 13	Week 26	Day 1	Week 13	Week 26
Male	5	NCa	91	43	NCa	84	22
	30	251	598	622	552	2160	1990
	60	320	585	558	848	7030	5280
Female	5	30	79	112	6	122	73
	30	451	480	475	376	2500	2540
	60	660	531	504	2210	5870	6830

NCa Not calculated, all serum concentrations were below the lower limit of quantification.

[Modified from NDA211150, Study Report (b) (4) HHL5000; page 12]

Study title: 9-Month Oral (Gavage) Repeat Dose Toxicokinetic Study in the Cynomolgus Monkey (Study no. (b) (4) HHL5001, GLP)

The toxicokinetic portion of the pivotal 9-month monkey toxicity study was repeated under GLP conditions after an internal audit of the bioanalytical data by the conducting laboratory determined the toxicokinetic component was not GLP compliant. Cynomolgus monkeys (3-4/sex/group) were administered pitolisant once daily for 9 months at 5, 12 and 30 mg/kg/day. Blood samples for TK analysis were collected on day 15 and during weeks 21 and 39.

Similar to the original study, isolated convulsions were observed in the HD 30 mg/kg/day group (1 male and 1 female). The single male that displayed convulsions on day 15 also had several instances of tremor and was ultimately prematurely terminated on day 57 due to severe clinical signs and worsening clinical condition including thin appearance and continued weight loss. In the LD and MD groups, no significant adverse clinical signs were noted except for loose/liquid feces. Mean body weight and body weight gain were not significantly affected by pitolisant in any dose group.

A summary of toxicokinetic parameters for pitolisant and the major metabolite BP2.951 are listed in the table below (Table 16). Pitolisant and its metabolites were absorbed rapidly with T_{max} ranging from 1 to 4 hours for parent and 1 to 2 h for BP2.951. Serum levels for pitolisant declined with a $T_{1/2}$ ranging between ~4 and 8 h. Systemic exposure to pitolisant tended to increase greater than dose proportionally for males but in a dose proportional manner in females. BP2.951 increased greater than dose proportionally in both males and females. Systemic exposure to pitolisant and its metabolites tended to decrease over the 9-month duration. There was no apparent significant sex difference in exposure to pitolisant or its metabolites. Serum levels of the metabolite BP2.951 was generally greater than that of the parent.

Table 16. Toxicokinetic parameters of Pitolisant and Metabolite BP2.951 following repeated oral administration of Pitolisant in 9-month Monkey Toxicokinetic Study

BF2.649 (parent)

Sex	Dose level	C_{max} (ng/mL)			AUC_{0-t} (h*ng/mL)		
	(mg/kg/day)	Day 15	Week 21	Week 39	Day 15	Week 21	Week 39
Male	5	24	15	9 82		59	24
	12	299	200	81	1380	942	375
	30	944	262 202		4980	1560	1150
Female	5	118	60	37	367	268	116
	12	289	118	72	1190	578	318
	30	517	244 172		2580	1260	774

BP2.951 (metabolite)

Sex	Dose level	C_{max} (ng/mL)			AUC_{0-t} (h*ng/mL)		
	(mg/kg/day)	Day 15	Week 21	Week 39	Day 15	Week 21	Week 39
Male	5	136	106 143 406 303				367
	12	586	401	405	1850	1230	1190
	30	1470	891	912	5250	3280	3690
Female	5	210	138 153 494 428				410
	12	630	303	385	1740 950		1120
	30	2390	1020 1280 7070 3770				4080

[Modified from NDA 211150, Study Report (b) (4) HHL5001; page 19]

6 General Toxicology

6.1 Single-Dose Toxicity

6.1.1 Single-Dose Studies in the Mouse

Single-dose toxicity studies by both the oral (Study no. T-264902, GLP) and i.v. route (Study no. T-264904, GLP) were carried out in Swiss OF1 mice.

Pitolisant was administered to Swiss mice (5/sex/dose) by oral gavage at 50 and 500 mg/kg. At 500 mg/kg, all but 1 animal died following severe clinical signs which occurred soon after administration and included seizure and prostration. The remaining HD female was found dead the day after drug administration. Necropsy of the HD animals

revealed only pulmonary hyperemia and one instance of internal autolysis in the female found dead. No mortalities or clinical signs were noted in the LD group.

In the preliminary i.v. study, Swiss mice (n=2/sex/dose) were administered pitolisant at 15, 20, and 30 mg/kg by tail vein injection. Mortality occurred at all doses with death of 3 of 4 animals in both the MD and HD groups and 1 of 4 in the LD group. In the definitive study, Swiss mice (n=10/dose) were administered pitolisant at 5 and 10 mg/kg. Death of 1 male occurred in the 10 mg/kg group. Clinical signs observed in males in the 10 mg/kg group included dyspnea, tachycardia and prostration. No clinical signs were noted in the 5 mg/kg male group or in females in either dose group. A slight decrease in mean body weight in LD males and in both sexes at the HD occurred 24-48 h post-dose, rebounding by 72 h and continued to increase throughout the observation period of 14 days. Gross pathology revealed no specific organ toxicities and the cause of death in both the preliminary and definitive studies was undetermined; however, it was speculated that deaths were related to disruption of respiratory function due to drug-related CNS effects.

6.1.2 Single-Dose Studies in the Rat

A single-dose toxicity study (Study no. T-264901, GLP) was done in Sprague-Dawley rats (5/sex/group) with administration of 50 and 500 mg/kg Pitolisant by oral gavage followed by a 14-day observation period. At the LD, no mortality, adverse clinical signs, or body weight effects were noted. At the HD, 3 out 5 females died. No cause of death was determined but mortality was attributed to test article. All 3 animals had pulmonary hyperemia but no other findings in gross pathology. Clinical signs included prostration in all males and 2 out 5 females. Rare occurrences of excessive salivation, motor incoordination and stereotypy behavior also occurred. A loss in body weight occurred 24 h post-dose (-7% M, -8% F). Body weight began to rebound at 48 h post-dose and continued to climb for the remainder of the study observation surpassing pre-dose body weight at 1-week post dosing.

A single dose toxicity study (Study no. T-264903, GLP) was done in Sprague-Dawley rats (range-finding 2/sex/group; definitive 5/sex/group) with i.v. administration of pitolisant at 10, 12, 15, 20 and 30 mg/kg. Death occurred at 30 (2/4), 20 (3/4), and 15 mg/kg (7/10) with clinical signs including prostration, trembling, tachycardia, excessive salivation, vocalization and seizures. No mortalities occurred at 12 and 10 mg/kg, however, similar clinical signs were observed to those in the higher dose groups, including seizures. No macroscopic findings were noted in any dose group.

6.2 Repeat-Dose Toxicity

6.2.1 Repeat Dose Studies in The Mouse

Study title: 4-Week Toxicity Study by the Oral Route (Gavage) in CB6F1-nonTgrasH2 Wild Type Mice

Study no.:	(b) (4) 36113 TSS
Study report location:	EDR, SDN 2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	09/10/2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BF-2649 (Pitolisant), Batch no. GPR01, 99.96%

Key Study Findings

- Hypoactivity occurred in all male and female mice in the MD and HD groups. Staggering gait occurred in MD and HD males and HD females. In the HD males, convulsions were observed in 5 out 10 mice.
- No effects on body weight, food consumption or hematology were noted.
- Significant increases in relative liver weights in MD and HD males and females ranging from 5-12% with correlated centrilobular hypertrophy.
- Based on minimal finding at the MD the **NOAEL is 75 mg/kg/day**. At the end of the 4-week study, the NOAEL dose corresponds to a maximum exposure (C_{max}) of 1705.15 and 1591.7 ng/mL, and a total exposure (AUC_{0-24h}) level of 8855.69 and 5535.41 ng*h/mL in males and females, respectively.

Methods

Doses:	30 (LD), 75 (MD), and100 (HD) mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral Gavage
Dose volume:	10 mL/kg (13.3 mL/kg for groups 3/7 and 4/8)
Formulation/Vehicle:	Suspension in RO purified water
Species/Strain:	Mouse / CB6F1-nonTgrasH2 from (b) (4)
Number/Sex/Group:	10/Sex/group
Age:	Approximately 10 weeks of age at commencement of dosing
Weight:	24.9-31.2 g Males; 19.7-22.4 g Females
Satellite groups:	Toxicokinetic satellite groups: 20/Sex/group for LD, MD, HD; 4/sex/group for controls
Unique study design:	Standard study design
Deviation from study protocol:	No study deviations noted

Observations and Results

Mortality
One animal was found dead in the 75 mg/kg satellite group on day 4 of dosing. Black discoloration foci were noted in the frontal lobe of the brain upon necropsy. Due to the death being a single incidence in the intermediate group it was deemed unlikely to be drug related.

Clinical Signs
Hypoactivity was the major clinical sign noted, affecting all animals in the intermediate and high dose groups. At the HD, additional clinical signs included clonic convulsions in in males (5/10) and staggering gait in both males (10/10) and females (5/10). Staggering gait was also noted in MD males (3/10). No clinical signs were noted for the LD group.

Body Weights
No significant changes in either body weight were noted.

Feed Consumption
No significant changes in food consumption were noted.

Hematology
No toxicologically significant changes in hematology parameters were noted. A full battery of hematology parameters was analyzed on blood samples collected from 5 animals from each group.

Clinical Chemistry
Significant increases in both cholesterol and triglycerides were noted; however, the changes were of low magnitude and a clear dose-response was not established. An adequate battery of clinical chemistry parameters was evaluated for 5 animals from all groups (separate 5 from those used for hematology).

Urinalysis
Urinalysis was not conducted in this study

Gross Pathology
Black foci in the frontal lobe of the brain of 1 male found dead in the 75 MD satellite group. No other drug-related macroscopic finds were noted for any dose group.
Organ Weights
Slight increases in liver weights occurred in the MD and HD groups.

Histopathology
Microscopic examination of a comprehensive battery of organs and tissues was performed on all control and HD animals. Livers and testes were also examined from the LD and MD males.

Adequate Battery
Yes

Peer Review

Yes; a peer review was conducted for at least 20% of slides from control and HD animals and for any identified target organs.

Histological Findings

Centrilobular hypertrophy was observed in males in the MD and HD groups which correlated with increased liver weights. Additional microscopic changes included increased incidence and severity of pale basophilic granular bodies in the tubular lumens of the testes of MD and HD males with unknown toxicological significance.

Special Evaluation

No special evaluations were conducted.

Toxicokinetics

TK parameters for Pitolisant on day 1 and at the end of the 4-week study are listed below (Table 17). After oral exposure, Pitolisant was rapidly absorbed (T_{max} = 0.25) and plasma levels increased dose proportionally. The major metabolite was BP1.2526.

Results:

Table 17. Toxicokinetic parameters following oral administration of BF2.649 in CB6F1-nonTgrasH2 Wild Type Mice at weeks 1 and 4.

Sampling period	Kinetic parameter	Dose-level BF2.649 (mg/kg/day) and sex					
		30		75		100	
		Male	Female	Male	Female	Male	Female
Week 1	C_{max} (ng/mL)	897.35	960.75	2252.15	1909.35	2470.5	4081.65
	t_{max} (h)	0.25	0.25	0.25	0.50	0.50	0.25
	$AUC_{(0-t)}$	4055.14	1308.27	8428.68	7630.27	15674.80	12656.89
Week 4	C_{max} (ng/mL)	981.25	963.45	1705.15	1744.5	2295.4	1591.7
	t_{max} (h)	0.25	0.25	0.50	0.25	0.50	0.25
	$AUC_{(0-t)}$	4243.79	6158.39	8855.69	5535.41	9675.81	8604.93

[Excerpted from NDA211150, Study Report (b) (4) 36113; page 37]

Dosing Solution Analysis

All dosing formulations were within ±15% of the target concentration value.

6.2.2 Repeat Dose Studies in The Rat

Study title: BF-2649 Repeated Dose Range Finding Toxicity Study in Rats (7days, Study no. T-264906, GLP)
Sprague-Dawley rats (5/sex/dose) were administered Pitolisant at 5, 35, 150, and 250 mg/kg by oral gavage once daily for 7 days. At the high dose (HD) of 250 mg/kg/day, 9 out 10 animals died between days 3 and 6. Clinical signs included, labored breathing and salivation in a majority of animals with a lower incidence of prostration, tremor and lethargy. Clinical signs were first observed within 1-hour post-dose and started on day 3 of dosing. No clinical signs were observed at the lower doses of Pitolisant. Mean body weight gain was reduced in all doses, however, the change was only statistically significant in males at 150 mg/kg/day. The MTD is 150 mg/kg.

Study title: 11-Day Repeat Oral (Gavage) Study of BF2.649 with Toxicokinetic Blood/Brain Sampling in Rats (Study no. R-B126-BF2.649, Non-GLP)
Sprague-Dawley rats (8/sex/timepoint) were administered Pitolisant at 60 mg/kg either as a single oral dose or once daily for 11 days. Blood and brain samples were collected at 15, 30, 60 min and 3, 8 and 24 h for PK analysis. No test-article related deaths occurred. One animal died shortly after dosing as a result of the dosing procedure. No clinical signs were noted in a majority of animals with either the single or repeated administration of Pitolisant. On day 11, a single animal was observed prostrated 15 min post-dose and recovered by 60 min. Serum levels of Pitolisant increased between day 1 and 11 with a ratio of 3.8 and 3.3 for C_{max} and AUC, respectively. Similarly, levels of Pitolisant in the brain were also increased after repeated dosing with C_{max} and AUC ratios of 2.5 and 4, respectively. The major metabolite, BP1.2526, also showed a slight increase in both serum and brain levels with a ratio of ~ 1.6 between day1 and 11. Metabolite BP2.951 showed slight accumulation in serum, however, it was below the limit of quantitation (BLOQ) in brain. Serum and brain levels of metabolite BP1.2525 were relatively consistent between single and repeated administrations. Regardless of dosing frequency, the parent was BLOQ in the serum by 24 h timepoint but still detectable in brain. All metabolites were BLOQ by 24 h in both serum and brain. **Due to only limited clinical signs, 60 mg/kg/day was considered the NOAEL**. At day11, this dose corresponded to the following exposure levels of Pitolisant in serum and brain, respectively; C_{max} is 312.46, 9667.99 ng/mL and $AUC_{0\text{-}24h}$ is 1070.3, 45732.1 ng*h/ mL. The exposure levels of the main metabolite, BP1.2526, in serum and brain were; C_{max} is 882.91, 11953 ng/mL and $AUC_{0\text{-}24h}$ is 11848.4, 174312.5 ng*h/mL, respectively.

Study title: BF-2649 – 13-Week Repeated-Dose Toxicity Study P.O. Rats (Study no. T-264907, GLP)
Sprague-Dawley rats (10/sex/dose; 5/sex/dose for TK) were administered pitolisant at 5, 30, and 150/100/75 mg/kg by oral gavage once daily for 13 weeks. Due to mortality, the HD group at 150 mg/kg dose was reduced to 100 mg/kg/day and an additional HD recovery group was added at this dose. The dose in HD and HD recovery groups were subsequently reduced again to 75 mg/kg/day. Several animals were found dead; 1/sex each at 100 and 150 mg/kg/day; and 1 M at 75 mg/kg/day. No cause of death could be determined in these animals, but possible drug-related toxicity could not be ruled out. Several clinical signs were noted in the HD group many of which occurred infrequently in a small number of animals. A comparison of reported clinical signs between the HD

group and the separate HD recovery group (Did not receive doses at 150 mg/kg) showed a reduction in the types of clinical signs and their frequency indicating possible sensitization due to the exposure at 150 mg/kg. No clinical signs were noted in the lower dose groups aside from single animal occurrences. Some clinical signs of note in the HD group are; abundant yellowish feces which occurred in most of the animals, aggressive behavior in males and excitation in females which did not resolve by the end of the study, and uncontrolled movements in 4 females and 3 males. Uncontrolled movements of the head and fore paws accompanied by jumps were noted usually 30 min after dosing. Reduction in the HD to 75 mg/kg temporarily resolved this finding (7 days) but eventually the behavior returned in affected animals as well as appeared in additional animals. There was a significant reduction (~14%) in body weight gain in males of the HD recovery group when compared to the recovery control group. During recovery, body weight gain exceeded that of corresponding controls. Females in the HD recovery group gained more weight compared to corresponding controls. This effect reversed during recovery phase. Food consumption was only reduced at the intermediate time point (7 weeks). No significant test-article related changes in hematology or urinalysis parameters were noted. Significant elevation in AST and ALT values was noted in the HD males, however, a single animal accounted for the majority of the effect. HD recovery males showed no drug-related effects on AST or ALT. All other clinical chemistry parameters were within normal ranges. Changes in liver enzymes could be related to the dose-related increase in liver weights in HD males. By the end of recovery phase, liver weights returned to control levels. Although not statistically significant, a 15% reduction in heart weight in HD males was recorded. No correlated histological changes were noted but moderate accumulation of Pitolisant in the heart has been reported. Generally, no test-article related macro- or microscopic changes were noted in all dose groups aside from sporadic and common findings in animals of this species and age. However, in the HD animals that died, hemorrhagic and edematous areas within the lung were noted. Additionally, although histopathological changes in lung, including lipoid pneumonia are a common finding, an increased incidence and severity were noted in the HD males and females. **The NOAEL dose = 30 mg/kg/day**, with corresponding serum levels of pitolisant at the end of the 13-week study; C_{max} is 140.9 ± 121.7 ng/mL and $AUC_{0\text{-}24h}$ is 222.6 ± 179 ng*h/mL.

Study title: 6 Month Oral Repeat Dose Toxicity Study in the Rat with a 4 Week Recovery Period

Study no.:	(b) (4) HHL1006
Study report location:	EDR, SDN 2
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	05/31/2005
GLP compliance:	Yes, Partial (TK non-GLP)
QA statement:	Yes
Drug, lot #, and % purity:	Pitolisant (BF-2649), Batch no. GP001, 99.95%

Key Study Findings

- Severe clinical signs including convulsions occurred in both sexes in the HD group after the dose was escalated from 75 to 100 mg/kg. After the dose was reverted back, convulsions continued to occur sporadically in several animals and the HD group was prematurely sacrificed on Day 59 of dosing.
- A new HD group was established at 60 mg/kg/day. Minimal clinical signs were noted (salivation and noisy breathing) up to Day 61. On day 89 through the end of dosing, convulsions and tremors were noted in a number of animals of both sex.
- Mean time to onset of convulsions was 32 and 39 min post-dose for males and females, respectively. No further convulsions were observed by 2 to 3 hours after dosing.
- Due to excessive clinical signs at the HD of 60 mg/kg/day, the NOAEL is considered to be 30 mg/kg/day. The corresponding exposure levels, at week 26, are 274 and 338 ng/mL for C_{max} and 392 and 489 ng*h/mL for AUC_{0-24h} in males and females, respectively.

Methods

Doses:	5, 30/40, 60, 75/100 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral Gavage
Dose volume:	10 mL/kg (13.3 mL/kg for groups 3/7 and 4/8)
Formulation/Vehicle:	Suspension in RO purified water
Species/Strain:	Rat / Sprague-Dawley from (b) (4)
Number/Sex/Group:	25/Sex Control and High dose groups and 20/Sex for Mid and Low dose groups; 5/Sex of Control and High Dose (60 mg/kg) Recovery group
Age:	Approximately 7 weeks of age at commencement of dosing
Weight:	189-264 g Males; 138-191 g Females
Satellite groups:	Toxicokinetic satellite groups: 12/Sex/dose
Unique study design:	Standard study design
Deviation from study protocol:	No deviations were noted in original study report. An internal investigation conducted after the final study report was released uncovered anomalies in the bioanalytical data. A study report amendment indicated samples were re-processed and the new data are believed to not affect toxicokinetic parameters by more than 5%. Therefore, the original toxicokinetic analysis was not adjusted.

Observations and Results

Mortality

All animals in the initial HD group of 75 (100) mg/kg/day were prematurely sacrificed on day 59 (males) and day 62 (females) due to the severity of clinical signs and poor animal health. The recovery animals of the concurrent control group were also sacrificed. In the new HD group of 60 mg/kg/day a total of 4 animals (2 males and 2 females) were prematurely sacrificed on days 119, 162, 153, and 155 due to excessive clinical signs. A total of 5 animals (1 control, 3 MD, 1 HD) were also prematurely sacrificed due to procedural related issues.

Clinical Signs

Due to a lack of clinical signs in the initial HD group of 75 mg/kg/day, doses were increased for MD (30 to 40 mg/kg) and HD (75 to 100 mg/kg) between days 29 and 33. After the dose increase, adverse clinical signs including convulsions, labored breathing, abnormal gait and subdued behavior were noted for several males in the HD group. Abnormal gait and subdued behavior were also noted in a few females in the HD group with 2 females having a single instance of convulsions on day 32. Distended abdomen was noted for some males and up to 17 females. The doses were reverted to the

original levels on day 49 with a 7-day wash-out period of no dosing. During that period no clinical signs were noted. Upon resuming dosing, at least one incidence of convulsion was recorded in 13/25 male and 7/25 female animals until animals were prematurely sacrificed on day 59 due to severe clinical signs and moribund condition. Additional clinical signs included salivation and abnormal gait.

In the new HD group of 60 mg/kg/day, at least one incidence of convulsions were noted in 18 males and 15 females from day 89 occurring sporadically throughout the study period. Sporadic occurrences of tremor were also noted for a majority of females (23/25) and males (16/25). Additional clinical signs included salivation, protruding eyes, and noisy breathing. The mean time to convulsions was 39 min in males and 32 min in females and mean time to absence of convulsions was after 1 h 43 min for males and 1 h 30 min for females post-dose. In the MD group occasional increased salivation was the only clinical sign noted. No clinical signs were noted in the LD group. No clinical signs were noted during the recovery phase in any dose group.

Body Weights
A decrease in body weight gain occurred in the HD (75/100 mg/kg) females, which is likely due to the deteriorating condition of these animals, however, such decrease did not occur in males. These body weight changes did not correlate to effect on food intake. Body weight gain in the 60 mg/kg/day group increased compared to controls. No change in bodyweight gain was noted in the MD or LD groups.

Feed Consumption
A slight increase in food consumption in the 60 mg/kg/day was noted which correlates with the increase in body weight gain noted in the males. All other groups showed no effect on food consumption.

Ophthalmoscopy
No drug-related findings. Ophthalmoscope examination of all animals occurred prior to the start of treatment and at weeks 12/13 and 25/26.

ECG
Not conducted

Hematology
A decrease in white blood cells (lymphocytes) was noted in females in the 75/100 mg/kg/day group at week 9. An increase in prothrombin time (PT) in males and females at week 14 and a decrease in activated partial thromboplastin time (APTT) in males at week 14 and week 26 were noted in the 60 mg/kg/day group. In the LD (males) and MD (males and females) group a slight increase in reticulocytes at week 26. All other significant changes were within the historical control range and were deemed not toxicologically significant. A full battery of hematology parameters was analyzed on blood samples collected from control and HD groups at weeks 13/14 and 26 and at the end of the recovery period.

Clinical Chemistry
All changes were considered not toxicologically significant due to a lack of dose relationship, inconsistency between sexes and falling within historical control ranges. An adequate battery of clinical chemistry parameters was evaluated for all control and HD group animals.

Urinalysis
No drug-related findings. Urine samples were collected from 10 animals/sex/group, in control and HD groups, for analysis at weeks 13/14 and 26 and at the end of the recovery period. An adequate battery of urinalysis parameters was evaluated.

Gross Pathology
Pale foci in the lungs of 60 mg/kg/day females were noted. A full necropsy was performed on all study animals including premature decedents.

Organ Weights
In males of the 75/100 mg/kg/day group prostate weights were decreased and liver weights were increased and In females, adrenals and kidney weights were increased. In the 60 mg/kg/day group, relative liver, kidney and adrenals weights were increased in both sexes. Absolute and relative adrenal weight was increased compared to controls in the MD group. The following organs were weighed: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, salivary glands, spleen, testes / epididymides, thymus gland, thyroids / parathyroid, and uterus.

Histopathology
Microscopic examination of a comprehensive battery of organs and tissues was performed on all unscheduled decedents, controls, and HD group animals. Further examination of LD and MD groups were conducted once findings were identified in the HD group.

Adequate Battery
Yes

Peer Review
Yes

Histological Findings
Target organs of toxicity included liver, kidney, adrenals, and lung. In the 60 mg/kg/day dose group, a small increase of minimal to slight nephropathy was noted in both males and females. Additionally, females (6/18) displayed diffuse cortical hypertrophy in the adrenal glands, which persisted in 2/5 animals after the recovery period. This finding was not present in the LD and MD groups or HD males. Alterations in the mucosal layer of the duodenum were noted in both males (7/18) and females (9/17) in the 60 mg/kg/day dose group. A dose-related slight hepatocellular alteration (cellular basophilia) was observed in 30 mg/kg/day (6/19 Males, 3/20 Females) and 60 mg/kg/day (15/17 Males, 13/18 Females) groups. Scattered vacuolation in the liver was

also noted in both males (7/17) and females (4/19), in the 60 mg/kg/day group. Increased incidence and severity of focal alveolar macrophages in the lungs was noted in females (15/18) at 60 mg/kg/day. This finding correlated with the pale foci noted in gross pathology and remained in all recovery animals. Males displayed a slight increase in incidence but was minimal in severity.

Special Evaluation

An expanded brain histopathology in animals dosed 60 mg/kg/day using specific neuronal and glial stains was conducted due to convulsions and CNS signs (Study no. R-BF2.649-TROTTIER-001, non-GLP). Sections from three brain areas including the frontal cortex, cerebral cortex with hippocampus, and cerebellum with medulla oblongata were stained with cresyl-violet, Fluoro-Jade, and for glial fibrillary acid protein (GFAP). Rats that underwent experimental cerebral ischemia were used as a positive control for the neurodegeneration indicator, Fluoro-Jade. Results from all three special stains did not demonstrate any histopathological findings in the sampled brain regions of pitolisant dosed animals indicating no evidence of treatment-related neurodegeneration or neuroinflammation.

Toxicokinetics

Blood samples were collected from satellite animals during weeks 13 and 26 of the study. After repeated exposure to Pitolisant, plasma exposure levels increased greater than dose-proportional especially between 5 and 30 mg/kg/day doses. Maximum plasma exposure occurred between 0.167 to 0.5 h post-dose and declined thereafter with a terminal half-life ($T_{1/2}$) of 1.15 to 7.33 h post-dose. No apparent sex difference in plasma exposure was recorded. After repeated dosing between 13 and 26 weeks, there appeared to be a slight accumulation. See Table 18 for a summary of TK parameters.

Reviewer Comment*: After release of the final study report, an internal investigation by the conducting laboratory uncovered anomalies in the bioanalytical data. A submitted study report amendment indicated samples were re-processed and the new data are believed to not affect toxicokinetic parameters by more than 5%. Therefore, the original toxicokinetic analysis was not adjusted, however, the TK portion of the study was deemed non-GLP compliant. A subsequent GLP 6-month TK study* (Study no. (b) (4) HHL5000, GLP) *in rats was conducted. The review of that study can be found under* ***Toxicokinetics in Section 5*** *of this NDA review.*

Table 18. Toxicokinetic parameters of Pitolisant at weeks 13 and 26 for male and female rats following repeated oral administration of Pitolisant at 5, 30, and 60 mg/kg/day

Week 13

Males

Dose (mg/kg/day)	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (hr*ng/mL)	λ_z (1/hr)	$t_{1/2}$ (hr)
5	7.44	0.333	6.32	NC+	NC+
30	312	0.167	250	0.179	3.88
60	778	0.333	1350	0.0945	7.33

Females

Dose (mg/kg/day)	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (hr*ng/mL)	λ_z (1/hr)	$t_{1/2}$ (hr)
5	12.3	0.167	9.34	NC+	NC+
30	335	0.167	330	NC+	NC+
60	976	0.333	1400	NC+	NC+

Week 26

Males

Dose (mg/kg/day)	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (hr*ng/mL)	λ_z (1/hr)	$t_{1/2}$ (hr)
5	34.5	0.167	16.9	0.603	1.15
30	274	0.500	392	0.0994	6.97
60	673	0.333	1520	0.152	4.56

Females

Dose (mg/kg/day)	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (hr*ng/mL)	λ_z (1/hr)	$t_{1/2}$ (hr)
5	11.8	0.500	14.1	NC+	NC+
30	338	0.333	489	0.247	2.80
60	789	0.167	2000	0.152	4.55

[Modified from NDA211150, Study Report (b) (4) HHL1006; pages 671 and 672]

Dosing Solution Analysis
All dosing formulations were within ±10% of the target concentration value.

6.2.3 Repeat Dose Studies in the Dog

Study title: 14-Day Dose-Range-Finding Study in Dogs and Monkeys; Oral Administration (Study no. CD01/7766T, GLP)

A multiple ascending-dose study was carried out in a single beagle dog and Cynomolgus monkeys to determine appropriate doses for the nonrodent repeat dose toxicity studies. In the dog, Pitolisant was administered once daily at 15, 30 and 60 mg/kg by oral gavage. The low and mid dose were administered each for 7 days. The high dose was only administered for 1 day. At the high dose of 60 mg/kg/day, the single animal was euthanized due to excessive clinical signs including clonic and tonic convulsions, inability to stand, ataxia and head swaying. Clinical signs first appeared 17 min after dosing. By 3.5 h after dosing, some clinical signs remained including slight ataxia, head movements and tremors. Emesis was noted at all doses. At 30 mg/kg/day, excitement and rigidity was noted 1 h post-dose and resolved by 2.5 h post-dose. A single monkey was administered 15, 30, 60 and 80 mg/kg once daily by oral gavage. The first 3 dose levels were administered for 7 days each and the high dose of 80 mg/kg was administered for 4 days. At the high dose, the animal was euthanized after 4 days of dosing due to clonic convulsions. Emesis occurred after each of the 4 doses of 80 mg/kg. No effects on body weight were evident and the only drug related changes noted were increased heart, liver and adrenal gland weights and microscopic findings in the gastric mucosa. A group of 3 monkeys were then administered 40 mg/kg/day for 29 days. At this dose, emesis and tremors occurred in all animals. A single female monkey had 2 instances of clonic convulsions (Day 6 and 24) and sporadic motor incoordination. A separate female had a single instance of slight head movements. Other drug related effects included a decrease in erythrocyte, hemoglobin and hematocrit levels and slightly increased serum glucose and ALT/GOT levels. Increased adrenal weights were noted in a single female. The monkey was selected as the non-rodent species for

further nonclinical testing. Due to the presence of convulsions at 40 mg/kg, the MTD was determined to be 30 mg/kg for the monkey.

6.2.4 Repeat Dose Studies in the Monkey

Study title: 13-Week Oral Toxicity Study P.O. in Cynomolgus Monkeys with a 4-Week Recovery Period

Study no.:	CD01/7989T
Study report location:	EDR, SDN 2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	02/13/2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BF-2649 (Pitolisant), Batch: PAN201BLLINF, 98.8%

Key Study Findings

- No drug-related mortalities or premature termination of animals occurred
- Tremors occurred in 4/6 males and 1/6 females at the HD of 30 mg/kg/day through week 3 in males and week 5 in the single female
- Due to only rare occurrence of emesis, the NOAEL is considered to be 12 mg/kg/day. The corresponding exposure levels, at week 13, are 204 ng/mL for C_{max} and 932 ng*h/mL for AUC_{0-24h}.
- MTD was 30 mg/kg/day

Methods

Doses:	5, 12, and 30 mg/kg
Frequency of dosing:	Once Daily
Route of administration:	Oral Gavage
Dose volume:	4 mL/kg
Formulation/Vehicle:	Suspension in distilled water
Species/Strain:	Monkey/Cynomolgus
Number/Sex/Group:	4/sex/dose
Age:	23-27 months old
Weight:	M (2.60-2.70 kg); F (2.15-2.28 kg)
Satellite groups:	Recovery Animals; 2/sex/dose for control and 30 mg/kg/day groups
Unique study design:	Standard study design
Deviation from study protocol:	No significant protocol deviations were noted that would impact study integrity.

Observations and Results

Mortality
All animals survived to scheduled termination.

Clinical Signs
Tremors were noted in 4 males at the HD of 30 mg/kg/day with 2 animals having tremors only during the first week of dosing and the remaining 2 displayed tremors up to 3 weeks of dosing and one of these animals also had one instance of clonic convulsions after the second administration. One female in the HD group also exhibited tremors lasting up to 5 weeks of dosing. Additional clinical signs in the HD group included occasional emesis and increased salivation. No signs of tremors or convulsions occurred in the MD or LD groups. Rare instances of emesis occurred in MD group. No test-article related clinical signs were note in the LD group animals.

Body Weights
No changes in body-weight gain were noted.

Feed Consumption
Food intake was not recorded.

Ophthalmoscopy
No ocular changes were noted. Ophthalmoscope examination of all animals occurred prior to the start of treatment and at weeks 6 and 13 and end of the recovery period.

ECG
No adverse test-article related changes in cardiovascular parameters were noted. ECG recording were performed before and 45 min after administration on days 42/86 for males and 43/87 for females and at the end of recovery period. The 45 min timepoint was selected to minimize the consequences of possible excessive CNS effects including tremors and convulsions which occurred usually between 1 and 2 hours post-dose. However, it may be possible that exposures did not reach maximal levels by 45 min since toxicokinetic analysis identified a T_{max} between 1 and 4 hours.

Hematology
An increase in segmented neutrophils occurred at the interim 6-week sampling in the LD and MD, reaching significance only in the MD group. No change in the HD group occurred. No difference was observed at the 13-week timepoint. During recovery, a significant increase in eosinophil counts was noted in the HD group. None of these changes were of toxicological significance. A full battery of hematology parameters was analyzed on blood samples collected prior to start of treatment, weeks 6 and 13 and at the end of the recovery period.

Clinical Chemistry
Decreased inorganic phosphate was the only significant change in blood chemistry noted, however, the change was minimal in magnitude and did not show dose-response. An adequate battery of clinical chemistry parameters was evaluated.

Urinalysis
No test-article related changes were noted. Urine samples were collected for analysis prior to the start of treatment and at weeks 5 and 12 and at the end of the recovery period. An adequate battery of urinalysis parameters was evaluated.

Gross Pathology
No test-article related adverse changes were noted. A full necropsy was performed on all study animals.

Organ Weights
No significant changes in organ weights were noted. The following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pancreas, pituitary, prostate, salivary glands, seminal vesicles, spleen, testes / epididymides, thymus gland, thyroids / parathyroid, and uterus.

Histopathology
Microscopic examination of a comprehensive battery of organs and tissues was performed on all unscheduled decedents, controls, and HD group animals. Further examination of LD and MD groups were conducted once findings were identified in the HD group.

Adequate Battery
Yes

Peer Review
Yes

Histological Findings
No test-article related histological changes were noted.

Special Evaluation
No special evaluations were conducted.

Toxicokinetics
After oral administration of Pitolisant, rapid absorption occurred with a T_{max} of 1 – 4 h. The terminal half-life ($T_{1/2}$) ranged from approximately 3 to 5 hours (Table 19). Systemic exposure increased greater than dose proportional. There was no significant difference in systemic exposures between males and females. No apparent accumulation occurred over the 13-week study. Plasma levels of BF-2649 were measured on days 1, 50, and 91 immediately before dosing and at 0.5, 1, 4, 8, 12, and 24 hours post-treatment.

Table 19. Pitolisant Toxicokinetic parameters during 13-Week Chronic Treatment in Monkeys

Parameter	Day 1	Day 50	Day 91	Average (± SEM)
Dose 5 mg/kg/day PO N = 8				
AUC0-24h (ng/mL*h)	78.3	74.9	81.0	78.1 ± 29.0
C_{max} (ng/mL) ± SEM	23.9 ± 8.7	14.5 ± 5.4	18.7 ± 6.4	19.0 ± 6.6
T_{max} (h)	0.9	1.8	2.8	1.8 ± 0.3
T½ (h)	2.5	3.6	2.7	2.9 ± 0.5
Dose 12 mg/kg/day PO N = 8				
AUC (ng/mL*h)	848	852	932	877 ± 146
C_{max} (ng/mL) ± SEM	213 ± 41	184 ± 42	204 ± 38	200 ± 36
T_{max} (h)	1.7	1.8	2.1	1.9 ± 0.5
T½ (h)	3.7	5.2	5.0	4.7 ± 0.2
Dose 30 mg/kg/day PO N = 12				
AUC (ng/mL*h)	2914	2042	2057	2337 ± 444
C_{max} (ng/mL) ± SEM	530 ± 87	328 ± 58	339 ± 50	399 ± 58
T_{max} (h)	1.2	3.3	3.5	2.7 ± 0.3
T½ (h)	4.6	5.3	4.8	4.9 ± 0.3

Average values for each parameter are calculated from the N values obtained at D1, D50 and D91.
Source (b) (4) CD01/7989T, Appendix IX, Toxicokinetic Parameters Section

[Excerpted from NDA211150, Toxicology Written Summary; page 38]

Dosing Solution Analysis

All dosing formulations were within ±10% of the target concentration value.

Study title: 9 Month Oral (Gavage) Repeat Dose Toxicity Study in the Cynomolgus Monkey with a 4 Week Recovery Period

Study no.:	(b) (4) HHL1005
Study report location:	EDR, SDN 2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	05/17/2005
GLP compliance:	Yes, Partial (TK non-GLP)
QA statement:	Yes
Drug, lot #, and % purity:	Pitolisant, Batch GPR01, 99.5%

Key Study Findings

- No drug-related mortalities or premature termination of animals were noted.
- Sporadic rare incidences of convulsions were noted in 3 males and 1 female at the high dose of 30 mg/kg/day. Convulsions were also associated with occasions of tremors, lateral recumbency and unsteady gait.
- Toxicokinetic analysis indicate that animals that displayed convulsions generally had higher plasma levels of Pitolisant.
- Due to minimal clinical signs, the **NOAEL is considered to be 12 mg/kg/day**. The corresponding exposure levels, at week 39, are 92.8 and 129 ng/mL for C_{max} and 455 and 536 ng*h/mL AUC_{0-24h} in males and females, respectively.

Methods

Doses:	0, 5, 12, and 30 mg/kg (2-week dose escalation schedule for the 12 and 30 mg/kg dose groups)
Frequency of dosing:	Daily
Route of administration:	Oral Gavage
Dose volume:	4 mL/kg
Formulation/Vehicle:	Sterile water
Species/Strain:	Monkey / Cynomolgus
Number/Sex/Group:	4/Sex/Group
Age:	1 to 2 years
Weight:	M (1.97 – 2.49 kg); F (1.95 – 2.36 kg)
Satellite groups:	2/Sex/Dose – 4-week recovery for control and high dose groups
Unique study design:	No
Deviation from study protocol:	Test formulation was used up to 12 days after preparation although stability was only established for 9 days. A subsequent stability test was done to confirm up to 14-day stability for the test substance. During stability testing only a single aliquot from the top and bottom of each concentration was tested instead of the 2 aliquots listed in the protocol. This deviation does not impact the integrity of the study. An internal investigation conducted after the final study report was released uncovered anomalies in the bioanalytical data. A study report amendment indicated samples were re-processed and the new data are believed to not affect toxicokinetic parameters by more than 5%. Therefore, the original toxicokinetic analysis was not adjusted.

Observations and Results

Mortality

No drug-related deaths or unscheduled terminations were noted. One control animal was sacrificed on day 11 due to adverse clinical signs including hunched posture, subdued behavior and cold body surface.

Clinical Signs

Sporadic single incidences of convulsions accompanied with unsteady gait and lateral recumbency were noted in 3 males (Days 15, 16 and 56) and 1 female (Day 21) and on multiple days in 1 female (Days 87 and 182) in the 30 mg/kg group (Table 20). Convulsions occurred between 46 and 70 min after dosing and lasted 30 to 60 min. TK analysis suggests that convulsions generally occurred during high plasma levels of the

test article (Table 21). Additional clinical signs noted in the high dose group were tremors in 1 M on day 16 and tremors and agitation in 1 F on day 20. These signs were initially observed between 42 and 57 min post-dose and subsided by 69 min post-dose. Vomiting and loose feces were noted for all groups receiving Pitolisant with increasing incidence with higher doses. Increased salivation was also observed in the HD group.

Table 20. Summary of Animals Displaying Convulsions/Tremors in 9-Month Oral Toxicity Study in Monkey

Group/ sex	Animal Number	Day Number	Actual time of dosing	Observations Post Dose	Convulsions	Tremors	Time from dosing to 1st convulsion or Tremor (Hr:Min)	Actual observation time when no more convulsions or tremors noted	Time from dosing to observation when no more Convulsions or Tremors noted (Hr:Min)
4M	401	56	8.23	9.33	1	N	1.10	10.00	1.47
4M	407	15	8.47	9.33	1	N	0.46	9.53	1.06
4M	411	16	8.54	9.51	1	Y	0.57	10.02	1.08
4F	408	87	8.00	9.06	1	N	1.06	9.12	1.12
4F	408	182	8.23	9.10	1	N	0.47	9.13	0.50
4F	410	20	8.14	8.56	0	Y	0.42	9.14	1.00

[Excerepted from NDA211150, Study Report (b) (4) HHL1005; page 228]

Table 21. Day 15 (or Week 22 / Days 148/149) Toxicokinetic Parameters in Primates Treated at 30 mg/kg/day

Animal Number	AUC (ng.h/ml)	Cmax (ng/ml)	Clinical signs	
401M	8660	1510	Convulsions	Day 56
403M	3200	513		
405M	2620	492		
407M	15700	4250	Convulsions	Day15
409M	6790	1120		
411M	5840	1130	Convulsions	Day16
			Tremors	Day16
400F	4780	605		
402F	3190	837		
404F	1750	446		
406F	5780	1070		
408F	6190 (4060)	901 (856)	Convulsions	Day 87 & 182
410F	1760	452	Tremors	Day 20
			Agitation	Day20

[Modified from NDA211150, Study Report (b) (4) HHL1005; page 32]

Body Weights
Body weight was minimally affected with the LD and MD males showing a decrease (6 and 5 %, respectively) compared to controls. No change was seen in the high dose males. Body weight gain was also variably affected, with all dose groups in the males and the MD group in females showing slight decreases (<10%) and slight increases in the LD and HD groups (3 and 12%, respectively). Interestingly, during the recovery period, mean body weight gain was reduced (50%) compared to controls in the HD males without change in food intake; no explanation was provided. No difference from controls were noted in the females. The effects on bodyweight are minimal and considered not drug related due to the lack of dose-response and inconsistencies between the sexes.

Feed Consumption
Due to the housing method, individual food consumption could not be quantified.

Ophthalmoscopy
No drug-related findings. Opthalmoscope examination of all animals occurred prior to treatment and at weeks 21 and 40 and at the end of the recovery period.

ECG
No drug-related findings. ECG from standard limb leads (I, II, and III) and augmented leads (AVR, AVL and AVF) were recorded in all animals prior to start of treatment and before dosing and 2 h post-dose at weeks 21 and 39.

Hematology
At week 15, changes in hematology parameters included increased WBC, neutrophil and eosinophil counts at the HD in both sexes. Neutrophils remained elevated (up to 87%) in HD females at weeks 28 and 41. During the recovery period no significant changes were noted. Adequate battery of hematology and coagulation parameters were evaluated prior to the start of dosing and at weeks 15, 28 and 41 and at the end of the recovery period.

Clinical Chemistry
A dose-related decrease in cholesterol levels (up to 35%) was noted in males at weeks 15, 28, and 41 and remained reduced compared to controls during the recovery period. A decrease in cholesterol was also noted in females at all sampling timepoints, though the effect was lower in magnitude and normalized during recovery. An adequate battery of clinical chemistry parameters was evaluated.

Urinalysis
Increased osmolality was noted at week 28 in the HD group. Increased presence of blood in urine samples from HD females was also noted during week 41. At the end of the recovery period, no difference compared to controls in blood pigment was noted. No associated histological changes were noted. The toxicological significance of this finding

is unknown. Urine samples were collected prior to the start of treatment and during weeks 15, 28 and 41 of dosing and at the end of the recovery period. An adequate battery of urinalysis parameters was evaluated.

Gross Pathology
No drug-related findings.

Organ Weights
Increased spleen and uterus weights were noted in females at 30 mg/kg/day compared to controls. Uterus weights at end of recovery phase were comparable to controls, however, spleen weights remained elevated. The following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary glands, spleen, submandibular lymph nodes, testes / epididymides, thymus, thyroids / parathyroid, and uterus.

Histopathology
Microscopic examination of a comprehensive battery of organs and tissues was performed on all study animals.

Adequate Battery
Yes

Peer Review
Yes

Histological Findings
All histological changes appear to be common background findings expected to occur in this strain monkeys at these ages and do not appear to be test-article related.

Special Evaluation
No special evaluations were conducted.

Toxicokinetics
After oral administration, Pitolisant was rapidly absorbed with a T_{max} between 1 to 2 h in both male and female monkeys. Plasma levels of Pitolisant increased slightly greater than dose proportional with minimal accumulation after repeated dosing. The terminal half-life ($T_{1/2}$) ranged between 2.20 and 7.4 h. Inter-animal variability in systemic exposure levels of Pitolisant tended to be high (up to 159%). There was no apparent significant difference in exposures levels of Pitolisant between males and females. Systemic exposure also appeared to decrease between day 15 to week 22 and week 39 (Table 22).

Reviewer Comment*: After release of the final study report, an internal investigation by the conducting laboratory uncovered anomalies in the bioanalytical data. A submitted study report amendment indicated samples were re-processed and the new data are believed to not affect toxicokinetic parameters by more than 5%. Therefore, the original toxicokinetic analysis was not adjusted, however, the TK portion of the study was*

deemed non-GLP compliant. A subsequent GLP 9-month TK study (Study no. (b) (4) HHL5001, GLP) *in monkeys was conducted. The review of that study can be found under* ***Toxicokinetics in Section 5.2*** *of this NDA review*

Table 22. Toxicokinetic Parameters of Pitolisant Following Repeated Oral Administration in a 9-Month Toxicity Study in Monkeys

	Males, Day 15			Females, Day 15		
Dose (mg/kg/day)	C_{max} (ng/mL)	t_{max}+ (hr)	AUC_{0-24} (ng.hr/mL)	C_{max} (ng/mL)	t_{max}+ (hr)	AUC_{0-24} (ng.hr/mL)
5	82.5 (83.4)	1.06 (1.00-1.12)	285 (67.7)	117 (57.8)	1.50 (1.00-2.00)	623 (88.4)
12	292 (41.3)	2.00 (1.03-2.00)	1290 (40.9)	403 (12.6)	1.00 (1.00-2.00)	1720 (10.2)
30	1130 (93.7)	1.00 (1.00-2.00)	5970 (73.5)	679 (38.4)	1.00 (1.00-4.00)	3450 (62.1)
	Males, Week 22			Females, Week 22		
Dose (mg/kg/day)	C_{max} (ng/mL)	t_{max}+ (hr)	AUC_{0-24} (ng.hr/mL)	C_{max} (ng/mL)	t_{max}+ (hr)	AUC_{0-24} (ng.hr/mL)
5	20.2 (149)	2.00 (2.00-2.00)	103 (111)	110 (89.1)	2.00 (1.00-2.02)	425 (98.9)
12	116 (93.7)	2.00 (2.00-2.00)	551 (107)	164 (41.9)	2.00 (2.00-2.00)	714 (41.1)
30	382 (30.4)	2.00 (1.00-4.02)	1890 (40.4)	584 (52.0)	2.00 (1.00-2.00)	2540 (57.3)
	Males, Week 39			Females, Week 39		
Dose (mg/kg/day)	C_{max} (ng/mL)	t_{max}+ (hr)	AUC_{0-24} (ng.hr/mL)	C_{max} (ng/mL)	t_{max}+ (hr)	AUC_{0-24} (ng.hr/mL)
5#	9.61 (38.6)[3]	2.00 (1.00-4.00)[3]	53.5 (4.78)[3]	61.8 (120)	2.00 (1.00-2.00)	245 (159)
12	92.8 (82.3)	2.00 (2.00-4.00)	455 (74.8)	129 (69.3)	2.00(1.00-2.02)	536 (53.5)
30	411 (59.1)	2.00 (2.00-4.00)	1930 (49.9)	391 (105)	2.00 (1.00-2.00)	1830 (104)

N = 4 (5 and 12 mg/kg/day) or 6 (30 mg/kg/day), unless stated otherwise [N].
+ = Median (range), # excluding Animal 201; data considered anomalous.

[Excerpted from NDA211150, Study Report (b) (4) HHL1005; page 359]

Dosing Solution Analysis
All dosing formulations were within ±10% of the target concentration value

6.2.5 Additional Toxicology Studies with Metabolites

Study title: BP1.8054 – 14-Day Preliminary Oral (Gavage) Toxicity Study in the Sprague-Dawley Rat (Study no. AB20728, Non-GLP)

Sprague-Dawley rats (5/sex/group with TK at 6/sex/group) were administered BP1.8054, a human specific metabolite, at 30, 100, and 300 mg/kg/day once daily for 14 days by oral gavage. BP1.8054 up to the highest dose of 300 mg/kg/day was well tolerated with no test-article related mortalities, clinical signs, or histopathology. Slight changes in hematology and coagulation parameters were recorded including increased

WBC counts (50%) in HD males, decreased RBC counts (-9%) and hemoglobin (-7%) in MD and HD males, and increased prothrombin time in both HD males (+1.7 s) and females (+4.8 s). Minimal changes in serum chemistry at the HD in both females and males included increased glucose and urea and decreased protein (+5%) and cholesterol (-30%). **The NOAEL was the HD of 300 mg/kg/day**. The NOAEL dose corresponded to the following exposure levels (Table 23).

Table 23. BP1.8054 Toxicokinetic Parameters After a Single and Repeated Dosing of 300 mg/kg

Day of treatment	Sex	AUC_{0-24h} (ng/mL*h)	T_{max} (h)	C_{max} (ng/mL)	AUC Ratio Male/Female	AUC ratio Day 13/Day 0
Day 0	Male	66611	0.5	9708	1.44	/
	Female	46239	0.5	14097		/
Day 13	Male	47980	0.5	7143	0.65	0.72
	Female	73314	0.5	20636		1.59

[Excerpted from NDA211150, Study Report AB20728; page 9]

Study title: BP1.8054 – 13-Week Oral (Gavage) Toxicity Study in the Sprague-Dawley Rat Followed by a 4-Week Treatment-Free Period (Study no. AB20729)

Sprague-Dawley rats (10/sex/group; 6/sex/group for TK; and 5/sex/group for recovery) were administered BP1.8054, a human specific metabolite, at 30, 100 and 300 mg/kg by oral gavage once daily for 13 weeks. A group of control and HD animals were also maintained treatment-free for a 4-week recovery period. No drug-related mortalities were reported. A single male animal (100 mg/kg/day) was found dead on day 90. Necropsy revealed a ruptured right median lobe of the liver with suspected blood present. This death is believed to be due to accidental injury and not drug related. No clinical signs were noted beyond sporadic single animal occurrences of decreased activity and tilted head. Decreased body weight gain (-10%) was noted for the HD group. No effect on food consumption was noted. As seen in the 14-day preliminary study, prothrombin time was significantly increased in both males (+3.2 s) and females (+3.9 s) in the HD group after 13 weeks of treatment, however, the effect resolved by the end of the recovery period. Minimal yet statically significant changes in clinical chemistry parameters included decreased triglycerides in HD male and female animals, increased urea in males at all doses and HD females and increased glucose in males at all dose levels. These changes were fully reversible in females and partially reversible in males during the recovery period. Histological examination revealed only inflammatory changes in the kidney with tubular basophilia and cortical tubular dilation which resolved by end of the recovery period. **The NOAEL was determined to be the HD of 300 mg/kg/day**. The NOAEL dose corresponded to the following exposure levels (Table 24)

Table 24. BP1.8054 Toxicokinetic Parameters during a 13-Week Chronic Treatment in Rats

Sex	Dose (mg/kg/day)	C_{max} (ng/mL)			AUC_{0-t} (ng/mL*h)		
		Day 1	Week 4	Week 13	Day 1	Week 4	Week 13
BP1.8054							
Male	30	1632	1097	1762	6588	4734	6809
	100	5666	4527	6600	27521	21389	25205
	300	16801	13061	13671	113167	73128	66698
Female	30	2750	2784	3561	7137	6568	11489
	100	7839	8206	11245	33373	35779	43915
	300	21454	18329	22219	131290	103723	118485

Study title: BP1.2526 – Pharmacokinetic Study by Intravenous Route (Bous) in Cynomolgus Monkeys (Study no. (b) (4) 32800, GLP)

Cynomolgus monkeys (*non-naïve*) were administered metabolite BP1.2526 by bolus peripheral vein injection at 1, 3, 6, 7, 8 and 10 mg/kg (free base) in an ascending dose paradigm with all 4 animals receiving the bottom two doses, 3 days apart, and each individual animal receiving one of the remaining doses 3 to 8 days later. Mortality and clinical signs were recorded. Blood samples were obtained at 5 min, 15 min, 0.5, 1, 2, 4, 8 and 24 h after dosing for PK analysis.

No mortality occurred in the study. At doses ≥ 7 mg/kg, hyperthermia and CNS clinical signs occurred including ataxia, ventral or lateral recumbency and tonic seizures. Clinical signs were observed immediately after dosing and lasted for approximately 30 min. At lower doses, hyperthermia occurred in all animals returning to physiologic values within a few hours of dosing. Pharmacokinetic data is summarized in the table below (Table 25).

Reviewer Comment: Metabolite BP1.2526 is a major circulating metabolite in the rat that has been suggested by the Applicant to be responsible alone or in part for the convulsions observed in rats and monkeys due to convulsions occurring after direct i.v. administration of the metabolite in rat (see summary of studies below). In the monkey, BP1.2526 is of low abundance (C_{max} = 53.4 ng/ml) after repeated oral administration of pitolisant at doses that cause convulsions (30 mg/kg pitolisant; Study no. (b) (4) HHL5001). IV administration of BP1.2526 failed to elicit convulsions in monkeys at an i.v. dose of 3 mg/kg (free base) with a corresponding C_{max} of 1109.58 ng/ml. These data suggest that convulsions occurring after oral administration of pitolisant, in monkeys, are most likely not due to formation of the metabolite BP1.2526.

Table 25. Pharmacokinetic Parameters after IV Administration of BP1.2526 in Monkeys

Dose (mg/kg)	Cmax (ng/mL)	T ½ (h)	C_0 (ng/mL)	AUC_{0-t} (ng*h/mL)	AUC_{0-inf} (ng*h/mL)	V_{ss} (mL/kg)	CL (mL/h/kg)
Mean (n=4)							
1	343.22	0.907	335	379	417	3000	2432
3	1109.58	1.048	1210	1224	1317	3251	2283
Single Animal							
6	2056.81	1.52	2454	2586	2638	3967	2275
7	2224.14	1.75	1954	2793	2902	4876	2412
8	2410.69	1.68	2225	2937	3007	4347	2660
10	3915.10	1.74	5522	5945	6122	3031	1633

[Modified from NDA 211150, Pharmacokinetics Written Summary; pages 79, 80]

Convulsions Related to Pitolisant and its Metabolites

A series of non-GLP single-dose oral and i.v. studies in rats were conducted to investigate the ability of pitolisant and its major rat metabolite, BP1.2526 to produce convulsions and how convulsions related to serum and brain concentrations of both. The studies briefly summarized below demonstrate that both pitolisant and its major circulating metabolite in rat, BP1.2526, induce convulsions when administered i.v. alone. These studies suggest that, in rat, convulsions occurring after oral administration of pitolisant may be due, at least in part, to the metabolite BP1.2526; however, the parent drug, pitolisant, can produce convulsions on its own without the formation of BP1.2526.

In male Sprague-Dawley rats, pitolisant was administered i.v. at 10 and 20 mg/kg and surviving animals were sacrificed 15 min after dosing (Study no. B115-BF2.649, Non-GLP). Convulsions followed by death occurred within 1-min post-dose in both the LD (1/4) and HD (3/4) groups. Additional clinical signs included prostration and increased respiratory rate which were also observed in animals that survived to the 15 min post-dose in the absence of convulsions. Significantly higher serum and brain concentrations of pitolisant were measured in the animals that died after convulsions compared to animals that survived to 15 min post-dose (Table 26). Minimal levels of metabolites were measured indicating the CNS-clinical signs, including convulsions, were related to the parent drug alone.

Table 26. Pitolisant Levels in Serum and Brain Tissue 15 Minutes Post-Dose Versus After Premature Death Following i.v. Administration of Pitolisant in Rats.

Plasma			Brain		
Rats Sacrificed at the 15-min Timepoint					
Dose	BF2.649		Dose	BF2.649	
	ng/mL	SEM		ng/g	SEM
10 mg/kg	714.87	90.7	10 mg/kg	29756.17	2733.34
20 mg/kg	547.00		20 mg/kg	24422.50	
Rats Dead Before the 15-min Timepoint					
Dose	BF2.649		Dose	BF2.649	
	ng/g	SEM		ng/g	SEM
10 mg/kg	2280.00		10 mg/kg	49812.50	
20 mg/kg	6504.33	739.01	20 mg/kg	100924.00	7423.54

[Modified from NDA211150, Study Report B115-BF2.649; page 12]

A subsequent study investigated the proconvulsant activity of the 2 major rat metabolites, BP1.2525 and BP1.2526 in Sprague-Dawley rats (Study no. R-B104-BF2.649, Non-GLP). BP1.2526 was administered i.v. at 10, 22.5 and 30 mg/kg (free base). Mortality in both the MD (1/8) and HD (5/8) groups occurred from immediately following drug administration up to 2 min post-dosing. No clinical signs were noted in those animals that died. In the surviving animals from the MD and HD groups, convulsions occurred within 5 min of exposure in addition to prostration and salivation. No clinical signs were noted at the LD. Significantly higher serum levels of BP1.2526, at the HD, occurred in animals that died compared to those that displayed convulsions but survived (Table 27). However, this pattern was not as absolute with respect to brain levels of BP1.2526.

Table 27. Mortality, Clinical Signs, and Serum and Brain Levels of BP1.2526 following i.v Administration in Sprague-Dawley Rats

Animal Identification	Compound	Dose mg/kg	BP1.2525		BP1.2526		Mortality and clinical signs
			Plasma ng/ml	Brain ng/g	Plasma ng/ml	Brain ng/g	
1	BP1.2526A	10	311.2	695.6	1351.0	15411.0	No Clinical Signs
2		10	90.0	438.1	1820.8	14880.7	No Clinical Signs
3		10	47.2	n.d.	1902.5	n.d.	No Clinical Signs
4		10	88.4	452.4	2534.4	17246.7	No Clinical Signs
5	BP1.2526A	10	91.8	389.6	2155.5	16185.3	No Clinical Signs
6		10	113.8	440.0	1873.7	12372.7	No Clinical Signs
7		10	50.7	502.0	1595.5	17140.0	No Clinical Signs
8		10	48.4	506.6	1424.9	15343.7	No Clinical Signs
9	BP1.2526A	22.5	148.4	1050.2	5193.0	32988.5	Prostration
10		22.5	109.5	940.4	3685.1	36716.1	Salivation, Chewing, Convulsions 4min post dose
11		22.5	143.8	788.5	3751.1	32538.7	Salivation, Chewing, Convulsions 4min post dose
12		22.5	11.4	451.3	6201.0	39790.2	Mortality (time of death: 2 min post dose)
13	BP1.2526A	22.5	103.1	781.3	4412.4	28576.1	Salivation, Chewing, Convulsions 3.5min post dose
14		22.5	98.5	817.0	3259.1	27411.6	Salivation, Chewing, Convulsions 5min post dose
15		22.5	180.6	734.3	4700.7	24392.7	Prostration
16		22.5	161.6	773.1	5482.0	27794.8	Prostration
17	BP1.2526A	30	374.7	581.1	>50000	39255.1	Mortality (time of death immediately post dose)
18		30	28.0	760.3	14152.3	39834.8	Mortality (time of death: 1.5 min post dose)
19		30	235.5	824.9	4527.3	31977.8	Salivation, Chewing, Convulsions 4min post dose
20		30	237.6	1013.7	5110.0	41322.8	Salivation, Chewing, Convulsions 5min post dose
21	BP1.2526A	30	65.9	595.4	27527.4	51308.2	Mortality (time of death immediately post dose)
22		30	21.2	615.8	21550.9	46409.4	Mortality (time of death immediately post dose)
23		30	121.8	1282.7	4900.2	41559.9	Salivation, Chewing, Convulsions 3.5 min post dose
24		30	33.9	541.4	19899.7	40847.9	Mortality (time of death immediately post dose)

[Excerpted from NDA211150, Study Report R-B104-2-649; page 16]

Intravenous administration of BP1.2525 did not cause clinical signs at any of the tested (1, 2 and 10 mg/kg), however, the study report notes in the results section that higher doses of BP1.2525 caused mortality without convulsions (this data was not included in the study report). The results also showed that the mean brain levels of BP1.2525 (8,541 ± 329 ng/g) at the HD, exceeded the brain levels of BP1.2525 (4066 ± 247 ng/g) achieved following oral dosing of pitolisant at the threshold pro-convulsant dose of 75 mg/kg (study B103, not submitted); suggesting that the convulsions elicited by oral exposure of Pitolisant is unlikely to be due to the metabolite BP1.2525. In contrast, brain levels of BP1.2526 (31,311 ± 2,110 ng/g at MD) were similar to those achieved after oral dosing of pitolisant at 75 mg/kg (37651 ± 4884 ng/g), indicating that convulsions observed following oral administration of pitolisant may potentially be due alone or in part to this metabolite.

The proconvulsant activity of additional metabolites (BP1.3484, BP1.3474, BP2.951, BP2.927, BP2.928, BP2.941, BP1.3534A, and BP1.3002) of pitolisant were investigated in Study no. R-B127-BF2.649, Non-GLP. All metabolites were administered i.v. at doses between 10 and 60 mg/kg. No convulsions were noted at any dose level. Minor clinical signs including subdued behavior and chewing were noted for some of the metabolites (BP2.941, BP1.3474, and BP1.3002). **Reviewer Comment:** Metabolite BP1.3484 is the major circulating metabolite of pitolisant measured in humans and monkeys. The high plasma levels attained in this study with no apparent convulsions far exceed those that occurred following oral administration in monkeys in the 6-month toxicity study where sporadic convulsions were observed, indicating that this metabolite is most likely not responsible for producing convulsions.

A single dose toxicity study (Study no. (b) (4) VGU-5011, GLP) was done in Sprague-Dawley rats with oral administration of pitolisant at 75, 150, 250, and 400 mg/kg, to determine the relationship between the convulsions and serum and brain levels of the parent drug and its major metabolites. At doses ≤ 250 mg/kg clinical signs included subdued behavior, abnormal gait, hunched posture, irregular breathing and increased salivation, however, no convulsions were noted during the observation time up to 30 min post-dose. At the high dose of 400 mg/kg, convulsions occurred in 8 of 20 rats. These rats also had the following signs in addition to the above: protruding eyes, dilation of pupils, prostration, pale color, and cold body surface. One animal in HD was found dead approximately 25 min after convulsions, rapid breathing, dilation and protruding of the eyes and prostration. Brain levels of pitolisant and the two major metabolites (BP1.2525 and BP1.2526) were up to 15 times those measured in serum. The third metabolite (BP2.951) showed poor penetration into the brain. Comparison of convulsing versus non-convulsing animals at 400 mg/kg revealed *generally* higher levels of pitolisant and metabolites in both serum and the brain in the former (Table 28; not the large SD).

Table 28. Serum and Brain levels of Pitolisant and major metabolites in Sprague-Dawley Rats After Single Oral Dose of Pitolisant at 400 mg/kg

	Dose		Analyte (ng/mL in serum or ng/g in brain tissue)			
	400 mg/kg, p.o. rats		BF2.649	BP2.951	BP1.2525	BP1.2526
Serum	Non convulsing	Mean	256	52	205	792
	n = 8	SD	118	25	52	263
	Convulsing	Mean	607	108	241	1110
	n = 12	SD	407	54	48	184
Brain	Non convulsing	Mean	3769	15	1270	10236
	n = 8	SD	1705	0	361	3049
	Convulsing	Mean	8916	18	1523	13766
	n = 12	SD	4892	5	273	1358

[Excerpted from NDA211150, Study Report (b) (4) VGU-5011; page 23]

Another single dose i.v. study (Study no. ZNA14542.001, GLP) was done in Sprague-Dawley rats with i.v. administration of pitolisant, and 2 of its major metabolites (BP1.2526 and BP2.951), to determine the relationship between clinical signs, specifically convulsions, and the serum and brain levels of the parent drug and the major metabolites. In the DRF segment, pitolisant was administered at 10 and 14 mg/kg. Similar clinical signs including convulsions and tremors were observed at both dose levels but 1 rat in HD was euthanized due to severe clinical signs. Metabolite BP1.2526 was injected i.v. at 21 and 32 mg/kg with convulsions and tremors as well as prostration, irregular breathing and protruding eyes noted at both doses, but one animal died at 32 mg/kg. Metabolite BP2.951 was injected i.v. at 30 and 41 mg/kg. Tremors were noted at 41 mg/kg but no convulsions. Clinical signs were limited to irregular

breathing at the lower dose. In the main study, pitolisant at 10 mg/kg resulted in convulsions (7/15), irregular breathing (8/15) and sporadic incidences of tremors. BP1.2526 at 21 mg/kg induced convulsions (15/15), irregular breathing (10/15), abnormal gait (12/15) and tremors (10/15) and resulted in one death. No clinical signs were noted for BP2.951 at 41 mg/kg. The associated brain levels of Pitolisant and its metabolites are listed below (Table 29). In summary a single i.v. administration of either pitolisant or its major rat metabolite, BP1.2526, high brain exposure levels caused convulsions. Conversely, a main human metabolite, BP2.951, did not penetrate the rat brain effectively, resulting in low brain exposure and failure to induce convulsions.

Table 29. Brain levels of Pitolisant, BP2.951, BP1.2525, and BP1.2526 in Sprague-Dawley Rats After Single i.v. Administration.

Group	Compound Dose	BF2.649	BP2.951	BP1.2525	BP1.2526
		Concentration (ng/g)			
1	Control	BLOQ	BLOQ	BLOQ	BLOQ
2	BF2.649 10 mg/kg	9985.6 ± 419.3	BLOQ	BLOQ	54.0 ± 25.9
3	BP1.2526 21 mg/kg	BLOQ	BLOQ	262.75 ± 35.7	29631.9 ± 1302.4
4	BP2.951 41 mg/kg	BLOQ	981.6 ± 37.6	BLOQ	BLOQ

BLOQ below the limit of quantification. Mean ± s.e.m. of 15 rats per group with individual value set at 0 when the value is BLOQ.

[Excerpted from NDA211150, Study Report ZNA14542.001; page 22]

7 Genetic Toxicology

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

Study no.:	CD01/7754T
Study report location:	EDR, SDN 2
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	05/31/2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Pitolisant, PAN 01/01, 99.3%

Key Study Findings

Pitolisant was negative for mutagenicity in a valid Ames assay

Methods

Strains:	Salmonella typhimurium: TA-1535, TA-1537, TA-98 and TA-100; E Coli: WP2uvrA
Concentrations in definitive study:	For Salmonella strain: 2500, 1250, 625, 312.5, 156.25, and 78.13 µg/plate; For E coli strain: 1250, 625, 312.5, 156.25, 78.13 µg/plate.
Basis of concentration selection:	Dose selection based on a range-finding study in TA-100 and WP2uvrA tester strain in absence of S-9 activation
Negative control:	Double distilled water
Positive control:	-S-9 (µg/plate): 4-nitro-O-phenylendiamin (1, 10), sodium azide (1,10), 9-aminoacridine (5, 50), 4-nitroquinoline oxide (0.5, 1). +S-9: 2-aminoanthracen (1 and 10)
Formulation/Vehicle:	Solution in double distilled water
Incubation & sampling time:	Both studies used the pre-incubation for 20 min followed by 72 h incubation at 37°C. 10% S-9 (Aroclor 1254-Induced rat liver homogenate)

Study Validity

Based on the following attributes the study is considered valid. The appropriate number and types of tester strains were used in the study. Dose selection was based on a range finding study with the top dose showing cytotoxicity. A minimum of 3 non-cytotoxic concentrations were used for all strains in the initial and confirmatory studies.

Appropriate positive and negative controls produced expected results. Each study utilized 3 replicate plates per concentration.

Results

Pitolisant was fully soluble in water up to 500 mg/mL and remained in solution during successive dilutions. Cytotoxicity occurred at concentrations ≥ 2500 and 1250 µg/plate in salmonella and Ecoli tester strains, respectively. Data from the initial and confirmatory studies showed no positive increases in the mean number of revertants in any strain, with or without metabolic activation. Positive controls increased revertants well above the required 3-fold increase over negative vehicle control.

Based on these study results, Pitolisant was determined to be **negative** for mutagenicity under the current study conditions.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Cell Mutation Assay at the Thymidine Kinase Locus (TK+/-) in Mouse Lymphoma L5178Y Cells with BF-2649

Study no.:	(b) (4)-702800
Study report location:	EDR, SDN 2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	07/30/2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Pitolisant (BF-2649), PAN 01/01, 99.9%

Key Study Findings

Pitolisant was considered **non-mutagenic** in a valid mouse lymphoma assay.

Methods

Cell line:	L5178Y Mouse Lymphoma Cells
Concentrations in definitive study:	Experiment 1: 2.5, 5, 10, 20, 40, 55 µg/mL (+/-S9); Experiment 2: 1.3, 2.5, 5, 10, 15, 20 (-S9)
Basis of concentration selection:	Dose selection was based on a range finding study with doses ranging from 27.3 to 3500 µg/mL ±S9 for 4h and -S9 for 24 h incubation times.
Negative control:	Deionized water
Positive control:	Methylmethane sulfonate (13 µg/mL -S9) ; 3-methylcholanthrene 3 µg/mL +S9)
Formulation/Vehicle:	Solution in deionized water
Incubation & sampling time:	4 Hours +/- Activation, 24 hours - Activation

Study Validity

Based on the following attributes the study is considered valid. Two independent experiments with two replicate cultures each were conducted. A minimum of four concentrations were analyzed. Positive controls increased mutant frequency 2-fold or greater. Both positive and negative control values were within the historical control data ranges for the conducting laboratory.

Results

In the range finding experiment, significant cytotoxicity (RSG <10% compared to negative controls) and/or precipitation occurred at doses ≥109.4 µg/mL after 4 and 24 h incubations ± S9 activation. In the first mutation frequency experiment, the concentrations of Pitolisant ranged from 2.5 to 55 µg/mL ±S9 with a 4h incubation. Greater than 2-fold increase in mutant colony frequency occurred after 4h treatment of 20 µg/mL -S9 and 40 µg/mL +S9. Data from the 4h HD group of 55 µg/mL +S9 was rejected due to low cloning efficiency (<10%). Change in mutant colony frequency did not occur in a parallel culture under identical conditions. Due to the irreproducibility and lack of dose relationship the results of experiment 1 are considered negative. In experiment 2, a 24h incubation with Pitolisant at doses of 2.5 to 55 µg/mL -S9 resulted in significant toxicity at all doses tested and the experiment was terminated prior to the generation of mutagenicity data. Toxicity data for this experiment were not provided in the study report. Experiment 2 was repeated with a lower concentration range of 2.5 to 20 µg/mL of Pitolisant. Mutant colony frequency did not increase significantly after 24h incubation -S9.

Based on the results from the two independent experiments, Pitolisant was determined to be **negative** for mutagenicity under the current study conditions.

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

Study title: BF-2649. Micronucleus Assay in Mice

Study no:	(b) (4) T-264905
Study report location:	EDR, SDN 2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	07/03/2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Pitolisant (BF-2642), PAN 01/01, 99.84

Key Study Findings

Pitolisant did not induce an increase in micronucleated PCEs. However, the study was considered inadequate due to the assessed single dose level exceeding the MTD due to mortality.

Methods

Doses in definitive study:	150 mg/kg
Frequency of dosing:	Single Dose
Route of administration:	Oral Gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Solution in water
Species/Strain:	Mouse/Swiss OF1
Number/Sex/Group:	5/sex/group
Basis of dose selection:	Dose selection was based on acute toxicity studies in mice with maximum nonlethal dose of 100 mg/kg and minimum lethal dose of 200 mg/kg
Negative control:	Water
Positive control:	Cyclophosphamide (50 mg/kg)

Study Validity

The study was deemed invalid due to only a single dose concentration being used which exceeded the MTD and resulted in the death of 3 animals.

Results

A single dose of 150 mg/kg was administered orally to Swiss mice and the frequency of micronucleated PCEs was assessed at 24 and 48h post-dose. Drug-related deaths of 3 mice were recorded, and replacement animals were used. Direct cytotoxicity was noted with a decrease in the PCE/NCE ratio, which reached statistical significance in females at the 48h sampling time.

Pitolisant did not increase the frequency of micronucleated PCEs compared to the negative control.

Due to the use of a single dose level and that dose exceeding the MTD, the study is considered **inadequate**.

Study title: In Vivo Mammalian Erythrocytes Micronucleus Test Performed in Mouse Bone Marrow (Two Treatments, One Sampling Time)

Study no:	(b) (4) 131003
Study report location:	EDR, SDN 2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	12/11/2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Pitolisant (BF2.649), Batch126223, 100.3%

Key Study Findings

Pitolisant was **negative** for clastogenicity in a valid *in vivo* mouse micronucleus assay at doses up to 50 mg/kg.

Methods

Doses in definitive study:	12.5, 25, 50 mg/kg/day
Frequency of dosing:	Daily for 2 days
Route of administration:	Oral Gavage
Dose volume:	20 mL/kg
Formulation/Vehicle:	Solution in distilled water
Species/Strain:	Mouse/Swiss OF1
Number/Sex/Group:	5/sex/group
Satellite groups:	3/sex/group
Basis of dose selection:	Dose selection was based on a preliminary DRF study. In the DRF study, two daily oral doses of 32, 50, 80 and 200 were tested. Doses ≥ 80 mg/kg exceeded the MTD due to excessive clinical signs (e.g. convulsions) and death.
Negative control:	Distilled water
Positive control:	Cyclophosphamide (50 mg/kg, i.p.)

Study Validity

Based on the following attributes the study is considered valid. The appropriated doses were selected based on a DRF study. Significant systemic exposure was confirmed by TK analysis of Pitolisant and its major metabolites (BP2.951, BP1.2516 and BP1.2525). The frequency of micronucleated PCEs in the negative control group was within historical control ranges and positive control significantly increased the number of micronucleated cells.

Results

In the preliminary dose range finding study, excessive clinical signs including convulsions and death occurred at doses ≥ 80 mg/kg. At 50 mg/kg/day, slight decreases in spontaneous motor activity and respiratory distress were noted. Therefore, the high dose of 50 mg/kg/day was selected for the definitive study. In the definitive study, similar clinical signs to the preliminary study were noted at 50 mg/kg/day. The PCE/NCE ratio did not change after exposure to Pitolisant indicating no direct bone marrow cytotoxicity.

Adequate systemic exposure was confirmed by TK analysis of Pitolisant and its major metabolites. At the HD of 50 mg/kg, the C_{max} of 2767 ng/mL was approximately 38X the human C_{max} at the MRHD of 35.6 mg.

Oral exposure of Pitolisant did not increase the frequency of micronucleated PCEs compared to the negative control at any concentration.

7.4 Other Genetic Toxicity Studies

The genotoxic potential of several metabolites, including the human specific metabolite, BP1.8054, was assessed by the bacterial Ames assay. The results of these studies are listed in the table below (Table 30). In summary, all tested metabolites were found to be negative for mutagenicity in valid Ames assays under the described study conditions.

In addition to the Ames test, the genotoxic potential of metabolite BP1.8054 was assessed in an *in vitro* micronucleus assay using cultured human lymphocytes. BP1.8054 was determined to be **non-clastogenic** in a valid study. The study is summarized below.

Study title: BP1.8054: *In Vitro* Mammalian Cell Micronucleus Test on Cultured Human Lymphocytes (Study no. (b) (4) -140906, GLP). Two independent studies were conducted with cultured lymphocytes being treated either for 4 hours with a 24 h recovery period ± S9 metabolic activation or 24 h continuous treatment -S9 activation. The concentrations of BP1.8054 (in DMSO) used in the definitive studies were 0.125, 0.25, 0.5 and 1 mM and were selected based on a preliminary toxicity study. BP1.8054 did not significantly increase the frequency of micronucleated cells above the concurrent negative solvent controls in either the short or continuous treatment studies. Both the negative and positive (mitomycin C, cyclophosphamide, and griseofulvin) control groups were within the historical ranges established by the conducting laboratory.

BP1.8054 was considered **negative** for clastogenicity under the described study conditions.

Table 30. Summary of Bacterial Ames Studies for Metabolites of Pitolisant

Test Article	Doses (µg/plate)*	GLP Compliance	Valid Study	Conclusions	Study Number
BP2.951	up to 5000	yes	yes	**negative**	(b) (4) 091011
BP1.2526	up to 5000	yes	yes	**negative**	(b) (4) 35831 MMO
BP1.8054	up to 3000	yes	yes	**negative**	(b) (4) 140905
BP1.3484	up to 5000	yes	yes	**negative**	(b) (4) 170117
BP1.3473	up to 1500	yes	yes	**negative**	(b) (4) 170116
BP1.8186	up to 5000	yes	yes	**negative**	(b) (4) 170118
BP1.10556	up to 5000	yes	yes	**negative**	(b) (4) 170115
BP1.10749	15 to 5000	yes	yes	**negative**	(b) (4) 170605

* Highest dose tested in at least one strain in the definitive studies

8 Carcinogenicity

8.1 105-Week Carcinogenicity Study by the Oral Route (Gavage) in Rats

Study no.:	(b) (4) 35834-TCR
Study report location:	EDR, SDN 2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Dosing initiation 8/31/2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Pitolisant, 0001208253 & 02514540001
CAC concurrence:	Yes

Key Study Findings

- There were no significant drug-related neoplastic findings following daily oral administration of pitolisant up to 30 mg/kg/day for 105 weeks.

- Increased incidence of hepatocellular adenomas in male rats at the mid dose of 15 mg/kg/day. Hepatocellular adenoma is considered a common neoplastic finding in the rat; therefore, this finding is considered not significant (p = 0.0401) by pair wise comparison to controls according to the statistical reviewer Hepei Chen.
- Survival analysis (see the statistical review by Hepei Chen for details) showed a statistically significant increase in mortality at the high dose of 30 mg/kg/day in male rats.
- At the high dose of 30 mg/kg/day, the corresponding plasma concentration level of pitolisant is 1014.5 ng*h/mL, which is approximately 1.3 times the MRHD of 40 mg/day, based on AUC.

Adequacy of Carcinogenicity Study

The rat carcinogenicity study is considered adequate based on appropriate route of administration, dose selection based on dose-range finding studies and with ECAC concurrence, and sufficient number of animals for adequate statistical analysis.

Appropriateness of Test Models

The rat model, Crl CD (SD), is an appropriate species and strain with adequate historical control data to assess long-term carcinogenicity potential.

Evaluation of Tumor Findings

The incidence of hepatocellular adenomas was increased in males at 15 mg/kg (MD). Independent statistical review of the results by Hepei Chen from the Division of Biometrics indicates that the increase incidence did not reach statistical significance for a common tumor (p-value = 0.0401). Since the incidence was non-significant compared to controls and a dose response was not established the finding is considered incidental and not drug-related. In agreement with the Applicant’s statistical analyses, no other statistically significant neoplastic findings were noted.

Methods

Doses:	0 (vehicle control), 5, 15, 30 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Solution in sterile water
Basis of dose selection:	Dose selection was based on the 3-month and 6-month toxicology studies in Sprague-Dawley rats. For both males and females, the HD of 30 mg/kg/day was recommended by the ECAC based on MTD due to seizures and death at 60 mg/kg/day (ECAC concurrence, July 9, 2009).
Species/Strain:	Rat/Sprague-Dawley (Crl CD (SD) IGS BR) from (b) (4)
Number/Sex/Group:	60/sex/group
Age:	5-6 weeks old
Animal housing:	All animals pair housed by sex and group
Paradigm for dietary restriction:	No dietary restrictions; all animals had free access to food and tap water *ad libitum*
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	Toxicokinetic satellite: 12/sex/group for LD, MD, HD groups; 2/sex/group for controls
Deviation from study protocol:	No significant protocol deviations were noted that would impact study integrity.

Observations and Results

Mortality

At week 101, the number of surviving animals in the female HD group decreased to 20 and dosing in this group was suspended. Dose stoppage followed pre-specified criteria recommended by the Division and the ECAC.

At terminal necropsy, the number of surviving animals were 32, 24, 26 and 22 for males; and 26, 21, 22, and 18 for females in the control, LD, MD and HD groups, respectively (Table 31). Survival analysis was conducted by the statistics reviewer (Hepei Chen; Figure 2, 3). A significant increase in mortality in HD males occurred compared to controls (p-value = 0.0282). The number of surviving animals at the end of the study was adequate to perform meaningful statistical evaluation.

Table 31. Cumulative Mortality in the 105/106-Week Rat Carcinogenicity Study

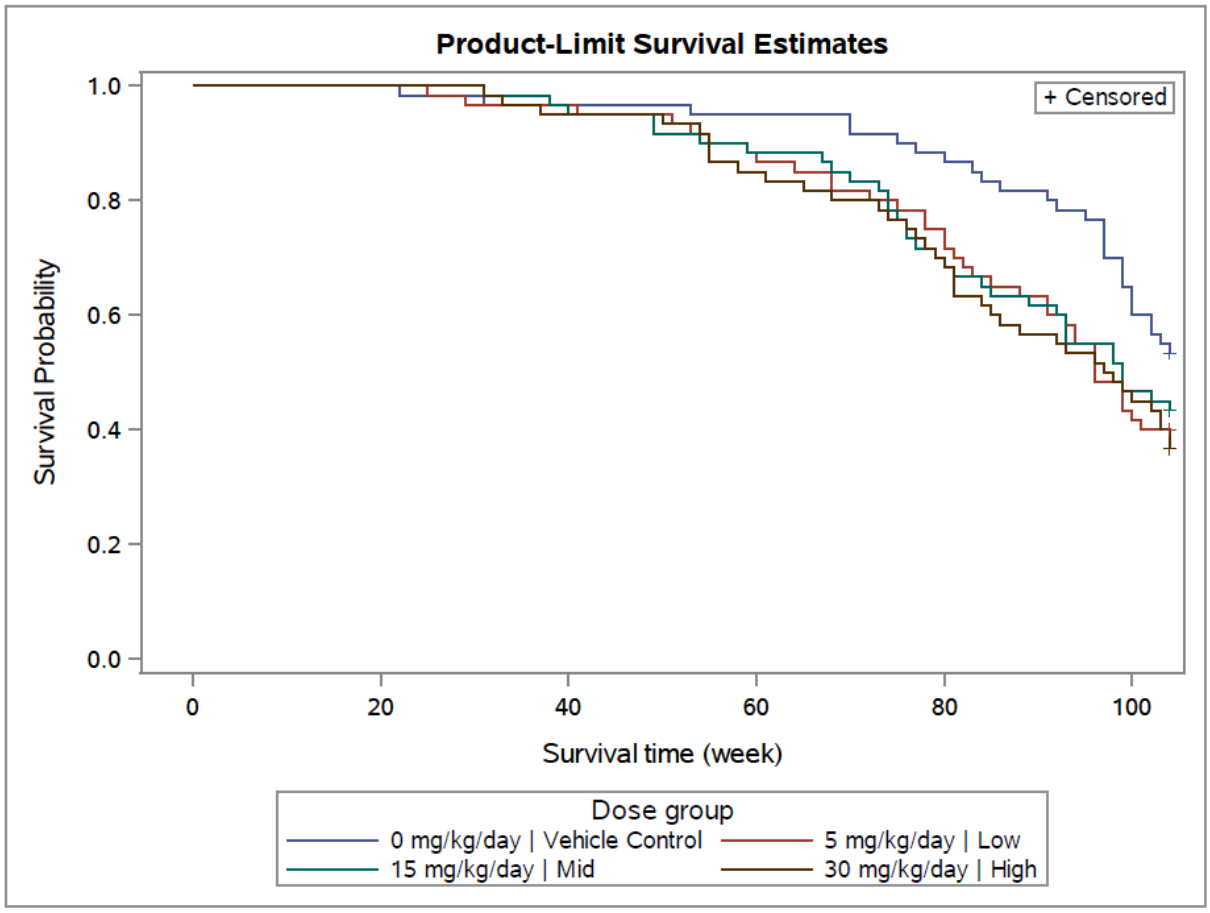
Sex	Male				Female			
Dose-level (mg/kg/day)	0	5	15	30	0	5	15	30
Group	1	2	3	4	1	2	3	4
n	60	60	60	60	60	60	60	60
. found dead	13	21	17	24	11	10	8	13
. sacrificed prematurely	15	15	17	14	23	29	30	29
Total number of deaths (weeks 1 to 106)	28	36	34	38	34	39	38	42

n: number of principal animals at study start.

[Excerpted from NDA211150, Study Report (b) (4) 35834-TCR; page 44]

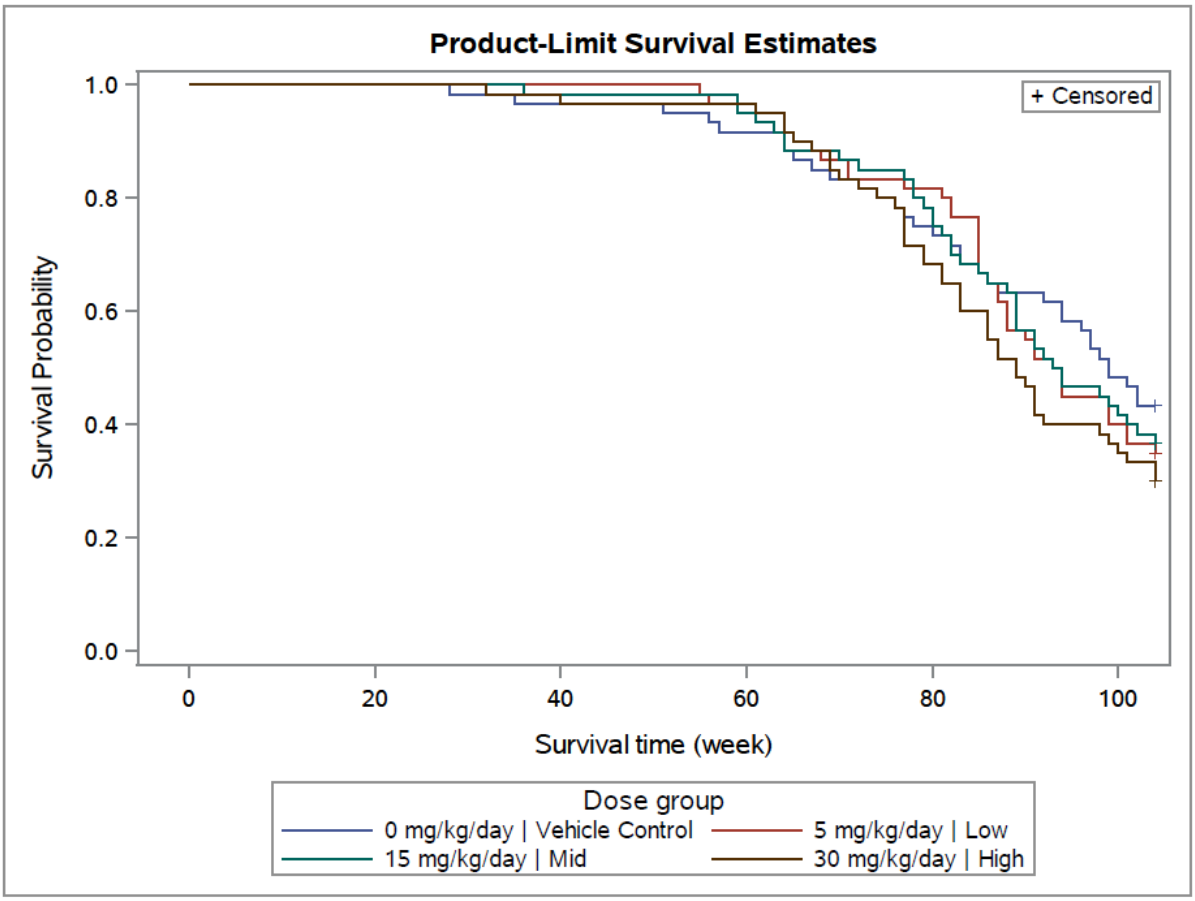
The causes for unscheduled mortality and early termination were generally comparable across all dose groups and are consistent with common causes of mortality for the age and strain of the test species. The most common causes of mortality were pituitary and mammary gland tumors (Table 32)

Figure 2. Kaplan-Meier Survival Functions for Male Rats in 105-Week Rat Carcinogenicity Study



[Excerpted from Hepei Chen's Statistical Review of Rat Carcinogenicity Study; page 24]

Figure 3. Kaplan-Meier Survival Functions for Female Rats in 105-Week Rat Carcinogenicity Study



[Excerpted from Hepei Chen's Statistical Review of Rat Carcinogenicity Study; page 25]

Table 32. Summary of Most Common Causes of Mortality in the 105-Week Rat Carcinogenicity Study

Sex	Male				Female			
Groups	1	2	3	4	1	2	3	4
Dose-level (mg/kg/day)	0	5	15	30	0	5	15	30
n	60	60	60	60	60	60	60	60
Pituitary gland adenoma; pars distalis	10	6	3	6	15	22	21	16
Mammary gland; fibroadenoma	-	-	-	-	6	3	5	6
Mammary gland; adenocarcinoma or adenocarcinoma arising in a fibroadenoma	-	-	-	-	1	5	2	6
Urogenital inflammation	3	2	3	2	-	-	-	-
Lymphoma; malignant	1	2	-	-	1	-	1	2
Uterus; adenocarcinoma	na	na	na	na	-	3	1	-
Subcutis; malignant sarcomatous tumors (fibrosarcoma, schwannoma, sarcoma NOS, malignant fibrous histiocytoma)	1	2	-	-	1	2	-	2
Hindleg ulcer/inflammation	2	1	3	1	-	1	-	-
Not evident	5	12	8	16	4	-	3	5
Dosing-related death	2	-	4	3	-	-	1	1

n: number of principal animals at study start.
*: occurring in three or more animals of 1 group/sex.
-: not observed.
na: not applicable.
NOS: Not Otherwise Specified.

[Excerpted from NDA211150, Study Report (b) (4) 35834-TCR; page 46]

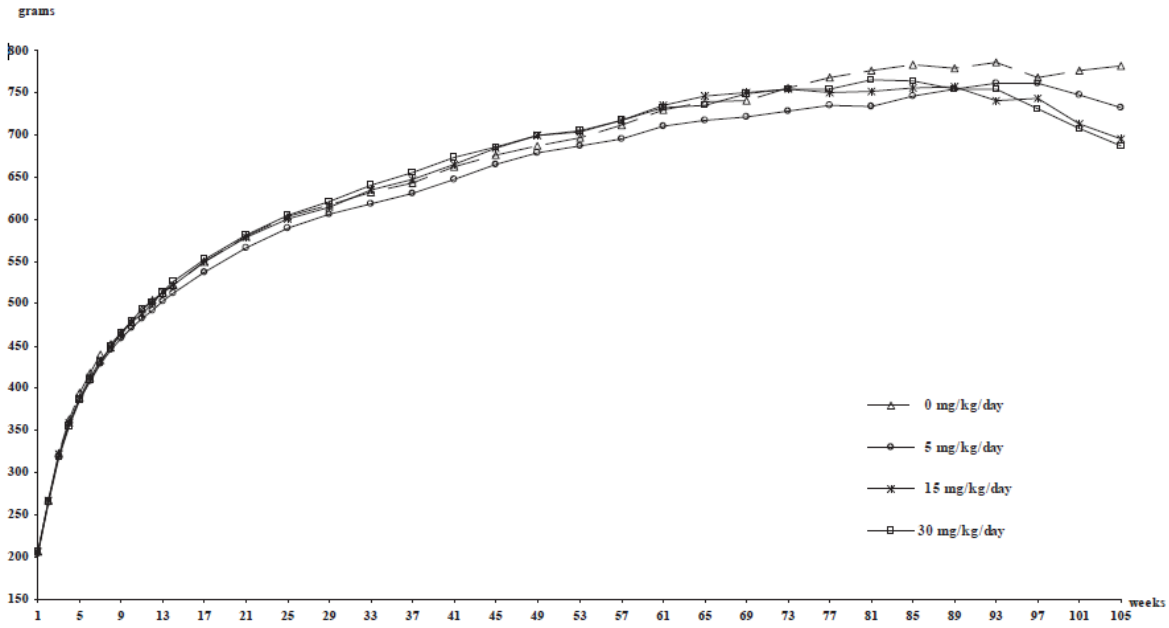
Clinical Signs

The main drug-related clinical signs included increased salivation, alopecia of head, soiled urogenital region and nodosities of the tail. A slight increase in convulsive episodes was observed in HD males and LD and HD females. Although the increase in convulsions lacked a dose-response in females, convulsions have been observed in rats at higher doses in the chronic toxicology studies and are therefore likely drug-related, particularly at the HD. However, some instances of convulsion in the LD female group occurred prior to dosing or during general handling and may be incidental.

Body Weights

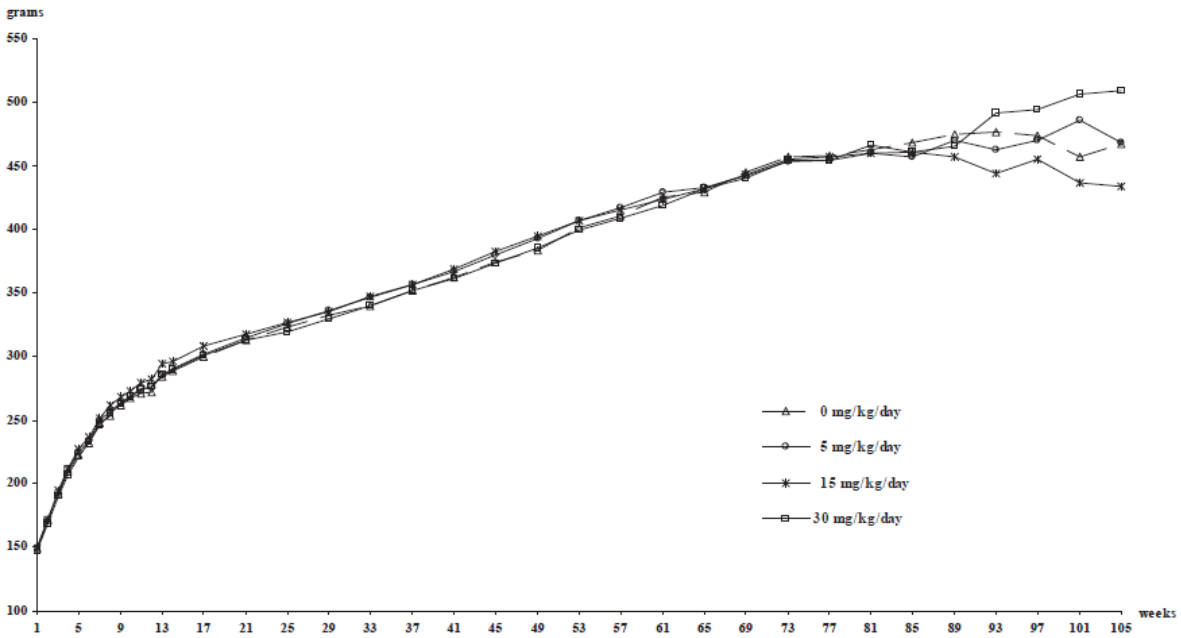
Overall, body weight remained similar for all groups throughout most of the study. By the end of the study, however, a significant reduction in body weight gain of 14.4% and 16.6%, compared to controls, occurred in males of the MD and HD groups, respectively (Figure 4; Table 33). The effects on bodyweight gain were most notable starting week 81 and progressed till the end of the study (week 105). Consequently, absolute body weight was significantly lower than controls in males starting at week 101 in the HD group and at week 105 in MD group. At the end of the study body weight in the MD and HD groups were reduced by 11% and 12%, respectively. No significant effects on body weight or body weight gain were observed at any dose in females (Figure 5).

Figure 4. Change in Mean Body Weight of Males in the 105-Week Rat Carcinogenicity Study



[Excerpted from NDA211150, Study Report (b) (4) 35834-TCR; page 63]

Figure 5. Change in Mean Body Weight of Females in the 105-Week Rat Carcinogenicity Study



[Excerpted from NDA211150, Study Report (b) (4) 35834-TCR; page 63]

Table 33. Mean Body Weight and Mean Body Weight Change in the 105-Week Rat Carcinogenicity Study

Sex	Male				Female			
Dose-level (mg/kg/day)	0	5	15	30	0	5	15	30
Group	1	2	3	4	1	2	3	4
Body weight change								
. Weeks 1/14	317	309	316	320	141	142	148	143
. Weeks 14/25	82	77	78	79	34	35	31	29
. Weeks 25/53	96	96	105	99	80	80	79	80
. Weeks 53/81	73	55	52	58	68	66	60	69
. Weeks 81/97	-3	17	-1	-18	-2	9	-4	22
. Weeks 97/105	-14	-21	-26	-51*	-17	-13	-25	-9
. Weeks 1/105	577	527	494*	481**	320	323	285	359
% difference from controls	-	*-8.7*	*-14.4*	*-16.6*	-	*+1*	*-10.9*	*+12.2*
Body weight								
. Week 1	206	204	206	207	149	149	149	147
. Week 13	513	503	514	514	284	285	294	286
. Week 25	604	590	601	605	323	326	327	319
. Week 53	697	687	704	705	401	407	407	399
. Week 77	769	735	751	754	458	454	457	454
. Week 93	786	761	741	754	477	463	444	491
. Week 101	776	748	714	708*	457	486	437	506
. Week 105	782	732	696*	688**	467	468	434	509
% difference from controls	-	*-6.4*	*-11.0*	*-12.0*	-	*+0*	*-7.0*	*+9.0*

Statistical significance compared to group 1 control: *: p<0.05, **: p<0.01. - : not applicable.

[Excerpted from NDA211150, Study Report (b) (4) 35834-TCR; page 49]

Food Consumption

Food consumption tended to be slightly higher compared to controls in both the male and female HD groups (Table 34). The increase was significant from week 6 in females and week 9 in males with an overall increase of 9% and 5%, respectively. Changes in food consumption did not appear to correlate with mean body weight changes.

Table 34. Mean Food Consumption (g/animal/day) in the 105-Week Rat Carcinogenicity Study

Sex	Male				Female			
Dose-level (mg/kg/day)	0	5	15	30	0	5	15	30
Group	1	2	3	4	1	2	3	4
. Week 1	29.0	28.6	28.8	28.7	19.9	19.8	20.1	19.8
. Week 6	29.3	28.2	28.3	29.8	19.9	20.3	20.8	21.1*
. Week 9	28.7	28.1	28.6	30.2*	20.2	20.8	20.3	21.6*
. Week 13	28.0	27.5	28.8	29.7**	19.8	20.1	20.4	21.3**
. Week 28	28.0	27.3	27.0	29.0	19.2	19.6	19.3	21.1**
. Week 52	28.2	28.5	28.7	30.8**	22.1	22.5	22.8	23.6*
. Week 76	27.9	28.1	29.1	30.3**	22.4	23.0	22.4	23.8
. Week 104	28.9	28.3	26.1	29.9	23.6	26.4	22.7	27.1
Mean Weeks 1 to 104	28.6	28.2	28.7	29.9	20.9	21.5	21.3	22.7
% from controls	-	*+1*	*0*	*+5*	-	*+3*	*+2*	*+9*

-: not applicable.
Statistically significant from controls: *: $p<0.05$, **: $p<0.01$.

[Excerpted from NDA211150, Study Report (b) (4) 35834-TCR; page 49]

Gross Pathology

A slight increase in the number of animals with enlarged livers and kidneys were noted in HD males; however, no correlated microscopic findings were noted. Enlarged ears were observed in slightly higher numbers of HD males. The increase in ear size was correlated to microscopic chondroplasty which is normally associated with regenerative hyperplasia and fibrosis of the ear cartilage after traumatic injury. These macroscopic findings are most likely incidental and not drug-related.

Histopathology

Peer Review

An internal peer review was conducted of at least 10% of the histological slides from each group and for all slides of identified target organs and tumors.

Neoplastic

The statistical analysis of tumor incidence was conducted by Hepei Chen from the Division of Biometrics. An increased incidence of hepatocellular adenomas was found in males from the MD group (Table 35); however, the increase was not statistically significant compared to controls for a common tumor. Based on the lack of statistical significance by both trend analysis and pairwise comparison and the finding only occurring in MD males, this finding was considered incidental and not drug-related.

Table 35. Summary Table of Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship and/or Pairwise Comparisons

Organ name	Tumor name	0 mg Vehicle (C) P - Trend	5 mg Low (L) P - C vs. L	15 mg Mid (M) P - C vs. M	30 mg High (H) P - C vs. H
Male					
Liver	Adenoma, Hepatocellular	0/60 (50)	0/60 (42)	4/60 (42)	0/60 (41)
		0.3355	NC	0.0401 $	NC
Female					
Mammary Glands Are	Adenocarcinoma	5/60 (44)	12/60 (46)	6/60 (44)	13/60 (45)
		0.0807	0.0640	0.5000	0.0353 @
	Adenocarcinoma Arising In Fibroadenoma	4/60 (44)	7/60 (44)	5/60 (43)	3/60 (40)
		0.7128	0.2605	0.4852	0.4461
	Adenocarcinoma/Adenocarcinoma Arising In Fibroadenoma	8/60 (45)	17/60 (48)	10/60 (45)	14/60 (45)
		0.2502	0.0454 @	0.3964	0.1098
Thyroid Glands	Adenoma, C Cell	1/60 (43)	7/60 (43)	4/60 (44)	1/60 (40)
		0.7816	0.0288 @	0.1874	0.7346
	Carcinoma, C Cell	1/60 (43)	1/60 (43)	0/60 (42)	0/60 (39)
		0.8015	NC	0.4941	0.4756
	Adenoma, C Cell/ Carcinoma, C Cell	2/60 (43)	8/60 (44)	4/60 (44)	1/60 (40)
		0.8844	0.0482 @	0.3492	0.4726

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
$ = Statistically significant at 0.05 level in rare tumor for test of pairwise comparison;
@ = Not statistically significant at 0.01 level in common tumor for test of pairwise comparison;

[Excerpted from Hepei Chen's Statistical Review of Rat Carcinogenicity Study; page 7]

Non-Neoplastic

In the HD male group, an Increased incidence of lung granulomas (HDM 19/60 vs 9/60 for male controls) and increased myeloid cells in the bone marrow (HDM 23/60; Male Controls 13/60) compared to controls were recorded. These findings were considered most likely incidental due to comparable incidence between female control and HD groups. Other non-neoplastic findings were of comparable frequency across all groups and are considered common background findings for the age and strain of test animal.

Toxicokinetics

After repeated oral administration, systemic exposure of pitolisant increased rapidly with maximum exposures occurring within 0.33 hours after administration. Plasma concentrations tended to increase in a more than dose-proportional manner. Exposure levels were generally higher at week 52 than week 26. No significant sex difference in pitolisant exposure was observed. Toxicokinetic parameters for pitolisant are summarized in the table below (Table 36). **Reviewer Comment:** The TK parameters for week 104 are based on a single animal per timepoint for each group and are not considered reliable.

Table 36. Toxicokinetic Parameters Following Oral Administration of Pitolisant in the 105-Week Rat Carcinogenicity Study

Sampling period	Dose mg/kg/day	Sex	t_{max} h	C_{max} ng/mL	AUC_{0-t} ng.h/mL	t h	C_{max} / Dose	AUC_{0-t} / Dose	Male / Female C_{max}	Male / Female AUC_{0-t}
Week 26	5	M	1	13.35	14.2	2	2.7	2.8	1.5	2.3
	5	F	0.333	9.10	6.21	2	1.8	1.2	-	-
	15	M	0.5	46.93	48.2	2	3.1	3.2	0.4	0.6
	15	F	0.167	119.54	78.9	2	8.0	5.3	-	-
	30	M	0.167	236.66	358	24	7.9	11.9	1.2	0.5
	30	F	0.333	201.70	652	24	6.7	21.7	-	-
Week 52	5	M	0.333	25.74	12.3	2	5.1	2.5	1.1	0.9
	5	F	0.333	23.87	13.1	2	4.8	2.6	-	-
	15	M	0.333	156.54	329	24	10.4	21.9	1.0	3.2
	15	F	0.333	160.99	102	2	10.7	6.8	-	-
	30	M	0.333	413.41	884	24	13.8	29.5	1.0	0.8
	30	F	0.333	422.09	1145	24	14.1	38.2	-	-
Week 104	5	M	*0.333*	*6.84*	*3.87*	*1*	*1.4*	*0.8*	*0.6*	*3.0*
	5	F	*0.5*	*10.85*	*1.28*	*0.5*	*2.2*	*0.3*	-	-
	15	M	*0.5*	*139.06*	*923*	*24*	*9.3*	*61.5*	*2.0*	*12.5*
	15	F	*0.333*	*67.87*	*73.9*	*2*	*4.5*	*4.9*	-	-
	30	M	*0.333*	*181.82*	*1565*	*24*	*6.1*	*52.2*	*na*	*na*

M: male; F: female, na: not applicable.
Italic: result given for information only (N=1 or 0 per time-point).

[Excerpted from NDA211150, Study Report (b) (4) 35834-TCR; page 52]

Dosing Solution Analysis
All dosing formulations were within ±15% of the target concentration value

8.2 26-Week Carcinogenicity Study by the Oral Route (Gavage) in CB6F1 TgrasH2 Mice

Study no.:	(b) (4) 35833-TCS
Study report location:	EDR, SDN 2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Dosing initiation 7/15/2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Pitolisant, 0001208253, 98.7%
CAC concurrence:	Yes

Key Study Findings

- There were no significant drug-related neoplastic findings following daily oral administration of pitolisant up to 75 mg/kg/day for 26 weeks in Tg.rasH2 mice.

- Increased incidence of bronchi-alveolar neoplasms in both low and mid dose males and high dose females. This finding was not statistically significant by pairwise comparison or trend analysis.
- At the high dose of 75 mg/kg/day, the corresponding plasma concentration level of pitolisant on week 26 is 8562 ng*h/mL, which is approximately 10.6 times the MRHD of 40 mg/day, based on AUC.

Adequacy of Carcinogenicity Study

The 26-Week Tg.rasH2 mouse carcinogenicity study is considered adequate based on appropriate route of administration, dose selection based on dose-range finding study and with ECAC concurrence, and sufficient number of animals for adequate statistical analysis. A positive control group (N-methyl-N-nitrosourea, 75 mg/kg i.p.) demonstrated sensitivity of the test system.

Appropriateness of Test Models

The Tg.rasH2 mouse is an acceptable model. Retrospective quantification of the major human metabolite BP1.8054 indicates sufficient levels in the mouse for adequate carcinogenicity evaluation.

Evaluation of Tumor Findings

Independent statistical review of study results by Hepei Chen from the Division of Biometrics indicates no statistically significant increase in tumor incidence compared to controls in either male or female Tg.rasH2 mice. This conclusion agreed with the Applicant’s analyses.

Methods

Doses:	0 (vehicle control), 15, 30 and 75 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Solution in sterile water
Basis of dose selection:	Dose selection was based on the 4-week dose-range finding study in CB6F1-nonTg.rasH2 mice. For both males and females, the HD of 75 mg/kg/day was selected based on MTD due to CNS clinical signs at 100 mg/kg/day (ECAC concurrence, April 14, 2010,).
Species/Strain:	Mouse/CB6F1-Tg.rasH2 from (b) (4)
Number/Sex/Group:	25/sex/group
Age:	8 weeks old; Older than traditionally used (5-6 weeks) for sufficient body weight to dose
Animal housing:	Individually housed
Paradigm for dietary restriction:	No dietary restrictions; all animals had free access to food and tap water *ad libitum*
Dual control employed:	Positive control; N-methyl-N-nitrosourea (Single i.p. dose at 75 mg/kg) in isotonic saline (0.9% NaCl)
Interim sacrifice:	No
Satellite groups:	Toxicokinetic satellite: 20/sex/group for LD, MD, HD groups; 4/sex/group for controls
Deviation from study protocol:	No significant protocol deviations were noted that would impact study integrity.

Reviewer Comment: At the pre-NDA meeting held on September 7, 2016 with the Division of Neurology Products (DNP), the division requested additional information to determine if separate carcinogenicity studies with the major human metabolites, BP1.8054 and BP1.9733 were needed. The major human metabolite, BP1.8054, was not observed after oral dosing in rats or monkeys and was not quantified in the Tg.rasH2 mouse carcinogenicity study. The metabolite BP1.9733, is a glucuronide metabolite but it was originally unclear if this metabolite was an acyl glucuronide, which would be of greater toxicological concern. The Applicant submitted additional nonclinical information (IND 111842; SDN10) containing retrospective quantification of metabolite BP1.8054 in serum samples from the 26-week Tg.rasH2 carcinogenicity study and structural information on BP1.9733. The following Comments were sent to the Sponsor (11/21/16; by email) based on the review of this information by the nonclinical reviewer from DNP, Dr. Melissa Banks-Muckenfuss:

1. *Based on the summary data provided, the carcinogenic potential of BP1.8054, a major human metabolite, appears to have been adequately assessed in the 26-*

week study in tg.rasH2 mouse. However, a final determination will be a matter of review.

2. *We agree that a carcinogenic assessment of BP1.9733, a major human metabolite, is not needed, based on your confirmation of its structure as an alkyl glucuronide.*

Observations and Results

Mortality

No treatment-related mortalities were reported. A total of 2 males (1 control and 1 LD) and 2 females (2 LD) died in the main study. The single male in the LD group was prematurely terminated due to severe clinical signs including hunched posture, hypoactivity, locomotor difficulties and decreased grasping reflex. The cause of poor clinical condition in this animal was undetermined; however, similar signs have been observed in animals from previous toxicology studies and were attributed to the drug. The other mortalities were due to either incorrect drug administration or spontaneous tumors (malignant lymphoma and splenic hemangiosarcoma). The number of surviving animals at the end of the study were adequate to perform meaningful statistical evaluation.

Clinical Signs

Limited drug-related clinical signs were observed in a small number of predominantly male animals, which included hunched posture, hypoactivity and half-closed eyes (Table 37). Single incidence of clonic convulsions were reported for 1 male and 1 female in the HD group.

Table 37. Summary Table of Relevant Clinical Signs in the 26-Week Tg.rasH2 Mouse Carcinogenicity Study

Sex	Male					Female				
Dose-level (mg/kg/day)	0	15	30	75	MNU	0	15	30	75	MNU
Group	1	2	3	4	5	1	2	3	4	5
Hunched posture		1	2	3	3	1		1		5
Dyspnea			1	2		1			2	3
Piloerection		1		1						1
Thin appearance			1	1				1		
Half-closed eyes				3					1	
Clonic convulsions				1					1	
Hypoactivity		1		3						
Abdominal breathing		1								
Enopthalmos		1								
Lacrimation		1								
Nodosities (scrotum, anus, vulva, urogenital region)					3	2				3

MNU: N-methyl-N-nitrosourea .

[Excerpted from NDA211150, Study Report (b) (4) 35833-TCS; page 43]

Body Weights

Overall bodyweight was only minimally affected by drug treatment (Table 38). In males, slight but significant reduction in body weight (4%) compared to controls was noted in the LD group, whereas body weight change was slightly but significantly higher in the HD group. In females, body weight and body weight gain were generally lower in treated versus controls groups but these changes were not significant by the end of the study.

Table 38. Mean Body Weight and Mean Body Weight Change in the 26-Week Tg.rasH2 Mouse Carcinogenicity Study

Sex	Male					Female				
Dose-level (mg/kg/day)	0	15	30	75	MNU	0	15	30	75	MNU
Group	1	2	3	4	5	1	2	3	4	5
Body weight change (g)										
. Week 1 to 26/27 (Days 182/183)	4.0	3.6	4.3	5.0*	3.8	3.8	3.3	3.2	3.2	3.8
Body weight (g)										
. Week 1 (Day 1)	23.2	22.4*	22.8	22.7	22.9	18.4	18.2	18.4	18.5	18.5
. Week 5 (Day 29)	25.1	24.3	24.4	24.7	23.4**	20.6	19.8**	20.0	19.9*	19.0**
. Week 9 (Day 57)	26.2	25.1**	26.1	26.0	25.7	21.0	20.6	20.6	20.6	21.1
. Week 13 (Day 85)	26.6	25.4*	26.1	26.4	26.3	21.6	20.7*	21.2	20.6**	21.6
. Week 20 (Day 134)	26.9	25.8	26.6	27.4	26.0	22.2	20.9**	21.3**	21.3*	22.3
. Week 26/27 (Day 182/183)	27.2	26.0*	27.1	27.7	26.4	22.2	21.6	21.6	21.7	22.8
% from controls	-	*-4*	*-0*	*+2*	*-3*	-	*-3*	*-3*	*-2*	*+3*

MNU: N-methyl-N-nitrosourea.
Statistically significant from controls: *: $p<0.05$, **: $p<0.01$.

[Excerpted from NDA211150, Study Report (b) (4) 35833-TCS; page 45]

Food Consumption

In general, food consumption in males was slightly lower in drug-treated groups compared to controls and reached significance at various time points throughout the treatment period (Table 39). Food consumption changes correlated with body weight changes. Food consumption in female drug-treated groups was comparable to controls.

Table 39. Mean Food Consumption (g) in the 26-Week Tg.rasH2 Mouse Carcinogenicity Study

Sex	Male					Female				
Dose-level (mg/kg/day)	0	15	30	75	MNU	0	15	30	75	MNU
Group	1	2	3	4	5	1	2	3	4	5
. Week 1	5.8	5.5	5.5	5.0**	5.5	4.9	5.1	4.8	4.9	5.7**
. Week 4	5.1	4.9	5.2	4.6**	4.9	4.8	4.7	5.1	4.9	4.4**
. Week 8	5.1	4.8	4.7*	4.9	4.9	4.9	4.9	4.8	5.1	4.9
. Week 12	4.8	4.5*	4.5*	4.5*	4.7	4.7	4.4	4.4	4.6	4.6
. Week 15	4.7	4.4	4.4*	4.7	4.4	4.4	4.3	4.5	4.5	4.8
. Week 19	4.6	4.5	4.4	4.7	5.0	4.5	4.3	4.3	4.4	4.6
. Week 25	4.5	4.2	4.3	4.4	5.2	4.3	4.4	4.2	4.4	5.8**
Mean weeks 1 to 25	*5.0*	*4.7*	*4.8*	*4.7*	*5.0*	*4.8*	*4.6*	*4.6*	*4.7*	*5.0*

MNU: N-methyl-N-nitrosourea.
Statistically significant from controls: *: p<0.05, **: p<0.01.

[Excerpted from NDA211150, Study Report (b) (4) 35833-TCS; page 45]

Gross Pathology

A significant and dose-related increase in absolute and relative (to body weight) liver weight was noted for both male and females at terminal necropsy (Table 40). The increased liver weight generally correlated to microscopic findings of increased incidence of hepatocellular hypertrophy in treated animals (
Table 42).

Table 40. Liver Weights (% from controls) in the 26-Week Tg.rasH2 Mouse Carcinogenicity Study

Sex	Male			Female		
Group	2	3	4	2	3	4
Dose-level (mg/kg/day)	15	30	75	15	30	75
Exam. animals / Num. of animals	24/25	25/25	25/25	23/25	25/25	25/25
Body weight	-3	-1	+6	0	0	-2
- *Liver*						
. absolute	-1	+4	+13**	+8**	+13**	+15**
. relative	+3	+5**	+7**	+9**	+14**	+17**

Statistically significant from controls: **: p<0.01.
The significance concerned the organ weights values and not the percentages.

[Excerpted from NDA211150, Study Report (b) (4) 35833-TCS; page 49]

Histopathology

Peer Review

An internal peer review was conducted of at least 10% of the histological slides from each group and for all slides of identified target organs and tumors.

Neoplastic

There were no significant drug-related increases in the incidence of neoplastic findings in either males or females of any dose group. An increased incidence of bronchio-alveolar adenoma in LD and MD males and bronchio-alveolar carcinoma in HD females were observed (Table 41); however, these were not statistically significant by either pairwise comparison to controls or by trend analysis. Both neoplastic findings are considered common in Tg.rasH2 mice. The statistical analysis and evaluation of tumor incidence was conducted by Hepei Chen from the Division of Biometrics, these conclusions agreed with the Applicant's analyses

In the positive control group, a high incidence of anticipated neoplastic findings in the thymus, forestomach, skin and Harderian glands demonstrated the sensitivity of the Tg.rasH2 mouse model for carcinogenicity evaluation.

Table 41. Incidence of Bronchi-Alveolar Adenoma and Carcinoma in 26-Week Tg.rasH2 Mouse Carcinogenicity Study

Sex	Male				Female			
Group	1	2	3	4	1	2	3	4
Dose-level (mg/kg/day)	0	15	30	75	0	15	30	75
Number of animals	25	25	25	25	25	25	25	25
. Adenoma; bronchio-alveolar	**0**	**1 (4%)**	**1 (4%)**	**0**	**0**	**0**	**0**	**0**
. Historical control data (Takaoka *et al*., 2003)	mean: 7.2% range: 0-20.0%				Data not shown			
. Historical control data (Kanno *et al*., 2003)	mean: 5.0%				Data not shown			
. Carcinoma; bronchio-alveolar	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2 (8%)**
. Historical control data (Takaoka *et al*., 2003)	Data not shown				mean: 1.7% range: 0-7.1%			
. Historical control data (Kanno *et al*., 2003)	Data not shown				mean: 3%			

[Excerpted from NDA211150, Study Report (b) (4) 35833-TCS; page 51]

Non Neoplastic

Liver
Increased incidence of hepatocellular hypertrophy was recorded in treated animals. The effect was dose-related in males (Appears this way on original
Table 42) and correlated with increased liver weights (Table 40). In females the finding was only present in the HD group; however, all drug treated female groups showed an increase in liver weights.

Testes
Increased incidence of pale basophilic granular bodies in the tubular lumens of the tests was noted in the HD males (Table 43). A similar finding was observed in the 4-week dose-range finding study in CB6F1-nonTgrasH2 wild type mice at doses of 75 and 100 mg/kg/day pitolisant. Although the increased incidence of testicular findings is likely drug-related, the presence of the basophilic bodies in the testes of control animals and a lack of an established dose relationship suggests this finding is of low toxicological significance.

Reviewer Comment: Decreased sperm motility and increased incidence of abnormal sperm morphology were observed in the rat fertility study; however, no apparent macroscopic histopathological findings were noted and no effect on fertility occurred. Combined, these findings may indicate the testes as a potential target of toxicity for the drug.

All other non-neoplastic findings occurred with similar frequency across all groups and are considered common background findings for the age and strain of test animal.

Table 42. Incidence of Hepatocellular Hypertrophy in the 26-Week Tg.rasH2 Mouse Carcinogenicity Study

Sex	Male				Female			
Group	1	2	3	4	1	2	3	4
Dose-level (mg/kg/day)	0	15	30	75	0	15	30	75
Number of animals	25	25	25	25	25	25	25	25
Liver								
. Hepatocellular hypertrophy	1	2	11	23	0	0	0	7

[Excerpted from NDA211150, Study Report (b) (4) 35833-TCS; page 55]

Table 43. Incidence of Testicular Basophilic Bodies in 26-Week Tg.rasH2 Mouse Carcinogenicity Study

Sex	Male			
Group	1	2	3	4
Dose-level (mg/kg/day)	0	15	30	75
Number of animals	25	25	25	25
Testes				
. Basophilic bodies	10	9	12	20

[Excerpted from NDA211150, Study Report (b) (4) 35833-TCS; page 55]

Toxicokinetics

Significant exposure to pitolisant and its major metabolites was demonstrated after daily oral administration in Tg.rasH2 mice. Systemic exposure of pitolisant increased rapidly with maximum exposure occurring within 0.25 to 0.5 hours after administration. Plasma concentrations tended to increase more than dose-proportional between the lower doses and less than dose-proportional between the higher doses. Exposure levels decreased over the duration of the study with drug concentrations being lower at week 26 compared to week 4 and 1. No significant sex difference in pitolisant exposure was observed. Toxicokinetic parameters for pitolisant are summarized in the table below (Table 44).

Table 44. Toxicokinetic Parameters of Pitolisant Following Oral Administration in the 26-Week Tg.rasH2 Mouse Carcinogenicity Study

Sampling period	Sex	Male			Female		
	Dose-level BF2.649 (mg/kg/day) Kinetic parameter	15	30	75	15	30	75
Week 1	C_{max} (ng/mL)	914	2454	3853	560	2171	3793
	t_{max} (h)	0.5	0.25	0.25	0.5	0.25	0.25
	AUC_{0-t}	1724	8190	16654	1055	9359	13031
Week 4	C_{max} (ng/mL)	816	1629	2384	1048	1903	2426
	t_{max} (h)	0.5	0.25	0.25	0.25	0.25	0.25
	AUC_{0-t}	3031	**6950**	14678	3088	12677	12639
Week 26	C_{max} (ng/mL)	491	1021	2584	480	650	1634
	t_{max} (h)	0.5	0.25	0.25	0.5	0.5	0.25
	AUC_{0-t}	**952**	**6679**	9896	996	4010	7228

AUC_{0-t} in ng.h/mL.

[Excerpted from NDA 211150, Study report (b) (4) 35833 – Amendment 1; page 6]

Dosing Solution Analysis

All dosing formulations were within ±15% of the target concentration value

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

9.1.1 BF-2649. Study of Toxicity on Fertility and Early Embryonic Development in Rats by Oral Administration

Study no.:	CD04/9381T
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	07/13/2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Pitolisant, GP001, 99.95%

Key Study Findings

- A single male in the MD group was found dead after 44 days of dosing; no cause of death was determined.
- Adverse clinical signs were observed in most HD males and females and included decreased motor activity and muscle tone, abnormal gait and excessive salivation. Majority of males also presented with involuntary movements, spasms and straub tail.
- During pregnancy, a slight but significant decrease in body weight gain (6%) compared to controls observed in the HD group without a corresponding decrease in food consumption.
- Non-significant decreases in mating, fertility and pregnancy indices observed in the HD group.
- A dose-related increase in post-implantation losses (16% in HD vs. 4% in controls) with a corresponding decrease in percentage of live conceptus, however, these differences were not statistically significant. One HD female had a 100% post-implantation loss.
- A decrease in sperm motility and an increase in sperm abnormalities in 4 males each in the MD and HD groups. These changes did not affect male fertility.
- The NOAEL for male and female fertility and early embryonic development is 30 mg/kg/day, which corresponds to exposures 1X the MRHD of 40 mg/day based on AUC in the 6-month repeat dose rat TK study (Study no. (b) (4) HHL5000) .

Methods

Doses:	0 (Vehicle Control), 30 (LD), 52 (MD) and 90 (HD) mg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral Gavage
Formulation/Vehicle:	Distilled water
Species/Strain:	Rat / HSD: Sprague Dawley from (b) (4)
Number/Sex/Group:	24/Sex/Group
Satellite groups:	None
Study design:	Female SD rats were dosed once daily with pitolisant for 14 days prior to mating, throughout mating, and up through Gestational Day (GD) 8 of pregnancy (Day 0 established by either a sperm positive vaginal smear or presence of vaginal plug). Females were sacrificed on GD 15. Male SD rats were dosed once daily with pitolisant for 4 weeks prior to mating and throughout the mating period. Males were sacrificed after 80% of females were confirmed pregnant. Mating pairs consisted of animals from the same treatment group. Dose levels were selected based on a 90-day repeat dose toxicity study in rats where 90 mg/kg/day caused mild toxic effects, 30 mg/kg was the NOAEL and was selected as the low dose. The middle dose of 52 mg/kg/day is the geometric mean.
Deviation from study protocol:	No significant protocol deviations were noted that would impact study integrity.

Observations and Results

Mortality

No treatment-related deaths were recorded. A single MD male was found dead after day 44 of dosing with no cause of death determined. Therefore, in the absence of deaths in the higher dose this death is unlikely drug related. An additional 5 unscheduled deaths occurred in the study. One HD male died on day 30 due to a dosing error with the animal receiving twice the intended dose (180 mg/kg). Two animals in both the LD and HD group, 1 male and 1 female in each, died and the deaths were attributed to dosing error and supported by the presence of reddish enlarged lungs and other signs of potential gavaging error.

Clinical Signs

Pitolisant at 90 mg/kg/day produced a number of clinical signs, including decreased motor activity, abnormal gait, spasms, involuntary movements, straub tail, hunched posture, bronchial wheezing and excess salivation. Most clinical signs were first

observed between 15 and 45 min after administration and resolved by 2 to 6 h post-dose. A single HD animal presented with sporadic convulsions on day 9 of dosing. Excessive salivation was the most prevalent clinical sign noted in the MD and LD groups with occasional instances of bronchial wheezing and hunched posture.

Body Weight

During the pre-mating period, body weight gain in HD females was significantly higher than in the control group (~ +5%). During pregnancy, HD females had a slight but significant reduction in body-weight gain (~ - 6%) compared to controls. There was no-treatment related effect on body weight gain in the LD and MD females or in males.

Food Consumption

Prior to mating, both sexes in the HD group had a significant increase in food consumption compared to controls at week 2 for females and week 4 for males (approximately +18% for both sexes). After mating, significantly increased food consumption persisted in HD males (~13-20%), however in females the increase was only significant at week 2 of pregnancy (~ 9%).

Toxicokinetics

Toxicokinetics not performed

Dosing Solution Analysis

All dosing formulations were within specified range of ±10% of the target concentration value.

Necropsy

Dose-related increases in reddish discoloration of the stomach and/or duodenum mucosa were found in with 1, 3, and 7 males in the LD, MD and HD groups, respectively; the source or cause was not explained. No drug-related macroscopic necropsy findings were noted in females in any dose group.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

No drug-related effects were observed on estrous cycle or mating times in any dose group. Although not statistically significant, a reduction in mating, fertility and pregnancy indices were recorded in the HD group (Table 45). Two HD females failed to mate after 21 days. Reduced sperm motility due to a significant increase in the number of animals with non-progressive sperm motility was noted in the MD (4 out of 23 males) and HD (4 out of 22 males) groups. In addition, a significant increase in the number of sperm abnormalities including sperm with isolated or misshapen heads or bent and degenerating tails were observed. Nevertheless, these sperm abnormalities did not significantly affect male fertility. A dose-related increase in the percentage of post-implantation losses with an associated decrease in percentage of live conceptus occurred in the mid and high dose groups, however the changes in both parameters were not statistically different than controls (Table 46). No significant changes in reproductive organ weights in either male or females.

Table 45. Summary Table of Female Mating Performance

TREATMENT DOSE mg/kg/day		Mean mating time (days)	No. of females per group	No. of females mated+	No. of pregnant females	No. of females with live conceptus	Mating index %	Fertility index %	Pregnancy index %
CONTROL -	MEAN SD n	3.6 3.76 24	24	24	24	24	100.0	100.0	100.0
BF-2649 30	MEAN SD n	4.2 3.61 23	23	23	23	23	100.0	100.0	100.0
BF-2649 52	MEAN SD n	2.6 1.56 24	24	24	24	24	100.0	100.0	100.0
BF-2649 90	MEAN SD n	4.2 3.86 21	23	21	18	17	91.3	85.7	94.4

+: Female with presence of spermatozoa or vaginal plug

Logrank test χ^2 test

Level of significance:
*: $\alpha < 0.05$ in comparison with the Control group
**: $\alpha < 0.01$ in comparison with respect Control group

[Excerpted from NDA211150, Study Report CD04/9381T; page 53]

Table 46. Summary Table of Female Fertility Parameters

MACROSCOPIC OBSERVATIONS AT HYSTERECTOMY
(Females sacrificed on day 15 of pregnancy)
Mean values

TREATMENT DOSE mg/kg/day		FEMALE	OVARIES		UTERUS		OVARIES	UTERUS					
		Body weight (g)	Absolute weight (mg)	Relative weight % (x100)	Weight without fetus (g)	Weight with fetus (g)	No. corpora lutea	No. total implantations sites	No. of live conceptus	No. of dead conceptus	% preimplantation loss	% postimplantation loss	% of live conceptus
CONTROL	MEAN SD n	301.6 14.75 24	169.40 28.685 24	5.64 1.062 24	3.98 0.643 24	19.89 2.850 24	16.5 1.77 24	14.8 1.66 24	14.3 1.99 24	0.5 0.83 24	9.44 9.150 24	3.87 6.451 24	96.13 6.451 24
BF-2649 30	MEAN SD n	297.0 18.47 23	168.20 40.951 23	5.65 1.305 23	4.22 0.750 23	20.95 1.629 23	15.7 1.40 23	15.0 1.07 23	14.3 1.02 23	0.7 0.71 23	4.38 6.217 23	4.25 4.613 23	95.75 4.613 23
BF-2649 52	MEAN SD n	298.1 16.91 24	188.40 28.369 24	6.33 0.955 24	3.79 1.023 24	18.41 4.574 24	15.8 2.01 24	13.8 2.72 24	12.5 3.36 24	1.3 1.49 24	11.66 17.586 24	10.53 14.504 24	89.47 14.504 24
BF-2649 90	MEAN SD n	300.9 16.31 18	183.92 42.607 18	6.12 1.404 18	3.65 0.834 18	18.04 5.261 18	16.9 1.57 18	14.9 2.47 18	12.3 4.41 18	2.6 4.25 18	11.42 14.118 18	16.44 25.919 18	83.56 25.919 18

Dunnett's test Bonferroni test

Level of significance:
*: $\alpha < 0.05$ in comparison with the Control group
**: $\alpha < 0.01$ in comparison with the Control group

[Excerpted from NDA211150, Study Report CD04/9381T; page 57]

9.2 Embryonic Fetal Development

9.2.1 Rabbit Embryonic and Fetal Development

Study Title: Preliminary Study of Effects on Embryo-Fetal Toxicity in Rabbits by Oral Administration: Dose Range Determination (Study no. CD04/9120T, GLP)

A preliminary dose-range finding study was conducted in pregnant New Zealand White (NZW) rabbits (n=4 to 5) with oral administration of pitolisant at 100, 120, 140, 160 and 200 mg/kg/day from gestation day (GD) 6 to 18, inclusive. A total of 3 unscheduled deaths occurred, 2 at 200 mg/kg after 2 and 6 doses and 1 at 160 mg/kg after 1 dose. One female in the 100 mg/kg/day group was terminated on day 16 of pregnancy due to vaginal blood loss over 3 days resulting from abortion. Clinical signs at the highest dose of 200 mg/kg included decreased muscle tone and motor activity, spasms, opisthotonos, prostration, and cyanosis. In addition to spasms and opisthotonos, convulsions occurred in 1 female after a single administration of 160 mg/kg and the animal died shortly after. No adverse clinical signs at doses ≤ 140 mg/kg/day except for vaginal blood loss in 1 female at 100 mg/kg/day due to abortion. Maternal body weight loss occurred at 160 mg/kg/day (-3%) and 200 mg/kg/day (-10%). After dosing cessation, body weight recovered in the 160 mg/kg/day group but not in the 200 mg/kg/day group. Food consumption correlated with changes in body weight in the highest 2 dose groups. At necropsy, gastric and renal findings were most notable affecting 1 to 2 animals at each dose level except for 120 mg/kg/day. These findings included dark discoloration and erosion of the gastric mucosa and pale coloration of the kidneys and congestive-like renal medulla. Fetal body weight was decreased in the 160 and 200 mg/kg/day groups by 19% and 31%, respectively. Both post-implantation losses and % total resorptions were substantially increased at 200 mg/kg/day. Major and minor malformations were recorded in 1 fetus at 200 mg/kg/day and a total of 5 fetuses from two litters at 160 mg/kg/day. Based on the result of the preliminary study the high dose selected for the main study was 150 mg/kg/day.

Study Title: Study for Effects on Embryo-Fetal Development in Rabbits by Oral Administration (Study no. CD05/999OT, GLP)

Pitolisant was administered orally to pregnant NZW rabbits at 30 (LD), 67 (MD), and 150 mg/kg/day (HD) from gestation day (GD) 6 to 19, inclusive. There were no drug-related mortalities. Two HD females aborted, one on GD 21 and one on GD 25. Additional clinical signs included single incidences of decreased muscle tone and bronchial wheezing. At the HD, a slight decrease in body weight (-2%) occurred during the dosing-period with a correlated decrease in food consumption. After cessation of dosing, both body weight change and food consumption were similar to or higher than controls. No drug-related clinical signs or effects on body weight or food consumption were noted in the LD and MD groups. Similar to the preliminary study, maternal necropsy findings were limited to reddish discoloration of the gastric mucosa and renal medulla. An increase in post-implantation losses and % total resorptions with a corresponding decrease in number of live fetuses occurred in the MD group, however, these effects were not statistically significant and were not observed in the LD or HD groups. Two fetuses from different litters, in the HD group, had major malformations including cleft palate and anasarca. One fetus also had severely dilated cerebral ventricles. An additional fetus had an ectopic kidney, which is considered a minor malformation. Two fetuses, 1 in the LD and 1 in the MD group had minor malformations including shortened tail and paw hyperflexion. In the control group, two fetuses from the same litter had major malformations including forelimb amelia and phocomelia,

adactyly, heart, lung and kidney abnormalities and spina bifida. One fetus from a separate litter had minor malformation of the hind limbs. Delay in skeletal development in the HD compared to controls was indicated by reduced ossification of skull, vertebra and pelvis and variations in sternebra and ribs. No malformations were recorded in the LD or MD groups. Toxicokinetic analysis conducted in this study demonstrated relatively low bioavailability of pitolisant by the oral route with a C_{max} of 53.8 ng/mL and AUC of 283.3 ng*h/mL at the HD of 150 mg/kg/day. In a separate TK study, low bioavailability was confirmed after oral administration of pitolisant at 67 mg/kg/day to pregnant rabbits (Table 47). In rabbits, orally administered pitolisant is rapidly converted to the main metabolite, BP2.951 and a secondary metabolite, BP2.941. BP2.951 is present at relatively low levels in humans (2-4%, Clinical Pharmacology Summary, page 110). Based on the results of this study alternative routes of exposure were investigated in rabbits to achieve higher exposure levels to pitolisant.

Table 47. Toxicokinetic Parameters for Pitolisant and its Main Metabolites in Pregnant Female Rabbits After Single and Repeated Oral Administration at 67 mg/kg/day

	Day 6 of Gestation		Day 19 of Gestation	
	C_{max} (ng/mL)	AUC (ng/mL*h)	C_{max} (ng/mL)	AUC (ng/mL*h)
Pitolisant	13.9	8.2	7.22	NA
BP2.941	42.5	53.7	149	148
BP2.951	1893	5300	2606	6244

AUC_{0-t} for pitolisant, AUC_{0-4h} for BP2.941 and AUC_{0-24h} for BP2.951
NA = not applicable

[Excerpted from NDA211150, Toxicology Written Summary; page 58]

Study Title: Toxicity, Pharmacokinetics in Female Rabbits by Oral, Intravenous, Subcutaneous and Intramuscular Routes (R-BF2-649-XL-017, non-GLP)

A preliminary study was conducted to investigate the TK and toxicity of pitolisant in NZW rabbits after oral, i.v., subcutaneous (s.c.) and intramuscular (i.m.) administration (Table 48). Relatively low plasma levels of pitolisant with high levels of the metabolite, BP2.951, was confirmed after a single oral administration at 67 mg/kg. Following i.v. administration of 1 mg/kg pitolisant, high plasma levels of the parent were measured with only negligible amounts of any metabolites. By the s.c. route, high plasma exposure levels of pitolisant at doses of 10, 15 and 30 mg/kg, were attained. The plasma levels of the main metabolites, BP2.941 followed by BP2.951, were significantly lower than the parent. Acute toxicity occurred at 30 mg/kg, with significant CNS clinical signs including abnormal head posture, abnormal ear movement, and convulsion followed by prostration. Clinical signs occurred within 15 min of drug administration and resolved by 1-hour post-dosing. No adverse clinical signs were recorded at 10 or 15 mg/kg pitolisant. Following i.m. administration, high plasma levels of pitolisant are

attained with lower levels of the main metabolites, BP2.941 followed by BP2.951. At 20 mg/kg pitolisant, i.m., CNS-related clinical signs were recorded in 2 out of 6 animals. One presented with loss of posture and one with convulsions. Premature death occurred immediately after administration in one animal, which may be due to a possible dosing error (i.v. instead of i.m.). Based on the results of this study the i.m. dosing route was selected for subsequent embryo-fetal studies in rabbits.

Table 48. Summary Table of Toxicity and Pharmacokinetic Parameters after PO, IV, IM and SC Administration of Pitolisant in Rabbits

Acute s.c. toxicity	No toxic signs at 10 and 15 mg/kg Transient and reversible CNS toxic signs (salivation, head posture, tonic and clonic convulsions) at 30 mg/kg	
Chronic (16 days) i.m. toxicity	At 20 mg/kg transient and reversible CNS toxic signs (loss of posture, convulsive episods) in two occasions only	
Pharmacokinetics :		
1 mg/kg, i.v.	BF2.649 C_{max} = 1456 ± 125 ng/mL BP2.941 C_{max} = 7.3 ± 0.1 ng/mL	AUC_{0-8h} = 454 ± 122 ng/mL*h AUC_{0-8h} = 7.3 ± 0.6 ng/mL*h
67 mg/kg, p.o.	BF2.649 C_{max} = 30.6 ± 9.8 ng/mL BP2.951 C_{max} = 1323 ± 256 ng/mL => oral bioavailability ~ 1%	AUC_{0-24h} = 333 ± 214 ng/mL*h AUC_{0-24h}= 5114 ± 1198 ng/mL*h
10 mg/kg, s.c.	BF2.649 C_{max} = 499 ± 115 ng/mL BP2.941 C_{max} = 78 ± 13 ng/mL BP2.951 C_{max} = 11.6 ± 0.3 ng/mL => s.c. bioavailability ~ 36%	AUC_{0-24h} = 1610 ± 113 ng/mL*h AUC_{0-24h} = 303 ± 13 ng/mL*h AUC_{0-24h} = 120 ± 8 ng/mL*h
20 mg/kg, i.m. (at Day 16)	BF2.649 C_{max} = 886 ± 177 ng/mL BP2.941 C_{max} = 85 ± 7 ng/mL BP2.951 C_{max} = 44 ± 7 ng/mL => i.m bioavailability ~ 32%	AUC_{0-8h} = 2862 ng/mL*h AUC_{0-8h} = 364 ng/mL*h AUC_{0-8h} = 220 ng/mL*h

[Excerpted from NDA211150, Study Report R-BF2-649-XL-017; page 3]

Study Title: Preliminary Study for Effects on Embryo-Fetal Development by Intramuscular Injection in Rabbits (Study no. 36787 RSL**, non-GLP)**

A preliminary dose-range finding study was conducted in pregnant NZW rabbits (n=5) with intramuscular administration of pitolisant at 5 (LD), 10 (MD) and 20 (HD) mg/kg/day from gestation day (GD) 6 to 18, inclusive. No unscheduled deaths occurred in the study. Clinical signs were limited to local thickening at the injection site. A single female in the HD group appeared emaciated with an absence of feces and blood in the bedding. No abnormal findings recorded in this female at necropsy and no apparent fetal toxicity. During the dosing period significant body weight loss occurred in the MD (-12.5%) and HD (-16%) groups. In the LD group, mean body weight gain was reduced by 71% compared to controls. During the dose-free period body weight gain increased compared to controls in the MD and HD groups, however, mean body weight was still

significantly lower compared to controls. A corresponding decrease in mean food consumption occurred throughout the dosing phase but food consumption exceeded controls during the dose-free period. No macroscopic findings were recorded at necropsy. Pre-implantation losses increased at all doses; however, the losses were within normal control ranges except for the LD group where a single female had 89% pre-implantation loss. Post-implantation losses were also increased in the HD due higher resorptions but again were within normal control ranges. Mean fetal body weight in the HD group was slightly lower (-6%) compared to controls. No malformations or variations recorded in any dose group. Based on the results of this study, 16 mg/kg/day was selected as the high dose in the main rabbit embryo-fetal development study.

Study Title: Study for Effects on Embryo-Fetal Development by Intramuscular Injection in Rabbits

Study no.:	36788 RSL
Study report location:	EDR, SDN 2
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	06/23/2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Pitolisant, 0001208253, 99.92%

Key Study Findings

- 2 females in the HD group and 1 female in the MD group were prematurely sacrificed. 1 HD female was sacrificed due to substantial weight loss and emaciated appearance. The other HD female and the MD female were sacrificed due to abortion.
- 2 females in the HD group had a single incidence of convulsions. Additional clinical signs included emaciated appearance and lack of feces.
- Body weight gain was significantly reduced with an overall body weight loss of 3% and 6% compared to body weight at start of dosing in the MD and HD groups, respectively. During dose-free period, body weight gain was significantly higher in both the MD and HD groups compared to controls indicating recovery. Changes in body weight correlated with changes in food consumption.
- Pre-implantation losses were slightly higher in the HD group compared to controls leading to a significant decrease in the mean number of implantation sites and mean total fetuses per litter. The number of abortions also increased in the MD (N=2) and HD (n=2) groups.
- No drug-related external or visceral abnormalities were noted. Skeletal developmental delay in the HD group was indicated by incomplete ossification of the sternebra (8 fetuses from 4 litters) and forepaw (19 fetuses out of 7 litters) and increased incidence of fused sternebra (6 fetuses from 6 litters) and supernumerary ribs.

- The NOAELs for maternal toxicity and embryo fetal development are 4 and 8 mg/kg/day, respectively. The corresponding exposures based on AUC are 0.6X and 1.3X the MRHD of 40 mg/day, respectively.

Methods

Doses:	0 (vehicle control), 4 (LD), 8 (MD), and 16 mg/kg (HD)
Frequency of dosing:	Daily
Dose volume:	0.333 mL/Kg total volume
Route of administration:	Intramuscular injection into 2 sites
Formulation/Vehicle:	Solution in Sterile Saline
Species/Strain:	Rabbit/New Zealand White
Number/Sex/Group:	20/Group
Satellite groups:	Toxicokinetic satellite group: 4/Group
Study design:	Pregnant rabbits were administered pitolisant at 0, 4, 8 and 16 mg/kg/day by IM injection from gestation days (GD) 6 to 18, inclusive. Necropsy/cesarean was performed on GD 29. Standard in life and terminal parameters were evaluated including mortality, clinical signs, body weight, food consumption, macroscopic necropsy and fetal examinations. Doses were chosen based on significant maternal body weight loss in preliminary dose-range finding studies.
Deviation from study protocol:	No significant protocol deviations were noted that would impact study integrity.

Observations and Results

Mortality

Two females in the HD group were prematurely sacrificed; 1 on GD 24 due to substantial body weight loss, absence of feces (GD21-GD24) emaciated appearance and 1 on GD 25 due to abortion. One female in the MD group was prematurely sacrificed on GD 25 due to abortion. Both females sacrificed due to abortion had significant weight loss with associated reduction in food consumption.

Clinical Signs

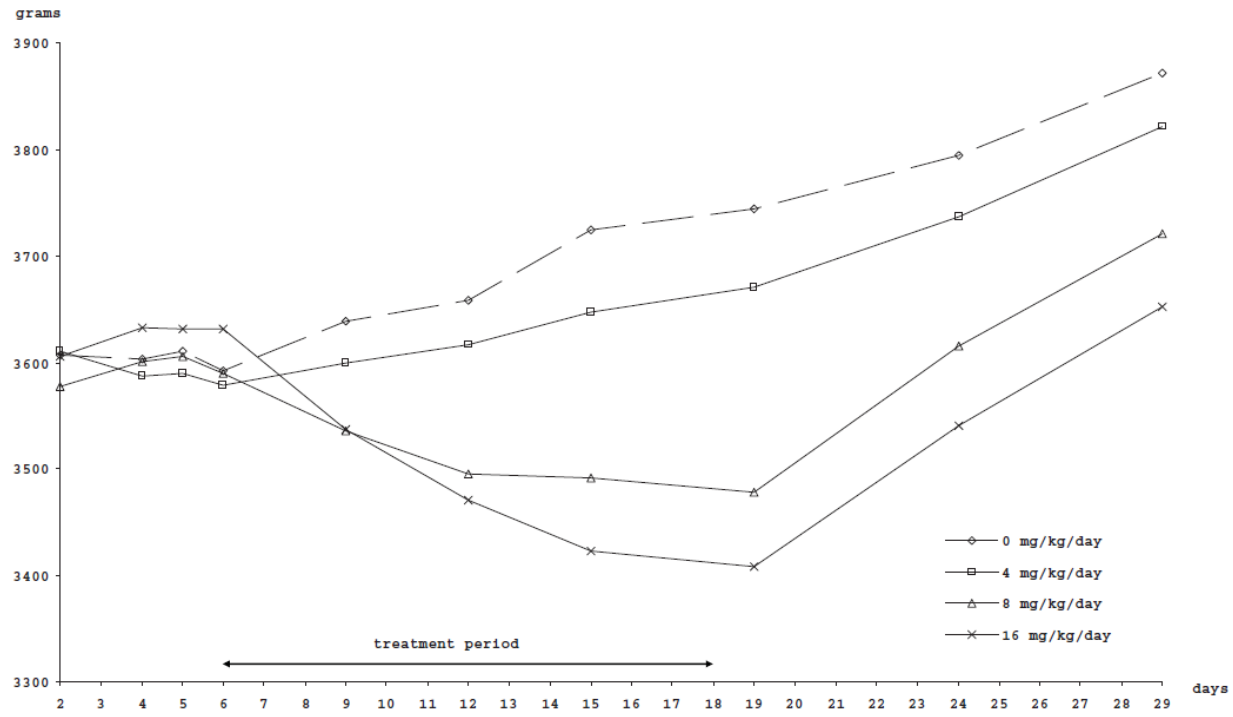
At the HD, 2 females had a single incidence of convulsions (GD11 and GD18). Additional drug-related clinical signs included emaciated appearance (2 at HD) and absence of feces (5 at HD; 4 at MD).

Body Weight

A significant reduction in body weight gain compared to controls resulted in body weight loss in the MD (-3%) and HD (-6%) groups during the dosing phase (GD 6-18) (Figure

6, Table 49). After cessation of dosing (GD 19-29), body weight gain was significantly higher than controls indicating recovery; however, absolute mean body weight in the HD group was still significantly lower (-6 %) than controls. Changes in body weight corresponded to changes in food consumption.

Figure 6. Maternal Mean Body Weight of Rabbits During Pregnancy



[Excerpted from NDA211150, Study Report 36788; page 41]

Table 49. Summary Table of Maternal Mean Body Weight and Body Weight Change in the Rabbit During Pregnancy

Dose-level (mg/kg/day)	0	4	8	16
Mean body weight (g)				
DG 6	3592	3579 (0%)	3590 (0%)	3631 (1%)
DG 9	3639	3600	3536	3537
DG 12	3658	3616	3495*	3470**
DG 19	3744	3670 (-2%)	3478# (-7%)	3408# (-9%)
DG 24	3795	3737	3615*	3541#
DG 29	3872	3821 (-1%)	3721 (-4%)	3652** (-6%)
Mean body weight change (g)				
DG 6 - 9	+47	+21	-54#	-94#
DG 6 - 19	+152	+91	-111#	-223#
DG 19 - 24	+51	+67	+136**	+133**

Statistically significant *versus* control *: p<0.05, **: p<0.01, #: p<0.001 (ANOVA + Dunnett test)
In brackets: differences from controls.

[Excerpted from NDA211150, Study Report 36788; page 36]

Food Consumption

Dose-related reduction in food consumption occurred during the dosing phase (GD 6-19) with differences from controls reaching significance in the MD and HD groups (Table 50). After cessation of dosing, food consumption rebounded, surpassing control levels. The changes in food consumption corresponded to body weight changes.

Table 50. Summary Table of Mean Food Consumption in the Rabbit During Pregnancy

Food consumption (g/rabbit/day)

Dose-level (mg/kg/day)	0	4	8	16
DG 6 - 9	153	140	105#	81#
DG 9 - 12	148	139	79#	62#
DG 12 - 15	112	110	53#	44#
DG 15 - 19	130	123	70**	61#
DG 19 - 24	124	145	148	150
DG 24 - 29	108	119	141*	145**

Statistically significant *versus* control *: p<0.05, **: p<0.01, #: p<0.001 (ANOVA + Dunnett test).

[Excerpted from NDA211150, Study Report 36788; page 37]

Toxicokinetics

Significant systemic exposure to pitolisant was achieved with a maximum exposure occurring within 15 min to 1 h after IM administration (Table 51). After repeated dosing, C_{max} tended to increase less than dose proportionally, while AUC tended to increase more than dose proportionally. There was no apparent accumulation of pitolisant after

repeated dosing; however, metabolite BP2.951, was 1.95-fold higher on GD18 compared to GD 6 at the HD.

Table 51. Toxicokinetic Parameters for Pitolisant and Its Metabolites in Pregnant Rabbits After Single and Repeated IM Administration

Timepoint	Dose (mg/kg/day)		Pitolisant[1]			BP2.941[1]			BP2.951[1]			BP1.3473[2]			BP1.3484[2]		
			4	8	16	4	8	16	4	8	16	4	8	16	4	8	16
Gestation Day 6	T_{max}	h	0.25	0.50	1.00	1.17	0.75	1.00	2.00	2.00	3.09	1.00	2.00	4.00	8.00	8.00	8.00
	C_{max}	ng/mL	261	291	453	25.6	51.3	107	8.80	19.9	35.1	366	382	680	265	502	1691
	AUC_{0-t}	ng/mL*h	561	1135	2874	95.0	215	554	48.3	121	392	2988	4797	9442	4481	8022	25761
Gestation Day 18	T_{max}	h	0.25	0.41	0.38	0.50	0.54	0.38	2.15	2.00	2.00	0.50	2.00	2.00	8.00	4.00	8.00
	C_{max}	ng/mL	200	333	608	43.6	99.4	175	10.1	21.5	40.7	296	389	541	256	571	1990
	AUC_{0-t}	ng/mL*h	466	1074	2974	113	354	964	48.6	107	395	2001	3647	7310	4266	10021	29838

Timepoint	Dose (mg/kg/day)		BP1.8186[2]			BP1.10556[2]		
			4	8	16	4	8	16
Gestation Day 6	T_{max}	h	nc	nc	2.00	1.00	1.00	1.00
	C_{max}	ng/mL	nc	nc	0.32	346	467	772
	AUC_{0-t}	ng/mL*h	nc	nc	0.5	1727	3395	8127
Gestation Day 18	T_{max}	h	nc	nc	2.00	0.5	2.0	2.0
	C_{max}	ng/mL	nc	nc	1.10	250	414	845
	AUC_{0-t}	ng/mL*h	nc	nc	6.5	1398	3278	8321

[1] Source: (b) (4) 36788 RSL, Section 3.2
[2] Source: Bioprojet-Biotech R-B452-2.649

[Excerpted from NDA211150, Toxicology Written Summary; page 60]

Dosing Solution Analysis

All dosing formulations were within specified range of ±15% of the target concentration value.

Necropsy

No drug-related macroscopic findings noted at necropsy

Cesarean Section Data

A dose-related reduction in mean gravid uterus weight was recorded with a significant difference from controls at the HD (-16%). Abortions occurred in a total of 4 dams, 2 each in the MD and HD groups (Table 52). A slightly lower number of corpora lutea were recorded at the HD; however, this is not considered drug-related due to their establishment prior to start of dosing on GD6. Pre-implantation losses were also slightly increased at the HD, resulting in slight but significant reduction in the number of implantations and the number of fetuses compared to controls.

Table 52. Summary Table of Cesarean Parameter Data

Parameter	Dose (mg/kg/day)			
	0 (vehicle)	4	8	16
Females with live fetuses	19	20	18	17
Mean corpora lutea	12.4	12.1	11.4	10.8
Mean implantation sites	10.9	10.4	10.1	8.9*
Mean preimplantation loss (%)	11.4	14	11.2	17.3
Mean Total Fetuses	10.3	10.3	9.6	8.5*
Mean live fetuses (%)	92.5	97.3	89.7	92.4
Mean dead fetuses	5	1	9	4
Abortions	0	0	2	2

*$p<0.05$

[Adapted from NDA211150, Study Report 36788; pages 61-62]

Offspring (Malformations, Variations, etc.)
No drug-related effects on mean fetal body weight, sex ratio, or on incidence of external or soft tissue abnormalities or variations recorded in any dose group. At the HD, skeletal variations including incomplete ossification of sternebre and forepaws and increased incidence of supernumerary ribs was recorded. There was also an increase in the number of fetuses with fused sternebre (6 fetuses from 6 litters). The skeletal findings indicate delayed skeletal development, which is most likely related to maternal toxicity. No drug-related skeletal development changes were observed in the LD and MD groups.

9.2.2 Rat Embryonic and Fetal Development

Study Title: Preliminary Study of Embryofetal Toxicity in Rat (Study no. RR-040120-01, GLP)

A preliminary dose-range finding study was conducted in Sprague-Dawley rats (n=6-10) with oral administration of pitolisant at 30, 75, 100, 140 and 180 mg/kg/day from gestation day (GD) 6 to 17, inclusive. No mortalities or adverse clinical signs were recorded at doses ≤ 100 mg/kg/day. A total of 11 treatment-related deaths occurred, 9 (90%) at the highest dose of 180 mg/kg/day and 2 (22%) at 140 mg/kg/day. Significant adverse clinical signs including unsteady gait, convulsions, mydriasis, lethargy, bronchial murmur and excessive salivation affected a majority of animals in the top 2 dose groups. During the dosing period, mean body weight gain was decreased by 24% and 44% compared to controls at 100 and 140 mg/kg/day, respectively, reaching statistical significance only at 140 mg/kg/day. After cessation of dosing, mean body weight gain slightly recovered at 100 mg/kg/day, but remained statistically lower at 140 mg/kg. Food consumption changes correlated with changes in body weight gain. At

necropsy no significant macroscopic findings were recorded. Dose-related reduction in fetal weight was recorded reaching significance in the 100 and 140 mg/kg/day dose groups. At 140 mg/kg/day, embryo-fetal toxicity occurred with increased number of dead fetuses (10 from 1 litter), increased early resorptions, altered sex ratio (more males than females), and increased incidence of major and minor malformations (all from same litter). Doses ≥ 140 mg/kg/day in pregnant rats resulted in significant maternal and embryofetal toxicity. The high dose selected in the subsequent main embryo-fetal development study in rats was 110 mg/kg/day.

Study Title: Study for Effects on Embryo-Fetal Development in Rats by Oral Administration

Study no.:	RR-040142-01
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	5/6/2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Pitolisant, GP-001, 99.96%

Key Study Findings

- No drug-related mortalities after oral administration of pitolisant up to 110 mg/kg/day from GD6 to GD17.
- One instance of convulsions occurred in the HD group with this animal displaying additional clinical signs including piloerection and straub tail. In the 110 mg/kg/day group, 2 females had single incidence of convulsions shortly after drug administration on GD 10 and GD 17. Yellow feces was the most prominent clinical sign affecting 7 animals in the 110 mg/kg/day group.
- During dosing period significant decreases in mean body weight change of -17.5% and -26.2%, occurred in 90 and 110 mg/kg/day groups, respectively. After cessation of dosing, body weight gain rebounded to control levels in the 90 mg/kg group but remained lower at 110 mg/kg. Changes in body weight gain corresponded to changes in food consumption.
- No fetal mortalities, early and late resorptions or abortions recorded. No drug-related malformations observed.
- Due to significant decreases in food consumption and body weight gain at the higher 2 doses the NOAEL for maternal toxicity was 52 mg/kg, which is approximately 6X and 2X the MHRD based on C_{max} and AUC, respectively. The NOAEL for fetal toxicity was the highest dose tested of 110 mg/kg/day corresponding to an exposure 12X and 4X based on C_{max} and AUC, respectively.

Methods

Doses:	0 (vehicle control), 30 (LD), 52 (MD), 90 (HD), and 110 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/Kg
Route of administration:	Oral Gavage
Formulation/Vehicle:	Solution in distilled water
Species/Strain:	Rat/Sprague-Dawley OFA
Number/Sex/Group:	16/group
Satellite groups:	Toxicokinetic satellite groups: 6/ group/timepoint for LD, MD, and HD; 3/group/timepoint for control
Study design:	Pregnant rats were administered pitolisant at 0, 30, 52, and 90 mg/kg/day by oral gavage from gestation days (GD) 6 to 17, inclusive. Due to a lack of significant toxicity, additional groups were added; a 110 mg/kg/day dose group plus a concurrent control group. Necropsy/cesarean was performed on GD 20. Standard in life and terminal parameters were evaluated including mortality, clinical signs, body weight, food consumption, macroscopic necropsy and fetal examinations. Doses were chosen based on significant maternal toxicity in preliminary dose-range finding studies.
Deviation from study protocol:	No significant protocol deviations were noted that would impact study integrity.

Observations and Results

Mortality

No drug-related mortality was recorded in any dose group. A total of 3 unscheduled deaths occurred in the 90 mg/kg/day groups, 2 females in TK satellite group and 1 female in the main study group. All 3 deaths were attributed to dosing error as evidence by necropsy findings including fluid in the thoracic cavity and/or perforation of the esophagus.

Clinical Signs

In general, the majority of animals presented with no significant drug-related clinical signs. In a single female in the 90 mg/kg/day group, piloerection, straub tail, and convulsions occurred between 6 and 30 min post-dose after 12 administrations. This animal was found dead in cage the following day and death was attributed to dosing error. In the 110 mg/kg/day group, convulsions occurred in 2 females (GD 10 and GD 17) within 30 min following administration. Additional clinical signs in this group were also noted including bronchial wheezing, decreased activity, excitation and yellow feces.

Majority of these clinical signs affected 1 to 2 animals except for yellow feces which was recorded in 7 animals.

Body Weight

Maternal mean daily body weight gain during the dosing phase was significantly lower compared to controls in both the 90 (17.5%) and 110 mg/kg/day (26.2%) dose groups. After cessation of dosing, body weight change was similar to controls in the 90 mg/kg/day group, but in the 110 mg/kg/day, mean body weight gain remained lower (-16%) although not statistically significant. The changes in body weight gain corresponded to significant changes in food consumption in both groups during the dosing and post-dosing phases. Body weight was not significantly affected in the LD and MD groups.

Feed Consumption

Mean absolute daily food intake during the dosing phase was significantly lower compared to controls for both the 90 (13%) and 110 mg/kg/day (~16%) groups. The lower food intake during dosing corresponded with a reduction in mean body weight change. During dosing-free period, daily food intake recovered; although still slightly lower than controls (-5%) the difference was not statistically significant. The recovery in food intake paralleled the recovery in body weight change. In the 110 mg/kg/day group, food consumption remained significantly lower than controls after cessation of dosing, which correlated with a continued lower body weight change during this period. Feed consumption in the LD and MD groups was similar to controls.

Toxicokinetics

After oral administration, pitolisant was rapidly absorbed with peak serum concentrations being reached within 10 to 30 min (Table 53). Serum levels increased after repeated dosing compared to a single dose (C_{max}; 2-3.5 fold and AUC; 2.6 – 5.6 fold) indicating accumulation after multiple doses (Figure 7). Both C_{max} and AUC levels tended to increase dose-proportionally.

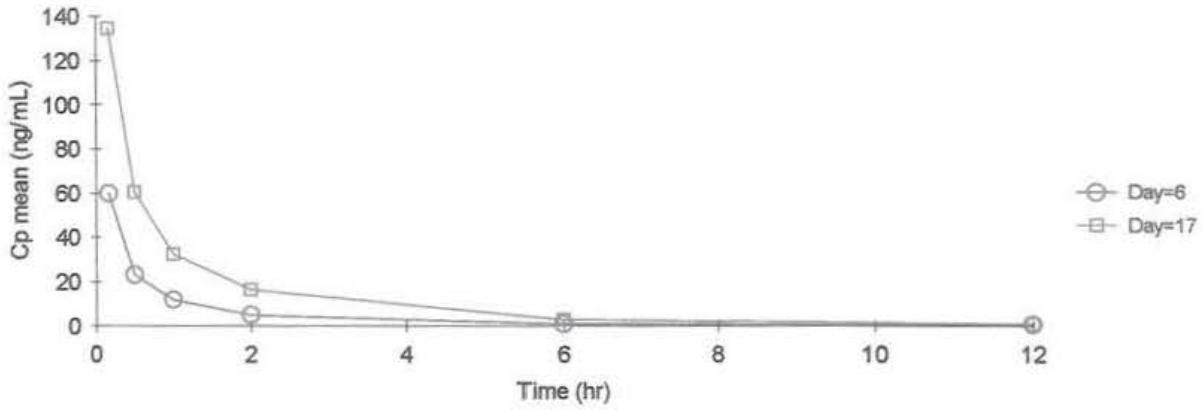
Table 53. Summary of Toxicokinetic Parameters for Pitolisant in Pregnant Rats After Repeated Oral Administration

Tabla 46. Pharmacokinetic parameters

Dose (mg/kg)	Day	$T_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/mL)	SE_C_{max} (hr*ng/mL)	C_{max}_D (kg*ng/mL/mg)	AUC_{last} (hr*ng/mL)	SE_AUC_{last} (hr*ng/mL)	AUC_{last}_D	Vz_F (L/kg)	Cl_F_obs (L/hr/kg)
30	6	3.06	0.17	59.99	24.9	2	54.22	15.03	1.81	2285.79	517.92
30	17	2.61	0.17	134.83	46.15	4.49	143.49	25.9	4.78	765.95	203.3
52	6	2.87	0.17	160.63	61.43	3.09	229.87	85.18	4.42	902.38	217.99
52	17	4.33	0.17	428.09	49.53	8.23	476.53	52.1	9.16	621.43	99.54
90	6	5.16	0.17	306	97.34	3.4	401.24	56.53	4.46	1421.24	190.77
90	17	3.43	0.5	736.92	472.45	8.19	1878.13	654.79	20.87	217.94	44.1
110	6	3.95	0.17	250.41	151.54	2.28	532.29	124.42	4.84	1056.3	185.41
110	17	3.48	0.5	872.79	102.94	7.93	2953.77	377.95	26.85	167.61	33.41

[Excerpted from NDA211150, Study Report RR-040142-01; page 60]

Figure 7. Mean Plasma Concentration of Pitolisant After Single and Repeated Oral Administration of 30 mg/kg.



[Excerpted from NDA211150, Study Report RR-040142-01; page 51]

Dosing Solution Analysis

No data on dosing solution analysis were presented in this study report.

Necropsy

No drug-related macroscopic findings noted at necropsy

Cesarean Section Data

No abortions, fetal mortalities, or late resorptions occurred in any dose group (Table 54). Increased early resorptions were recorded in the LD and 90 mg/kg/day groups; however, these were not statistically significant, and no dose-relationship was established. Mean fetal weight was slightly lower in the 90 mg/kg/day group compared to controls but this difference was not statically significant.

Table 54. Summary Table of Cesarean Parameter Data

Table 22: Hysterectomies: Implant data.

			Exp. group			
			A	B	C	D
Litters examined		Count	N=23	N=22	N=21	N=22
Total abortions		Count	N=0	N=0	N=0	N=0
Implants by litter		Total sum	331	293	302	308 ▪
		Mean	14.4	13.3	14.4	14.0
		SD	(1.5)	(2.0)	(2.2)	(1.7)
Alive fetuses	Bearing litters	Count	N=23	N=22	N=21	N=22
	Absolute frequency	Total sum	320	272	293	293
		Mean	13.9	12.4	14.0	13.3
		SD	(1.7)	(2.9)	(1.9)	(1.8)
	Relative frequency by litter	Mean	96.7%	92.6%	97.2%	95.2%
		SD	(6.0)	(14.8)	(4.2)	(5.9)
Dead fetuses	Bearing litters	Count	N=0	N=0	N=0	N=0
	Absolute frequency	Total sum	0	0	0	0
Late resorptions	Bearing litters	Count	N=0	N=0	N=0	N=0
	Absolute frequency	Total sum	0	0	0	0
Early resorptions	Bearing litters	Count	N=7	N=10	N=7	N=10
	Absolute frequency	Total sum	11	21	9	15
		Mean	.5	1.0	.4	.7
		SD	(.8)	(2.0)	(.7)	(.8)
Sex ratio (males %)		Mean	51.6%	49.7%	50.1%	53.6%
		SD	(11.5%)	(16.8%)	(15.7%)	(15.7%)
Fetal weight (g)	Males + Females	Mean	3.77	3.86	3.87	3.65
		SD	(.20)	(.25)	(.31)	(.48)
	Males	Mean	3.86	3.94	3.99	3.74
		SD	(.19)	(.25)	(.30)	(.49)
	Females	Mean	3.66	3.75	3.77	3.54
		SD	(.23)	(.29)	(.31)	(.50)
Placental weight (g)	Males + Females	Mean	.521	.521	.553	.550
		SD	(.045)	(.053)	(.043)	(.054)
	Males	Mean	.531	.529	.565	.550
		SD	(.052)	(.054)	(.047)	(.047)
	Females	Mean	.511	.509	.543	.548
		SD	(.050)	(.058)	(.036)	(.062)

▪ Total sum Implants= Total alive fetuses + Total early resorptions

[Excerpted from NDA211150, Study Report RR-040142-01; page 33]

Offspring (Malformations, Variations, etc.)

No increase incidence compared to controls in external, visceral, or skeletal malformations and variations were recorded for any dose group.

9.2.3 Additional Embryo-Fetal Development Study

Study Title: Quantification of BP1.8054 in Rabbit Serum From "Study for Effect on Embryo-Fetal Development by Intramuscular Injection in Rabbits (R-B365-1-8054, Non-GLP)

To determine if sufficient exposure levels were attained for the major human metabolite, BP1.8054, in pregnant rabbits following intramuscular administration of pitolisant, plasma levels of BP1.8054 were measured in serum samples collected in the rabbit embryo-fetal development study (Study no. 36788 RSL). At the NOAEL of 8 mg/kg for embryo-fetal toxicity in the embryo-fetal development study, plasma levels of metabolite BP1.8054 corresponded to 0.73X and 0.25X the MRHD of 40 mg/day (human AUC = 338 ng*h/mL) based on AUC at GD 6 and GD18, respectively (Table 55). At the HD of 16 mg/kg/day, significant maternal toxicity occurred with minor effects on reproductive parameters including a slight increase in pre-implantation loss and a reduction in number of implantations and live fetuses. Fetal effects were limited to delayed skeletal development. The exposure to the metabolite BP1.8054 at the HD of 16 mg/kg corresponds to 1.2X and 0.6X the exposure at the MRHD based on AUC.

Table 55. Toxicokinetic Parameters for BP1.8054 in Rabbit Serum from Embryo-Fetal Development Study 36788 RSL

Dose	8 mg/kg/day (NOAEL dose)		16 mg/kg/day (Maternal toxicity)	
Day of Gestation	6	18	6	18
C_{max} (ng/mL)	65.4	20.1	57.6	21.7
T_{max} (h)	1	0.5	1	2
AUC_{0-24} (ng/mL*h)	248	86	407	208

[Excerpted from NDA211150, Study Report R-B365-1-8054; page 32]

Study Title: BP1.8054 – Dose Range-Finding Study by the Oral Route (Gavage) in the Pregnant Rat (Study no. AB20815, Non-GLP)

A preliminary dose-range finding study was conducted in Sprague-Dawley rats (n=6-10) with oral administration of the major human metabolite, BP1.8054, at 30 (LD), 100 (MD) and 300 mg/kg/day (HD) from gestation day (GD) 6 to 17, inclusive. No mortalities or treatment related clinical signs noted for any dose group. Mean body weight gain and mean food consumption were both slightly lower (-6% and -11%, respectively) in the HD group compared to controls during the dosing period. After cessation of dosing both

body weight gain and food consumption recovered. No macropscopic findings noted at necropsy. Pre- and post-implantation parameters and fetal body weight were similar between treated and control groups. A single fetus in the MD group had an external malformation (omphalocele). No other malformation or variations were noted. The C_{max} and AUC plasma levels of BP1.8054 at the HD of 300 mg/kg/day were 22179 ng/mL and 62232 ng/mL*h, respectively. Base on the results of this study, the HD selected for the main embryo-fetal development study with BP1.8054 was 300 mg/kg/day.

Study Title: BP1.8054 – Embryo-Fetal Development Study by the Oral Route (Gavage) in the Rat (Study no. AB20816, GLP)

BP1.8054 was administered orally to pregnant female Sprague-Dawley rats (n=22) at 30, 100 and 300 mg/kg/day from gestation day (GD) 6 to 17, inclusive. No mortalities or clinical signs noted for any dose group. Despite transient dose-related decreases in mean food consumption, no significant effect on mean body weight was recorded. No macroscopic findings noted at necropsy for any dose group. Slight increases in pre- and post-implantation losses were recorded in the LD group but these parameters were not statistically different from controls and/or were within historical control ranges. Mean fetal body weight was slightly but significantly lower in the LD (-6) and HD (-7%) groups. The decrease in mean fetal body weight in the LD group was deemed incidental due to atypically low body weights in two fetuses from separate litters that affected the group mean. There were no external, visceral, or skeletal malformations in any dose group. Consistent with the slight decrease in fetal body weight, a delay in ossification was noted in the HD group. Due to no maternal and fetal toxicity, the NOAEL was the HD of 300 mg/kg with plasma levels of BP1.8054 corresponding to 920X and 472X the MRHD based on C_{max} and AUC, respectively (Table 56).

Table 56. Toxicokinetic Parameters of Metabolite BP1.8054 in Pregnant Female Rats

	Toxicokinetic Parameters (gestation day 17) Mean ± s.d.	
Dose (mg/kg/day PO)	**C_{max} (ng/mL)**	**AUC_{last} (ng/mL*h)**
30	4,078 ± 937	7,669
100	12,585 ± 3,321	39,423
300	36,979 ± 14,075	159,465

[Excerpted from NDA211150, Toxicology Written Summary; page 56]

9.3 Prenatal and Postnatal Development

9.3.1 BF-2649. Study for Effects on Pre- and Postnatal Development, Including Maternal Function in Rats by Oral Administration

Study no.:	CD04/9189T
Study report location:	EDR, SDN 2
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	01/20/2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Pitolisant, GP001, 99.95%

Key Study Findings

- Treatment related mortality occurred after oral administration of pitolisant at 120 mg/kg/day. The surviving animals in the 120 mg/kg group and the entire 60 mg/kg dose group were euthanized after 9 to 10 drug administrations. Two new dose groups were started, 90 mg/kg/day and 52 mg/kg/day.
- At the new HD of 90 mg/kg/day, treatment-related mortality occurred with 7 of 9 deaths resulting from dystocia during delivery.
- Clinical signs in the HD included decreased motor activity, decreased muscle tone, abnormal gait, bronchial wheezing, pallor, spasms and convulsions. Only minimal clinical signs effecting 1 to 2 animals were recorded at the MD of 52 mg/kg/day.
- A slight but significant reduction (-6%) in body weight gain, which correlated to a reduction in food consumption, was recorded in the HD group.
- A severe reduction in the viability index occurred in the HD group due to the loss of 7 dystocic females, fully stillborn litters in 3 additional females and death of all remaining pups due to a lack of milk production or maternal neglect. In the MD group, two females failed to nurse their pups for 2-3 days with one litter not surviving. Three additional litters were mostly devoured.
- At the HD, 18 pups from 4 separate litters had cleft palate and 5 pups from 2 litters displayed abnormal limb flexure.
- F1 generation body weight, at birth, was significantly reduced in the MD (-18%) and HD (-24%) groups compared to controls and remained lower throughout the lactation and post-weaning periods.
- At the MD, physical development was affected with a decrease in (~10%) naso-anal length and a delay in developmental parameters including incisor eruption, eye and ear opening and testes decent.
- Minimal effects on motor and behavioral activity in the MD group, with a slight decrease in the percentage of pups with positive pre-weaning surface and air righting reflex and a consistent increase in post-weaning locomotor activity.
- The NOAEL for maternal toxicity and developmental toxicity of the F1 generation was 30 mg/kg/day. Based on TK analysis in the 6-month repeat dose toxicity study in rats, exposures at the NOAEL dose of 30 mg/kg/day, in females, corresponds to ~1X the exposure at the MRHD of 35.6 mg, based on AUC.

Methods

Doses:	0 (vehicle control), 30 (LD), 52 (MD), 60, 90 (HD) and 120 mg/kg/day (60 and 120 mg/kg/day groups were suspended after 9-10 administrations due to severe maternal toxicity; The new mid and high dose groups of 52 and 90 mg/kg/day were added as replacement groups)
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Solution in distilled water
Species/Strain:	Rat / Sprague Dawley
Number/Sex/Group:	24/Group
Satellite groups:	None
Study design:	Pregnant rats were orally administered pitolisant once daily from implantation on gestation day (GD) 6 through lactation day (LD) 21. Standard parameters were evaluated in the F0 generation including mortality, clinical signs, body weight, food consumption, parturition and lactation. In the F1 generation; physical, sensory and motor development, reproductive capacity and social behavior were evaluated. Body weight and litter size were also recorded for the F2 generation.
Deviation from study protocol:	No significant protocol deviations were noted that would impact study integrity.

Observations and Results

F_0 Dams:

Survival

Treatment-related mortality occurred in both the original HD of 120 mg/kg/day and the new HD of 90 mg/kg/day. At 120 mg/kg, a total of 5 animals died after receiving between 2 to 9 doses. The surviving animals in this group and in the 60 mg/kg/day group were euthanized. In the new HD group of 90 mg/kg, a total of 9 animals died, 7 of which were due to dystocia. During the lactation period, the remaining dams from the HD group were euthanized due to lack of viable pups. An additional 9 deaths combined from all dose groups occurred and deaths were attributed to dosing errors; evidenced by reddish liquid in thoracic cavity, foamy content in trachea or perforations of the esophagus.

Clinical Signs

Severe clinical signs including decreased motor activity, decreased muscle tone, abnormal gait, bronchial wheezing, pallor, spasms and convulsions were recorded in the 120 and 90 mg/kg/day dose groups. In the 60 mg/kg/day group, sporadic instances

of clinical signs similar to those observed at higher doses were noted, minus convulsions. Only minor clinical signs in a few animals were recorded in the MD and LD groups.

Maternal Body Weight
In the HD pregnant females, body weight gain tended to be lower than controls resulting in a slight but significant reduction (6%) in mean body weight compared to controls on day 21 of pregnancy. Reduction in body weight correlated with a reduction in food intake. Body weights during lactation were not recorded in the HD group due to a lack of viable F1 offspring after 4 days postpartum and subsequent termination of females in this group. At the MD, body weight gain was similar to controls during pregnancy; however, tended to be lower during lactation and resulted in a reduced mean body weight compared to controls, which was significant on day 16 of lactation. Again, the reduction in body weight gain during lactation in the MD group correlated to a reduction in food intake.

Food Consumption
Although not significant, reduction in food consumption was recorded in HD pregnant females during the second and third week of pregnancy and in MD females during lactation. The reduction in food intake in these two groups correlated to a reduction in body weight gain.

Maternal Necropsy
Macroscopic examination revealed gastrointestinal findings in the HD group including dark discoloration in the stomach mucosa (15/21 animals), dilation of the stomach (14/21) and all three sections of the small intestine (most prevalent in jejunum; 17/21) with the presence of yellowish liquid. Additional findings included, reddish discoloration of the pancreas (11/21) and renal medulla (4/21) as well as a reduction in the size of the thymus (14/21). Similar findings listed above were noted in 1 to 2 animals from the MD group except for reddish renal medulla which effected 6 out 23 animals. The most prominent finding in the LD group was reddish discoloration of the pancreas effecting 3 out 22 animals.

Natural delivery and litter observations
The pregnancy index was significantly reduced (-57%) in the HD group due to severe reduction in the number of liveborn pups (Table 57). Seven females died during delivery due to dystocia. In 3 of the surviving females, the complete litter was delivered still born. The surviving pups in the HD group died or were devoured within the first 3 days of birth due to a failure in nursing either from a lack of milk production or maternal neglect. In the MD group, mating performance was similar to controls with all females giving birth to live pups (Table 58). However, the viability index at 4 days postpartum significantly decreased due to alteration in maternal behavior. One female lacked milk production and did not nurse, resulting in total loss of the litter by 3 days postpartum. Another female failed to nurse for the first two days after delivery; however, the pups survived. Three additional MD females devoured a significant portion of delivered pups. Mating

performance in the LD group was similar to controls and all dams nursed their offspring normally.

Table 57. Reproductive capacity of F_0 Generation

TRATMENT DOSE mg/kg/day	No. of females with positive mating	No. of pregnant females	No. of females with live pups	Pregnancy index %
CONTROL	24	22	22	100.0
BF-2649 30	26	22	21	95.5
BF-2649 52	24	23	23	100.0
BF-2649 90	22	21 ①	9	42.9*

χ^2 test

Level of significance:
*: $p < 0.05$ in comparison with the Control group

①: Seven females died because of dystocic delivery and two as a result of the treatment

[Excerpted from NDA211150, Study Report CD04/9189T; page 57]

Table 58. Mating performance in F_0 generation

TREATMENT DOSE mg/kg/day		No. of live-born pups on day 0-1 postpartum			No. of stillborn pups on day 0-1 postpartum			No. of live pups on day 4 postpartum			No. of live pups on day 4 postpartum after litter reduction			No. of live pups on day 21 postpartum			Index of live-born pups	Viability index on day 4 postpartum	Viability index on day 21 postpartum	Number of implantation sites	Postimplantation losses (%)
		Males	Females	Total	Males	Females	Total	Males	Females	Total	Males	Females	Total	Males	Females	Total					
CONTROL	MEAN	5.5	6.5	12.1	0.2	0.2	0.4	5.4	6.4	11.8	3.5	4.1	7.6	3.5	4.0	7.5	97.43	97.19	99.24	13.5	9.35
	SD	2.76	2.52	3.37	0.50	0.39	0.73	2.70	2.54	3.45	1.14	0.87	1.18	1.14	0.84	1.26	4.992	5.111	3.553	3.36	11.258
	n	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
BF-2649 30	MEAN	6.1	5.7	12.0	0.2	0.5	0.7	6.2	5.9	12.1	4.1	3.9	8.0	4.1	3.8	7.9	90.07	95.85	98.21	14.2	18.48
	SD	2.00	2.45	3.24	0.52	0.83	0.88	1.47	2.05	2.07	0.65	0.65	0.00	0.70	0.70	0.36	21.024	8.013	4.482	3.00	20.319
	n	22	22	22	20	20	22	21	21	21	21	21	21	21	21	21	22	21	21	22	22
BF-2649 52	MEAN	5.6	6.5	12.4	0.5	0.4	1.1	4.7	5.5	10.2	3.6	3.7	7.4	3.6	3.7	7.4	92.40	79.71*	100.00	14.9	15.95
	SD	1.56	2.39	2.37	0.86	0.67	1.68	2.18	3.07	4.17	0.85	1.42	1.53	0.85	1.42	1.53	10.701	26.689	0.000	1.69	14.859
	n	23	23	23	22	22	23	23	23	23	22	22	22	22	22	22	23	23	22	23	23
BF-2649 90	MEAN	1.8*	1.5*	3.2*	4.4*	3.9*	8.0*	0.0*	0.0*	0.0*	-	-	-	-	-	-	30.92*	0.00*	-	14.3	74.83*
	SD	2.79	1.81	3.98	3.47	3.35	5.16	0.00	0.00	0.00	-	-	-	-	-	-	36.350	0.000	-	2.38	29.551
	n	11	13	13	11	13	13	9	9	9	-	-	-	-	-	-	13	9	-	19	13

Bonferroni test

Level of significance:
*: $p < 0.05$ in comparison of the Control group

[Excerpted from NDA211150, Study Report CD04/9189T; page 58]

F1 Generation:

Body Weight

Mean body weight at birth was significantly reduced in the MD (-18%) and HD (-24%) groups compared to controls and remained significantly lower (-12% at PD21) in the

surviving MD pups prior to weaning (Figure 8). Post weaning, significantly lower mean body weight persisted in males (up to day PD84) and females (up to day PD63) (Figure 9). Mean body weight in the LD group was similar to controls at birth and throughout the lactation and post-weaning periods.

Figure 8. Group Mean Body Weight for F1 Generation Rats During Lactation Period

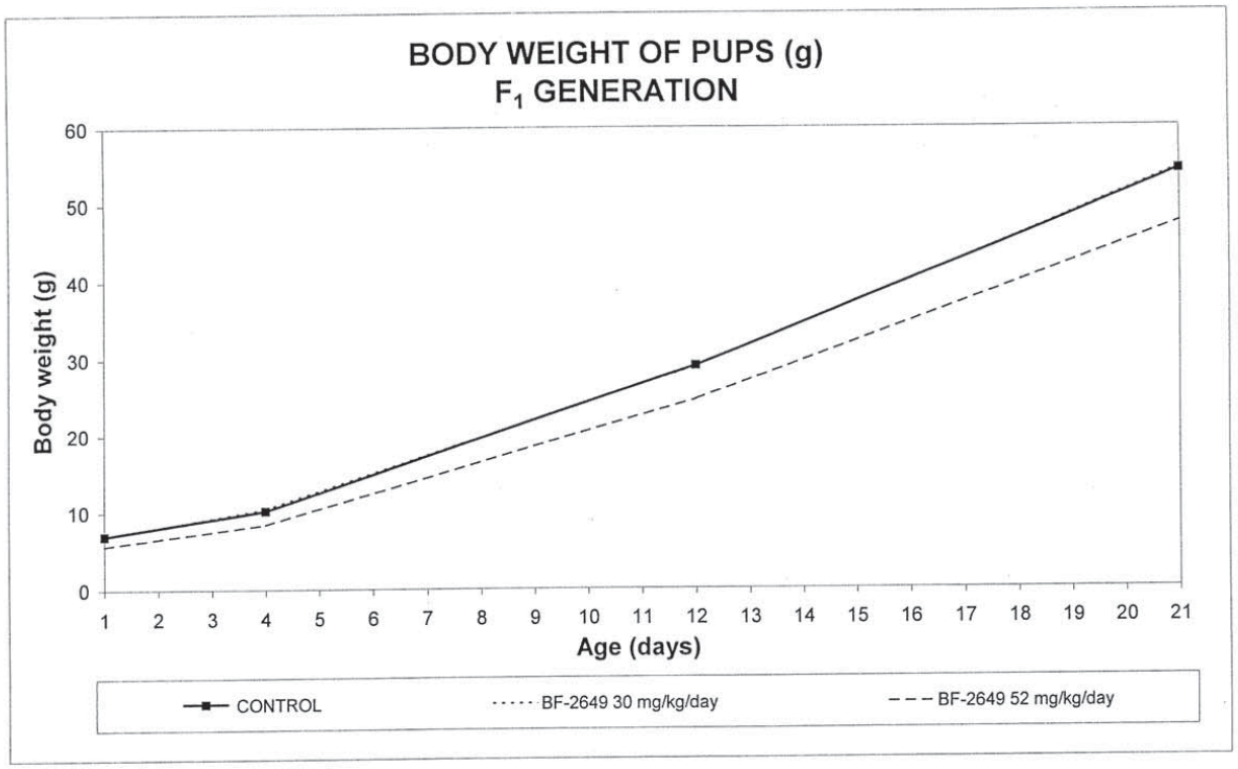
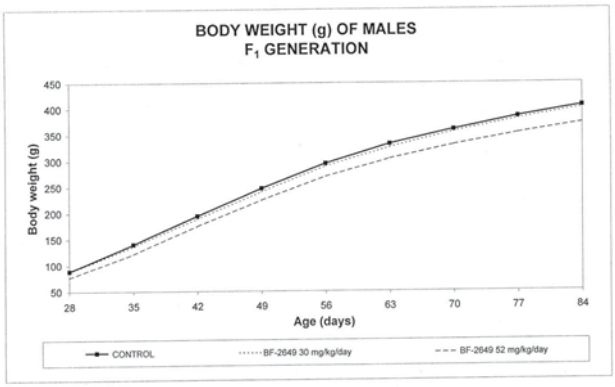
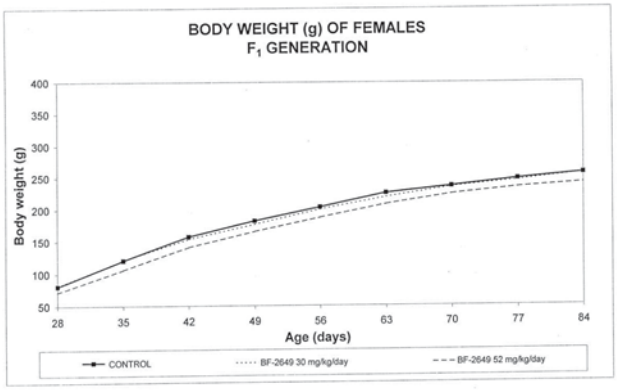


Figure 9. Group Mean Body Weight for F1 Generation Male and Female Rats Post Weaning





Food Consumption

Post-Weaning food consumption was also consistently lower; however, the difference was not statically significant and as the pups aged the difference in consumption between treated and control groups diminished. Food intake in the LD pups was similar to controls.

Physical appearance and development

At the HD, a total of 18 pups from 4 separate litters had cleft palate and a total of 5 pups from two separate litters had abnormal limb flexure. No physical alterations were noted in the MD and LD groups. Overall mean pup size as measured by the naso-anal length was significantly lower (-9 to -10%) in the MD group compared to controls over the first 30 days postpartum; however, when measured at day 60, the mean naso-anal length in the MD group was similar to controls (Table 59). Several physical development parameters, including incisor eruption, descent of the testes, and eye and ear opening, were slightly but significantly delayed in the MD group. No effects on physical development were observed at the LD. Due to lack of surviving pups, physical development in the HD group could not be measured.

Sensory, Motor, and Behavioral Development

No drug-related effects on auditory and visual responses and corneal and pupillary reflexes were recorded. On day 1 post-partum, the percentage of pups with surface righting reflex was significantly lower in the MD group; however, this parameter was similar to controls by day 4. At day 17 of lactation, a slight but significant reduction in the percentage of pups with righting in air flex was lower than controls but was similar to controls on day 21 and beyond. In open field activity, both male and females in the MD group consistently displayed increased locomotion which reached significance on days 21, 24, and 60 after birth. Other significant alterations in open-field behavior parameters were either of minimal magnitude, not consistently affected, and were only recorded in one sex.

Mating/Fertility/Nursing

No drug-related effect on mating, gestation, or pregnancy parameters were observed. All F1 generation females nursed normally.

Necropsy
No macroscopic necropsy findings in the F1 generation rats

F2 Generation Litter Observations:

No drug-related effects on litter size, viability, pup body weight, or sex ratio were observed.

Table 59. Summary Table of Physical Development Parameters for F1 Generation Rats

TREATMENT DOSE mg/kg/day		Age (days) pinna detachment	hair growth	incisor eruption	opening of the ears	opening of the eyes	descent of the testes	opening of the vagina	Naso-anal length (cm) age (days) 1	4	12	30	60	85
CONTROL	MEAN	2.51	3.66	8.80	12.56	16.00	22.72	37.85	5.40	6.38	9.11	14.76	20.63	22.57
	SD	0.745	0.511	1.028	1.005	0.772	0.994	3.724	0.237	0.358	0.547	0.656	0.644	0.466
	n	22	22	22	22	22	22	22	22	22	22	22	22	22
BF-2649 30	MEAN	2.25	3.49	8.93	12.54	16.02	22.90	36.16	5.33	6.39	9.12	14.98	20.79	22.47
	SD	0.509	0.440	0.696	0.873	0.798	0.982	2.813	0.193	0.170	0.308	0.368	0.744	0.824
	n	21	21	21	21	21	21	21	21	21	21	21	21	21
BF-2649 52	MEAN	2.99	3.79	9.79*	13.83*	16.96*	24.09*	39.43	5.01*	5.91*	8.37*	14.26*	20.20	22.21
	SD	0.752	0.333	0.828	0.647	0.668	1.534	4.163	0.203	0.249	0.325	0.484	0.981	0.935
	n	22	22	22	22	22	22	21	23	22	22	22	22	22

[Excerpted from NDA211150, Study Report CD04/9189T; page 71]

10 Special Toxicology Studies

10.1 Phototoxicity

Study Title: Assessment of Phototoxicity Potential of Pitolisant (BF2.649) (Study no. R-BF2.649-MC-001)

A phototoxicity assessment was made for pitolisant hydrochloride by measuring the UV/visible (290-700 nm) spectrum of pitolisant at a concentration of 1 mM in either methanol or phosphate buffer saline (pH 7.4). The absorption maximum wavelength for pitolisant in either solvent was 290 nm and the molar extinction coefficient for pitolisant was calculated to be 68 and 67 L/mol/cm, respectively. According to ICH S10, compounds with a MEC of less than 1000 L/mol/cm do not pose a significant photo safety concern and addition phototoxicity studies are not required.

11 Integrated Summary and Safety Evaluation

Pitolisant is a new molecular entity under development for the treatment of excessive daytime sleepiness and cataplexy in adult patients with narcolepsy. An adequate nonclinical package was submitted under NDA 211150 to allow for a thorough nonclinical safety assessment of pitolisant. For consistency, the nonclinical doses are expressed as salt form unless stated otherwise.

Pharmacology

In vitro radioligand binding assays demonstrated high binding affinity of pitolisant to both human and mouse histamine-3 (H3) receptors (K_i = 1 -2.4 and 5.7 - 14 nM, respectively). In functional assays, pitolisant was shown to be both a potent inverse agonist (EC_{50} of 1.5 nM) and a competitive antagonist (K_B = 0.31 nM). Pitolisant does not bind appreciably to other histamine receptors (H1, H2, or H4 receptors; Ki ≥ 10 µM). In an off-target screen against a panel of receptors and transporters, high binding affinity was noted at sigma 1 and sigma 2 receptors with K_i's in the low nanomolar range similar to the binding affinity to the H3 receptor. Moderate binding was also observed at 5-HT_{2A} and dopamine D3 receptors (K_i = 544 and 380 nM, respectively). Although pitolisant demonstrates moderate to high affinity for these off-target sites, functional assays for these receptors showed minimal functional potency of pitolisant in comparison to that of the primary target. An off-target screen of several prominent human metabolites showed only low antagonist activity (Ki = 271 nM) of metabolite BP2.951 at the serotonin 5-HT_{2A} receptor. Due to relatively low brain penetration by BP2.951, significant off-target activity of BP2.951 at the 5-HT_{2A} receptor is unlikely.

In vivo, the ability of pitolisant to enhance histaminergic neurotransmission was evaluated in both mice and rats through quantification of brain tele-methylhistamine (t-MeHA) levels, the main neuronal histamine metabolite. Following administration of a single oral dose of pitolisant, t-MeHA levels increased significantly with a maximum effect at 90 minutes and a return to control levels by 6 h post-dose. After repeated dosing for up to 17 days, the magnitude of response remained similar to that elicited after a single administration, indicating an absence of tolerance. The calculated ED_{50}s were approximately 2 and 3 mg/kg in mice and rats, respectively. *In vivo* brain microdialysis studies in mice and rats also demonstrated effects of pitolisant on additional neuronal systems including the dopaminergic, noradrenergic and cholinergic systems. In male Wistar rats, pitolisant (10 mg/kg, i.p.) significantly increased extracellular dopamine (+219%), noradrenaline (+208%) and acetylcholine (+293%) levels in the prefrontal cortex and acetylcholine (+269%) in the hippocampus. However, pitolisant did not increase extracellular dopamine levels in the striatum, specifically the nucleus accumbens, a region associated with drug abuse potential. It is unclear if activation of non-histaminergic neurons by pitolisant is due to a direct effect through blockade of H3 heteroreceptors expressed on these neurons or a secondary effect of increased histamine and activation of H1 or H2 receptors.

The wake promoting activity of pitolisant was evaluated in mice and cats through continuous EEG/EMG recoding. In both species, single oral doses (up to 10 mg/kg in cats and 20 mg/kg in mice) increased the duration of wakefulness with an associated

decrease in slow wave sleep and paradoxical sleep (REM). In addition to enhanced wakefulness, pitolisant also caused a decrease in the number of narcoleptic attacks and a reduction in the total duration of narcolepsy when administered in the narcolepsy mouse model (Orexin$^{-/-}$). In the MPTP cat model of parkinsonism, where significant hypersomnia is observed, pitolisant significantly increased wakefulness and suppressed slow wave and REM sleep.

The safety pharmacology of pitolisant was evaluated in the cardiovascular, respiratory, and CNS systems. Pitolisant is a moderate hERG channel inhibitor with an IC_{50} of 1.32 µM. However, in *in vivo* cardiovascular studies in the rat, rabbit and dog, minimal to no effect on QTc intervals and no pro-arrhythmic potential of pitolisant were observed. In telemetered dogs, no QTc effects were observed up to 14 times the maximum recommended human dose of 35.6 mg, based on C_{max}. Respiratory function in anesthetized rats with i.v. infusion up 6 mg/kg of pitolisant was minimally affected with significantly increased tidal volume occurring at 4 and 6 mg/kg, which corresponds to 1 times the MRHD, based on mg/mm^2. In the CNS, pitolisant produced slight sedation with pronounced core muscle hypotony. Pitolisant was also proconvulsant after pentylenetetrazol (PTZ) challenge in mice with increase spasm and tremors at 4 times MRHD and convulsions at 7 times the MRHD, based on mg/m^2.

Pharmacokinetics

Following oral administration of ^{14}C-pitolisant in rats and monkeys, pitolisant and its metabolites were rapidly and effectively absorbed with nearly complete absorption of radioactivity with a T_{max} ranging from 0.25 to 1.5 h. Oral bioavailability of pitolisant, however, was relatively low at 1.5% and 27% in rat and monkey, respectively. This is likely due to extensive first pass metabolism in the liver. Once absorbed, pitolisant and its metabolites are widely distributed to systemic tissues with an apparent volume of distribution approximately 10-fold greater than total body water. In addition to GI tract tissues, high levels of radioactivity occurred in liver and kidney in both species as well as adrenals and pancreas in rats and prostate and seminal vesicles in monkeys. High concentrations of radioactivity were measured in the pigmented portion of the eye (Uveal tract) and levels of radioactivity were greater in pigmented skin/fur compared to non-pigmented tissue; indicating some affinity for melanin. After oral administration of ^{14}C-pitolisant to pregnant rats, radioactivity was measurable in fetal tissue with peak levels occurring at 30 min post-dose. No retention in fetal tissue was observed. Pitolisant is extensively metabolized with a complex species-specific metabolic profile resulting in < 2% recovery of unchanged pitolisant. Pitolisant is primarily metabolized through oxidation followed by glucuronidation and glycine conjugation. The main metabolizing enzyme is CYP2D6 with potential auxiliary metabolism through CYP3A4. The majority of metabolites are inactive at H3 receptors, however, residual activity is present in some metabolites that undergo oxidation alone (BP1.2526). Metabolism is most similar between humans and monkeys with BP1.3484 being the most prominent circulating metabolite in both. Elimination occurs relatively rapidly with a terminal half-life ranging from 1-7 h and a majority of radioactivity (92.1%) being recovered by 24 h after administration. The primary route of elimination is through metabolism followed by urinary excretion in humans and monkeys. In rat, radioactivity is equally eliminated

through urine and feces. Radioactivity was measurable in the milk of lactating rats at concentrations 1 to 3 times higher than measured in plasma.

Toxicology

General Toxicology

Single dose toxicity studies were conducted with pitolisant in mice and rats by both the oral and i.v. routes. Repeat dose toxicity studies were conducted with oral administration of pitolisant in mice up to 4-weeks, in rats up to 6-months and in monkeys up to 9 months in duration. Repeat dose studies were also conducted with oral administration of the disproportionate major human metabolite BP1.8054 in rats up to 13 weeks in duration. Additional studies were conducted to investigate convulsions using single dose i.v. administration of metabolites in both rats and monkeys.

Single dose studies in mice and rats demonstrated the CNS as a major target organ of toxicity for pitolisant. Convulsions followed by mortality occurred soon after oral and i.v. administration of pitolisant in both mice and rats at 500 mg/kg (Oral) and at doses ≥ 10 and ≥ 15 mg/kg (i.v.) in mice and rats, respectively. Additional clinical signs included prostration, tachycardia and increase respiration.

Repeat-dose toxicity studies in mice, rats and monkeys confirmed CNS-related clinical signs including convulsions were the most prominent toxic effect of pitolisant. Convulsions in all species were first observed shortly after administration near T_{max} and usually resolved within 2 to 3 hours after administration. Convulsions were not observed after discontinuation of dosing.

In a 4-week study in mice, pitolisant at 75 and 150 mg/kg/day caused hypoactivity and staggering gait with convulsions occurring in half the mice at the HD. The only other significant effect of pitolisant was increased liver weights (5-12%) at 75 and 150 mg/kg/day with a corresponding microscopic finding of centrilobular hypertrophy.

In rats, pitolisant was administered orally for 13-weeks at 5, 30 and 150 mg/kg/day. Due to mortality the HD was reduced to 100 mg/kg and subsequently reduced again to 75 mg/kg with mortality occurring at both 100 and 75 mg/kg. At ≥ 75 mg/kg/day, prostration, trembling, straub tail and uncontrolled movements with jumping were observed. Similar effects were observed in the 6-month rat study where severe clinical signs including convulsions occurred at 100 mg/kg and convulsion persisted after the dose was reduced to 75 mg/kg. Due to the severity of clinical signs the entire group was prematurely terminated. At 60 mg/kg, convulsions were observed starting on day 89 and persisted sporadically throughout the duration of the study. The cause for the delay in the appearance of convulsions at 60 mg/kg is unclear, however, C_{max} for pitolisant increased between day 1 and week 13 by approximately 40% and 100% in females and males, respectively. By week 26 exposure levels decreased but remained higher than on day 1. Due to the 3-month delay in the appearance of convulsions, an expanded brain histopathology evaluation was conducted using specialized stains and markers to reveal any potential brain lesions. No histopathological findings in the tested brain

regions were observed indicating an absence on neuronal degeneration or inflammation. Additional histopathological findings were observed in the lung consisting of pale foci associated with focal increases in alveolar macrophages. The finding occurred in the majority of females at 60 mg/kg and was still present after the recovery period. The Applicant speculates that due to its cationic amphiphilic nature, pitolisant associates with phospholipids forming micelles that can be recognized by macrophages. The toxicological significance is unclear. The NOAEL was 30 mg/kg/day corresponding to 9 and 1 times the MRHD, based on C_{max} and AUC, respectively.

In monkeys, pitolisant was administered orally for 9-months at 5, 12, and 30 mg/kg/day. At 30 mg/kg/day, CNS-related toxicity occurred with tremors, unsteady gait and convulsions occurring sporadically throughout the study. The NOAEL was 12 mg/kg/day, corresponding to 1 and 0.4 times the MRHD, based on C_{max} and AUC, respectively.

Due to low systemic exposure to the major human metabolite **BP1.8054** after oral administration of pitolisant in toxicological species, separate nonclinical studies with direct dosing of this metabolite were conducted in rat and nonclinical safety of this metabolite was adequately assessed. Significant systemic exposure was attained with all other human metabolites after oral administration of pitolisant resulting in adequate assessment of these metabolites in the nonclinical studies.

Mutagenicity and Carcinogenicity

Pitolisant was not genotoxic in an adequate battery of valid genotoxicity studies including bacterial Ames, in vitro mouse lymphoma, and in vivo micronucleus assays.

Pitolisant did not significantly increase drug-related neoplastic findings in either rats or mice as assessed in the carcinogenicity studies. In Tg.rasH2 mice, pitolisant was administered orally for 26 weeks at doses up to 75 mg/kg/day, which is ~9 times the MRHD of 40 mg/day based on mg/mm^2. The mice displayed similar clinical signs as seen in the general tox studies including hypoactivity, hunched posture and single incidence of convulsions in 2 animals (1 M and 1 F in HD group). The corresponding plasma exposure levels (AUC) after 26 weeks of repeated dosing at 75 mg/kg/day were 9896 and 7228 ng*h/mL in male and female mice, respectively, which are approximately 12 and 9 times the MRHD of 40 mg/day (based on human AUC value of 810 ng*h/mL at steady state). In rats, pitolisant was administered orally for 105 weeks at doses up to 30 mg/kg/day which corresponds to AUC plasm levels of 1145 and 884 ng*h/mL (PK taken at week 52) in male and female rats, respectively, which are approximately 1 times the MRHD of 40 mg/day (based on human AUC value of 810 ng*h/mL at steady state) for both males and females.

Reproductive and Developmental Toxicology

In a rat fertility study, pitolisant was administration orally at doses of 30, 52 and 90 mg/kg/day prior to and throughout mating in males and females and continued through early gestation in females. These doses correspond to approximately 7, 13 and 22

times the MRHD of 40 mg/day, based on mg/m^2. CNS related clinical signs were primarily observed at 90 mg/kg/day and included hypoactivity, decrease muscle tone, abnormal gait, hunched posture, spasms, involuntary movements, and a single instance of convulsions. A dose-related increase in the percentage of post-implantation loss with associated decrease in percentage of live conceptus occurred at 52 and 90 mg/kg/day. Reduced sperm motility and abnormal sperm morphology occurred at 52 and 90 mg/kg/day without significant effects on fertility. The NOAEL for male and female fertility was 30 mg/kg/day, which is approximately 7 times the MRHD, based on mg/m^2.

The embryo-fetal developmental toxicity of pitolisant was assessed in rat and rabbits following administration during organogenesis. Oral administration of pitolisant in rats and intramuscular administration in rabbits produced maternal toxicity at doses ≥ 2 and ≥ 1 times the MRHD of 40 mg/day, respectively, based on AUC. Pitolisant was not teratogenic in either rats or rabbits at doses up to 4 and 1 times the MRHD, respectively, based on AUC. However, delayed skeletal development was observed in rabbits at 4 times the MRHD, based on AUC.

In pregnant rats, pitolisant was orally administered during organogenesis, at 30, 52, 90 and 110 mg/kg/day. No mortalities occurred. Single incidences of convulsions occurred at 90 (1 female) and 110 mg/kg/day (2 females). Significant reduction in body weight gain at 90 (17.5%) and 110 mg/kg/day (26.2%) occurred with a correlated decrease in food consumption. No adverse effects on embryo-fetal development were noted. The NOAEL for embryo-fetal toxicity was 110 mg/kg/day with a corresponding plasma exposure level (AUC) of 2953 ng*h/mL, which is 4 times the MRHD of 40 mg/day.

In pregnant rabbits, pitolisant was orally administered during organogenesis, at 30, 67 and 150 mg/kg/day. Limited maternal toxicity occurred at 150 mg/kg/day, consisting of weight loss (-2%) correlated with decreased food consumption. The incidence of malformations was similar between treated and control groups, however, skeletal development was delayed at 150 mg/kg/day with reduced ossification and variations in sternebra and ribs. Due to rapid conversion of pitolisant to metabolite BP2.951 (a minor metabolite in humans), relatively low plasma levels of pitolisant were attained. A subsequent embryo-fetal study was conducted in rabbits using intramuscular administration. Rabbits were administered pitolisant IM at 4, 8 and 16 mg/kg/day during organogenesis. Maternal toxicity occurred in both the 8 and 16 mg/kg/day groups, with two HD females being prematurely sacrificed due to moribund condition. Convulsions occurred in 2 females in the HD group. Significant body weight loss of 3% and 6% occurred in the MD and HD groups, respectively, which rebounded after cessation of dosing. Body weight changes correlated with changes in food consumption. A total of 4 dams, 2 each in the MD and HD groups aborted. Pitolisant was not teratogenic, however, delayed skeletal development (incomplete ossification and supernumerary ribs) was observed at 16 mg/kg/day. The NOAEL for maternal toxicity and embryofetal development are 0.6 and 1 times the MRHD of 40 mg/day, based on AUC.

In the pre-and post-natal developmental toxicity study, pitolisant was administered orally to pregnant rats from gestation day 7 through lactation day 20 post-partum at doses of

30, 52, and 90 mg/kg/day, which are 7, 13 and 22 time the MRHD, based on mg/m^2. Maternal toxicity occurred at 22 times the MRHD and included death due to dystocia, CNS clinical signs including convulsions, and significant decreases in body weight gain and food consumption. At this maternally toxic dose, fetal toxicity included stillbirths, postnatal pup mortality (due to lack of milk and/or failure of dam to nurse), and decreased pup length and weight. At the maternally toxic dose (22 times the MRHD), pitolisant was teratogenic, causing major malformations including cleft palate in 18 pups from 4 litters and abnormal limb flexure in 5 pups from 2 litters. Toxicity in the F1 generation occurred at the mid and high dose (≥ 13 times the MRHD), with delayed postnatal development including decreased body weight and length compared to controls and delayed incisor eruption and testes descent. Motor development (postural and righting reflexes) was also delayed between days 1 and 17 of lactation. No effect on sexual maturation or reproductive capacity of the F1 generation was observed. The NOAEL for developmental toxicity is the low dose, which is approximately 7 times the MRHD, based on mg/m^2.

Summary of Safety Margins at the NOAELs from Pivotal Studies

The safety margins at the reviewer-determined NOAELs in the pivotal nonclinical studies are summarized in the table below (Table 60)

Table 60. Safety Margins at the NOAEL from Pivotal Nonclinical Studies

Species	Duration	NOAEL (mg/kg/day)	Cmax (ng/mL)	AUC (ng*hr/mL)	Safety Margin (based on Cmax)	Safety Margin (based on AUC)
Human, MRHD 40 mg, Cmax: 73 ; AUC: 810						
General Toxicology Studies						
Rat (PO)	6-month	30	652	837	9	1
Monkey (PO)	9-month	12	77		1	0.4
Carcinogenicity						
Rat (PO)	105-week	30	418	1015	6	1
Tg.RasH2 Mouse	26-week	75	2584	8562	9*	9*
Reproductive and Developmental Toxicity Study						
Rat Seg I Fertility (PO)	Female	30	ND	ND	7*	1
	Male	30	ND	ND	7*	0.8
Rat Seg II (PO)	Maternal	52	428	477	6	0.6
	Embryo-fetal	110	873	2954	2	4
Rabbit Seg II (IM)	Maternal	4	608	1074	8	1
	Embryo-fetal	8	200	466	3	0.6
Rat Seg III (PO)	Maternal	30	ND	ND	7*	1$
	Developmental	30	ND	ND	7*	1$

* = Safety margins based on mg/m^2 body surface area

$ = Safety margins based on toxicokinetic data from the 6-month repeat-dose general toxicology study in rat

ND = Not determined due to lack of available toxicokinetic data in the study

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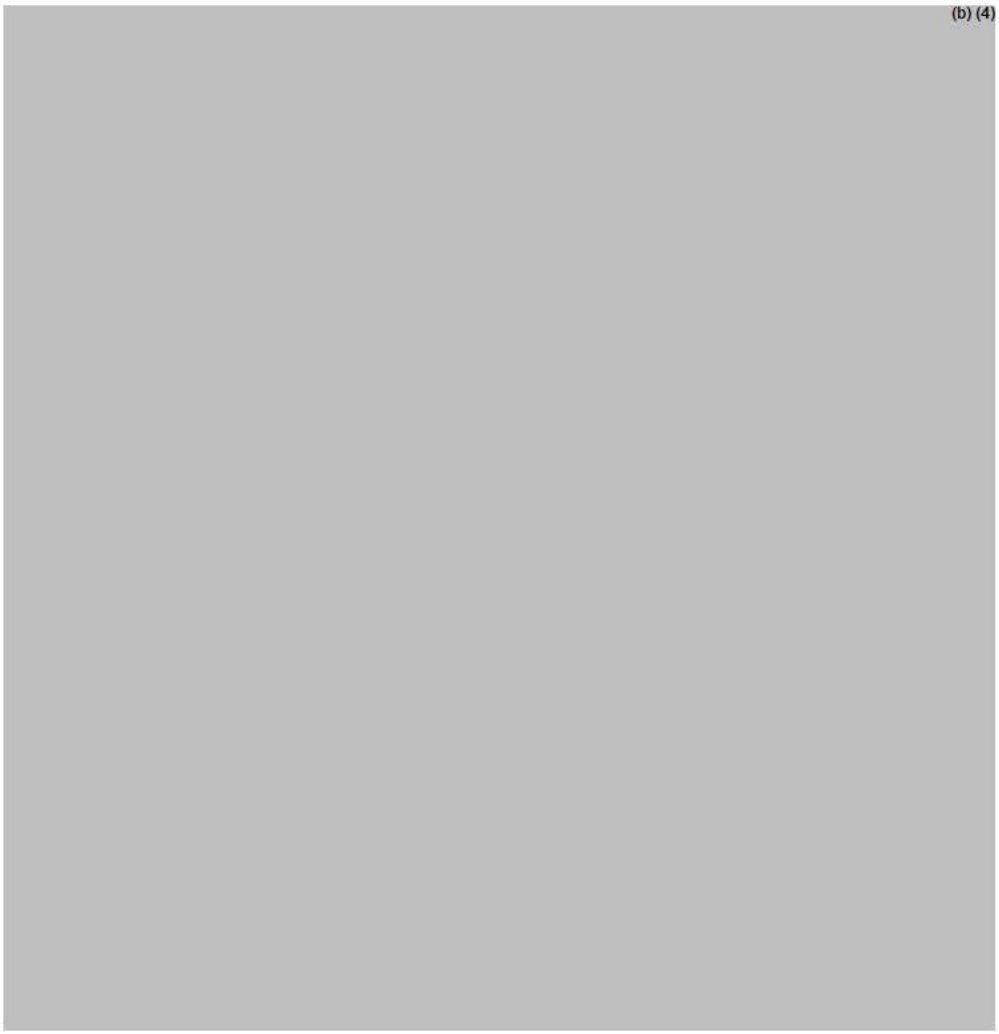
12 Appendix/Attachments

12.1 ECAC Meeting Minutes for the Rat Carcinogenicity Study Protocol



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12.3 ECAC Meeting Minutes for the Final Rat and Mouse Carcinogenicity Studies

Executive CAC Final Study Minutes
Date of Meeting: June 25, 2019

Committee: Karen Davis Bruno, PhD, OND IO,
Chair Tim McGovern, PhD, OND IO,
Member Ron Wange, PhD, OND IO,
Member
Whitney Helms, PhD, DHOT, Alternate Member
Aisar Atrakchi, PhD, DPP, Pharm/Tox Team Supervisor
James Miller, PhD, DPP, Presenting Reviewer

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

Application Type and Number(s): NDA 211150
Drug Name: Pitolisant - Tablet film coated
Sponsor: Bioproject Pharma

Background
NDA 211150 was submitted for priority review on December 14, 2018. This application seeks market approval for pitolisant, a histamine H3 receptor antagonist/inverse agonist, indicated for the treatment of excessive daytime sleepiness and cataplexy in adult patients with narcolepsy. Pitolisant was negative in an adequate battery of in vitro and in vivo genotoxicity assays. The final results from the 2-year rat and 26-week Tg.rasH2 mouse carcinogenicity studies were submitted with this NDA. The statistical analysis of tumor incidence was conducted by Hepei Chen from the Division of Biometrics and her conclusions concurred with the Applicant.

Mouse Carcinogenicity Study
TG.rasH2 mice (25/sex/group) received daily oral administration of pitolisant at 0 (sterile water), 15, 30, and 75 mg/kg for up to 26 weeks. The selected doses were based on a 4-week dose range finding study in CB6F1-nonTg.rasH2 mice and received prior ECAC concurrence. There were no statistically significant drug-related neoplastic findings in either male or female Tg.rasH2 mice after daily oral administration of pitolisant for up to 26 weeks.

Rat Carcinogenicity Study
Sprague-Dawley rats (60/sex/group) received daily oral administration of pitolisant at 0 (sterile water), 5, 15 and 30 mg/kg for up to 105 weeks. The selected doses were based on 3- and 6-month repeat dose toxicity studies and were in concurrence with ECAC recommendations. At week 101, dosing was suspended in the high dose female group after the number of surviving animals in this group dropped to 20. Dose stoppage followed pre-specified criteria in concurrence with ECAC recommendations. Survival analysis indicated a statistically significant increase in mortality in males at the high

dose, however, the number of surviving animals was adequate to perform statistical evaluation. There were no statistically significant drug-related neoplastic findings in either male or female rats after daily oral administration of pitolisant for up to 105 weeks.

Executive CAC Conclusions
Mouse:

- The Committee concurred that the carcinogenicity study was adequate, noting prior approval of the protocol.
- The Committee concurred that there were no drug-related neoplasms in the 26-week Tg.rasH2 mice study in either males or females.

Rat:

- The Committee concurred that the carcinogenicity study was adequate, noting prior approval of the protocol.
- The Committee concurred that there were no drug-related neoplasms in the 105-week rat study in either males or females.

Karen Davis Bruno, PhD
Chair, Executive CAC

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ROBEENA M AZIZ
07/01/2019 11:40:19 AM

KAREN L DAVIS BRUNO
07/01/2019 11:45:02 AM

12.4 Statistical Review for the final Carcinogenicity Review

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Science
Office of Biostatistics

Statistical Review and Evaluation

CARCINOGENICITY STUDIES

IND/NDA Number:	NDA 211150
Drug Name:	BF2.649
Indication:	Treatment of narcolepsy
Studies:	Carcinogenicity Studies in Rats for 104 Weeks and Mice for 26 Weeks
Applicant:	Sponsor: Bioprojet-Pharma 9 rue Rameau 75002 Paris, France Testing Facility for Rat Study: (b) (4)
Review Priority:	Standard
Biometrics Division:	Division of Biometrics - VI
Statistical Reviewer:	Hepei Chen
Concurring Reviewer:	Karl Lin, Ph.D.
Medical Division:	Division of Psychiatry Products
Reviewing Pharmacologist:	James Miller, Ph.D.
Keywords:	Carcinogenicity, Dose response

31 pages have been withheld as a duplicate copy of the "Statistical Review and Evaluation – Carcinogenicity Studies" which is located in the "Statistical Review(s) Section".

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/s/

JAMES A MILLER
07/23/2019 03:07:06 PM

AISAR H ATRAKCHI
07/25/2019 11:43:06 AM