CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

212801Orig1s000

NON-CLINICAL REVIEW(S)

Memorandum

PHARMACOLOGY/TOXICOLOGY MEMO TO FILE

Date:	19 Dec 19
NDA:	212801
Sponsor:	Novartis
Drug:	Osilodrostat
Reviewer:	D. Minck, Ph.D.

Purpose of memo: Based on the potential for [redacted] (b) (4), CMC has asked the sponsor to test 3 batches of API and drug product for [redacted] (b) (4) content (18 Nov 19). The sponsor responded 26 Nov 19 (SDN 38). The purpose of this memo is to provide the nonclinical assessment of the specifications for these derivatives proposed by the sponsor.

Assessment: The drug substance synthetic process involves reaction that may lead to the formation of [redacted] (b) (4) derivatives. The potential [redacted] (b) (4) derivatives are listed below:

[redacted] (b) (4)

The GC MS/MS method used by the sponsor has a LOQ of [redacted] (b) (4) ppm for each [redacted] (b) (4) (validation report VR38469 03 5001229.01). For all API batches tested, the levels were BLOQ so the sponsor did not test drug-product. Based on their results, the sponsor has identified the following specifications for the derivatives:

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(b) (4)

Since the Agency has identified specification limits for other (b) (4) ranging from (b) (4) (b) (4) ng/day, pharmtox agrees with the sponsor proposal for a (b) (4) ppm limit for the potential (b) (4) in osilodrostat as the corresponding daily dose (b) (4) ng/day) is below these levels.

Reference ID: 4537177

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/s/

DANIEL R MINCK
12/19/2019 03:02:21 PM

TODD M BOURCIER
12/19/2019 03:32:56 PM

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:	212801
Supporting document/s:	1 (0000)
Applicant's letter date:	7 Mar 19
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Indication:	Cushing's Disease
Applicant:	Novartis
Review Division:	DMEP
Reviewer:	D. Minck, E. Braithwaite
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1 Executive Summary

1.1 Introduction

Novartis seeks to market osilodrostat for the treatment of Cushing's Disease. Osilodrostat (LCI699) inhibits 11β-hydroxylase (CYP11B1), the enzyme that catalyzes the last step in the biosynthesis of cortisol in the adrenal gland, thereby leading to lower cortisol levels.

1.2 Brief Discussion of Nonclinical Findings

LCI699 was evaluated in a series of nonclinical studies to establish its' pharmacologic, pharmacokinetic, and toxicologic profile.

Inhibition of cortisol synthesis was evident in nonclinical studies. The IC_{50} for inhibition of CYP11B1 was 2.5 nM. A robust and more potent inhibition of aldosterone synthase (CYP11B2) was also evident (IC_{50} = 0.7 nM). LCI699 also inhibits aromatase, but at a higher concentration (IC_{50} = 1.7 µM) than that necessary to inhibit CYP11B1 and CYP11B2. However, this activity is still within 2-fold of C_{max}[1]. As a result, it is not possible to definitively attribute the effects observed in nonclinical studies to inhibition of a specific enzyme.

Adverse effects on the central nervous system (CNS) were seen at high doses in repeat dose toxicity studies in mice, dogs, and NHPs (≥ 26-fold the exposure at the MRHD across species), but were not observed in the single dose rat CNS safety pharmacology study. CNS findings were also noted at lower doses in the mouse carcinogenicity study (2-fold the exposure at the MRHD). The mechanism(s) inducing the CNS effects is not known.

Cardiovascular studies revealed adverse effects at high doses in animal studies. Observations included inhibition of the hERG current at > 50-fold the MRHD, prolongation of the QRS, QT, and QTc levels in dogs at 11-fold following intravenous though not oral dosing, and in NHPs at ≥15-fold the MRHD. In addition, Torsades de Pointes and ventricular tachycardia were seen in NHPs at 100-fold the exposure at the MRHD. However, a thorough QT clinical study revealed no relevant risk of QT prolongation at the MRHD of 30 mg bid (refer to Clinical review for details).

Pharmacokinetic and metabolic profiles were qualitatively similar between humans and nonclinical species used for evaluating the potential toxicity or osilodrostat thereby supporting the translatability of the findings from these studies. Osilodrostat is distributed into most tissues with the highest levels in the adrenal gland, brain, kidney, liver, pancreas, and spleen. LCI699 was found to bind melanin, but as osilodrostat is not strongly absorbed in the 290 nm to 700 nm range there is not a concern for sun sensitivity. Metabolism is primarily via oxidation and glucuronidation.

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[1] Throughout this review the Maximum Recommended Human Dose (MRHD) is considered 30 mg bid (60 mg/day) with a corresponding AUC of 1680 ng·hr/mL and C_{max} of 306 ng/mL. LCI699 total drug and free drug plasma concentrations of are approximately 1.3 µM and 0.8 µM, respectively, at C_{max}.

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Osilodrostat inhibited CYP enzymes 1A2, 2C19, 2D6, and 2E1 at exposures comparable to the exposure at the MRHD and inhibition of additional CYP enzymes (CYPs 2C19 and 2D6) occurred at multiples within 5-fold the clinical exposure at the MRHD; there was no inhibition by the primary metabolite. Clinically relevant inhibition of UCG1A1 also occurred with the metabolite.

The toxicity of osilodrostat was assessed in a series of repeat-dose toxicity studies that were conducted in mice, rats, and dogs, including chronic (rat and dog) and carcinogenicity (mouse and rat) studies. Effects on fertility were evaluated in the rat and embryofetal development was assessed in the rat and rabbit. Across studies, drug-related effects were observed at lower dosages as treatment duration increased. The primary target organs were the adrenal gland, liver, and female reproductive tissues. Adverse effects increased in severity with dosage and study duration, with most demonstrating recovery upon treatment cessation.

In the adrenal glands, increased weights with correlating microscopic changes were seen in multiple species. In rodents, increased adrenal weights, cortical hypertrophy, and/or vacuolation occurred at exposures that were >30-fold the exposure at the MRHD. In contrast, effects on adrenal weight and histopathology occurred at lower exposures in the dog as there were adrenal weight increases (>30% in females) evident at 0.2-fold the exposure at the MRHD, but with corresponding microscopic alterations only present at exposures ≥ 10-fold the exposure at the MRHD. These effects were reversible. The effects on the adrenal are likely related to the pharmacologic activity of the drug and may reflect an adaptive response, with the vacuoles possibly forming as a result of accumulation of precursor lipids secondary to the inhibition of cortisol synthesis.

Effects on rodent, but not dog, livers included increased weights, hypertrophy, and vacuolation, all of which were reversible. In the repeat dose rodent toxicity studies, one or more of these effects were observed at exposures ≥ 7-fold the exposure at the MRHD. However, there were no effects on liver enzymes until exposures were in excess of 100-fold the exposure at the MRHD. In the rodent carcinogenicity studies, these microscopic changes were observed at all dosages administered (≥ 2-fold the exposure at the MRHD). These effects are consistent with adaptive responses to the induction of metabolic enzymes (e.g., CYP2B1, CYP2B2, or UDP-GT) that correlate with hepatocellular tumors in the rodent carcinogenicity studies which could be rodent-specific effects since they were not seen in the dog. Alternatively, they could be related to the metabolite M34.5 which occurs at a higher level in rodents than in humans (and is not seen in dogs).

Observations in female reproductive tissues included reversible effects in the ovary (increased weights, follicular degeneration, prominent corpora lutea) and uterus (increased or decreased weights, atrophy) at exposures that were >0.3-fold those at the MRHD across species. As osilodrostat is also an inhibitor of aromatase (an important enzyme involved in the conversion of testosterone to estradiol), this pharmacologic activity may be the basis for the induction of some of the observed effects.

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Fertility studies revealed effects on female fertility and maintenance of pregnancy (e.g., increased time to mating, decreased mating and fertility, decreased implantation sites, increased resorptions, etc,) at exposures > 100-fold those at the MRHD. Embryofetal development studies revealed increased fetal resorptions and altered morphological development in rats and rabbits at exposures greater than 100-fold and 7-fold, respectively, the exposure at the MRHD. The pre-and postnatal toxicity study revealed prolonged gestation, dystocia, and adverse effects on fetal viability at an exposure that was 37-fold the exposure at the MRHD.

Osilodrostat was not considered genotoxic based on a battery of genotoxic assays.

In the 2-year mouse carcinogenicity study there were statistically significant increases in the incidences of hepatocellular adenomas and/or carcinomas, with a corresponding increase in the incidence of combined hepatocellular adenomas and carcinomas, in males at exposures 6-fold those at the MRHD; no drug-related neoplasms were seen in the female mouse. In the 2-year rat study there was an increase in hepatocellular adenomas in males at exposures 18-fold those at the MRHD and increases in hepatocellular adenomas and carcinomas (individual and combined tumors) in females at 65-fold those at the MRHD. These observations were also associated with drug-related increases in hepatocellular hypertrophy, consistent with a continuum of rodent liver hypertrophy progressing to tumorigenicity. There was also an increased incidence of thyroid follicular cell adenomas, with a corresponding increase in combined adenomas + carcinomas in male rats at exposures 55-fold those at the MRHD.

Based on the absence of a genotoxic signal, the hepatocellular tumors likely were induced by non-genotoxic mechanisms. This interpretation is supported by a gene-profiling study in rats that revealed induction of transcripts encoding CYP2B1, CYP2B2, and Ugt2B1 that are targets of constitutive androstane receptor (CAR) activators. Activators of the CAR are known to induce cell proliferation leading to tumors in both mice and rats, but not humans. There was also slight activation of transcripts for CYP1A1 which is consistent with an AhR mode of action that is relevant to humans. However, the activation was minimal, occurred only at the highest dose administered (90-fold the exposure at the MRHD), and occurred in the absence of increases in other AhR markers. Although the Sponsor did not definitively identify the MOA for tumor development in rodents, the overall weight of evidence indicates that the rodent liver neoplasms were most likely mediated by rodent-specific CAR activation which is not relevant in humans. Consequently, there is not a significant concern for human-relevant liver neoplasms at clinically relevant exposures.

LCI699 was determined to be a strong sensitizing agent (Category 1A) indicating there is the potential for an allergic response following skin contact.

Assessment of Clinical Relevance

The pharmacology studies demonstrated that osilodrostat has the desired cortisol lowering activity although aldosterone levels are also lowered and, at higher drug levels, may also lower estrogens secondary to aromatase inhibition. As a result, one may anticipate some

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degree of unintended pharmacology from potential reduction in aldosterone and estrogens and from any associated compensatory response.

Osilodrostat or its' primary metabolite has the potential to inhibit CYP enzymes 1A2, 2C19, 2D6, and 2E1and UCG1A1 at exposures comparable to the exposure at the MRHD indicating a potential for effecting the pharmacokinetics of drugs metabolized by these enzymes/transporters.

Nonclinical toxicology studies revealed effects in the adrenal, liver, female reproductive tissues, and on embryofetal development. To the extent that these observations are due to excessive reduction in cortisol, such changes would not be expected to occur in the context of Cushing's disease. In addition, the majority of effects occurred at exposures >10-fold the MRHD, although there were some effects at lower multiples. However, since only a limited number of patients are likely to require doses up to the proposed MRHD of 60 mg/day (refer to the Clinical review), the actual safety margins at the nonclinical NOAELs and LOAELs identified in healthy animals are likely to be at least 2-fold higher for the vast majority of patients that would be exposed to LCI699.

Effects on embryofetal viability and morphological development in rats and rabbits were evident, though sensitivity differed between the species. In rabbits, exposures <10-fold the MRHD were adverse to fetal development. Given the role of cortisol in human fetal development, fetal exposure to LCI699 may present a risk which will be addressed in labeling.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data support marketing approval of osilodrostat for the treatment of Cushing's Disease.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Comments on the Sponsor's proposed text for sections of the label containing nonclinical material (sections (b) (4) 6, 8, 12, and 13) are provided below.

(b) (4)

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6 ADVERSE REACTIONS

Clinical will need to comment on the appropriateness for identifying hypocortisolism and QT prolongation as adverse reactions. (b) (4)

(b) (4)

8. USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Based on the (b) (4) , the inclusion of text in Section 8.1 is appropriate. The proposed text will need to be revised.

8.2 Lactation

Minor revisions to proposed text will be made.

(b) (4)

8.4 Pediatric Use

(b) (4)

12 CLINICAL PHARMACOLOGY

(b) (4)

13 NONCLINICAL TOXICOLOGY

More information than necessary was proposed by the sponsor for inclusion. Section will need to be revised to focus on risk of carcinogenicity, mutagenicity, and impairment of fertility.

2 Drug Information

2.1 Drug

CAS Registry Number: 1315449-72-9

Generic Name: Osilodrostat

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Code Name: LCI699, LCI699 Phosphate, LCI699 Phosphate salt, LCI699-AZA, LCI699-D12, NVP-LCI699-AZA, NVP-LCI699-NX-1

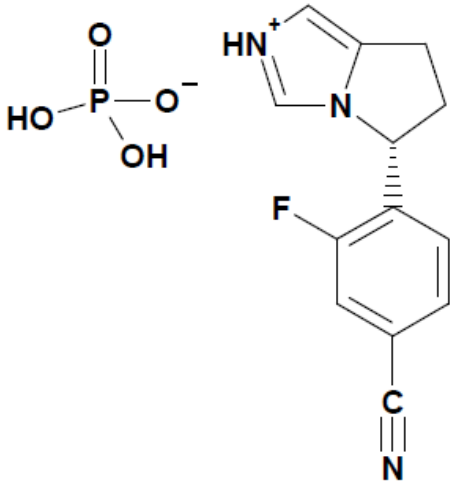
Chemical Name
CAS: 4-[(5R)-6,7-Dihydro-5H-pyrrolo[1,2-c]imidazol-5-yl]-3-fluorobenzonitrile phosphate

IUPAC: (5R)-5-(4-cyano-2-fluorophenyl)-6,7-dihydro-5H-pyrrolo[1,2-c]imidazolium dihydrogen phosphate

Osilodrostat has one chiral center and the *R*-enantiomer (b) (4) used as the drug. The *S*-enantiomer is considered an impurity and is limited to ≤ (b) (4) %. See section 2.5.

Molecular Formula/Molecular Weight: $C_{13}H_{10}N_3F{\cdot}H_2PO_4$ / 325.2 (b) (4) (salt), (b) (4) (base)

Structure or Biochemical Description:



Pharmacologic Class: Cortisol synthase inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 117489 supported this NDA.

(b) (4)

2.3 Drug Formulation

Osilodrostat is formulated as film-coated tablets in immediate release dosage forms for oral administration at strengths of 1, 5, and 10 mg. The composition of the drug product is summarized below:

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Table 1: Composition of Drug Product

Table 1-2 Composition of the drug product

Ingredients	Amount per 1 mg film-coated tablet (mg)	Amount per 5 mg film-coated tablet (mg)	Amount per 10 mg film-coated tablet (mg)	Function	Reference to standards
Tablet core					
Osilodrostat phosphate (corresponding to Osilodrostat free base)	1.4 (b) (4) (1.000)	7. (b) (4) (5.000)	14.3 (b) (4) (10.000)	Active ingredient	Novartis monograph
(b) (4) Microcrystalline cellulose				(b) (4)	Ph. Eur., USP/NF
Mannitol					Ph. Eur., USP/NF
Magnesium stearate					Ph. Eur., USP/NF
(b) (4) / Colloidal silicon dioxide					Ph. Eur., USP/NF
Croscarmellose sodium					Ph. Eur., USP/NF
Weight of tablet core:					
Film-coat [1]					
Hypromellose (b) (4)					Ph. Eur., USP/NF
Titanium dioxide (b) (4)					Ph. Eur., USP/NF
(b) (4) Ferric oxide (yellow)					Regulation (EU) 231/2012 [a], USP/NF
(b) (4) Ferric oxide (red)					Regulation (EU) 231/2012 [a], USP/NF
(b) (4) Ferrosoferric oxide					Regulation (EU) 231/2012 [a], USP/NF
(b) (4) Polyethylene glycol (PEG) 4000					Ph. Eur., USP/NF
Talc					Ph. Eur.. USP/NF
(b) (4)					Ph. Eur., USP/NF
Total coating weight:					
Total weight of film-coated tablet:	**95.00**	**118.00**	**234.00**		

(b) (4)

[2] E: Official European Union numbering system

(b) (4)

[a] Regulation (EU) 231/2012: Commission regulation (EU) laying down specifications for food additives

Sponsor table

2.4 Comments on Novel Excipients

All excipients are USP/NF and used at levels within that used previously for other products.

2.5 Comments on Impurities/Degradants of Concern

Multiple potential and actual impurities could be/were formed at various stages during the synthesis of osilodrostat. However, the vast majority were removed at later steps and relatively few were actually found in batches of drug substance or drug product. There are no significant safety concerns associated with the proposed impurity specification levels.

Impurities in drug substance were evaluated as per ICH Q3A for a compound to be administered at a dose as high as 60 mg/day (reporting threshold 0.05%, identification threshold 0.1%, and qualification threshold 0.15%) while drug product was evaluated in accordance with ICH Q3B (reporting threshold 0.1%, identification threshold 0.2%, and qualification threshold 0.3%).

The *S*-enantiomer (LCI698) is considered an impurity (impurity 531-07) in the drug substance. This impurity is an inhibitor of aldosterone synthase (CYP11B2) and aromatase.

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A level of (b) (4)% was qualified in nonclinical toxicology studies (batch 0650001) and a limit of ≤ (b) (4)% has been set for clinical batches.

In drug substance, the related substances (b) (4) were detected at levels up to (b) (4)%, below the levels qualified in toxicology studies (b) (4) respectively). The specification levels are set at (b) (4)% and (b) (4)%, respectively.

In drug product, the degradation products (b) (4) and (b) (4) were detected at levels < (b) (4)%. These were qualified in tox studies at levels of (b) (4)% and (b) (4)%, respectively. Impurity (b) (4) is a potential impurity that was qualified at a level of (b) (4)% that was observed in stressed batches of test substance but not under long term stability testing. The specifications for (b) (4) have been set at NMT (b) (4)%, (b) (4)%, and (b) (4)%, respectively, well within the limits qualified in toxicology studies. Refer to section 10.4 for details.

Several (b) (4) were reported during the manufacture of osilodrostat that are controlled to sufficiently low levels in the final product. (b) (4) (b) (4) tested positive in the *in vitro* reverse mutation mutagenicity assay (Ames test) but are limited to ≤ (b) (4)%. (b) (4) a known mutagen, is limited to ≤ (b) (4) ppm.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed patient population is individuals with Cushing's disease. The recommended starting dose is 2 mg bid with gradual titration (1 or 2 mg bid) until normal cortisol levels are achieved. The maximum proposed dose is 60 mg/day (30 mg bid). In patients receiving this dose (Study 2108), the C_{max} and AUC at steady state were 306 ng/mL and 1680 ng·hr/mL, respectively. Plasma protein binding in humans is approximately 37%.

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Table 2: Clinical Exposure Predictions – Study 2108

Table 11-9 PK parameters for osilodrostat on Day 15 (PAS)

Parameter	Statistics	Osilodrostat
AUCtau (ng*hr/mL)	n	19
	Mean (SD)	1680 (493)
	CV%	29.3
	Geo-mean	1620
	Geo-CV%	26.6
	Median	1570
	Min-Max	1050-3190
Cmax (ng/mL)	n	19
	Mean (SD)	306 (71.7)
	CV%	23.4
	Geo-mean	299
	Geo-CV%	23.0
	Median	294
	Min-Max	219-440
Tmax (hr)	n	19
	Median	1.01
	Min-Max	0.50-2.02

n = number of subjects with corresponding evaluable PK parameters.
Sponsor table

Because oral contraceptives were also administered in study 2108, the sponsor conducted pk modeling to estimate the exposures at various doses not using data from this study. In their modeling, the projected C_{max} and AUC at steady state were 232 ng/mL and 1372 ng·hr/mL, respectively following a 30 mg bid dosing regimen. These values were approximately 18% to 25% lower than those measured in study 2108. However, as the measured data are higher, their use provides a more conservative assessment for the determination of exposure multiples. For this reason, the data from study 2108 are used for all safety margin determinations in this review.

2.7 Regulatory Background

Summarized below are the major project milestones and interactions with the sponsor in which nonclinical issues were a key component:

- IND 117489 filed 30 May 13.
- (b) (4)
- End of Phase 2 (EOP2) meeting held 9 Oct 13. FDA agreed that conducted/proposed studies appear adequate. Also requested additional mechanistic studies to evaluate cardiovascular effects.
- Based on mortality in ongoing mouse carcinogenicity study, sponsor submitted a termination plan 29 Aug 14. FDA recommendations sent to sponsor 4 Sep 14.
- Sponsor submitted safety report regarding observations from rat and mouse carcinogenicity studies 2 Oct 15. Information request for studies investigating liver and thyroid tumor pathways sent to sponsor 17 Dec 15.

Reference ID: 4532599

- A preNDA meeting package was submitted 19 Jul 18. Included question on acceptability of nonclinical program. Preliminary comments sent to sponsor 17 Aug 18. Agency agreed that package appears adequate to support filing. Also included a comment that NDA needs to include support for proposed tumorigenic mechanism. The preNDA meeting was held 20 Aug 18; no further discussion of nonclinical issues.
- NDA submitted 7 Mar 19.
- Additional study investigating potential for AhR induced tumor formation requested at mid-cycle NDA review on 28 Aug 19.

3 Studies Submitted

3.1 Studies Summarized

Pertinent nonclinical studies supporting this marketing application have been fully reviewed within this document and/or were previously reviewed under IND (b) (4) 117489.

3.2 Studies Not Reviewed

Analytical method development/validation, acute toxicity, range-finding, and repeat dose toxicity studies other than the chronic toxicity studies are not summarized within this review. These studies may have been summarized in previous reviews of studies submitted to the IND (b) (4) filed for LCI699.

3.3 Previous Reviews Referenced

Most studies supporting this NDA were originally submitted to IND (b) (4) 117489 where they were reviewed by (b) (4) Dr. J Hawes (reviews of IND 117489 dated 28 Jun 13 and 3 Oct 13).

4 Pharmacology

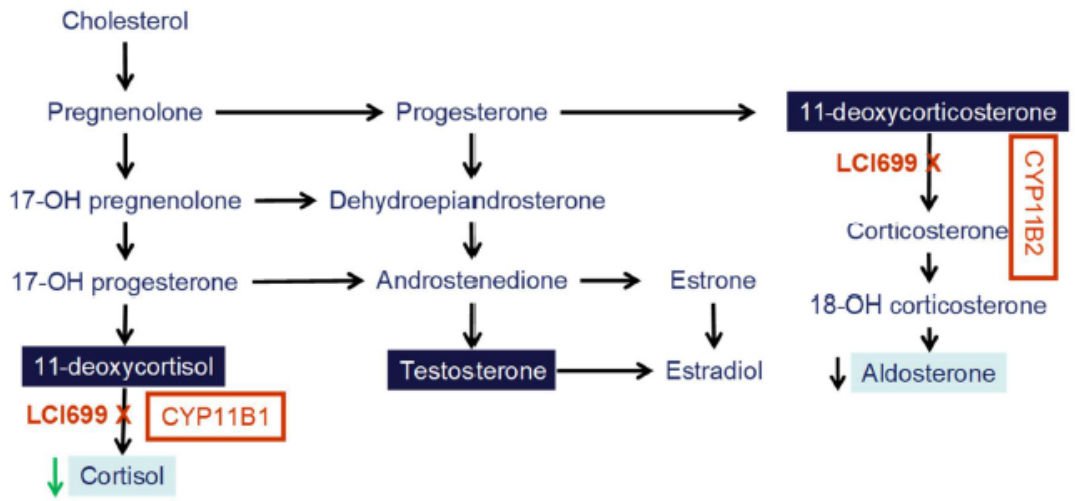
4.1 Primary Pharmacology

LCI699 is an inhibitor of CYP11B1 and CYP11B2 enzymes. It was originally developed as an inhibitor of aldosterone synthase (CYP11B2) for the treatment of diseases associated with high aldosterone levels (refer to section 4.2). However, clinical data revealed a more pronounced effect on cortisol levels (Studies CLCI699A2101 and CLCI699A2201). This results from the inhibition of the mitochondrial enzyme cortisol synthase (11β-hydroxylase, CYP11B1), the enzyme involved in the final step of cortisol synthesis, which is 95% identical in coding region and 93% identical in amino acid sequence to CYP11B2. The synthesis of aldosterone and cortisol, and the steps where LCI699 exerts its pharmacologic effects, are summarized in the sponsor figure below:

Reference ID: 4532599

Figure 1: Pathways Inhibited by LCI699

Figure 2-1 Site of action of osilodrostat (LCI699) in the aldosterone and cortisol synthesis pathways



Cortisol synthase (11β-Hydroxylase, CYP11B1) and aldosterone synthase (11β-Hydroxylase, CYP11B2) are intracellular enzymes located specifically within mitochondria. An *in vitro* cell-based enzyme assay was used to select potential inhibitors. CYP11B2 has high nucleotide sequence and amino acid homology with CYP11B1 i.e. the enzyme that is responsible for cortisol generation in humans. Using these screening assays, osilodrostat was identified as a potent inhibitor of CYP-11B1 and CYP11B2.

Sponsor figure

The observed reductions in cortisol levels led to the use of LCI699 for the treatment of Cushing's Disease. Because human data demonstrated pharmacologically mediated reductions in cortisol, minimal nonclinical pharmacology studies were performed with LCI699 to demonstrate this activity.

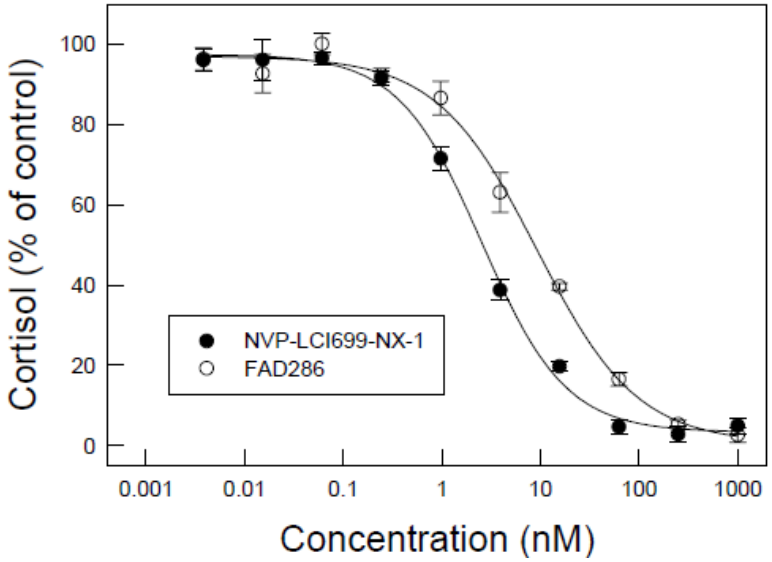
4.1.1 In Vitro Studies

The potency of LCI699 to inhibit the production of cortisol was evaluated in a Chinese hamster lung cell line (V79-4) that overexpresses human CYP11B1 (study RD-2007-51927). LCI699 showed a dose-dependent reduction in cortisol synthesis, reflecting inhibition of CYP11B1 that was greater than that seen with the positive control CYP11B1 inhibitor fadrozole (FAD286). The IC_{50} was calculated to be 2.5 nM, approximately 3.5-fold less potent than its inhibition of CYP11B2 (IC_{50} = 0.7 nM; see section 4.2.1). Data are summarized in the sponsor figure below.

Reference ID: 4532599

Figure 2: Inhibition of CYP11B1 Activity

Figure 3-1 Inhibition of human CYP11B1 activity by NVP-LCI699-NX-1 and FAD286 in vitro



Sponsor figure

The sponsor referenced a published article (authored by many sponsor staff) that summarizes the potency of LCI699 against CYP11B1 and CYP11B2 using rat and human recombinant enzymes and adrenal homogenates from rats and NHPs (Menard et al, 2014). These investigations revealed that LCI699 was more potent at inhibiting human CYP11B1 than that of other species. Data are summarized in the sponsor table below:

Table 3: Species Comparison of CYP 11B1 and 11B2 IC_{50} Values

Table 2-1 In vitro effect of osilodrostat on human rat and monkey enzymes

Assay	Aldosterone synthase (CYP11B2) IC50, nmol/L	11β-hydroxylase (CYP11B1) IC50, nmol/L
Recombinant human enzyme	0.7 ± 0.01 (6)	2.5 ± 0.1 (4)
Recombinant rat enzyme	160 ± 9 (3)	410 ± 5 (3)
Rat adrenal homogenate	802 (2)	3045 (2)
Monkey adrenal homogenate	12 ± 1 (6)	62 ± 5 (6)

Effect of osilodrostat on the inhibition of aldosterone synthase (CYP11B2) and 11β-hydroxylase (CYP11B1). All values are mean ± SEM (n) except for rat adrenal homogenate where only mean values from two experiments are presented. CYP, cytochrome P450; IC50, half maximal inhibitory concentration (Menard et al 2014).

Sponsor table

The sponsor also referenced published data (Creemers et al, 2016) demonstrating that LCI699 inhibits cortisol production in HAC-15 cells (a pluripotent cell line established from

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human adrenocortical carcinomas) and in primary adrenocortical adenoma cultures. The reported IC_{50} values were 39 and 66 nM, respectively.

4.1.2 In Vivo Studies

The pharmacology studies conducted with LCI699 focused on effects related to the inhibition of CYP11B2 and the synthesis of aldosterone are summarized in section 4.2.

The sponsor refers to published literature for some of the nonclinical pharmacologic support. The effects of LCI699 on corticosterone and aldosterone were assessed in rats (Menard et al, 2014 – sponsor employees were co-authors). Adrenal hormones were stimulated by angiotensin II (Ang II) or adrenocorticotropic hormone (ACTH). Treatment with LCI699 reduced the plasma concentrations of both aldosterone and corticosterone in a dose-dependent manner. The ED_{50} for inhibiting the Ang II-stimulated and ACTH-stimulated aldosterone responses were 0.6 mg/kg (EC_{50} = 127 nmol/L) and 1.1 mg/kg (EC_{50} = 771 nmol/L), respectively. The ED_{50} for inhibiting the ACTH-stimulated corticosterone response was 73 mg/kg (EC_{50} = 36 µmol/L).

The effects of LCI699 on basal and ACTH-stimulated plasma aldosterone concentration and plasma cortisol concentration were also evaluated in NHPs (Study RD-2010-50210). In this study, single doses of LCI699 at 5 - 150 µg/kg were followed 3 hours later by injection of ACTH. LCI699 inhibited the rise in aldosterone in a dose-related manner but there was no inhibition of the rise in cortisol. The estimated ED_{50} for inhibition of ACTH stimulated plasma aldosterone was 13 µg/kg.

Although the sponsor was unable to show an effect on cortisol levels in the NHP in their investigation, they did make reference to a published study (Weldon et al, 2016) in which doses of up to 1000 µg/kg of LCI699 were evaluated. Using these high doses, an EC_{50} of 1500 nM was determined for the ability of LCI699 to inhibit ACTH stimulated cortisol synthesis. The lack of an effect on cortisol levels in NHPs in the sponsor's study as compared to that reported in humans was hypothesized to be due to differences in the number of doses administered, timing of ACTH stimulation, and fasting status. However, no specific data addressing any of these concepts were provided.

4.2 Secondary Pharmacology

The effects of osilodrostat on aldosterone synthesis are considered secondary pharmacologic effects in patients with Cushing's Disease.

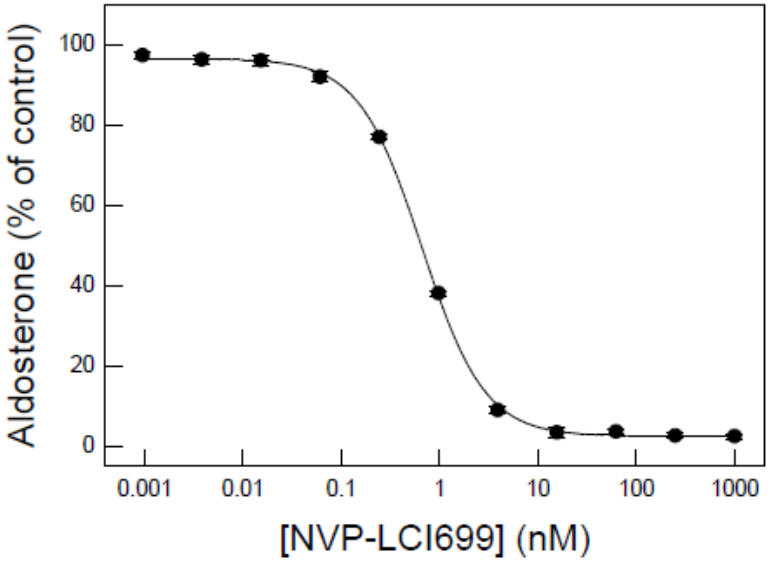
4.2.1 In Vitro Studies

The effect LCI699 had on CYP11B2 activity was evaluated in a Chinese hamster lung cell line (V79-4) that overexpresses human CYP11B2 (study RD-2009-50223). There was a dose-related inhibition of aldosterone production. The IC_{50} was calculated to be 0.7 nM, approximately 3.5-fold lower than the IC_{50} determined for inhibition of CYP11B1 (2.5 nM). Data are summarized in the sponsor figure below:

Reference ID: 4532599

Figure 3: Inhibition of CYP11B2 Activity

Figure 3-1 Effect of NVP-LCI699 on the activity of recombinant human CYP11B2 in vitro



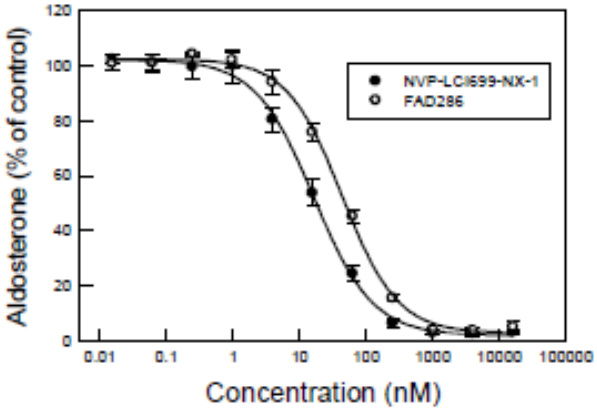
Recombinant human CYP11B2 preparation was incubated with indicated concentrations of NVP-LCI699 and 11-deoxycorticosterone at 25 °C for 2 hr. The production of aldosterone was measured by a scintillation proximity assay. All values are mean ± SEM (n = 6). The IC_{50} value for NVP-LCI699 is 0.7 ± 0.01 nM.

Sponsor figure

The effects of LCI699 on aldosterone production in a human adrenocortical carcinoma cell line (NCI-H295R) was evaluated (Study RD-2005-51167). A dose-related inhibition of aldosterone was noted as shown in the sponsor figure below:

Figure 4: Inhibition of Aldosterone Production

Figure 3-1 Inhibition of aldosterone production from NCI-H295R cells by NVP-LCI699-NX-1 and FAD286

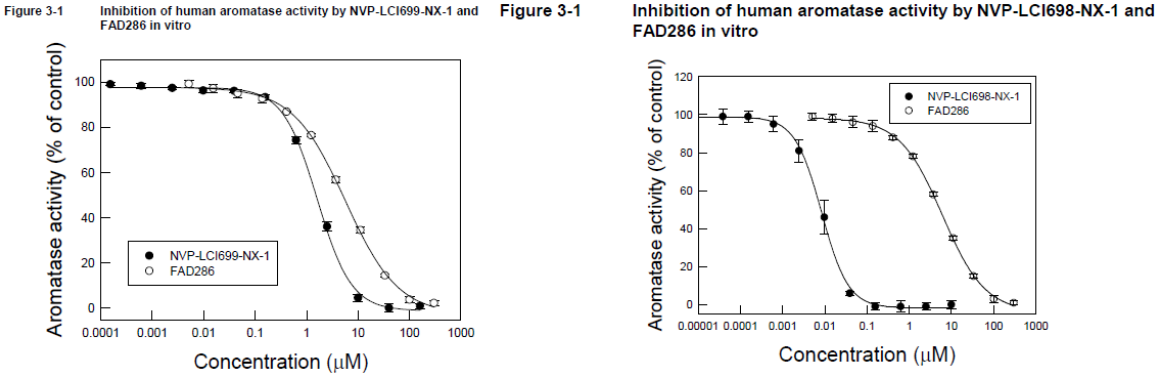


Sponsor figure

The IC_{50} was calculated to be 17 nM. In comparison, the IC_{50} for the aldosterone synthase inhibitor positive control FAD286 was 47 nM.

The effects LCI699 had on human aromatase activity, a member of the cytochrome P450 superfamily that catalyzes the conversion of androstenedione to estrone and testosterone to estradiol, was evaluated (Study RD-2007-51924). The IC_{50} for aromatase inhibition by LCI699 was calculated to be 1.7 µM under the conditions of this study. A similar study with LCI698, the (+) enantiomer of LCI699 that is considered an impurity, was found to inhibit human aromatase with a calculated IC_{50} of 9 nM (Study RD-2006-50293). Data from these two studies are shown in the sponsor figures below:

Figure 5: Inhibition of Aromatase Activity by LCI699 and Metabolite LCI698



FAD286 is the reference CYP11B2 inhibitor.
Sponsor figures

Off-target activity was evaluated in a panel of various receptors, transporters, ion channels, and enzyme binding assays at a concentration of 10 µM (Study RD-2013-50304). A greater than 50% inhibition was only evident for the histamine-1 receptor (64% inhibition; IC_{50} = 10 µM; Ki = 7.1 µM); inhibition greater than 50% was not seen against other targets evaluated.

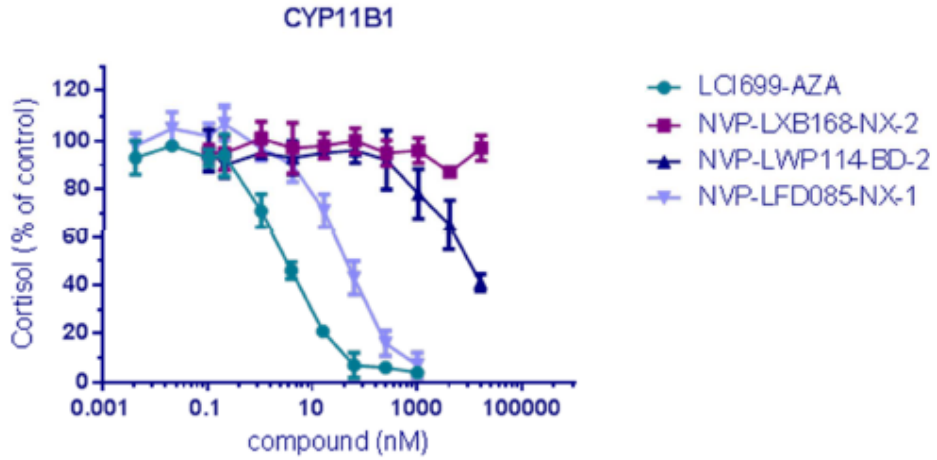
The primary circulating human metabolite M34.5 (LXB168) and the two minor circulating human metabolites M16.5 (LWP114), and M24.9 (LFD085) were evaluated for their potential to inhibit CYP11B1, CYP11B2, and human aromatase in a series of *in vitro* studies.

The effects of the 3 metabolites on CYP11B1 were evaluated in V79-4 Chinese hamster lung cells expressing human 11β-hydroxylase by measuring cortisol levels (Study RD-2014-00299). LCI699 dose-dependently inhibited CYP11B1 as shown by a reduction in cortisol levels. LXB168 had no effect while LWP114 was a weak inhibitor. LFD085 showed some activity but was still 12-fold less potent that LCI699. The inhibition curves and calculated IC_{50} concentrations are summarized in the sponsor figure and table below:

Reference ID: 4532599

Figure 6: Inhibition of CYP11B1 by Metabolites

Figure 3-1 NVP-LWP114 and NVP-LFD085 inhibit 11β-hydroxylase activity



Symbols represent mean ± SEM (n = 3).

Sponsor figure

Table 4: Inhibitory Potency of Metabolites Towards CYP11B1

Table 3-1 LCI699 metabolites are less potent inhibitors of 11β-hydroxylase than the parent compound

Compound	IC_{50} (nM)
LCI699-AZA	3.2 ± 0.2
NVP-LXB168-NX-2 (M34.5)	> 16,000
NVP-LWP114-BD-2 (M16.5)	10,700 ± 2,800
NVP-LFD085-NX-1 (M24.9)	40 ± 4

Data represent mean ± SEM (n = 3).

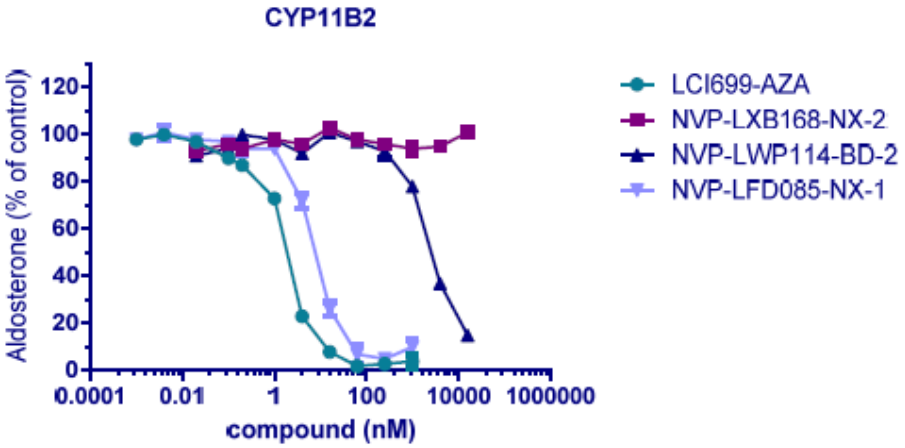
Sponsor table

A similar study design was utilized to evaluate the effects on CYP11B2 (Study RD-2014-00354). LCI699 and the metabolite LFD085 both inhibited CYP11B2 activity, with LFD085 being about 4-fold less potent than LCI699. LWP114 also showed inhibitory activity but was significantly less potent that LCI699. No activity was shown by LXB168. The inhibition curves and calculated IC_{50} concentrations are summarized in the sponsor figure and table below:

Reference ID: 4532599

Figure 7: Inhibition of CYP11B2 by Metabolites

Figure 3-1 NVP-LWP114 and NVP-LFD085 inhibit aldosterone synthase activity



Symbols represent mean ± SEM (n = 3).

Sponsor figure

Table 5: Inhibitory Potency of Metabolites Toward CYP11B2

Table 3-1 LCI699 metabolites are less potent inhibitors of aldosterone synthase than the parent compound

Compound	IC_{50} (nM)
LCI699-AZA	1.8 ± 0.1
NVP-LXB168-NX-2 (M34.5)	> 16,000
NVP-LWP114-BD-2 (M16.5)	3,392 ± 172
NVP-LFD085-NX-1 (M24.9)	7.0 ± 0.4

Data represent mean ± SEM (n = 3).

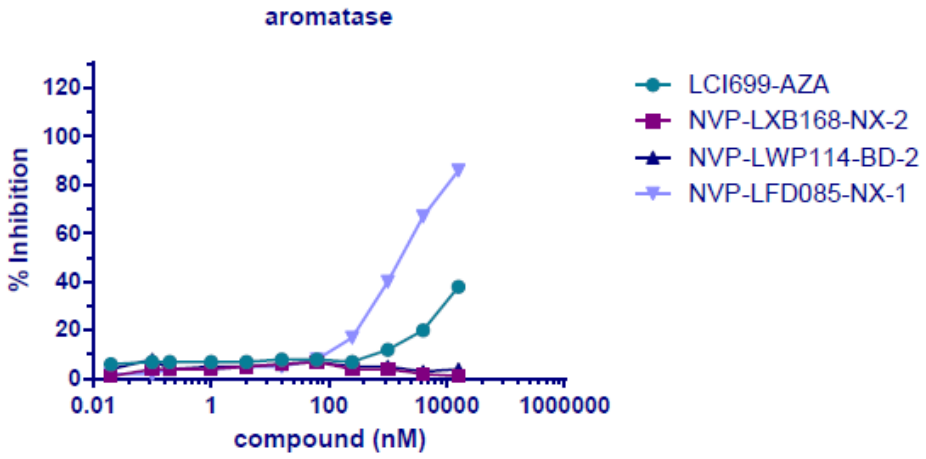
Sponsor table

CYP19 (aromatase) is a potential target of CYP11B1 and CYP11B2 inhibitors. In a study evaluating inhibition of aromatase activity, LCI699 showed minimal inhibition while metabolites LXB168 and LWP114 showed no inhibitory activity (Study RD-2014-00365). In contrast, metabolite LFD085 showed a dose-dependent inhibition of activity. The inhibition curves and calculated IC_{50} values are summarized in the sponsor figure and table below:

Reference ID: 4532599

Figure 8: Inhibition of Aromatase by Metabolites

Figure 3-1 NVP-LXB168 and NVP-LWP114 do not inhibit aromatase activity



Symbols represent mean ± SEM (n = 3).
Sponsor figure

Table 6: Inhibitory Potency of Metabolites Towards Aromatase

Table 3-1 Two of the three LCI699 metabolites tested are less potent inhibitors of aromatase than the parent compound

Compound	IC_{50} (µM)
LCI699-AZA	> 16
NVP-LXB168-NX-2 (M34.5)	> 16
NVP-LWP114-BD-2 (M16.5)	> 16
NVP-LFD085-NX-1 (M24.9)	1.8 ± 0.1

Data represent mean ± SEM (n = 3).
Sponsor table

The sponsor speculated that the potency of LFD085 was a result of contamination by its' enantiomer during the synthesis. This assessment was based on data from an earlier study that showed inhibition with a batch containing ~3% of the enantiomer (IC_{50} of 0.28 µM) but not with a batch that was purified (IC_{50} of 6.3 µM). None of the data from the earlier study were provided.

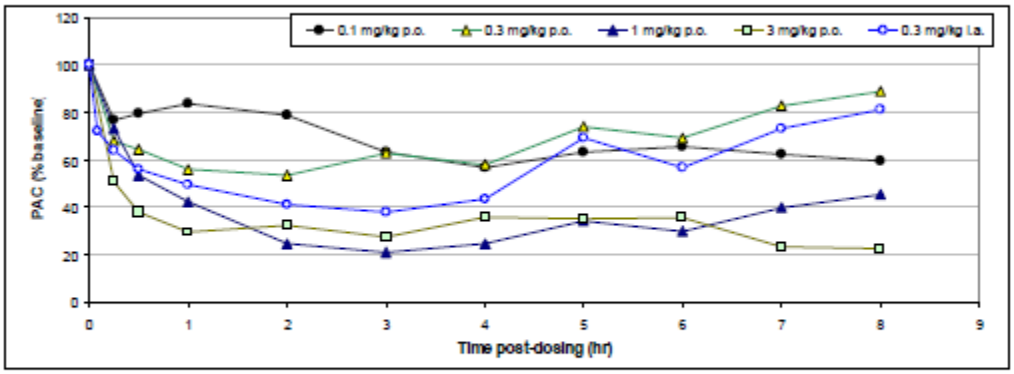
4.2.2 In Vivo Studies

A rat study was performed to evaluate the effect LCI699 has on plasma aldosterone concentrations (PAC) as compared to those induced by the positive control FAD286 (Study RD-2006-50315; also issued as Study RD-2007-51901). LCI699 was found to dose-, concentration-, and time-dependently lower PAC.

Reference ID: 4532599

Figure 9: Effect of LCI699 on Aldosterone Levels in the Rat

Figure 3-1 Time-course of PAC as a percent of baseline PAC in response to one i.a. and four p.o. doses of NVP-LCI699



Values represent the mean PACs expressed as a percent of baseline (i.e., PAC before dosing but one hour post-angiotensin II) for 1 i.a. and 4 p.o. doses of NVP-LCI699 over 8 hours post-dosing (SEM values omitted for clarity). NVP-LCI699 time- and dose-dependently lowered PAC that had been acutely elevated by angiotensin II. n=3 per group for each dose or administration route. i.a., intra-arterial; PAC, plasma aldosterone concentration; p.o., per os.

Sponsor figure

The ED_{50} and EC_{50} were calculated to be 2.5 µM and 127 nM, respectively.

Both AUC and oral bioavailability increased with dose, as summarized in the sponsor table below:

Table 7: Exposure and Oral Bioavailability

Table 3-3 Estimates of oral BAV and PK parameters of NVP-LCI699

Dose (mg/kg)	Route	$AUC_{(0-24\ h)}$ (nM•hr)	Oral BAV (%)	$t_{½}$ (hr)	CL (ml/hr/kg)	V_{ss} (ml/kg)
0.3	i.a.	3677	--	2.4±0.6	379±59	1240±50
0.1	p.o.	158	13±6	–	–	--
0.3	p.o.	487	13±2	–	–	--
1	p.o.	5100	42±5	–	–	--
3	p.o.	23187	63±8	–	–	--

Values are mean AUCs (0 to 24 hour post-dosing) or mean±SEM oral BAV, $t_{½}$, CL, or V_{ss}. The mean AUC for the i.a. dose was used to estimate the individual BAVs for each rat for each p.o. dose of NVP-LCI699 in this study. n=3 per group for each dose or administration route. AUC, area under the curve; BAV, bioavailability; CL, clearance; i.a., intra-arterial; p.o., per os; $t_{½}$, elimination half-life, V_{ss}, steady-state volume of distribution.

Sponsor table

4.3 Safety Pharmacology

Safety pharmacology studies were conducted to evaluate potential effects of LCI699 on the central nervous system, respiratory system, and cardiovascular system. The majority of the studies summarized below were previously reviewed by [redacted] (b) (4) J. Hawes.

Reference ID: 4532599

4.3.1 Neurological

Neurological function was evaluated in male rats administered single oral doses of 0, 0.5, 1, and 5 mg/kg (Study 0680190). Rats were evaluated for signs of neuropharmacologic activity pre-dose and at 0.25, 0.5, 0.75, 1, 2, 3, 4, and 24 hours after dosing. No effects were evident on any of the endpoints evaluated.

Although not safety pharmacology studies, multiple CNS signs were reported in repeat dose studies in mice and in a cardiovascular study in NHPs.

In the 13-week mouse toxicology study (reviewed under the IND), marked drug-related clinical signs consistent with CNS toxicity clinical signs including twitches, ataxia, aggression, changes in locomotor activity, hypersensitivity to touch, cold to touch, labored respiration, pale appearances/eyes, and salivation were associated with mortality at ≥100 mg/kg/day (≥160- and 104-fold the AUC and C_{max} exposures at the MRHD). Milder, non-adverse CNS-related effects including increased locomotor activity, hypersensitivity to touch, aggression, ptosis, and hunched posture were observed at ≥30 mg/kg (34- and 44-fold the AUC and C_{max} exposures, respectively, at the MRHD).

In a pilot study in male dogs (section 6.2), aggresssion, hypersensitivity to touch, ataxia, muscle tremors, and/or decreased activity were seen at dosages ≥ 15 mg/kg (40- and 29-fold the AUC and C_{max} exposures, respectively, at the MRHD).

In a cardiovascular study (section 4.3.3.2) in male NHPs, clinical signs of CNS toxicity including dilated pupils, disorientation, ataxia, head shaking, decreased activity, muscle twitches, tremors, chomping/chewing, and salivation were observed at 100 mg/kg (74- and 26-fold the AUC and C_{max} exposures, respectively, at the MRHD). The adverse clinical signs improved after 30 hours with supportive care. The NOAEL for CNS effects was 30 mg/kg with safety margins of 32- and 15-fold the AUC and C_{max} exposures at the MRHD.

4.3.2 Pulmonary

Effects on the pulmonary system were evaluated by plethysmography (heads-out) in male rats (Study 0680191). Single oral doses of 0, 1.5 or 5 mg/kg were administered with evaluations at pre-dose and 1, 2, 4, and 24 hours after dosing. There were no effects on respiratory rate, minute volume, or tidal volume.

4.3.3 Cardiovascular

Multiple *in vitro* and *in vivo* studies evaluating effects on cardiovascular endpoints were performed.

Reference ID: 4532599

4.3.3.1 In Vitro Studies

The effects of LCI699 on hERG channels was evaluated at concentrations of 1, 10, 30, 100, and 300 µM (0.2, 2.3, 6.8, 22.7, and 75 µg/mL, respectively; Study 0616814). LCI699 concentration-dependently inhibited hERG channels at >10 µM and the IC_{50} was calculated to be 54 µM (12.3 µg/mL) which is 42-fold higher than the steady state C_{max} for total drug (1.3 µM) predicted for 30 mg doses in humans. There was no inhibition of the relative surface expression of the hERG channel at concentrations up to 100 µM suggesting that LCI699 is not a hERG trafficking inhibitor (Study 1370701).

In a study evaluating the effects of LCI699 on 12 distinct cardiac ion channels expressed in mammalian cells (Study 1370651), inhibition in excess of 50% occurred for two of the channels. An IC_{50} of 103.6 µM and 95.0 µM was determined for Kir6.2/SUR2A and hKvLQT1/hminK, respectively. These were the only two channels tested at concentrations up to 300 µM; other channels evaluated were only tested up to a concentration of 100 µM.

The potential for LCI699 to effect repolarization (QT interval) and conduction was evaluated in isolated rabbit hearts using the Langendorff technique (Study 0518535) at concentrations of 0.1, 0.3, 1, 3, and 10 µM. The APD_{60} (monophasic action potential duration at 60% repolarization) was prolonged at concentrations $\geq$ 1 µM. At 10 µM, a delay in repolarization (based on APD_{60} prolongation) was seen in 2 of 3 trials suggesting that this concentration may be a threshold for proarrhythmic effects (approximately 8-fold higher than the predicted C_{max} values following repeated dosing in humans for total drug ($C_{max} \approx 1.3$ µM) and 13-fold higher for free drug (0.8 µM).

Effects on QRS duration, QT interval, $T_{p\text{-}e}$ (interval between the peek and the end of the T wave used as an index of transmural dispersion of repolarization and pro-arrhythmias), and Torsades de pointes (TdP) scores were evaluated in isolated rabbit heart ventricular wedge preparations (Study 1570263). LCI699 or the comparator SOM230 (pasireotide, a somatostatin analog approved for the treatment of Cushing's Disease) were administered alone or in combination and with various glucose concentrations. Treatment with LCI699 resulted in a concentration-related prolongation of the QT interval at all concentrations, $T_{p\text{-}e}$ at $\geq$ 1.4 µM, and TdP scores at concentrations $\geq$ 4.3 µM, but did not affect QRS duration (Table 8). Treatment with SOM230 alone resulted in statistically significant increases in QT duration at all concentrations evaluated. When SOM230 was co-administered with LCI699, there was not considered to be any potentiation of the LCI699 effects on QT interval. However, SOM230 appeared to potentiate the effects on $T_{p\text{-}e}$ and TdP scores. Compared to LCI699 alone, LCI699 at $\geq$ 14.3 µM with SOM230 at 0.3 µM resulted in prolonged $T_{p\text{-}e}$ as did LCI699 at 42.9 µM with SOM230 at 0.1 µM (Table 9). An increased incidence of early after depolarization (EAD) was only seen at the highest combination dose administered (Table 10). Glucose concentrations did not appear to potentiate any of the effects. These data are summarized in the sponsor tables below:

Reference ID: 4532599

Table 8: LCI699 Effects on Cardiovascular Endpoints

Table 6-3 LCI699

	Control	0.4 μM	1.4 μM	4.3 μM	14.3 μM	42.9 μM
QT (ms)	318.5±17.1	331.0±18.3*	347.0±19.1**	384.3±27.2**	493.3±47.2*	726.0±63.8**
T_{p-e}(ms)	58.8±5.7	61.8±6.2	67.0±7.0*	75.8±7.6**	87.3±4.3**	118.0±21.5*
QRS (ms)	31.5±0.9	31.3±1.0	31.3±0.6	31.0±0.7	31.5±1.0	31.5±0.6
QRS (ms)	31.3±0.9	31.3±0.9	31.3±1.0	31.0±0.7	31.3±0.6	31.3±0.5
TdP	0.0±0.0	0.0±0.0	0.3±0.3	1.8±0.3**	3.0±0.8*	4.3±1.3*
RPSEC	N	N	N	N	N	N

RSPC: reversal of the positive staircase effect on contractility; N: no effect; *: p<0.05; **p<0.01.

Sponsor table

Table 9: LCI699 with SOM230 Effects on Cardiovascular Endpoints

Table 6-4 LCI699+SOM230 (0.3 μM)

	Control	0.4 μM	1.4 μM	4.3 μM	14.3 μM	42.9 μM
QT (ms)	311.5±12.9	327.3±16.3*	348.5±17.4**	382.0±19.5**	497.5±15.4**	703.5±9.9**
T_{p-e}(ms)	56.8±6.6	62.3±7.8*	68.0±7.9**	79.0±7.5**	110.8±3.7**	132.0±6.2**
QRS (ms)	32.0±0.9	32.3±1.0	32.0±0.9	32.0±0.9	32.0±0.6	31.8±0.5
QRS (ms)	32.0±0.9	32.3±1.0	32.0±0.9	32.0±0.9	32.0±0.6	31.8±0.5
TdP Score	0.0±0.0	0.0±0.0	1.3±0.3*	2.5±0.5*	6.5±1.8*	6.8±0.9**
RPSEC	N	N	N	N	N	N

RSPC: reversal of the positive staircase effect on contractility; N: no effect; *: p<0.05; **p<0.01.

Table 6-5 LCI699+SOM230 (0.1 μM)

	Control	0.4 μM	1.4 μM	4.3 μM	14.3 μM	42.9 μM
QT (ms)	358.0±11.5	375.3±10.0	394.0±9.1*	442.8±15.4*	605.8±21.2**	884.5±43.0**
T_{p-e}(ms)	66.0±3.9	70.8±4.6	75.0±6.1	82.3±4.8*	92.0±4.3*	157.7±8.4*
QRS (ms)	32.5±1.0	32.5±1.0	32.5±1.0	32.5±1.0	32.5±1.0	32.5±1.0
QRS (ms)	32.5±1.0	32.5±1.0	32.5±1.3	32.5±1.0	32.5±1.0	32.5±1.0
TdP Score	0.0±0.0	0.0±0.0	0.3±0.3	1.8±0.5*	3.3±0.5**	5.5±0.9**
RPSEC	N	N	N	N	N	N

RSPC: reversal of the positive staircase effect on contractility; N: no effect; *: p<0.05; **p<0.01.

Sponsor tables

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Table 10: EAD Incidences with LCI699 and SOM230 Co-treatment

EAD Incidence

	C	0.4 µM	1.4 µM	4.3 µM	14.3 µM	42.9 µM
LCI699	0/4	0/4	0/4	0/4	0/4	1/4
LCI699+SOM230 (0.3 µM)	0/4	0/4	0/4	0/4	2/4	3/4
LCI699+SOM230 (0.1 µM)	0/4	0/4	0/4	0/4	0/4	1/4 with R on T
LCI699+SOM230 (0.1 µM) + Glucose 200 mg	0/4	0/4	0/4	0/4	0/4	2/4
LCI699+SOM230 (0.1 µM) + Glucose 400 mg	0/4	0/4	0/4	0/4	0/4	1/4
LCI699+SOM230 (0.1 µM) + Glucose 60 mg	0/4	0/4	0/4	0/4	0/4	0/4

Sponsor table

These data indicate that 0.3 µM SOM230 significantly potentiated LCI699-related proarrhythmia effects at LCI699 concentrations ≥14.3 µM, and that 0.1 µM SOM230 slightly potentiated LCI699-related proarrhythmia effects at an LCI699 concentration of 42.9 µM. However, since ≥14.3 µM LCI699 is approximately 11-fold higher than the predicted clinical C_{max} value for total drug (C_{max} ≈ 1.3 µM) and 18-fold higher for free drug (C_{max} ≈ 0.8 µM), co-treatment-related arrhythmias are not anticipated at clinical exposures.

4.3.3.2 In Vivo Studies

Single oral doses of LCI699 were administered to telemetered male dogs (Studies 0570242 and 0680192). There were no adverse cardiovascular changes in mean arterial, systolic, or diastolic pressure, heart rate, or ECG parameters in the GLP study at doses up to 5 mg/kg.

Continuous intravenous infusion of LCI699 at 50 mg/kg for 14 days in dogs (Study 0870526) was associated with QRS widening and QTc prolongation. These cardiac effects were seen at exposures corresponding to 12.4 µM free drug which is 23% of the hERG IC_{50} and is consistent with the proarrhthymia effects observed in the *in vitro* cardiovascular safety pharmacology studies. These effects were observed at concentrations 14-fold higher than the estimated free drug (0.8 µM) in humans administered the MRHD.

In a non-GLP study in NHPs, single ascending doses of 10, 30, and 100 mg/kg were administered to male and female (3/sex) NHPs (Study 1270320). Non-invasive jacket telemetry was used to record data. Severe clinical signs were observed at the HD that were summarized in section 4.3.1. Other relevant clinical signs at the HD included emesis that occurred at 1.5 to 3 hours post-dose, coinciding with t_{max}. There were no clear drug-related effects of body weight, but food consumption was decreased 25% to 75% during the 24 hours after each treatment at all doses. Dose-dependent QTc interval prolongation with increases in magnitude and duration was evident at 30 mg/kg (65 ms, ↑ 28%) and 60 mg/kg (93 ms, ↑39%) in males. Similar effects, but at a lessened magnitude, were also seen in females at 30 mg/kg (45 ms, ↑18%) and 60 mg/kg (39 ms, ↑15%). Across sexes, QTc interval prolongation occurred in 3/6 animals at the MD and in 5/6 animals at the HD. At the

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HD, QTc interval prolongations did not return to baseline levels by 24 hours post-dose (Figure 11). There were no significant effects on heart rate. One HD male also experienced drug-related abnormalities including non-conducted P wave and aberrant ventricular conduction. Another HD male exhibited drug-related premature ventricular complexes (PVCs), non-sustained ventricular tachycardia (NSVT) and Torsades de pointes (TdP). Overall, the NOEL for CV effects in NHPs was 10 mg/kg with C_{max} values of 1710 and 2300 ng/mL in males and females, respectively. Due to marked QTc interval prolongation and arrhythmias at 100 mg/kg, the maximum tolerated dose (MTD) was 30 mg/kg with C_{max} values of 5740 and 4540 ng/mL in males and females (19-fold and 15-fold the C_{max} at the MRHD, respectively).

Figure 10: Effects of LCI699 on QT Duration

Figure 1-6 Mean electrocardiographic data – QTcI - males

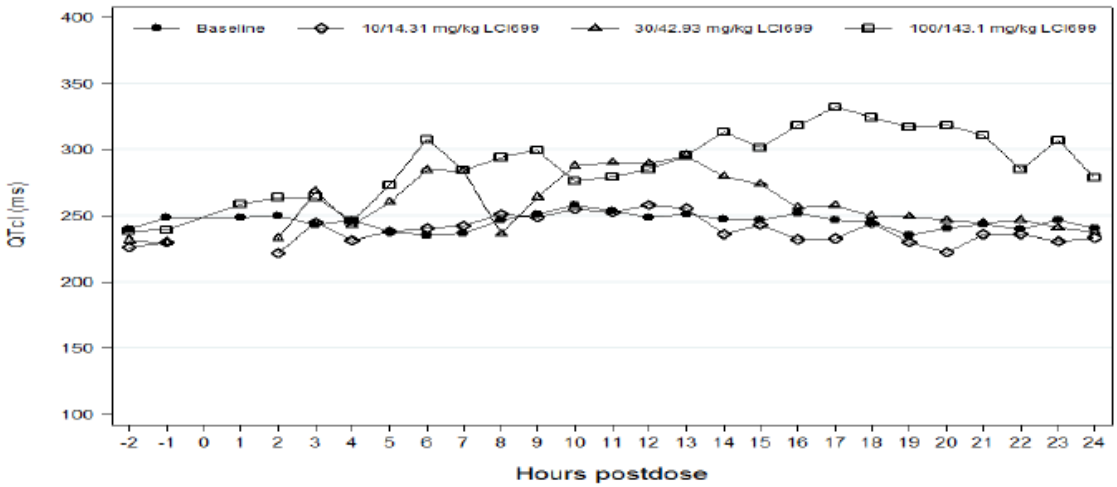
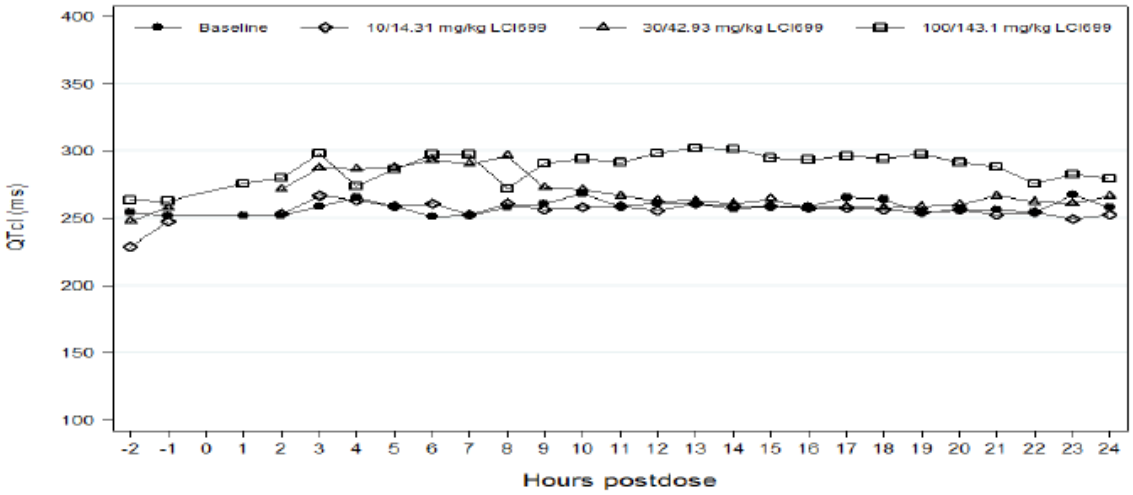


Figure 3-6 Mean electrocardiographic data – QTcI - females



Note: Dose levels are represented as the base/salt
Sponsor figures

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The effects of LCI699 (oral) with or without SOM230 (subcutaneous) treatment were evaluated in a series of studies in NHPs. ECG endpoints (non-invasive jacket telemetry) were included in a non-GLP 2-week ranging study in which 10 or 30 mg/kg of LCI699 or 1.6 mg/kg of SOM230 were administered (Study 1270612). Dose-dependent QTc interval prolongations were observed from 1 to 8 hours following treatment with LCI699; intervals were increased 28 ms (~11%) and 71 ms (~29%) over controls at 10 and 30 mg/kg, respectively. However, no effects were seen on PR or QRS intervals or heart rate and no arrhythmias were observed. No effects were evident on any cardiovascular endpoints following treatment with SOM230 alone.

In another NHP study, single doses of LCI699 at 10 or 30 mg/kg ± SOM230 at 1.6 mg/kg were administered using a Latin Square design (minimum of 4 days between treatments) in males (Study 1270713). An LCI699 alone dose of 10 mg/kg, SOM230 alone at 1.6 mg, or the combination of the two at the same doses had no effect on clinical signs, body temperature, blood pressure, or ECG parameters (implanted transmitters). Administration of LCI699 at 30 mg/kg with or without SOM230 led to transient mild decreases in systolic and diastolic blood pressures (≤18%) during the first hour post-dosing followed by slight increases (~10%) during the second hour post-dosing, and increases (~15%) in QT/QTc for up to 10 hours post-dosing. The effects were generally of greater magnitude with the combination than with LCI699 alone.

In a subsequent NHP study (Study 1570261), a single 30 mg/kg dose of LCI699 was administered alone or in combination with SOM230 at 0.129, 0.517, or 2.07 mg/kg. A 2.07 mg/kg dose of SOM230 by itself was also evaluated. Male NHPs were treated using a Latin Square design (ECG measurements were recorded using implanted transmitters). The administration of LCI699 with the MD and HD of SOM230 was associated with hypoactivity and recumbency which correlated with lower arterial blood and pulse pressures. Emesis and/or vomitus were observed in animals from all groups but the controls. The administration of LCI699 alone or combined with SOM230 resulted in increased QT and/or QTc times of up to 31% for up to 5 hours regardless of the SOM230 dose. Treatment with SOM230 by itself did not affect QT/QTc durations. However, SOM230 treatment at 2.07 mg/kg, either alone or in combination with LCI699, resulted in decreased (up to 14%) PR intervals for up to 5 hours. These data are summarized in the sponsor table below:

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Table 11: LCI699 and/or SOM230 Effects on Cardiac Interval Durations

Table 4.1: Largest covariate adjusted mean difference attributed to the test article compared with vehicle control article

Test article	Dose (mg/kg)	PR interval (msec)	QT interval (msec)	QTci interval (msec)
LCI699	30	NA	59 (24%)[b]	53 (23%)[b]
SOM230	2.07	-10 (-12%)[a]	NA	NA
LCI699/ SOM230	30/0.129	NA	78 (31%)[b]	62 (26%)[b]
LCI699/ SOM230	30/0.517	NA	57 (24%)[b]	53 (23%)[b]
LCI699/ SOM230	30/2.07	-12 (-14%)[a]	45 (18%)[b]	60 (25%)[b]

a Calculated at 30 minutes postdose
b Calculated from 0.75 through 5 hours postdose

Sponsor table

The administration of SOM230 at 2.07 mg/kg either alone or in combination with LCI699 was associated with decreases in systolic, diastolic, and/or mean arterial pressure or arterial pulse pressure, with the peak decreases ranging up to 40% through 30 minutes post-dose. These data are summarized in the sponsor table below:

Table 12: Effect of SOM230 (with or without LCI699) on Blood Pressure

Table 4.2: Largest covariate adjusted mean difference in arterial blood pressures compared with vehicle control article (30 minutes postdose)

Test article	Dose (mg/kg)	Systolic (mmHg)	Diastolic (mmHg)	Mean (mmHg)	Pulse pressure (mmHg)
SOM230	2.07	-40 (-37%)	-25 (-38%)	-34 (-40%)	-14 (-35%)
LCI699/ SOM230	30/2.07	-36 (-33%)	-21 (-31%)	-30 (-35%)	-15 (-36%)

Sponsor table

During the 1 to 4 hour post-dose period, increases of up to 26% in mean arterial pressure occurred in the LCI699 or MD combination group, but not the HD combination group. There were no effects on QRS duration, ECG waveforms, heart rates, or body temperature.

Exposure data were also collected as part of this study. Exposure to LCI699 was considered comparable between groups regardless of the co-administered SOM230 dosage. Exposure to SOM230 increased in a dose-proportional manner; the presence of LCI699 had no apparent effect on exposure. Exposure date for LCI699 and SOM230 are shown in the sponsor tables below:

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Table 13: Individual Animal Exposures to LCI699 and SOM230

Table 4.5: Mean (n=4 or 5) toxicokinetic parameters of LCI699 in male monkey plasma

	AUC_{0-24h}		AUC_{0-24h} /Dose		C_{max}		C_{max}/Dose		T_{max}	
Study Day	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
29#	42000	16400	1400	549	3450	957	115	31.9	4.00	2.45
36#	34300	14900	1140	497	3200	1340	107	44.6	2.58	1.02
39#	41300	20200	1380	673	4070	1910	136	63.7	1.67	1.03
43#	53500	28400	1780	947	4850	2270	162	75.6	3.67	1.63

TK parameters: ng*h/mL for AUC_{0-24h}, (ng*h/mL)/(mg/kg) for AUC_{0-24h}/Dose, ng/mL for C_{max}, (ng/mL)/(mg/kg) for C_{max}/Dose, and h for T_{max}.
= Dose levels: 30 mg/kg LCI699 for Day 29, 30 mg/kg LCI699 and 0.1 mg/kg SOM230 for Day 36, 30 mg/kg LCI699 and 0.4 mg/kg SOM230 for Day 39, and 30 mg/kg LCI699 and 1.6 mg/kg SOM230 for Day 43.

Table 4.6: Mean (n= 6) male monkey plasma SOM230 concentrations (ng/mL)

	0.5 h		1 h		3 h		7 h		24 h	
Study Day	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
32*	3580	431	3930	493	2440	620	799	255	81.2	20.9
36*	314	84.4	261	67.8	155	40.0	45.1	9.67	8.40	3.26
39*	1140	167	838	120	597	121	160	41.4	15.2	6.72
43*	3780	672	3950	1230	3220	788	1360	545	103	57.7

* = Dose levels: 2.07 mg/kg SOM230 for Day 32, 30 mg/kg LCI699 and 0.129 mg/kg SOM230 for Day 36, 30 mg/kg LCI699 and 0.517 mg/kg SOM230 for Day 39, and 30 mg/kg LCI699 and 2.07 mg/kg SOM230 for Day 43.

Sponsor tables

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

5.1.1 Absorption

LCI699 is rapidly absorbed, reaching t_{max} by ~1 hour after oral administration in rats and dogs (~2 hours in humans). Oral bioavailability is high (calculated to be >100%), although the doses used for intravenous administration were different from those used for oral administration. The apparent terminal half-life was 1.3 hours in rats and 2.2 hours in dogs as compared to a terminal half-life of 4 to 5 hours in humans. Mean pharmacokinetic parameters following a single dose are summarized in the sponsor table below:

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Table 14: Pharmacokinetic Summary

Table 3-3 Mean pharmacokinetic parameters of LCI699 in plasma of animals (rat, dog) after single p.o. administration

Species (Reference)	Gender, (n)	Dose[a)] (mg/kg)	Tmax (h)	Cmax (ng/mL)	AUClast (ng/mL·h)	Tlast (h)	AUCinf (ng/mL·h)	T1/2 (h)	Apparent F* (%)
Rat (DMPK R0600206-A)	m,(3)	3	0.83	791	3720	12	3730	1.3	122
Dog (DMPK R0700721)	m,(3)	8	0.5	5550	30100	24	30200	2.2	117
Human (Study CLCI699C2101)	m, (5)	0.673[b)]	2.0[c)]	313	3050	48[c)]	3050	3.98[c)]	n.d.

n.d. = not determined; * Apparent F was calculated using two different doses (i.v. / p.o.)

a) Dose refers to free compound (MW: 227.2 g/mol), nominal dose

b) 50 mg dose/subject, mean body weight 74.3 kg

c) Median

Source: Rat - [Table 2.6.5.13A-DMPK R0600206-A], Dog - [Table 2.6.5.13B-DMPK R0700721], Human [CLCI699C2101]

Sponsor table (Pharmacokinetic Written Summary)

5.1.2 Distribution

The distribution of LCI699 was evaluated by quantitative whole-body autoradiography in pigmented (LEH) and albino (Han Webster) male rats (Study DMPK R0600206-B). Following oral administration, LCI699 is widely and rapidly distributed to tissues, reaching peak levels in most tissues by 1 hour post-dose. Tissues with the highest exposure ($AUC_{0-\infty}$) in the pigmented rat were the adrenal gland, bile, eye, Harderian gland, kidney, liver, pancreas, skin, small intestine, glandular stomach, and uveal tract. The highest tissue to blood ratios were also found in these tissues. The C_{max} concentrations of radio-labelled material in the brain and spinal cord were 70% and 20% higher, respectively, than C_{max} blood concentrations indicating penetrance into the CNS at exposures higher than blood levels. In the albino rat, tissue distribution was evaluated in individual animals at different timepoints following dose administration. In general, the highest levels were found in the adrenal gland, Harderian gland, kidney, liver, and spleen. LCI699 has a significant affinity toward melanin in the eye, skin, and the uveal tract as the level in these tissues from pigmented rats was higher than in these tissues from the albino rat.

The *in vitro* blood distribution was evaluated in mice, rats, dog, NHP, and humans (Studies DMPK R1200424 and DMPK R0600208). Across species, the blood to plasma concentration ratios were less than 1 (0.94, 0.83, 0.93, 0.94, 0.85 in mice, rat, dog, NHP, and human, respectively), indicating that LCI699 was more distributed to plasma. Binding to plasma protein was comparable between nonclinical species (31.8% in mice, 35.9% in rats, 26.8% in dogs, 26.6% in NHPs) and humans (36.7%).

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5.1.3 Metabolism

The *in vitro* metabolism of ^{14}C-LCI699 was investigated in rat, dog, NHP, and human hepatocytes (Study DMPK R0600207-01). Although there was some variation in the pathways between species, metabolism was primarily via oxidation, glucuronidation, ribose conjugation, and N-methylation. The metabolites identified in humans were also observed in one or more nonclinical species.

The *in vivo* metabolism was determined in the mouse, rat, dog, and human (Studies DMPK R1200350, DMPK R0600206-A, DMPK R0700721, CLCI699C2101). Although the levels of the metabolites differed between species, all circulating human metabolites were detected in one or more nonclinical species. The oxidative metabolite M34.5 (LXB168) is the most abundant human metabolite, representing 41.8%-60.9% of the total radioactivity in humans. In rats, it accounted for 61% of the radioactivity which is approximately 1.1-fold the exposure in humans. The glucuronide M16.5 (LWP114) accounted for >10% of total radioactivity in 2 out of 5 human subjects indicating that it can also be a disproportional human metabolite. It was qualified in the 13-week mouse toxicology study at the NOAEL with a safety margin of approximately 7-fold the projected exposure at the MRHD based on total radioactive dose percentage estimates. The oxidative metabolite M24.9 (LFD085) accounted for 3.8%-8.8% of the radioactivity in the patients evaluated. In accounted for approximately 10%, 5%, and 15% of the radioactivity in mice, rats, and dogs, respectively.

5.1.4 Excretion

The majority of excretion in the rat, dog, and human is by the urinary route which accounts for ~79%, ~86%-91%, and ~91% of a radiolabeled dose in these species, respectively (Studies DMPK R0600206-A, DMPK R0700721, CLCI699C2101). Fecal excretion accounted for ~17%-27%, ~6%-8%, and <2% of the dose in rats, dog, and humans, respectively. The amount of unchanged LCI699 excreted in the urine of rats, dogs, and humans was comparable across species (ranging from approximately 5%-10% of the dose) indicating that metabolism is the major elimination pathway for the 3 species evaluated.

5.2 Potential for Drug Interactions

5.2.1 Inhibitory Activity

The potential for LCI699 to induce or inhibit various enzymes/transporters or act as a substrate was evaluated in a series of *in vitro* studies (DMPK studies R0700727, R1200914, R1300116, R1300021, R1300841, R1701176, R1300066, and R1701177).

- LCI699 did not inhibit P-gp, OAT1, OATP1B1, OATP1B3, OCT1, BSEP, BCRP, or MRP2 transporters. For these transporters, the IC_{50}, when able to be calculated, was $\geq$100 µM, well above the C_{max} (306 ng/mL, 1.3 µM) at the MRHD of 30 mg.
- IC_{50} values of 45 µM and 38 µM were calculated for inhibition of the OAT3 and MATE2K transporters, respectively; these were not of significant clinical concern.
- For the transporters OCT2 and MATE1, IC_{50} values of 8.6 µM and 5.6 µM, respectively, were calculated for their inhibition. The clinical risk for inhibition is considered minimal.

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Data are summarized in the reviewer created table below:

Table 15: Transporter Inhibition by LCI699

Transporter	Highest concentration evaluated (µM)	IC_{50} value (µM)	Maximum Inhibition (%)
P-gp	50 & 100	ND	none
OAT1	100	>100	29.1
OAT3	100	45	66.9
OATP1B1	100	ND	none
OATP1B3	100	ND	none
OCT1	100	>100	23.2
OCT2	100	8.59	91.6
MATE1	100	5.57	80.6
MATE2K	100	37.7	77.6
BSEP	5 & 50	ND	none
BCRP	25 & 50	ND	none
MRP2	50	ND	none

ND – not determined
Based on data in Table 3-7 of Clinical Pharmacology Overview

A similar series of studies (DMPK R1701155, 1701176, R1701177) were also conducted with the major human metabolite M34.5 (LXB168)

- LXB168 showed no inhibitory activity towards P-gp, BSEP, BCRP, or MRP2
- LXB168 inhibited OAT1, OAT3, and MATE1, with calculated IC_{50} values of 19.9, 3.23, and 67.6 µM, respectively, indicating the potential for interactions with OAT3.

Data are summarized in the reviewer created table below:

Table 16: Transporter Inhibition by Major Metabolite M34.5 (LXB168)

Transporter	Highest concentration evaluated (µM)	IC_{50} value (µM)	Maximum Inhibition (%)
P-gp	50	>50	11.6%
OAT1	80	19.9	86.8%
OAT3	50	3.23	97.2%
OATP1B1	80	ND	16.6%
OATP1B3	80	ND	4.02%
OCT1	80	ND	3.52%
OCT2	80	ND	10.4%
MATE1	80	67.6	53.1%
MATE2K	80	ND	19%
BSEP	5 & 50	>50	4.8%
BCRP	50	ND	none
MRP2	50	ND	none

ND – not determined

The potential for LCI699 to inhibit various CYP enzymes was evaluated in a series of *in vitro* studies (DMPK R0600401 and R1600265). These studies demonstrated that LCI699:

- has inhibitory potential towards CYPs 1A2, 2C19, 2D6, and 2E1
- has weaker inhibitory potential towards CYPs 3A4/5, 2B6, 2C9, CYPs 2C8 and 2A6

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- showed an apparent time-dependent inhibition towards CYP2C19, but not for CYPs 1A2, 2C9, 3A4/5, or 2D6 (studies DMPK R1600265, R0600401, and R1600741)

These data are summarized in the sponsor table below:

Table 17: Inhibition of CYP Enzymes by LCI699

Table 3-6 Inhibitory potency of osilodrostat for cytochrome P450 enzymes (in vitro) and basic DDI risk assessment

CYP Enzyme	IC_{50}	K_I or $IC_{50}/2$ (µM)	TDI parameters K_I (uM); k_{inact} (min^{-1})	FDA $R_1 = 1+[I]/K_I$ [I] = $C_{max,u}$ (risk ≥ 1.02)	FDA R_2 [I] = $C_{max,u}$ (risk ≥ 1.25)
CYP1A2	~ 1	0.776		1.84	
CYP2A6	No inhibition	n.d.		n.d.	
CYP2B6	20	10		1.06	
CYP2C8	~ 100	50		1.01	
CYP2C9	~ 40	20		1.03	
CYP2C19	~ 4	4.63	52.3; 0.026	1.14	45.1
CYP2D6	~ 4	1.97		1.33	
CYP2E1	~ 2	0.482		2.35	
CYP3A4/5	~ 6.5	3.25 (K_I =7)		1.20	

n.d.= not determined

$R_2 = (k_{obs} + k_{deg})/k_{deg}$, where $k_{obs} = k_{inact} \times 50 \times C_{max,u}/K_I + 50 \times C_{max,u}$

$C_{max,ss}$ used was 232 ng/mL with an fu_p of 0.636 (unbound Cmax,ss of 0.649 µM) (Table 3-2), 30 mg dose

Source: [DMPK R0600401] and [DMPK R1600265]

Sponsor table from Summary of Clinical Pharmacology

The potential for the M34.5 metabolite to inhibit CYP enzymes was also evaluated. No inhibition of CYPs 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, or 3A4/5 was seen at a concentration of 100 µM (DMPK R1400089).

Both LCI699 and the M34.5 metabolites were evaluated for their ability to inhibit human UDP-glucuronosyltransferase 1A1 (UGT1A1) and 2B7 (UGT2B7). Inhibition of the UCG1A1 was evident with both LCI699 (IC_{50} = 30 µM) and the M34.5 metabolite (IC_{50} = 0.7 µM). No inhibition of UGT2B7 was seen with either compound at concentrations up to 100 µM. Consequently, there is a potential for interactions between M34.5 and UGT1A1 substrates at the MRHD concentration (0.85 µM).

5.2.2 Induction Activity

The potential for LCI699 to induce various CYP enzymes was evaluated in a series of *in vitro* studies (DMPK R1200233, R1400331). Treatment of human hepatocytes with various concentrations of osilodrostat and then measuring the levels of mRNA for the various CYP enzymes revealed an induction of CYPs 1A2 and 2B6 (EC_{50} values of 191 and 136 µM, respectively). There was also some induction of CYP 3A4 at a higher concentration (EC_{50} = 375 µM). No induction was reported for CYP 2C9. In a similar study with the metabolite M34.5 (DMPK R1800303), induction was seen for CYPs 2B6 and 3A4, but only at high concentrations (EC_{50} values of 125 and 275 µM, respectively). No induction of CYPs 1A2 or

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2C9 was observed. Overall, no drug-related induction of any of the CYP enzymes evaluated would be expected at clinically-relevant exposures.

5.3 Toxicokinetics

Toxicokinetic data are summarized within the toxicology study reviews.

6 General Toxicology

6.1 Single-Dose Toxicity

Key study observations from single dose studies are tabulated below:

Table 18: Summary of Acute Toxicity Studies

Study Type / Study #	Dosages	Notable Observations
Mouse		
Acute Mouse Study 0680189	0, 50, 100, 125, 150 mg/kg 10 mL/kg	150 mg/kg: Mortality, adverse clinical signs. 125 mg/kg: NOAEL
Pilot Dog Study 0570241	30, 15, 7.5 mg/kg in decreasing manner (same animals used with multiday washout between dose levels	30 mg/kg: weight loss ≥ 15 mg/kg: Adverse clinical signs 7.5 mg/kg: NOEL

6.2 Repeat-Dose Toxicity

The toxicity of LCI699 was evaluated in sub-chronic and chronic toxicity studies that were originally reviewed by (b) (4) J. Hawes. The results of the longest duration study in the primary species supporting this application are included in this review.

6.2.1 Rodent

6.2.1.1 26-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with LCI699 in Rats with a 13-Week Recovery Period

Study no.:	0870522
Study report location:	EDR (SDN 1)
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	LCI699, batch 0652003, 99.7% purity

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Methods

Doses:	0, 0.2, 2, 20 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Reverse osmosis water
Species/Strain:	Rat / Crl:WI(Han)
Number/Sex/Group:	20
Age:	7 – 8 weeks
Weight:	♂ 215-259 g; ♀ 165-199 g
Satellite groups:	10/sex at C and HD for recovery
Unique study design:	No
Deviation from study protocol:	None affecting study integrity

Key Study Findings: Increases in body weight gain and food consumption were noted in females at 20 mg/kg. Target organs identified were the liver, adrenal glands, ovary, and prostate. Observations in the liver included increased size and weight with microscopic findings of cytoplasmic vacuolation in both sexes at the 20 mg/kg. Increased adrenal gland weight correlating with hypertrophy of the zona fasciculata was evident in the adrenal cortex of 20 mg/kg females. Increased ovarian weights occurred at $\geq$ 2 mg/kg and increased incidence of prominent corpora lutea was seen at 20 mg/kg. Also observed were decreased prostate weights with no correlative microscopic findings in 20 mg/kg males. With the exception of decreased prostate weight, all findings were reversible. Based on the incidence and severity of findings, the NOAEL was considered the MD of 2 mg/kg. At this dose, the AUC_{0-24} was 6170 and 5150 ng·hr/mL in males and females, respectively.

Observations and Results

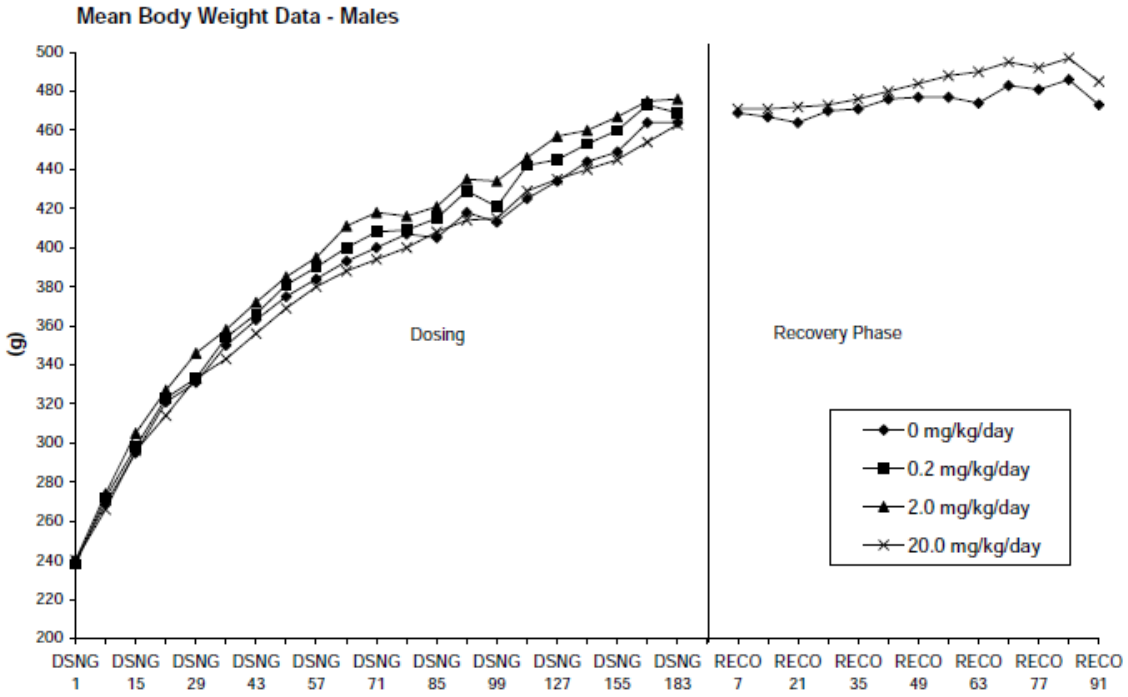
Mortality: There were 4 deaths that occurred following blood collection (1 C male, 2 C females, 1 HD female). No lesions were found postmortem. All deaths were blood collection related.

Clinical Signs: No drug-related effects noted.

Body Weights: Periods of increased or reduced weight gain were seen during the dosing period in males at the MD/HD, with the effects sometimes reaching statistical significance. However, the alterations in weight gain did not result in differences in absolute body weights. In females, increases or reductions in weight gain were also evident in various groups during the treatment period that attained statistical significance. The periods of increased weight gain resulted in increased absolute body weights at the HD that were statistically significant. Weight gain over the entire dosing period was also increased (~35%) in HD females, with weights at the end of dosing being 10% heavier than concurrent controls. There were no effects on weight parameters during or following recovery. Weight data are summarized in the sponsor figures below:

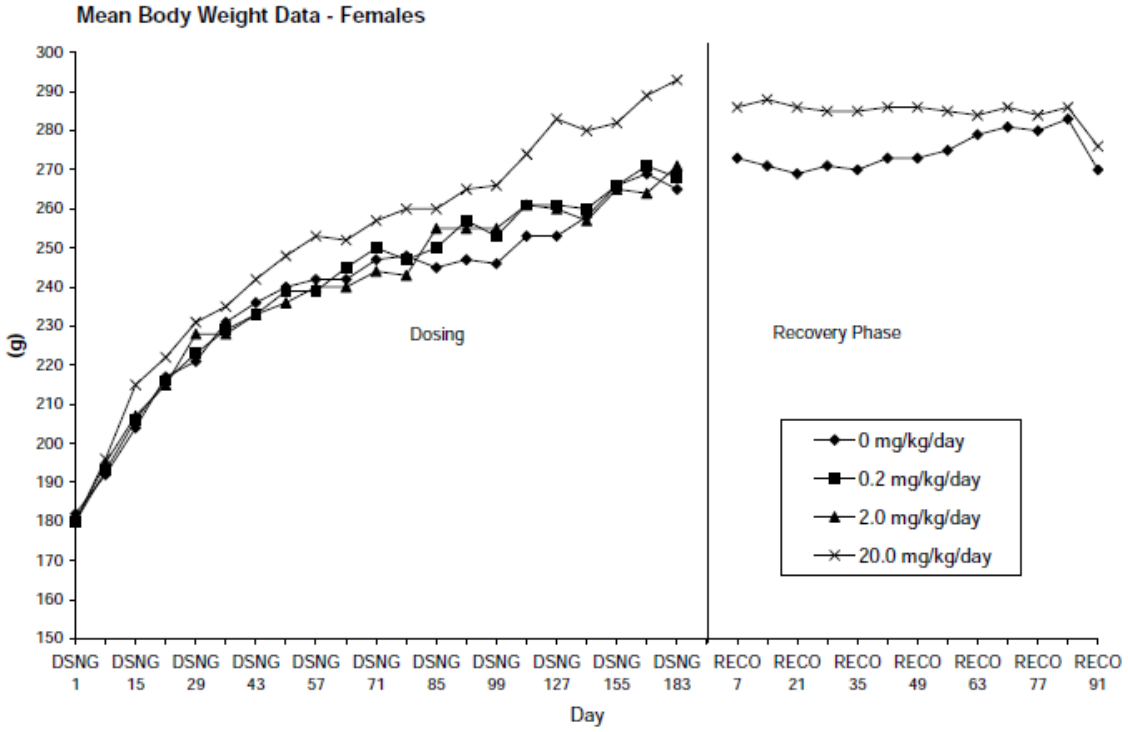
Reference ID: 4532599

Figure 11: Body Weights in 26-Week Study (Male) - Rat



Sponsor figure

Figure 12: Body Weights in 26-Week Study (Female) - Rat



Sponsor figure

Reference ID: 4532599

Feed Consumption: At the HD, food consumption was increased (~8%) from weeks 20 – 24 in males (statistically significant). In females, increased food consumption (up to ~21%) started to be evident beginning at week 2 and continuing through the remainder of the treatment period, with many of the intervals reaching statistical significance. The increased food consumption correlated with the heavier body weights in this group.

Ophthalmoscopy: No drug-related effects observed.

Hematology: There were no adverse drug-related effects on hematologic or coagulation parameters. Statistically significant effects included slight increases in RBC, HGB, WBC, LYM, and Fib in females at 20 mg/kg, decreases in MCV and MCH in females at 20 mg/kg, and decreased platelets at all doses in males. Other effects observed that reached statistical significance were only seen transiently. The observed effects were of minimal magnitude and not considered toxicologically significant.

Clinical Chemistry: Slight reductions in TP and ALB were observed in females at 20 mg/kg that were reversible. Other statistically significant effects were only observed transiently. None of the observed effects were considered adverse.

Urinalysis: No significant drug-related effects.

Gross Pathology: No significant drug-related effects.

Organ Weights: Drug-related increases in the weights of the liver, ovaries, and adrenal gland were evident at the HD in one or both sexes relative to controls. For the liver, absolute and adjusted (for body and brain weights) weights were increased in both sexes that correlated with microscopic observations of hepatocellular hypertrophy and vacuolation. Increased ovarian weights correlated with prominent corpora lutea. Absolute, but not brain or body weight adjusted, increased adrenal weights in females were correlated with zona fasciculata hypertrophy. Decreases in prostate weight without microscopic correlates occurred at the HD and persisted through recovery. The mean differences from control values at the HD are summarized in the reviewer table below:

Table 19: Organ Weight Changes in 26-Week Study - Rat

Mean Organ Weights – gram (%)						
Organ	20 mg/kg					
	Male			Female		
	Absolute	R-Body	R -Brain	Absolute	R-Body	R -Brain
Liver	+26.5	+26.7	+26.2	+30.4	+15.8	+29.7
Ovaries	-	-	-	+39.2	+22.0	+38.0
Adrenal	-	-	-	+18.2	-	-
Prostate, dosing	-16.4	-15.4	-16.7	-	-	-
Prostate, recovery	-19.2	-20.6	-19.3	-	-	-

R: relative weight.
All % deviations from concurrent control are statistically significantly different (P <0.05)

Reference ID: 4532599

Histopathology:

Adequate Battery: Yes

Peer Review: Yes

Protocol identified tissues from all animals in the C and HD groups and from animals found dead were microscopically examined. The adrenal glands, cervix, liver, ovaries, oviduct, uterus, vagina, and lesions from the LD and MD group were also examined microscopically. Adrenal glands, liver, ovary, and lesions were evaluated from all recovery animals.

Drug-related microscopic alterations were noted at the HD in the liver of both sexes and in the adrenal glands and ovaries of females.

In the liver, cytoplasmic vacuolation characterized as centrilobular and extending to midzonal/periportal areas, and centrilobular hypertrophy were observed in both sexes at the HD. Hepatic changes were generally of greater severity and occurred at a higher incidence in males relative to females. At the recovery phase necropsy the liver findings were resolved in all but a single male.

In the adrenal cortex, minimal to slight hypertrophy of the zona fasciculata was observed in all females at the HD. This finding was absent in the recovery group animals.

There was an increase in the incidence of prominent corpora lutea in the ovaries at the HD. A slight increase was also observed at the MD. This effect was reversible.

The incidence and severity of these findings is tabulated in the sponsor table below:

Reference ID: 4532599

Table 20: Microscopic Observations in 26-Week Study - Rat

Incidence and average severity of LCI699-related microscopic findings at the dosing phase

	LCI699 mg/kg/day							
Sex	Males				Females			
Dose level	0	0.2	2.0	20.0	0	0.2	2.0	20.0
Liver								
Hypertrophy, Hepatocyte								
Minimal	0	0	0	1	0	0	0	7
Slight	0	0	0	7	0	0	0	8
Moderate	0	0	0	11	0	0	0	0
Average Severity	0.0	0.0	0.0	2.5	0.0	0.0	0.0	1.2
Vacuolation, Hepatocyte								
Minimal	0	0	0	2	0	0	0	7
Slight	0	0	0	2	0	0	0	3
Moderate	0	0	0	14	0	0	0	0
Average Severity	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.7
Total Examined	20	19	20	19	20	20	20	20
Adrenal cortex								
Hypertrophy, Zona fasciculata								
Minimal	0	0	0	0	0	0	0	4
Slight	0	0	0	0	1	0	0	16
Average Severity	0.0	0.0	0.0	0.0	0.1	0.0	0.0	1.8
Total Examined	20	20	20	20	20	20	20	20
Ovary								
Prominent Corpora Lutea								
Slight	-	-	-	-	4	4	6	14
Average Severity	-	-	-	-	0.4	0.4	0.6	1.4
Total Examined	-	-	-	-	20	20	20	20

Sponsor table

Toxicokinetics: After single or repetitive dosing, systemic exposures ($AUC_{0\text{-}24h}$) to LCI699 increased approximately proportional to the increase in dose in both sexes at all sampling intervals. Exposures to LCI699 on days 23 and 149 were similar to the exposure on day 1. No sex difference in exposure to test substance was noted. Time to maximum plasma concentration (t_{max}) was generally between 0.5 and 1 hour post-dose. Data are summarized in the sponsor table below:

Reference ID: 4532599

Table 21: Mean Exposure Data in 26-Week Study - Rat

Toxicokinetic parameters of LCI699 in rat plasma

Dose of LCI699 (mg/kg/day)	Study Day	Gender	AUC0-24h (ng*h/mL)	SE of AUC (ng*h/mL)	AUC0-24h/Dose (ng*h/mL/mg/kg/day)	SE of AUC/Dose (ng*h/mL/mg/kg/day)	Cmax (ng/mL)	Cmax/Dose (ng/mL/mg/kg/day)	Tmax (h)
0.2	1	Male	151	13.4	753	67.0	47.3	237	1
		Female	238	26.6	1190	133	77.5	388	0.5
	23	Male	200	11.6	999	58.0	54.7	274	0.5
		Female	295	32.2	1480	161	66.5	333	0.5
	149	Male	387	31.5	1940	158	72.2	361	0.5
		Female	350	22.7	1750	114	83.2	416	0.5
2	1	Male	3350	170	1680	85.0	808	404	1
		Female	4190	181	2090	90.5	939	470	0.5
	23	Male	4090	250	2050	125	752	376	0.5
		Female	4010	296	2010	148	965	483	0.5
	149	Male	6170	565	3090	283	869	435	0.5
		Female	5150	349	2580	175	931	466	1
20	1	Male	69800	2630	3490	132	5840	292	1
		Female	83300	5110	4160	256	6290	315	1
	23	Male	56600	1890	2830	94.5	5010	251	1
		Female	62400	2950	3120	148	5460	273	0.5
	149	Male	61900	2580	3100	129	5860	293	1
		Female	72700	2970	3630	149	6670	334	1

Sponsor table

The L-enantiomer (LCI698) was detected only in samples from animals dosed at ≥MD. Within the two highest dose groups, exposure to LCI698 increased approximately proportional to the dose. No apparent differences in exposure to LCI698 were observed between male and female rats or between days 1, 23 and 149. The overall exposure to the impurity LCI698 in mid and high dose group animals ranged from approximately 0.005% to 0.13% of the exposure to LCI699. Data are summarized in the sponsor table below:

Table 22: Mean Exposure Data for Enantiomer (LCI698) in 26-Week Study - Rat

Toxicokinetic parameters of LCI698 in rat plasma

Dose of LCI699 (mg/kg/day)	Study Day	Gender	AUC0-24h (ng*h/mL)	SE of AUC (ng*h/mL)	AUC0-24h/Dose (ng*h/mL/mg/kg/day)	SE of AUC/Dose (ng*h/mL/mg/kg/day)	Cmax (ng/mL)	Cmax/Dose (ng/mL/mg/kg/day)	Tmax (h)
2	1	Male	0.166	0.167	0.0831	0.0835	0.133	0.0665	1
		Female	2.34	0.873	1.17	0.437	0.698	0.349	0.5
	23	Male	0.779	0.588	0.390	0.294	0.412	0.206	0.5
		Female	3.07	0.195	1.54	0.0975	0.687	0.344	0.5
	149	Male	0.149	0.0861	0.0745	0.0431	0.298	0.149	0.5
		Female	1.52	0.407	0.761	0.204	0.644	0.322	1
20	1	Male	40.7	6.53	2.04	0.327	3.87	0.194	3
		Female	90.8	4.88	4.54	0.244	6.41	0.321	3
	23	Male	47.2	2.61	2.36	0.131	4.00	0.200	1
		Female	78.8	2.48	3.94	0.124	6.12	0.306	1
	149	Male	41.5	2.56	2.07	0.128	4.42	0.221	1
		Female	71.9	4.83	3.60	0.242	5.57	0.279	1

Sponsor table

Dosing Solution Analysis: The formulations were found to be chemically stable. The achieved concentrations of test substance in the formulation were in the range of 93% to 102% of targeted concentrations. Test substance was not identified in any of the vehicle control article formulations.

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6.2.2 Non-Rodent

6.2.2.1 39-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with LCI699 in Dogs with a 4-Week Recovery Period

Study no.:	0870521
Study report location:	EDR (SDN 1)
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	LCI699, batch 0652003, 99.7% purity

Methods

Doses:	0, 0.1, 1, 10 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Reverse osmosis water
Species/Strain:	Dog / beagle
Number/Sex/Group:	4
Age:	8 – 9 months
Weight:	♂ 7.6 – 10.1 kg; ♀ 6.5 – 9.2 kg
Satellite groups:	Additional 2/sex at C and HD for recovery
Unique study design:	No
Deviation from study protocol:	None affecting study interpretations.

Key Study Findings: Decreases in food consumption observed during the first 5 weeks of treatment correlated with fluctuations in body weights. Thereafter, there were no significant effects. In the adrenal cortex, atrophy and increased vacuolation of the zona glomerulosa occurred at an increased incidence at ≥ 1 mg/kg; these effects reversed or were in the process of reversing following the recovery period. Ovarian and uterine weights were also increased at ≥ 1 mg/kg but there were no microscopic correlates. The adrenal and reproductive organ findings are likely pharmacodynamic effects of drug-treatment and were not considered adverse. There also was an increased incidence of pituitary cysts in 10 mg/kg males and in all drug-treated female groups relative to controls but the relevance to treatment is uncertain since pituitary cysts are commonly seen in dogs. The NOAEL was considered the HD of 10 mg/kg. At this dose, the AUC_{0-24} was 22000 and 20300 ng·hr/mL in males and females, respectively.

<u>Observations and Results</u>

Mortality: There was no mortality

Clinical Signs: No adverse drug-related effects noted.

Body Weights: Although there were transient dose-independent changes in body weights of drug-treated animals, there were no consistent effects. Statistically significant increases

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in body weight gain were noted in HD males between Days 169 and 176 that were followed by significantly reduced weight gain between Days 183 and 190. Statistically significant decreases in weight gain were observed in HD females during the first week, but significantly increased between Days 92 and 99 followed by another decrease between Days 183 and 190. The inconsistent fluctuations in body weight reflect changes in food consumption and are not clearly drug-related. It is noted that mean body weights were 30% higher in HD females after the recovery period and correlate with an increase (↑48%) in food consumption. Data are summarized in the reviewer tables below:

Table 23: Body Weights in 39-Week Study (Male) - Dog

Absolute Body Weight/Weight Gain in grams[a] (% of control) - Male				
Day or Interval	Dosage (mg/kg/week)			
	0	0.1	1	10
Dosing Period				
1	8.9	8.5	8.5	8.6
29	8.7	8.7	8.7	8.8
92	9.1	9.2	9.0	9.4
183	9.6	10.2	9.8	10.2
274	9.6	10.4	9.7	10.3 (107%)
1 - 274	0.8	2.0	1.2	1.7
Recovery Period				
7	11.2	-	-	11.5
28	11.0	-	-	11.2

Table 24: Body Weights in 39-Week Study (Female) - Dog

Absolute Body Weight/Weight Gain in grams[a] (% of control) - Female				
Day or Interval	Dosage (mg/kg/week)			
	0	0.1	1	10
Dosing Period				
1	7.6	7.8	7.7	7.8
29	7.7	7.7	7.8	7.7
92	8.0	8.1	8.4	8.3
183	8.1	8.4	8.8 (109%)	8.9 (110%)
274	8.7	8.9	9.2 (106%)	9.1 (105%)
1 - 274	1.1	-	-	1.3 (118%)
Recovery Period				
7	7.7	-	-	9.5 (123%)
28	7.6	-	-	9.9 (130%)

Feed Consumption: Decreases in food consumption were observed during the first 5 weeks in males at the HD, reaching statistical significance during Week 5 (↓17%). Decreases in food consumption were observed during the first 2 weeks in females at the HD, reaching statistical significance during Week 1 (↓26%). Although fluctuations in food consumption correlate with fluctuations in body weights, there were no significant differences between drug-treated animals and controls in food consumption after the first 5 weeks of treatment. During the recovery period, food consumption was increased in HD females (mean weekly increases ranged from 15% to 48%).

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Ophthalmoscopy: No drug-related effects.

Hematology: There were no drug-related effects on hematologic or coagulation parameters.

Clinical Chemistry: No significant drug-related effects

Urinalysis: No significant drug-related effects.

Gross Pathology: No drug-related effects.

Organ Weights: The weights (absolute, body weight, and/or brain weight adjusted) of the ovaries and uterus were increased in MD and/or HD animals. Ovary weights were heavier at both the MD and HD and remained slightly heavier through the end of recovery, but none of the effects reached statistical significance. Uterine weights were increased at the MD and HD with those at the MD reaching statistical significance. Uterine weights remained increased at the end of recovery at the HD, but the increase was not statistically significant. The mean differences from control values are summarized in the reviewer table below:

Table 25: Organ Weights in 39-Week Study - Dog

Mean Organ Weights – gram (% of control)				
Organ	Female			
	Dosage (mg/kg)			
	0	0.1	1	10
Ovary, dosing	1.08	1.01	1.66 (154%)	1.85 (171%)
Ovary, recovery	1.13	-	-	1.50 (133%)
Uterus, dosing	4.68	3.56	14.47* (309%)	11.03 (236%)
Uterus, recovery	4.35	-	-	20.95 (482%)

Values are rounded by reviewer
*p <0.05

Histopathology:

Adequate Battery: Yes
Peer Review: Yes

Drug-related microscopic alterations were noted in the adrenal cortex at ≥ MD in both sexes. Microscopic findings included minimal to moderate atrophy along with minimal to slight vacuolation of the zona glomerulosa, with moderate diffuse atrophy observed in a single HD female. Atrophy of the zona glomerulosa was not seen after recovery, indicating reversal. However, minimal vacuolation was evident in 1 of 2 females at the HD following recovery, suggesting reduced, but persistent vacuolation. The incidences and severities of these observations are shown in the sponsor table below:

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Table 26: Microscopic Observations in Adrenal Gland in 39-Week Study - Dog

Incidence and severity of selected test article-related microscopic findings in the adrenal cortex - dosing phase sacrifice

Sex	Males				Females			
Dose level LCI699 (mg/kg/day)	0	0.1	1.0	10	0	0.1	1.0	10
Number examined	4	4	4	4	4	4	4	4
Adrenal Cortex								
Atrophy, Zona Glomerulosa								
Not Present	4	4	3	1	4	4	3	0
Minimal	0	0	0	2	0	0	1	2
Slight	0	0	1	1	0	0	0	1
Moderate	0	0	0	0	0	0	0	1
Vacuolation, Zona Glomerulosa, Increased								
Not Present	4	4	1	0	4	4	2	3
Minimal	0	0	3	3	0	0	2	0
Slight	0	0	0	1	0	0	0	1

Sponsor table

Additional microscopic observations of note included minimal bile duct hyperplasia and minimal hepatocyte pigment observed in a single LD female; these were not considered drug-related due to the singular occurrence and lack of a dose response. Moderate chronic-active inflammation of an epididymal vessel was observed in one HD male that was considered incidental based on the absence of vascular findings in any other animals.

Pituitary cysts were observed in all males at the HD and in half the females in each of the drug-treated groups but were not evident in any recovery animal. The relationship of this finding to drug-treatment is unknown given that pituitary cysts are spontaneously observed in this species. Data are summarized in the sponsor table below:

Table 27: Microscopic Observations in Pituitary Gland in 39-Week Study - Dog

Summary of Microscopic Observations - Dosing Phase - Final Phase Sacrifice

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males				Females			
Tissues With Diagnoses	Dosage group:	Ctls	2	3	4	Ctls	2	3	4
	No. in group:	4	4	4	4	4	4	4	4
Pituitary	Number examined:	4	4	4	4	4	4	4	4
	Unremarkable:	3	3	3	0	4	2	1	1
Cyst		1	1	1	4	0	2	2	2
Angiectasis		0	0	0	0	0	1	0	1
Infiltrate, Lymphocytes/Macrophages		0	0	0	0	0	0	1	0

Sponsor table

Toxicokinetics: Absorption was rapid with t_{max} values between 0.5 and 1.5 hours, with the time increasing with dose. Exposures increased up to 4-fold greater than increases in dose but there were no indications of accumulation. Instead, there appeared to be a trend for lower exposure upon repeated dosing at the HD, but not in the other groups. There were no apparent gender effects. Data for LIC699 are summarized in the sponsor table below:

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Table 28: Mean Exposure Data for LCI699 in 39-Week Study - Dog

Mean (n = 4) toxicokinetic parameters of LCI699 in dog plasma

Dose of LCI699 (mg/kg/day)	Study day	Gender	AUC_{0-24h} (ng*h/mL)		AUC_{0-24h}/dose (ng*h/mL) / (mg/kg/day)		C_{max} (ng/mL)		C_{max}/dose (ng/mL) / (mg/kg/day)		T_{max} (h)	
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0.1	1	Male	23.5	2.48	235	24.8	12.0	4.00	120	40.0	0.500	0.00
		Female	18.3	7.75	183	77.5	10.4	5.44	104	54.4	0.625	0.250
	22	Male	29.2	5.39	292	53.9	15.8	6.60	158	66.0	0.500	0.00
		Female	24.7	10.1	247	101	15.3	7.32	153	73.1	0.625	0.250
	267	Male	35.3	5.48	353	54.8	16.7	2.45	167	24.4	0.500	0.00
		Female	15.3	6.68	153	66.8	6.68	3.12	66.8	31.2	0.875	0.250
1	1	Male	692	37.1	692	37.1	326	64.2	326	64.2	0.500	0.00
		Female	588	166	588	166	230	109	230	109	0.625	0.250
	22	Male	673	161	673	161	277	116	277	116	0.500	0.00
		Female	550	198	550	198	222	82.7	222	82.7	0.625	0.250
	267	Male	822	87.1	822	87.1	268	74.9	268	74.9	0.500	0.00
		Female	542	76.5	542	76.5	223	68.3	223	68.3	0.500	0.00
10	1	Male	28700	6130	2870	613	5070	1100	508	110	0.875	0.250
		Female	31100	5450	3110	545	5480	997	548	99.7	0.750	0.289
	22	Male	25400	3940	2540	394	3720	892	372	89.2	1.50	1.00
		Female	23700	3600	2370	360	3710	233	371	23.3	0.875	0.250
	267	Male	22200	6630	2220	663	3770	923	377	92.3	1.00	0.00
		Female	20300	1140	2030	114	3850	235	385	23.5	1.00	0.00

Sponsor table

Exposure to the L-enantiomer LCI698 was only detectable at the HD. There was no accumulation of gender effect noted. Exposures were over 2500-fold lower than LCI699, ranging from 0.027 to 0.036% of LCI699 exposures. Similar to LCI699, a trend for reduced AUC and C_{max} exposures was apparent with repeated administration at the HD. Data are summarized in the sponsor table below:

Table 29: Mean Exposure to Enantiomer (LCI698) in 39-Week Study - Dog

Mean (n = 4) toxicokinetic parameters of LCI698 in dog plasma

Dose of LCI699 (mg/kg/day)	Study day	Gender	AUC_{0-24h} (ng*h/mL)		AUC_{0-24h}/dose (ng*h/mL) / (mg/kg/day)		C_{max} (ng/mL)		C_{max}/dose (ng/mL)/ (mg/kg/day)		T_{max} (h)	
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
10	1	Male	9.31	1.48	0.931	0.148	2.48	0.610	0.248	0.0610	0.750	0.289
		Female	8.76	1.45	0.876	0.145	2.61	0.399	0.261	0.0399	0.875	0.250
	22	Male	6.93	1.54	0.693	0.154	1.76	0.548	0.176	0.0548	1.00	0.00
		Female	7.71	3.91	0.771	0.391	1.58	0.100	0.158	0.00998	0.750	0.289
	267	Male	7.97	1.80	0.797	0.180	2.07	0.478	0.207	0.0478	1.00	0.00
		Female	7.19	0.551	0.719	0.0551	2.13	0.241	0.213	0.0241	0.875	0.250

Sponsor table

Dosing Solution Analysis: The formulations were stable. Test-article concentrations ranged from 94.6% to 101% of theoretical. The only exception was for one of the LD samples that was measured at 53.3% of claim. However, the second sample and both

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back-up samples ranged from 96.7% to 100% of claim. An investigation revealed that improper mixing before analysis was the likely reason for the low result. Consequently, the dosing formulations were considered acceptable.

6.3 Other Toxicology Studies

6.3.1 Combination Toxicology

6.3.1.1 A 13-Week Oral (Gavage)/Subcutaneous Injection (SC) Toxicity Study in the Rat

Study no.:	1170625
Study report location:	EDR (SDN 1)
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	LCI699, batch 0752004, 100% purity SOM230, batch R0002, 100% purity

Methods

Doses:	LCI699/SOM230 1.5/0.03, 5/0.1, 20/0.3, 20/-, -/0.3
Frequency of dosing:	Once daily
Route of administration:	LCI699 - oral gavage; SOM230 – sc injection
Dose volume:	LCI699 - 5 mL/kg; SOM230 – 1 mL/kg
Formulation/Vehicle:	Reverse osmosis water
Species/Strain:	Rat / Crl:WI(Han)
Number/Sex/Group:	10
Age:	7 – 8 weeks
Weight:	♂ 176-216 g; ♀ 123-165 g
Unique study design:	No
Deviation from study protocol:	None that impacted study integrity

Key Study Findings: The purpose of this study was to determine the toxicity of LCI699 in combination with pasireotide (SOM230), a somatostatin analogue used for the treatment of Cushing's disease. Activation of somatostatin receptors in the pituitary by SOM230 results in suppression of ACTH which is a key player in the HPA-axis that stimulates adrenal gland production of corticosteroid. Both LCI699 and SOM230 lead to reductions in mean urinary free cortisol levels, the primary endpoint in the pivotal Phase III studies in Cushing's patients, but by different mechanisms of action on different endocrine glands.

In males, the main findings were reduced weight gain/body weight, reductions in organ (adrenal, heart, kidney, liver, pituitary, spleen) weights, and microscopic alterations in the bone marrow and pituitary in MD and HD combination groups and SOM230 alone group, and microscopic changes in the liver of the HD combination group and LCI699 alone group. None of the effects were exacerbated by combination treatment as compared to those seen

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in either the LCI699 or SOM230 alone groups. For males, the NOAEL was considered to be the LD combination group (LCI699 1.5 mg/kg/SOM230 0.03 mg/kg).

For females, reduced weight gain/body weight occurred in the HD combination and SOM230 alone groups while decreased pituitary weights were seen in the MD and HD combination groups and the SOM230 alone group. Increased ovarian weights were evident in the MD and HD combination and LCI699 alone groups; uterine weights were also reduced in all groups administered LCI699, but the effects did not reach statistical significance. Microscopic alterations were noted in the adrenal glands in the LCI699 alone group, but not in any of the combination groups while liver and ovarian changes were seen in the HD combination and LCI699 alone groups. Hyperplasia/hypertrophy of the vaginal mucosa was evident only in the SOM230 alone group. The NOAEL was considered to be the MD combination group (LCI699 5 mg/kg/SOM230 1 mg/kg).

In summary, the combination of LCI699 and SOM230 did not appear to exacerbate any of the effects seen with either compound by itself. Furthermore, some of the effects seen with LCI699 alone (e.g., microscopic changes in the liver of males and females and in the adrenal glands and ovaries of females) occurred at a lower incidence and/or reduced severity in various combination groups. Exposure to LCI699 in the combination groups was similar to the exposures at similar doses reported in previous studies with LCI699 alone.

Observations and Results

Mortality: There was no mortality

Clinical Signs: No drug-related effects noted.

Body Weights: In males, lower weight gains and lower body weights became evident within two weeks of treatment initiation in the group administered SOM230 alone. Reduced weight gains resulting in lower body weights were also evident in the MD and HD combination groups, but the reductions were less than those seen with SOM230 alone. These effects persisted through study termination. Lower weight gains over the treatment period were also seen in the LD combination group, but there was no corresponding effect on absolute weights. Over the 13-week treatment period, weight gains were reduced approximately 19%, 40%, and 51% in the LD, MD, and HD combination groups, respectively, and 56% in the SOM230 alone group. These effects were statistically significant. As a result of the lower weight gains, absolute weights at study termination were reduced 8%, 19%, 22%, and 25% in the LD, MD, HD combination and SOM230 alone groups, respectively, with all effects reaching statistical significance with the exception of the LD combination. Data are summarized in the sponsor figure below:

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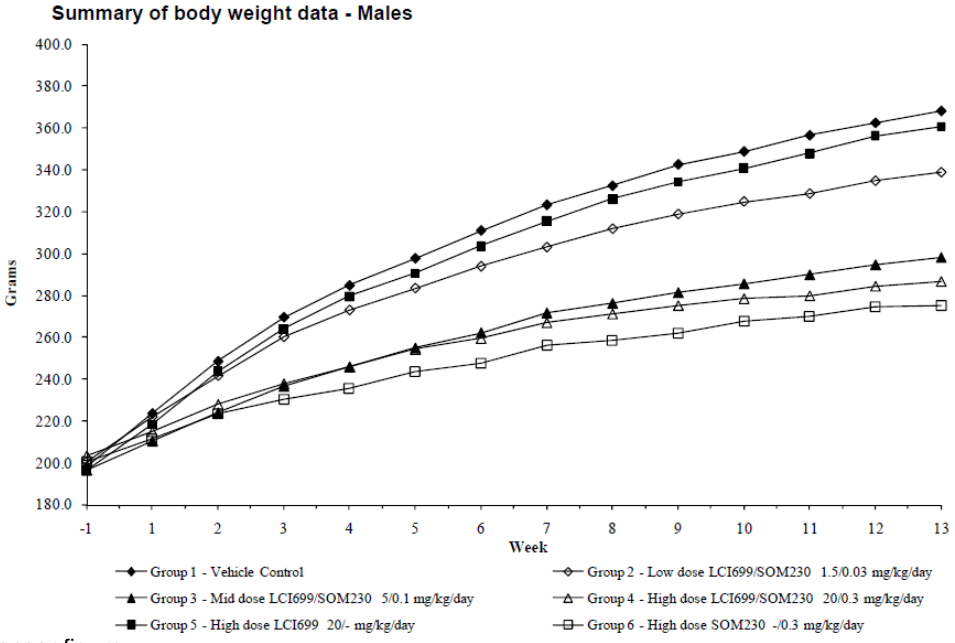
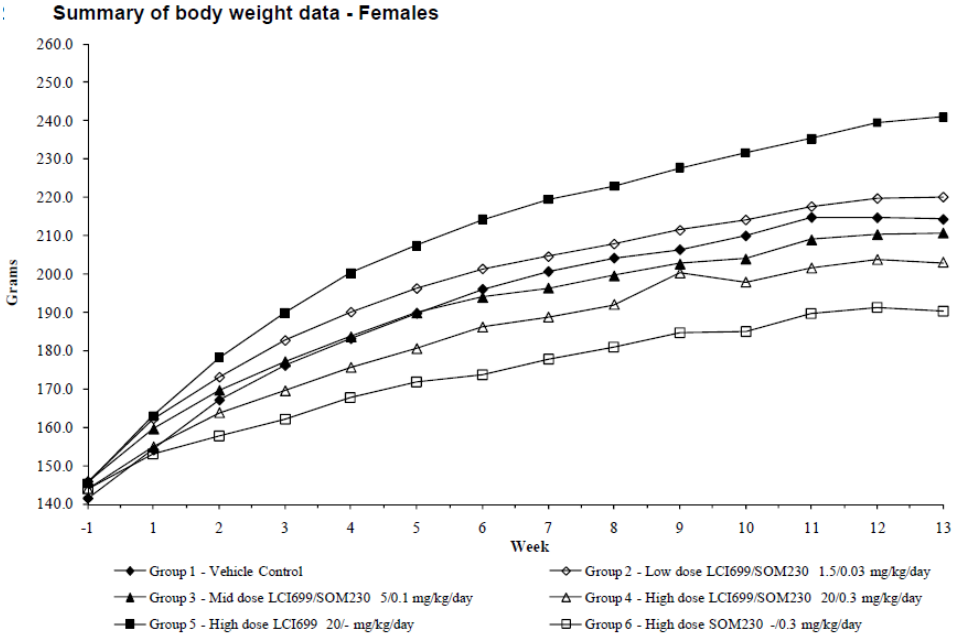
Figure 13: Body Weights in 13-Week Combination Study (Male) - Rat



Sponsor figure

In females, reduced weight gain was only seen in the SOM230 alone group where weight gain over the study was 36% lower, resulting in an absolute weight that was 11% lower at study completion. In females administered LCI699 alone, weight gain was increased 31% over controls and resulted in terminal weights that were 12% heavier than controls. These effects were statistically significant. Data are summarized in the sponsor figure below:

Figure 14: Body Weights in 13-Week Combination Study (Female) Rat



Sponsor figure

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In summary, co-administration of LIK699 and SOM230 appeared to attenuate the reductions in body weight/weight gain induced by SOM230 alone in both sexes.

Feed Consumption: No drug-related effects

Ophthalmoscopy: No drug-related effects.

Hematology: Alterations were seen in a number of parameters in males at combination doses ≥ MD and in all groups of females. Treatment with LCI699 alone resulted in increases in RBC, Hgb, Hct, and reticulocyte counts, and decreases in MCV and MHC. Treatment with SOM230 alone resulted in increased RBC counts and decreases in MCV, MHC, and reticulocyte counts. The changes observed were ≤ 22% different from controls and not considered adverse. There was no exacerbation of effects with co-administration of the two drugs. Data are summarized in the sponsor table below:

Table 30: Hematology Changes in 13-Week Combination Study - Rat

Table 4-1 Hematology changes in rats administered LCI699/SOM230 when compared to control

	Males				Females			
Dose (mg/kg/day):	5/0.1	20/0.3	20/-	-/0.3	1.5/ 0.03	20/0.3	20/-	-/0.3
Red blood cell count 106/µL								
Week 4	+5%	+4%	–	**+8%**	–	+5%	–	**+6%**
Week 14	–	–	+4%	+6%	**+5%**	**+7%**	**+6%**	**+5%**
Hemoglobin conc g/dL								
Week 4	–	–	–	**+4%**	–	–	–	–
Week 14	–	+5%	+4%	–	–	+4%	–	–
Hematocrit %								
Week 4	–	–	–	–	–	+4%	–	–
Week 14	–	–	+5%	–	–	**+6%**	–	–
Mean corpuscular volume fL								
Week 4	–	-4%	–	**-6%**	–	–	–	**-4%**
Week 14	–	–	–	**-4%**	–	–	**-4%**	**-5%**
Mean Hemoglobin concentration pg								
Week 4	–	–	–	-4%	–	–	**-4%**	**-4%**
Week 14	–	–	–	-4%	–	–	**-5%**	**-4%**
Absolute reticulocyte count 109/L								
Week 4	–	–	+22%	-19%	–	–	–	-15%
Week 14	–	–	**+19%**	–	–	**+20%**	**+18%**	–

A dash (–) indicates absence of change in group. Numerical values indicate fold change of enzyme activity or percent differences of treated group mean relative to control group mean. Numerical values in bold are statistically significant (P ≤ 0.05).

Sponsor table

Clinical Chemistry: Alterations were seen in multiple parameters in all groups. Increases in ALP, A/G ratio, Na, and Cl, and decreases in TP, Alb, Glob, CA, and Tri levels in one or both sexes appear to be related to SOM230 treatment as alterations were not evident in the LCI699 alone group. Decreases in CK seen in males appears to be related to LCI699 treatment. The magnitudes of these effects were not altered by co-administration with LCI699 or SOM230. The alterations in urea levels in females appears to be related to

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SOM230 treatment but is uncertain for males. The effects on Tri and CK levels were attributed to reductions in body weight. Data are summarized in the sponsor table below:

Table 31: Clinical Chemistry Alterations in 13-Week Combination Study - Rat

Table 4-2 Clinical biochemistry changes in rats administered LCI699/SOM230 when compared to control

	Males					Females				
Dose (mg/kg/day):	1.5/ 0.03	5/0.1	20/0.3	20/-	-/0.3	1.5/ 0.03	5/0.1	20/0.3	20/-	-/0.3
ALP activity U/L										
Week 4	–	–	–	–	–	–	1.23x	1.27x	–	1.19x
Week 14	–	–	–	–	–	1.18x	**1.44x**	**1.47x**	–	**1.46x**
Urea conc mg/dL										
Week 4	**18%**	**17%**	**32%**	–	–	–	–	**30%**	–	**21%**
Week 14	–	–	18%	–	–	–	–	–	–	10%
Total protein conc g/dL										
Week 4	–	–	**-8%**	–	–	**-10%**	**-9%**	**-12%**	**-6%**	**-8%**
Week 14	–	–	–	–	–	**13%**	**-14%**	**-13%**	**-7%**	**-12%**
Albumin g/dL										
Week 4	–	–	–	–	–	**-11%**	**-12%**	**-10%**	-7%	**-9%**
Week 14	–	–	–	–	–	**-15%**	**-14%**	**-11%**	**-8%**	**-13%**
Globulins g/dL										
Week 4	-8%	**-17%**	**-16%**	–	**-14%**	–	–	-13%	–	–
Week 14	**-11%**	**-21%**	**-18%**	–	**-23%**	-10%	**-13%**	**-16%**	–	–
Albumin/Globulin										
Week 4	9%	**24%**	**14%**	–	**21%**	–	–	–	–	–
Week 14	**16%**	**31%**	**24%**	–	**37%**	–	–	–	–	–
Calcium mg/dL										
Week 4	-3%	**-3%**	**-3%**	–	**-3%**	**-4%**	–	**-5%**	–	**-5%**
Week 14	–	–	–	–	–	–	–	–	–	–
Triglycerides g/dL										
Week 4	–	–	–	–	–	**-32%**	**-45%**	**-39%**	–	**-48%**
Week 14	–	–	–	–	–	**-44%**	**-50%**	-30%	-27%	**-51%**
Creatine kinase U/L										
Week 4	–	–	–	**-39%**	–	–	–	–	–	–
Week 14	-50%	-41%	-20%	–	–	–	–	–	–	–
Sodium mmo/L										
Week 4	–	–	–	–	**2%**	–	–	**2%**	–	**2%**
Week 14	–	–	–	–	**3%**	–	–	–	–	–
Chloride mmo/L										
Week 4	–	–	–	–	**2%**	**2%**	**2%**	**3%**	–	**4%**
Week 14	**2%**	**2%**	**3%**	–	**5%**	–	–	–	–	**4%**

A dash (–) indicates absence of change in group. Numerical values indicate fold change of enzyme activity or percent differences of treated group mean relative to control group mean. Numerical values in bold are statistically significant (P ≤ 0.05, 0.01 or 0.001).

Sponsor table

The co-administration of LCI699 and SOM230 was not considered to have exacerbated or attenuated any of the effects induced by either compound alone.

Hormone Analysis: Dose-related reductions were seen for IGF-1 levels in males administered SOM230 in combination with LCI699 were similar to those seen with SOM230 alone. In females, slight increases in IGF-1 levels were evident in all the combination treatment groups and the group administered LCI699 alone. In contrast, females administered SOM230 alone showed a reduction in IGF-1 levels.

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Serum corticosterone levels were reduced in all male combination groups and in all drug-treated female groups, but none of the effects attained statistical significance. Thus, LCI699 or SOM230 had no apparent interactive effects on corticosterone levels. The serum IGF-1 and corticosterone data are summarized in the sponsor table below:

Table 32: Hormonal Alterations in 13-Week Combination Study - Rat

Table 4-3 Hormone changes in rats administered LCI699/SOM230 when compared to control

	Males					Females				
Dose (mg/kg/day):	1.5/ 0.03	5/0.1	20/ 0.3	20/-	-/0.3	1.5/ 0.03	5/0.1	20/ 0.3	20/-	-/0.3
IGF-1 ng/mL										
Week 14	**-28%**	**-36%**	**-43%**	–	**-47%**	32%	24%	30% *(-24% 1/10)*	**49%**	*(-21% to -31% 4/10)*
Serum Corticosterone pg/mL										
Week 14	-28%	-38%	-25%	–	–	-35%	-42%	-32%	-36%	-27%

A dash (–) indicates absence of change in group. Numerical values indicate fold change of enzyme activity or percent differences of treated group mean relative to control group mean. Numerical values in bold are statistically significant (P ≤ 0.05, 0.01 or 0.001). *Numerical values in italic and brackets indicate percent differences of individual values relative to group mean values and the incidence of change, when the changes were not present in all animals of the group.*

Sponsor table

ACTH levels tended to be lower in most drug-treated groups, but the high variability and/or absence of a dose-response suggested these effects were unrelated to treatment. Urine corticosterone levels were increased in a dose-related manner in the combination treatment groups, but none of the effects reached statistical significance and there were multiple animals that samples could not be obtained from.

Urinalysis: The only effects seen were slight reductions in specific gravity (-16%) and creatinine concentration (-26%) in males administered SOM230 alone.

Gross Pathology: Enlarged adrenals were seen in 3 animals administered LCI699 alone that correlated with increased weights.

Organ Weights: In males, statistically significant increases or decreases in the absolute and/or adjusted (for body or brain) weights of the adrenals, kidney, pituitary, spleen, testis, and thyroid/parathyroid occurred in the MD/HD combination groups and in the group administered SOM230 alone. Kidney weights were also reduced in the LD combination group. In these organs, the absolute and adjusted weights were of the same magnitude. The magnitude was also similar between the various combination treated groups and the SOM230 alone group. No effects were seen in the group administered LCI699 alone. For the epididymis, heart, and liver, statistically significant reduced absolute and brain weight adjusted weights were seen in the MD/HD combination and SOM230 alone groups, but body weight adjusted weights were minimally increased, suggesting these effects were related to

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the lower body weights that occurred in these groups. The effects on organ weights were within the range of ± 30%. Data are summarized in the reviewer created table below:

Table 33: Organ Weight Changes in 13-Week Combination Study (Male) - Rat

Organ Weights (g) in Males (values rounded)							
Organ	Weight	Dosages (LCI699 / SOM230 in mg/kg)					
		0	1.5/0.03	5/0.1	20/0.3	20/0	0/0.3
Adrenal	Abs	0.045	0.041	0.037* (82%)	0.036* (80%)	0.050	0.036* (80%)
	Rel BW	0.012	0.012	0.013	0.013	0.014	0.013
	Rel BR	2.22	2.03	1.86 (84%)	1.83* (82%)	2.41	1.85 (83%)
Epididymis	Abs	1.11	1.10	1.09	1.07	1.07	1.03
	Rel BW	0.30	0.33	0.37*** (1.2x)	0.38*** (1.3x)	0.30	0.38*** (1.3x)
	Rel BR	54.2	54.1	54.3	54.0	52.1	53.0
Heart	Abs	0.99	0.95	0.87** (88%)	0.85*** (86%)	1.03	0.83*** (84%)
	Rel BW	0.27	0.28	0.29	0.30* (1.1x)	0.29	0.30** (1.1x)
	Rel BR	48.8	46.7	43.5* (89%)	42.8** (88%)	50.00	42.4** (87%)
Kidney	Abs	2.00	1.73** (87%)	1.57*** (79%)	1.56*** (78%)	1.99	1.45*** (73%)
	Rel BW	0.55	0.52	0.53	0.55	0.56	0.53
	Rel BR	98.1	85.2** (87%)	78.5*** (80%)	78.9*** (80%)	96.7	74.4*** (76%)
Liver	Abs	9.85	9.10	8.47** (86%)	8.52* (86%)	10.75	6.90*** (70%)
	Rel BW	2.69	2.72	2.87	2.99	3.01	2.52
	Rel BR	484	448	424* (88%)	430	522	354*** (73%)
Pituitary	Abs	0.010	0.009	0.008** (80%)	0.008** (80%)	0.010	0.008* (80%)
	Rel BW	0.0028	0.0029	0.0027	0.0028	0.0028	0.0030
	Rel BR	0.51	0.47	0.39* (76%)	0.40* (78%)	0.48	0.42
Spleen	Abs	0.58	0.51	0.44*** (76%)	0.41*** (71%)	0.58	0.45*** (78%)
	Rel BW	0.16	0.15	0.15	0.15	0.16	0.16
	Rel BR	28.6	25.2	22.0*** (77%)	20.9*** (73%)	28.0	22.8*** (80%)
Testis	Abs	3.46	3.52	3.53	3.50	3.70	3.29
	Rel BW	0.95	1.05	1.20*** (1.3x)	1.23*** (1.3x)	1.04	1.21*** (1.3x)
	Rel BR	170	173	176	177	180	169
Thyroid / Parathyroid	Abs	0.02	0.02	0.02	0.02	0.02	0.02
	Rel BW	0.0058	0.0068	0.0066	0.0074* (1.3x)	0.0070	0.0077* (1.3x)
	Rel BR	1.05	1.11	0.98	1.07	1.21	1.08
Rel BW – relative to body weight (%) Rel BR – relative to brain weight (%) *p<0.05, **p<0.01, ***p<0.001							

In females administered LCI699 alone, statistically significant absolute and adjusted (for body and/or brain weight) adrenal, kidney, liver, ovary/oviduct, and spleen weights were increased while pituitary weights were decreased. In the combination treatment groups, increases or decreases of similar magnitude were only seen for the ovary/oviduct and pituitary at the MD/HD. In contrast, reduced weights for these tissues, and the heart, were seen in the group administered SOM230 alone. Uterine weights were decreased in all groups administered LCI699 either alone or in combination, but the effect did not follow a dose response pattern and none of the effects reached statistical significance. Across organs, the alterations were ≤ 60%. Data are summarized in the reviewer-created table below:

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Table 34: Organ Weight Changes in 13-Week Combination Study (Female) - Rat

Organ Weights (g) in Females (values rounded)							
Organ	Weight	Dosages (LCI699 / SOM230 in mg/kg)					
		0	1.5/0.03	5/0.1	20/0.3	20/0	0/0.3
Adrenal	Abs	0.054	0.058	0.055	0.053	0.070*** (1.3x)	0.046 (85%)
	Rel BW	0.025	0.026	0.026	0.026	0.029	0.024
	Rel BR	2.90	3.04	2.91	2.87	3.68*** (1.3x)	2.50
Heart	Abs	0.75	0.75	0.69	0.72	0.78	0.65* (87%)
	Rel BW	0.35	0.34	0.33	0.35	0.32	0.34
	Rel BR	39.9	39.4	36.0	39.0	41.1	35.4
Kidney	Abs	1.35	1.39	1.30	1.35	1.50* (1.1x)	1.19** (88%)
	Rel BW	0.62	0.63	0.62	0.66	0.62	0.63
	Rel BR	71.6	72.8	67.8	73.1	78.7* (1.1x)	64.5* (90%)
Liver	Abs	6.16	6.13	5.79	6.03	7.89*** (1.3x)	5.04** (81%)
	Rel BW	2.86	2.79	2.75	2.95	3.26** (1.1x)	2.66
	Rel BR	327	320	303	327	415*** (1.3x)	274** (84%)
Ovary / Oviduct	Abs	0.12	0.14	0.15* (1.3x)	0.16** (1.3x)	0.19*** (1.6x)	0.11
	Rel BW	0.06	0.06	0.07* (1.2x)	0.08** (1.3x)	0.08*** (1.3x)	0.06
	Rel BR	6.60	7.28	7.80	8.42** (1.3x)	10.2*** (1.5x)	5.77
Pituitary	Abs	0.014	0.014	0.012* (86%)	0.011** (79%)	0.013	0.011** (79%)
	Rel BW	0.0067	0.0065	0.0057	0.0055* (82%)	0.0054* (81%)	0.0061
	Rel BR	0.76	0.75	0.62** (82%)	0.61** (80%)	0.69	0.62** (82%)
Spleen	Abs	0.45	0.49	0.43	0.40	0.49	0.37** (82%)
	Rel BW	0.21	0.23	0.21	0.19	0.20	0.19
	Rel BR	23.7	25.8	22.7	21.4	25.5	19.9
Thymus	Abs	0.29	0.32** (1.1x)	0.24	0.25	0.31** (1.1x)	0.25
	Rel BW	0.11	0.15** (1.4x)	0.12	0.12	0.13	0.13
	Rel BR	12.1	16.8** (1.4x)	12.8	13.6	16.5** (1.4x)	13.4
Uterus	Abs	0.73	0.57	0.47	0.61	0.47	0.66
	Rel BW	0.34	0.26	0.23	0.30	0.19	0.36
	Rel BR	38.7	29.8	24.8	32.9	24.5	36.0

ABS – absolute weight
Rel BW – relative to body weight (%)
Rel BR – relative to brain weight (%)
*p<0.05, **p<0.01, ***p<0.001

In summary, the co-administration of LCI699 and SOM230 did not appear to dramatically alter the effects on the majority of organ weight effects seen with either compound administered separately. For the liver, LCI699 and SOM230 appeared to have opposing effects such that the effects in the combination groups were attenuated to some extent.

Histopathology:

Adequate Battery: Yes

Peer Review: Yes

The adrenal glands, bone marrow, injection sites (of all groups administered SOM230), liver, ovary, pituitary, ovaries, uterus, vagina, and lesions from all group were examined microscopically. Other tissues were evaluated in the HD combination group and the groups administered LCI699 or SOM230 alone. Tissues from controls were also evaluated.

In males, hypocellularity of the bone marrow occurred at an increased incidence relative to controls in all drug-treated groups; the incidences were increased in the SOM230 alone and

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MD/HD combination groups as compared to the incidence in the LCI699 alone group. It is not clear if SOM230 coadministration exacerbated the effect of LCI699 or if LCI699 attenuated the effect of SOM230. Inflammation was evident at the injection site at similar incidences and severities in all combination groups and the group administered SOM230 alone, suggesting the effect is SOM230 mediated. In the liver, hepatocellular hypertrophy and/or vacuolation occurred in the LCI699 alone and HD combination group; this effect was considered LCI699-related. Decreased cytoplasmic mass was seen in the pituitary of MD/HD combo groups and in the SOM230 group, suggesting the effect is induced by SOM230. Data are summarized in the sponsor table below:

Table 35: Microscopic Observations in 13-Week Combination Study (Male) - Rat

Table 4-7 Incidence and severity of noteworthy histopathological changes - Males

Tissue/Finding	Sex	Males					
LCI699/SOM230 Dose (mg/kg/day)		0	1.5/0.03	5/0.1	20/0.3	20/-	-/0.3
Bone Marrow	No. examined	10	10	10	10	10	10
Hypocellularity: hematopoietic							
	Total number affected	1	3	6	9	3	10
	Minimal	1	3	6	9	3	9
	Slight	—	—	—	—	—	1
Injection site	No. examined	10	10	10	10	-	10
Inflammation							
	Total number affected	5	7	8	9	—	8
	Minimal	4	6	5	6	—	4
	Slight	1	1	3	3	—	4
Liver	No. examined	10	10	10	10	10	10
Hypertrophy: hepatocellular							
	Total number affected	—	—	—	—	6	—
	Minimal	—	—	—	—	6	—
Vacuolation: hepatocellular							
	Total number affected	—	—	—	3	10	—
	Minimal	—	—	—	3	5	—
	Slight	—	—	—	—	3	—
	Moderate	—	—	—	—	2	—
Pituitary	No. examined	10	9	10	9	10	9
Decrease cytoplasmic mass: acidophils							
	Total number affected	—	—	5	9	—	7
	Minimal	—	—	5	—	—	4
	Slight	—	—	—	5	—	3
	Moderate	—	—	—	4	—	—

Sponsor table

In females, cortical hypertrophy in the adrenal gland was seen in the group administered LCI699 alone, but not in any of the combination groups. At the injection sites, inflammation was seen at an increased incidence in the group given SOM230 alone and all combination groups as compared to the incidence in controls. As in males, this appears to be related to SOM230. Also, as in males, LCI699-related hepatocellular hypertrophy and/or vacuolation were only seen in the LCI699 alone group and the HD combination group. Ovarian follicular degeneration occurred at an increased incidence in the LCI699 alone group and in HD combination group, suggesting this effect is induced by LCI699. Hyperplasia/hypertrophy of the vaginal mucosa was only evident in the SOM230 alone treated groups.

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Microscopic observations in females are summarized in the sponsor table below:

Table 36: Microscopic Observations in 13-Week Combination Study (Female) - Rat

Table 4-8 Incidence and severity of noteworthy histopathological changes - Females

Tissue/Finding	Sex	Females					
LCI699/SOM230 Dose (mg/kg/day)		0	1.5/0.03	5/0.1	20/0.3	20/-	-/0.3
Adrenal	No. examined	10	10	10	10	10	10
Hypertrophy: cortical							
	Total number affected	—	—	—	—	4	—
	Minimal	—	—	—	—	4	—
Injection site	No. examined	10	10	10	10	-	10
Inflammation							
	Total number affected	5	6	5	7	—	10
	Minimal	5	6	4	7	—	10
	Slight	—	—	1	—	—	—
Liver	No. examined	10	10	10	10	10	10
Hypertrophy: hepatocellular							
	Total number affected	—	—	—	—	10	—
	Minimal	—	—	—	—	10	—
Vacuolation: hepatocellular							
	Total number affected	—	—	—	2	7	—
	Minimal	—	—	—	2	6	—
	Slight	—	—	—	—	1	—
Estrous cycle	No. examined	10	10	10	10	10	10
	Estrus	1	3	2	1	4	3
	Metestrus	1	1	2	3	2	—
	Diestrus	4	1	1	2	1	—
	Proestrus	4	5	5	4	3	7
Ovary	No. examined	10	10	10	10	10	10
Degeneration: follicular							
	Total number affected	—	—	—	3	6	1
	Minimal	—	—	—	1	3	—
	Slight	—	—	—	2	3	1
Vagina	No. examined	10	10	10	10	10	10
Hyperplasia/hypertrophy: mucosa							
	Total number affected	—	—	—	—	—	7
	Minimal	—	—	—	—	—	2
	Slight	—	—	—	—	—	5

Sponsor table

In summary, the incidence and magnitude of effects seen in the combination treatment groups were reduced as compared to those occurring in either the LCI699 or SOM230 alone treated groups. Although it is not possible to clearly state if the effects were attenuated by co-treatment, it is clear that co-administration did not exacerbate any of the observed effects.

Reference ID: 4532599

Toxicokinetics: Data were collected from 2 animals per timepoint from the controls and the three combination groups. Data were not collected from animals administered LCI699 or SOM230 alone so it is not possible to ascertain if co-administration altered exposures.

Exposure to LCI699 increased in a dose-proportional manner. Peak levels were reached as soon as 0.5 hours post-dose. There was no sex difference in exposure or evidence of accumulation. Exposures were comparable to that seen with LCI699 alone in other studies at the same dosages. Data are summarized in the sponsor table below:

Table 37: Mean Exposure to LCI699 in 13-Week Combination Study - Rat

Table 4-10 Mean (n = 2) rat plasma LCI699 toxicokinetic parameters

Dose of LCI699 (mg/kg/day)	Study day	Gender	AUC0-24h (ng*h/mL) value	Cmax (ng/mL)	Tmax (h)
1.5	1	Male	2450	634	1.0
		Female	2730	608	0.5
	77	Male	2690	999	1.0
		Female	3010	617	0.5
5	1	Male	12700	2100	0.5
		Female	15700	2170	1.0
	77	Male	11500	2340	0.5
		Female	13300	2130	0.5
20	1	Male	98000	7200	3.0
		Female	80400	5490	7.0
	77	Male	50000	6100	1.0
		Female	51600	4930	0.5

Sponsor table

Exposure to SOM230 increased in a dose-proportional manner. Peak levels were reached 0.5 to 1 hour after dose administration. Levels in males were 1.4 to 1.8-fold higher than females and were slightly higher on day 77 as compared to day 1 indicating some accumulation with repeated dosing. Data are summarized in the sponsor table below:

Table 38: Mean Exposure to SOM230 in 13-Week Combination Study - Rat

Table 4-12 Mean (n = 2) rat plasma SOM230 toxicokinetic parameters

Dose of SOM230 (mg/kg/day)	Study day	Gender	AUC0-24h (ng*h/mL) value	Cmax (ng/mL)	Tmax (h)
0.03	1	Male	108	34.4	1.0
		Female	66.1	27.9	0.5
	77	Male	143	61.1	1.0
		Female	79.6	45.5	0.5
0.1	1	Male	365	122	1.0
		Female	210	95.4	0.5
	77	Male	442	160	1.0
		Female	318	146	1.0
0.3	1	Male	947	237	0.5
		Female	651	186	0.5
	77	Male	1510	293	1.0
		Female	888	323	1.0

Sponsor table

Reference ID: 4532599

7 Genetic Toxicology

The sponsor evaluated LCI699 for genotoxic potential in an *in vitro* bacterial reverse mutation (Ames) assay, an *in vitro* micronucleus assay in cultured human peripheral lymphocytes, and in an *in vivo* micronucleus induction study and *in vivo* comet assay in rats.

7.1 In Vitro Assays

7.1.1 Reverse Mutation in Five Histidine-Requiring Strains of *Salmonella typhimurium* (study 0680235)

An Ames assay was performed to determine the potential for LCI699 to induce mutations in bacterial strains *TA98, TA100, TA102, TA1535,* and *TA1537*. There were no increases in the number of revertant cells at the concentrations evaluated (1.6, 8, 40, 156.25, 200, 312.5, 625, 1000, 1250, 2500, 5000 µg/plate across two experiments) in the presence or absence of S9 metabolic activation. Significant increases in the number of revertants were observed with the positive control substances but not the negative control, indicating that the study was valid.

7.1.2 Induction of Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes (study 0680236)

LCI699 was evaluated in this assay using human lymphocytes from pooled blood samples from three human donors in two experiments. Concentrations to be tested were selected based on a ranging study evaluating mitotic inhibition that revealed concentrations up to 2272 µg/mL could be evaluated in the presence and absence of S9. Chromosome aberrations were subsequently analyzed at 3 concentrations in the main experiments.

Treatment of cultures in the absence of S9 resulted in statistically significant increased incidences of cells with structural aberrations compared to negative controls in both cultures at concentrations $\geq$ 2000 µg/mL, with the incidences exceeding the historical negative control range at the highest concentration analyzed in Experiment 1 and the two highest concentrations in Experiment 2. Data are shown in the sponsor tables below:

Reference ID: 4532599

Table 39: Chromosome Aberration Assay – Structural Aberrations without S9

4.3.4.1 3 hour treatment -S-9, 17 hour recovery (3+17), Experiment 1
Donor sex: female

Table 4-7 Experiment 1, 3+17, -S-9, cells with structural aberrations

Treatment (µg base/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Significance §	MIH* (%)
Solvent	A	100	1	1		
	B	100	0	0		
	Totals	200	1	1		-
1400	A	100	2	0		
	B	100	0	0		
	Totals	200	2	0	NS	10
2000	A	100	5	4		
	B	100	3	3		
	Totals	200	8	7	$p \leq 0.05$	40
2272	A	100	11	9		
	B	100	14	13		
	Totals	200	25	22	$p \leq 0.001$	46
NQO, 5.00	A	60	20	20		
	B	56	20	20		
	Totals	116	40	40	$p \leq 0.001$	

Binomial Dispersion Test χ^2= 1.97, NS
§ Statistical significance (Appendix: Detailed summary of aberrations)
NS = not significant
Numbers highlighted exceed historical negative control range (Historical control data)
*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%
(where T = treatment and C = negative control)

4.3.4.3 3 hour treatment -S-9, 17 hour recovery (3+17), Experiment 2
Donor sex: female

Table 4-9 Experiment 2, 3+17, -S-9, cells with structural aberrations

Treatment (µg base/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Significance §	MIH* (%)
Solvent	A	100	1	1		
	B	100	1	1		
	Totals	200	2	2		-
1200	A	100	1	1		
	B	100	1	0		
	Totals	200	2	1	NS	17
2000	A	100	10	8		
	B	100	8	5		
	Totals	200	18	13	$p \leq 0.01$	44
2200	A	100	12	11		
	B	100	9	8		
	Totals	200	21	19	$p \leq 0.001$	56
NQO, 2.50	A	70	24	20		
	B	78	20	19		
	Totals	148	44	39	$p \leq 0.001$	

Binomial Dispersion Test χ^2= 2.27, NS
§ Statistical significance (Appendix: Detailed summary of aberrations)
NS = not significant
Numbers highlighted exceed historical negative control range (Historical control data)
*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%
(where T = treatment and C = negative control)

Sponsor tables

In the presence of S9 activation, statistically significant increased frequencies of cells with structural aberrations also occurred at concentrations ≥ 2000 µg/mL. The incidences exceeded the negative control historical range at the highest concentration tested in Experiment 1 and the two highest concentrations evaluated in Experiment 2. Data are shown in the sponsor tables below:

Table 40: Chromosome Aberration Assay - Structural Aberrations with S9

4.3.4.2 3 hour treatment +S-9, 17 hour recovery (3+17), Experiment 1
Donor sex: female

Table 4-8 Experiment 1, 3+17, +S-9, cells with structural aberrations

Treatment (µg base/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Significance §	MIH* (%)
Solvent	C	100	2	1		
	D	100	1	1		
	Totals	200	3	2		-
1400	A	100	1	1		
	B	100	0	0		
	Totals	200	1	1	NS	0
1750	A	100	3	3		
	B	100	0	0		
	Totals	200	3	3	NS	0
2272	A	100	9	8		
	B	100	5	5		
	Totals	200	14	13	$p \leq 0.01$	0
CPA, 6.25	A	41	20	20		
	B	67	21	20		
	Totals	108	41	40	$p \leq 0.001$	

Binomial Dispersion Test χ^2= 4.79, NS
§ Statistical significance (Appendix: Detailed summary of aberrations)
NS = not significant
Numbers highlighted exceed historical negative control range (Historical control data)
*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%
(where T = treatment and C = negative control)

4.3.4.4 3 hour treatment +S-9, 17 hour recovery (3+17), Experiment 2
Donor sex: female

Table 4-10 Experiment 2, 3+17, +S-9, cells with structural aberrations

Treatment (µg base/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Significance §	MIH* (%)
Solvent	A	100	0	0		
	B	100	0	0		
	Totals	200	0	0		-
1200	A	100	1	1		
	B	100	3	2		
	Totals	200	4	3	NS	21
2000	A	100	3	3		
	B	100	2	2		
	Totals	200	5	5	$p \leq 0.05$	30
2200	A	100	10	8		
	B	100	12	9		
	Totals	200	22	17	$p \leq 0.001$	40
2272	A	100	11	10		
	B	100	9	9		
	Totals	200	20	19	$p \leq 0.001$	49
CPA, 12.5	A	62	21	20		
	B	41	21	20		
	Totals	103	42	40	$p \leq 0.001$	

Binomial Dispersion Test χ^2= 0.67, NS
§ Statistical significance (Appendix: Detailed summary of aberrations)
NS = not significant
Numbers highlighted exceed historical negative control range (Historical control data)
*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%
(where T = treatment and C = negative control)

Sponsor tables

Reference ID: 4532599

The positive control substances (4-Nitroquinoline 1-oxide (NQO), cyclophosphamide (CPA) induced an increase in structural aberrations supporting the validity of the study.

For numerical aberrations, there were no increases in the frequencies in the absence or presence of S9 activation in Experiment 1. However, increases were seen in the presence or absence of S9 in Experiment 2 at all concentrations. These data for Experiment 2 are shown in the sponsor tables below:

Table 41: Chromosome Aberration Assay - Numerical Aberrations

Table-7 Numerical aberrations - 3+17 hours, -S-9, Experiment 2

Donor sex: female

Treatment (µg base/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	101	0	0	1	1	1.0
	B	100	0	0	0	0	0
	Total	201	0	0	1	1	0.5
1200	A	100	0	0	0	0	0
	B	102	0	0	2	2	2.0
	Total	202	0	0	2	2	1.0
2000	A	103	0	0	3	3	2.9
	B	102	0	0	2	2	2.0
	Total	205	0	0	5	5	2.4
2200	A	103	0	0	3	3	2.9
	B	102	0	0	2	2	2.0
	Total	205	0	0	5	5	2.4
NQO, 2.50	A	70	0	0	0	0	0
	B	79	0	0	1	1	1.3
	Total	149	0	0	1	1	0.7

** = Total cells examined for numerical aberrations

Numbers highlighted exceed historical negative control range (Appendix: historical control data and abbreviations and classification of observations)

For abbreviations and classification see Appendix: historical control data and abbreviations and classification of observations

Table-8 Numerical aberrations - 3+17 hours, +S-9, Experiment 2

Donor sex: female

Treatment (µg base/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
1200	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
2000	A	104	0	0	4	4	3.8
	B	102	0	0	2	2	2.0
	Total	206	0	0	6	6	2.9
2200	A	108	0	0	8	8	7.4
	B	105	0	0	5	5	4.8
	Total	213	0	0	13	13	6.1
2272	A	102	0	0	2	2	2.0
	B	102	0	0	2	2	2.0
	Total	204	0	0	4	4	2.0
CPA, 12.5	A	62	0	0	0	0	0
	B	41	0	0	0	0	0
	Total	103	0	0	0	0	0

** = Total cells examined for numerical aberrations

Numbers highlighted exceed historical negative control range (Appendix: historical control data and abbreviations and classification of observations)

For abbreviations and classification see Appendix: historical control data and abbreviations and classification of observations

Sponsor tables

The increased numerical aberrations were attributed to increased numbers of polyploid cells. However, the significance of these findings is not clear as the increases did not follow a concentration-related pattern, there were no increases seen in Experiment 1, increases were not seen with the positive control substances, and the detection of numerical aberrations was not a primary endpoint of the study.

Regardless of the effects on numerical aberrations, LCI699 was considered a clastogen as it induced increased numbers of structural aberrations in both the presence and absence of S9 metabolic activation.

7.1.3 Micronucleus Test In Vitro Using TK6 Cells (Study 0514061)

The clastogenic and/or aneugenic potential of LCI699 was evaluated in this non-GLP assay. TK6 cells were exposed to LCI699 for 3 hours with S9 metabolic activation (284, 568, 1136, 2272 µg/plate) or for 20 hours without S9 (71, 142, 284, 568 µg/plate). There was no increase in the percentage of cells with micronuclei under either condition at concentrations up to those resulting in > 50% reduction in cell growth. The positive controls induced increases in the percentage of micronuclei.

Reference ID: 4532599

7.2 In Vivo Assays

7.2.1 Rat Micronucleus Test (Study 0670426)

Rats were administered a single oral dose of LCI699 at doses of 30, 100, or 200 mg/kg or the positive control cyclophosphamide. Bone marrow was collected 24 hours (all groups) and 48 hours (C and HD) post-dose. There was no indication of an increase in the incidence of micronucleated immature erythrocytes or change in the proportion of immature erythrocytes following treatment with dosages up to 200 mg/kg. The study was considered valid as increase were observed with the positive control article. As a result, LCI699 was considered negative for evidence of genotoxicity in this assay.

7.2.2 In Vivo Alkaline Single Cell Gel Electrophoresis Analysis in Rat Liver and Peripheral Blood Leukocytes with LCI699 (Study 0612507)

The potential for LCI699 to induce DNA breaks in rat liver and leukocyte cells was evaluated in a Comet Assay. LCI699 was administered to rats at dosages of 26.2, 52.4, and 104.8 mg (base content)/kg (37.5, 75, 150 mg (net weight)/kg, respectively) by oral gavage at 24 and 6 hours prior to tissue sampling. There were no differences between LCI699-treated groups and controls for DNA migration in hepatocytes or leukocytes while increases were seen with the positive control MMS. The results did not show any evidence that LCI699 has the potential to induce DNA damage.

8 Carcinogenicity

8.1 A 104-Week Oral (Gavage) Carcinogenicity Study in Mice

Study no.:	1270062
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	LCI699, lot # 1212007, 100%
CAC concurrence:	(b) (4)

Reference ID: 4532599

Methods

Doses:	0, 3, 10, 30 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Ultra-pure water
Basis of dose selection:	Clinical signs of CNS toxicity (hypersensitivity to touch, ataxia, increased locomotor activity, twitches, labored respiration, abdominal distention, piloerection, lacrimation, pale or thin appearance, hunched posture, and dehydration) at 50 mg/kg and mortality at 200 mg/kg for both sexes in a 13-week dose range-finding study.
Species/Strain:	Mouse / Crl:CD-1 (ICR)BR
Number/Sex/Group:	70
Age:	6 weeks
Animal housing:	Up to 3/cage of same sex and dose group
Weight:	♂ 24.8 – 34.9g; ♀ 18.3 – 25.2g
Satellite groups:	10/sex/group for tk
Unique study design:	No
Deviation from study protocol:	None that effected study integrity

Key Study Findings

Neoplastic NOAEL: The neoplastic NOAEL was considered 3 mg/kg for males due to statistically significant increases in incidences of hepatocellular adenomas, carcinomas, and combined adenoma/carcinoma at higher doses. The NOAEL for females was 30 mg/kg due to absence of drug-related neoplastic findings.

Table 42: Exposure Multiples for Neoplastic Observations in CD-1 Mice

CD-1 Mouse Neoplastic Findings			Exposure Multiple [c]
Sex	NOAEL	AUC_{0-24h} (ng*h/mL)	AUC
♂	3 mg/kg/day	2,838 [a]	1.7 X
♀	30 mg/kg/day	52,000 [b]	31 X

[a] AUC_{0-24h} value was calculated from data in Study #: 0770695, a 13 week repeat dose study. The AUC_{0-24h} value for male mice treated with 10 mg/kg LCI699 on day 72 (9,460 ng*h/mL in males) was divided by 3.3 to estimate an AUC_{0-24h} value for mice treated with 3 mg/kg LCI699.
[b] AUC_{0-24h} for mice were based on data from Study #: 0770695, a 13 week repeat dose study (10 to 100 mg/kg/day, Day 72)
[c] MRHD = 60 mg/day or 30 mg twice daily (AUC_{0-t} = 1,680 ng*h/mL from Study: CLCI699C2108).

Non-neoplastic NOAEL: The non-neoplastic NOAEL for males and females was the highest dose examined (30 mg/kg/day) due to a lack of adverse non-neoplastic findings.

Reference ID: 4532599

Table 43: Exposure Multiples for Non-neoplastic Findings in CD-1 Mice

CD-1 Mouse Non-Neoplastic Findings			Exposure Multiple [c]
Sex	NOAEL	AUC_{0-24h} (ng*h/mL)	AUC
♂	30 mg/kg/day	61,500 [a]	37X
♀	30 mg/kg/day	52,000[b]	31X

[a]AUC_{0-24h} and C_{max} for mice were extrapolated based on data from Study #: 0770695, a 13 week repeat dose study (10 to 100 mg/kg/day, Day 72)
[b] MRHD = 60 mg/day or 30 mg twice daily (AUC_{0-t} = 1,680 ng*h/mL from Study: CLCI699C2108).

Adequacy of Carcinogenicity Study: The study design was considered adequate for evaluating the potential for LCI699 to induce carcinogenicity. All female groups were terminated beginning day 721 (week 103) when the number of surviving females in the control group reached 20.

Appropriateness of Test Model: The mouse was considered an acceptable test species.

Evaluation of Tumor Findings: There were statistically significant increases in liver tumors in males at dosages ≥ 10 mg/kg. Increased incidences of drug-related neoplasms were not observed in females at the dosages evaluated.

Observations and Results

Mortality: Both LCI699-dependent and LCI699-independent findings caused mortality in mice. At week 104, survival in drug-treated male LD and HD groups was slightly less than that of controls. In week 103, the number of surviving control females was reduced to 20 and all females were necropsied starting on day 721. Regardless, there were an adequate number of females available to evaluate the carcinogenic risk. Survival data are summarized in the sponsor table below:

Reference ID: 4532599

Table 44: Survival Rates at Various Study Timepoints - Mice

Table 4-1 Summary of survival ratios with % survival at periodic intervals

Study week	Males				Females			
	Dose (mg/kg/day)				Dose (mg/kg/day)			
	0	3	10	30	0	3	10	30
16	70 (100%)	70 (100%)	70 (100%)	69 (99%)	68 (97%)	70 (100%)	66 (94%)	67 (96%)
28	68 (97%)	70 (100%)	70 (100%)	69 (99%)	68 (97%)	68 (97%)	65 (93%)	66 (94%)
56	66 (94%)	65 (93%)	68 (97%)	63 (90%)	61 (87%)	64 (91%)	61 (87%)	58 (83%)
80	49 (70%)	48 (69%)	59 (84%)	47 (67%)	46 (66%)	51 (73%)	44 (63%)	42 (60%)
96	34 (49%)	36 (51%)	37 (53%)	28 (40%)	29 (41%)	38 (54%)	33 (47%)	28 (40%)
100	33 (47%)	31 (44%)	33 (47%)	24 (34%)	25 (36%)	32 (46%)	30 (43%)	23 (33%)
104	30 (43%)	25 (36%)	33 (47%)	17 (24%)	20 [a] (29%)	30 [a] (43%)	29 [a] (41%)	22 [a] (31%)

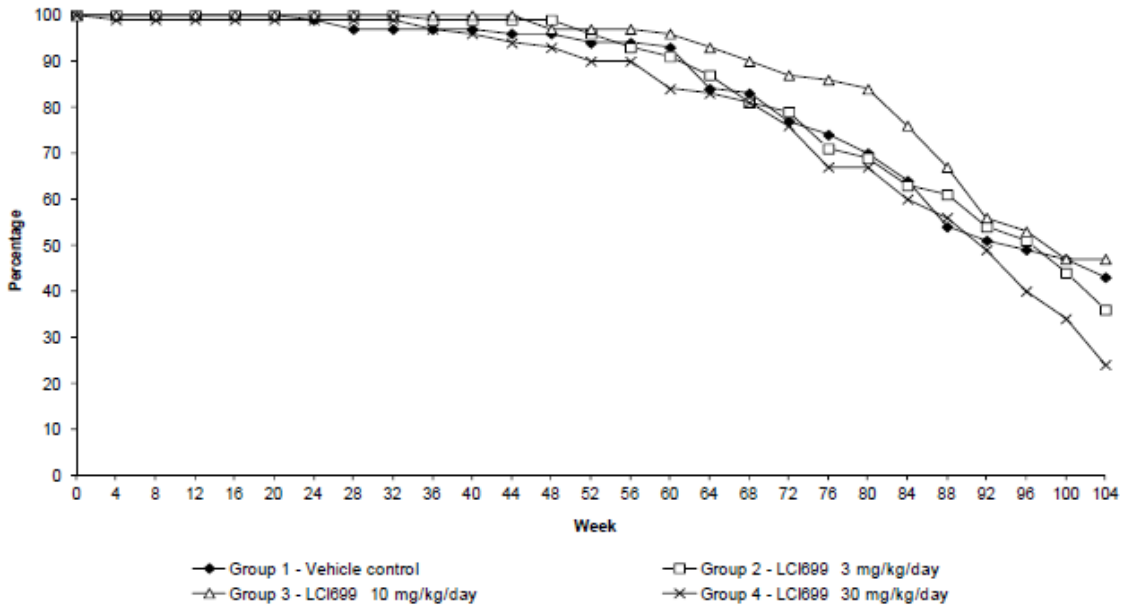
Note: n = 70 for each dose group.

a Groups 1-4 terminated on Day 721 (week 103).

Sponsor table

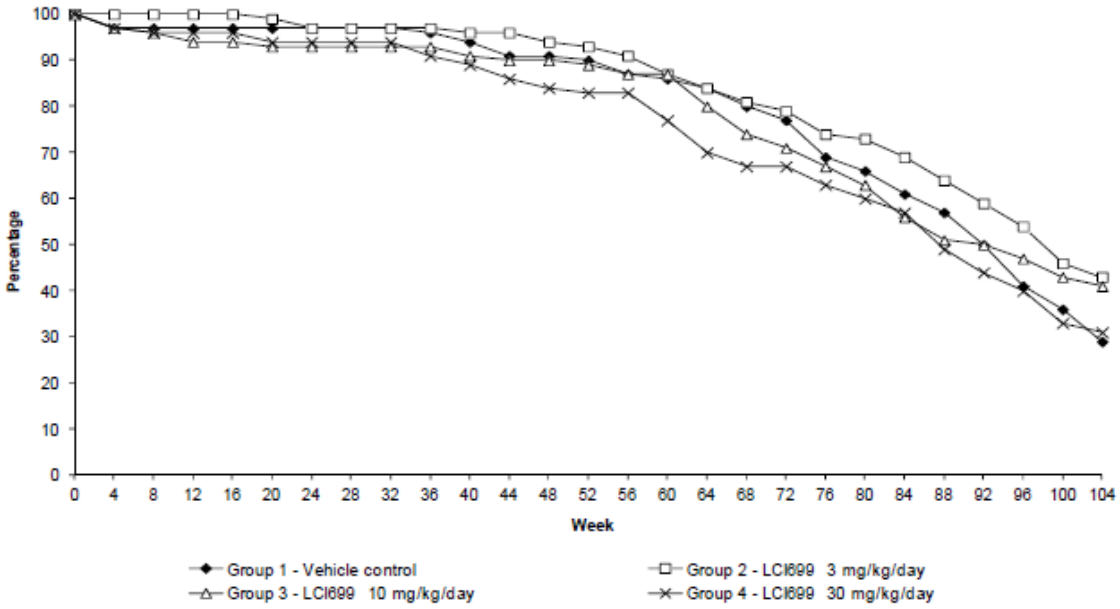
Survival curves for both males and females are shown below:

Figure 15: Survival Curve for Male Mice



Sponsor figure

Figure 16: Survival Curve for Female Mice



Sponsor figure

In HD males, an increased incidence of mortality (11/53) was observed due to hepatocellular adenomas/carcinomas. The slightly increased incidence of neoplastic fibrosarcoma (subcutis tumors) at doses ≥MD in male mice was considered incidental due to the frequency of this spontaneous tumor type in rodents (Greaves, P. et al, 2013). Lymphomas were the most frequent neoplastic finding, consistent with previous studies showing that lymphomas are one of the most common tumors in CD-1 mice (Ward, J. 2006). The most common non-neoplastic cause of death in both control and LCI699-treated male and female mice was urogenital tract changes (i.e. obstructive uropathy and nephropathy). The causes of early deaths are summarized in the sponsor table below:

Reference ID: 4532599

Table 45: Causes of Death in Mice

Table 4-3 Major causes of death in preterminal decedent animals

Sex	Males				Females			
Dose Level (mg/kg/day)	0	3	10	30	0	3	10	30
Number examined[a]	40	45	37	53	50	40	41	48
Undetermined	1	5	3	5	1	1	3	8
Accidental (including gavage accident)	1	0	3	0	2	1	4	6
Neoplastic:								
Hemolymphoreticular tumors:								
Histiocytic Sarcoma	0	3	2	1	3	2	2	2
Lymphoma	7	8	7	6	19	11	14	15
Hepatic tumors[b]	1	2	2	**11**	0	0	0	0
Lung tumors[c]	6	10	5	4	1	1	3	2
Subcutis tumors[d]	1	2	4	4	3	1	0	1
Vascular tumors[e]	2	2	3	1	1	1	0	0
Other Neoplastic:	6	3	3	4	3	4	2	2
Non Neoplastic: Urogenital tract changes[f]	9	7	3	11	8	13	6	5

Bold text indicates LCI699-related observations

a Total number of found dead or preterminally euthanized animals

b Hepatocellular adenoma/carcinoma

c Carcinoma: bronchioloalveolar

d Fibrosarcoma

e Hemangiosarcoma

f Obstructive uropathy and nephropathy

Sponsor table

Clinical Signs: Clinical signs of decreased activity, dehydration, prominent backbone, decreased muscle tone, etc, associated with poor or deteriorating condition were evident in animals prior to being found dead/euthanized. These signs occurred at comparable incidences between the groups. Dose-dependent trends toward increases were seen in mice that were hypersensitive to touch, hyperreactive (females), had shallow breathing (males), or had an increased respiratory rate. These findings are consistent with the 13-week repeat dose ranging study in mice. Convulsions (non-sustained) and/or tremors were also noted to occur at increased incidences in all drug treated male groups and HD females. The relationship of these signs to drug-treatment is uncertain, although twitches/convulsions have been reported in 2- and 13-week studies at dosages $\geq$ 100 mg/kg. Clinical signs occurring at increased number of animals in drug-treated groups are summarized in the reviewer created table below:

Reference ID: 4532599

Table 46: Clinical Signs - Mice

		Males				Females			
# animals		70	70	70	70	70	70	70	70
mg/kg/day		0	3	10	30	0	3	10	30
Hypersensitive	# of Observations	2	8	21	55	1	3	9	135
	# of animals	1	2	3	9	1	1	2	8
	Days from – to	548 555	555 667	562 731	121 731	287 287	682 695	136 556	17 721
Hyperreactive	# of Observations	1	-	1	3	1	-	1	22
	# of animals	1	-	1	2	1	-	1	4
	Days from - to	506 506	-	520 520	471 632	510 510	-	403 403	227 690
Breathing shallow	# of Observations	14	16	30	80	42	38	45	36
	# of animals	5	8	11	19	19	17	20	14
	Days from – to	422 447	454 581	457 731	527 597	258 723	153 695	42 730	77 722
Respiratory Rate Increased	# of Observations	9	22	27	31	12	26	30	77
	# of animals	4	7	9	9	5	5	10	9
	Days from – to	478 604	440 716	421 702	478 731	396 591	397 584	416 717	87 721
Convulsions (non-sustained)	# of Observations	-	-	5	2	1	1	4	8
	# of animals	-	-	2	2	1	1	1	3
	Days from – to	-	-	268 688	268 646	717 717	398 398	330 542	308 507
Tremors	# of Observations	-	11	8	10	4	6	6	12
	# of animals	-	8	6	7	4	6	6	10
	Days from – to	-	349 696	268 716	268 695	275 661	136 695	14 710	178 729

Palpable masses were observed at similar incidences in controls and all drug-treated groups. Other clinical signs observed in control and LCI699-treated mice included a firm abdominal inter-structure, dark/opaque/protruding eyeballs, cold to touch, fur discolorations, etc. These findings were not considered test article-related as they were observed at comparable incidences in all groups or at low incidences, occurred in the absence of a dose-related pattern, and/or were common observations in long term studies in mice.

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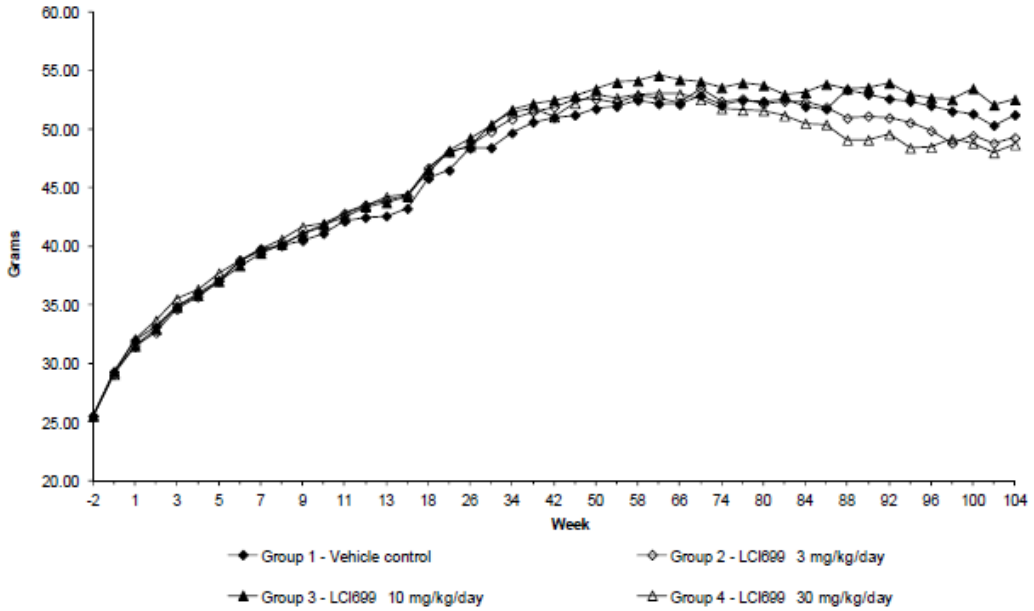
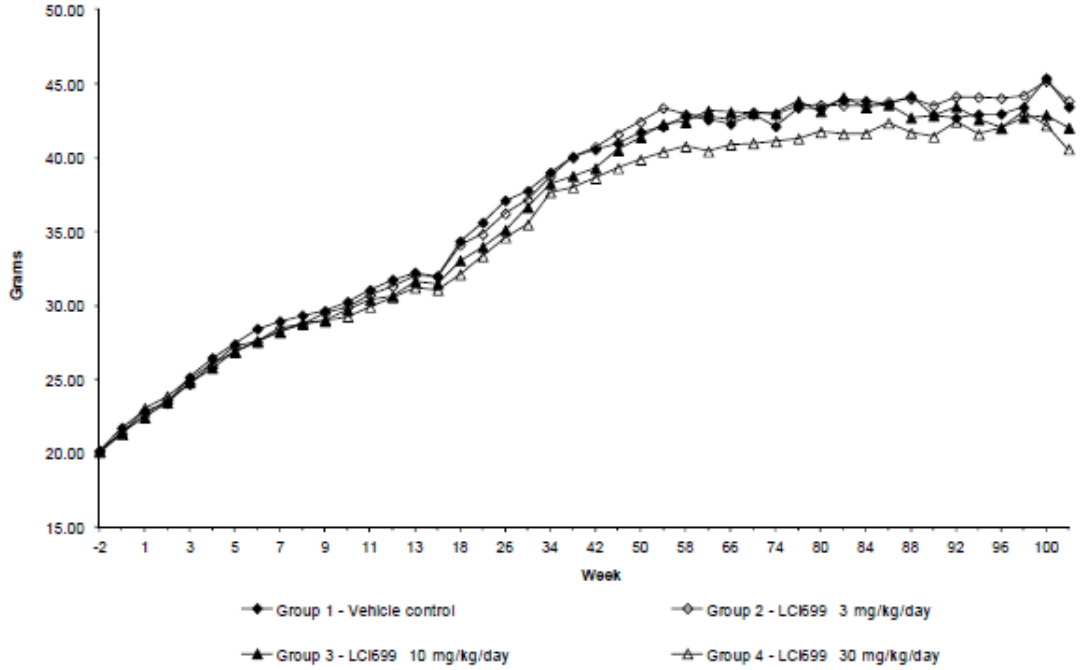
Body Weights: Effects on body weight were unremarkable. Decreases in body weight for LCI699-treated mice did not exceed 10% when compared to control mice at any dose or time point. Data are summarized in the sponsor body weight curves below:

Figure 17: Summary of Body Weight - Male Mice



Sponsor figure

Figure 18: Summary of Body Weight - Female Mice



Sponsor figure

Reference ID: 4532599

Feed Consumption: There were no effects on feed consumption.

Gross Pathology: Gross macroscopic findings were limited to the livers of male mice. Macroscopic findings included an increased incidence of liver enlargement at the HD, pale focus formation (≥ LD), and raised/nodule/mass focus formation (≥ LD) when compared with the control group. These findings correlated with hepatocellular altered cell foci and/or neoplasia and were similar to findings observed in male rats. Data are summarized in the sponsor table below:

Table 47: Drug-related Macroscopic Observations

Tissue/Finding Sex	Males				Females			
Dose (mg/kg/day)	0	3	10	30	0	3	10	30
Number of animals examined	70	70	70	70	70	70	70	70
Liver								
Enlargement	7	10	9	**15**	13	12	15	14
Focus pale	10	**17**	**18**	**17**	12	8	10	13
Focus raised/nodule/mass[a]	24	**33**	**45**	**48**	17	13	8	10

a Combined incidence

Bold text indicates LCI699-related observations

Sponsor table

Histopathology
Peer Review: Yes

Neoplastic: A drug-related increase in the incidence of neoplasms was only observed in males. There was a statistically significant increased incidence of hepatocellular adenomas and/or carcinomas at dosages ≥ MD and a numerical increase at the LD. There was also an increase incidence of foci of cellular alteration in basophilic, clear cell, and eosinophilic cells that were considered spontaneous by the sponsor. The incidences of these effects are summarized in the sponsor table below:

Reference ID: 4532599

Table 48: Incidence of Liver Tumors - Mice

Table 4-6 Incidence, severity and/or multiplicity of LCI699-related proliferative and neoplastic liver histopathological changes

Tissue/Finding	Sex	Males				Females			
	Dose (mg/kg/day)	0	3	10	30	0	3	10	30
Liver	Number examined	70	70	70	70	70	70	70	70
Hepatocellular adenoma									
	Total number affected	13	15	**29** [a]	**27** [a]	2	1	1	2
	Single	11	13	13	13	2	1	1	2
	Multiple	2	2	**16**	**14**	0	0	0	0
Hepatocellular carcinoma									
	Total number affected	3	8	7	**18** [a]	0	0	1	0
	Single	3	6	5	**14**	0	0	1	0
	Multiple	0	2	2	**4**	0	0	0	0
Combined incidence: Adenoma/carcinoma		16	23	**36** [a]	**45** [a]	2	1	2	2
Focus of cellular alteration: basophilic									
	Total number affected	2	2	6	7	0	0	0	0
	Minimal	1	1	0	2	0	0	0	0
	Mild	1	1	4	5	0	0	0	0
	Moderate	0	0	2	0	0	0	0	0
Focus of cellular alteration: clear cell									
	Total number affected	2	3	9	6	0	1	0	0
	Minimal	0	2	4	3	0	1	0	0
	Mild	1	0	1	2	0	0	0	0
	Moderate	1	1	3	1	0	0	0	0
	Marked	0	0	1	0	0	0	0	0
Focus of cellular alteration: eosinophilic									
	Total number affected	1	3	6	**11**	1	1	1	0
	Minimal	1	1	2	4	0	1	1	0
	Mild	0	2	2	6	1	0	0	0
	Moderate	0	0	2	1	0	0	0	0

[a] Statistically significantly different from control ($p \leq 0.01$; see Statistical Analysis Report for details)

Bold text indicates LCI699-related observations

Sponsor table

Statistical analysis (FDA) revealed a statistically significant increase in the incidence of hepatocellular adenomas (≥ 10 MD) and hepatocellular carcinomas (HD) by pairwise comparisons when compared to controls. Hepatocellular carcinomas and adenomas also showed a statistically significant increasing dose-response relationship across the control and treated groups when analyzed individually or in combination. The percentage of mice with hepatocellular adenomas at the MD (41%) and HD (39%) or hepatocellular carcinomas at the HD (26%) exceeded levels in historical control data for this facility (maximum levels were 23% for hepatocellular adenomas and 15% for hepatocellular carcinomas). The incidence of adenomas and carcinomas combined (46% and 51% in the MD and HD

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groups, respectively), also exceeded the level in controls (21%). No statistically significant increases were detected by trend wise or pairwise comparisons in females. The FDA statistical analysis is shown below:

Table 49: FDA Statistical Analysis of Tumors in Mice

Sex	Organ Name	Tumor Name	0 mg/kg Veh. Cont. (N=65) P - Trend	3 mg/kg Low (N=60) P - VC vs. L	10 mg/kg Med (N=65) P - VC vs. M	30 mg/kg High (N=65) P - VC vs. H
Male	Hemolymphoreticular Tissue	Histiocytic Sarcoma	0/70 (47) 0.7614	6/70 (49) 0.0151*	3/70 (53) 0.1449	1/70 (44) 0.4835
	Liver	Hepatocellular Adenoma	13/70 (50) 0.0023*	15/70 (49) 0.3874	29/70 (57) 0.0072*	27/70 (51) 0.0049*
		Hepatocellular Carcinoma	3/70 (48) 0.0001*	8/70 (49) 0.1060	7/70 (53) 0.2029	18/70 (49) 0.0002*
		Hepatocellular Adenoma/Carcinoma	15/70 (51) 0.0001*	21/70 (51) 0.1501	32/70 (58) 0.0057*	36/70 (55) 0.0002*
Female	Ovary	Luteoma	6/70 (45) 0.0413@	2/70 (50) 0.9796	4/70 (44) 0.8334	8/70 (40) 0.2963
	Uterus	Leiomyoma	1/70 (44) 0.0257@	1/70 (48) 0.7740	0/70 (44) 1.0000	4/70 (42) 0.1658
		Leiomyoma / Leiomyosarcoma	2/70 (44) 0.0348@	3/70 (48) 0.5416	1/70 (44) 0.8793	6/70 (42) 0.1181

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
@: not statistically significant at 0.005 for common in dose response relationship (trend) tests.
*: Statistically significant at 0.005 and 0.025 for common and rare tumors, respectively in dose response relationship (trend) tests and significance levels of 0.01 and 0.05 for common and rare tumors, respectively in pairwise comparisons.

FDA table

Non-Neoplastic: LCI699 treatment resulted in an increase in the incidence and severity of non-neoplastic changes in the liver. Centrilobular or diffuse hepatocellular hypertrophy tended to increase in incidence and/or severity (minimal to marked) with increasing dose, with the incidences reaching statistical significance in all drug-treated male groups and the HD female group. Minimal to marked bile duct hyperplasia (≥ MD in males and HD in females) affected a smaller number of LCI699-treated mice. These effects occurred at higher incidences in males as compared to their incidence in females. Additional observations in the liver including minimal to marked inflammation (focal or multifocal infiltrations of mixed inflammatory cells), minimal to mild single cell necrosis, and minimal to moderate reactive sinusoidal lining cells were typically seen at higher incidences and/or severity in LCI699-treated mice when compared to controls. Signs indicative of lipid accumulation included minimal to moderate pigment deposition in hepatocytes and Kupffer cells (suggestive of lipofuscin) and hepatocyte vacuolation consistent with intracytoplasmic lipid accumulation in HD females. The incidences and severity of these observations are summarized in the sponsor table below:

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Table 50: Non-Neoplastic Liver Observations - Mice

Table 4-9 Incidence and severity of LCI699-related non-neoplastic liver histopathological changes

Tissue/Finding	Sex	Males				Females			
	Dose (mg/kg/day)	0	3	10	30	0	3	10	30
Liver	Number examined	70	70	70	70	70	70	70	70
Hepatocellular hypertrophy									
	Total number affected	7	**18**	**42**	**36**	12	16	17	**21**
	Minimal	0	0	3	3	1	0	2	1
	Mild	4	4	22	14	9	10	14	7
	Moderate	3	14	12	17	1	6	1	13
	Marked	0	0	5	2	1	0	0	1
Hyperplasia: bile duct									
	Total number affected	0	0	**3**	**4**	0	0	0	1
	Minimal	0	0	2	3	0	0	0	0
	Mild	0	0	1	1	0	0	0	0
	Marked	0	0	0	0	0	0	0	1
Inflammation									
	Total number affected	9	**17**	**28**	**16**	8	12	15	11
	Minimal	8	13	19	10	8	11	13	8
	Mild	1	4	8	5	0	1	2	2
	Moderate	0	0	0	1	0	0	0	0
	Marked	0	0	1	0	0	0	0	1
Necrosis single cell									
	Total number affected	13	**28**	**24**	**36**	18	19	11	17
	Minimal	8	17	16	25	12	16	8	13
	Mild	5	11	8	11	5	3	3	4
	Moderate	0	0	0	0	1	0	0	0
Pigmentation									
	Total number affected	19	**29**	**39**	**40**	39	47	34	38
	Minimal	10	21	24	19	35	38	21	25
	Mild	8	6	14	14	4	8	11	12
	Moderate	1	2	1	7	0	1	2	1
Reactive sinusoidal lining cells									
	Total number affected	6	**14**	**13**	**22**	7	14	6	12
	Minimal	0	2	0	6	1	6	1	2
	Mild	6	11	11	13	5	8	5	9
	Moderate	0	1	2	3	1	0	0	1
Vacuolation hepatocellular									
	Total number affected	24	15	11	10	9	12	11	**20**
	Minimal	13	5	6	1	0	2	3	4
	Mild	8	7	4	5	7	5	6	9
	Moderate	3	3	1	4	2	4	2	7
	Marked	0	0	0	0	0	1	0	0

Bold text indicates LCI699-related observations

Sponsor table

Additional non-neoplastic, but proliferative, drug-related observations were also evident in the pituitary and thyroid gland of male mice only. Hyperplasia was observed in the pars distalis of the pituitary and in follicular cells of the thyroid in males administered dosages ≥ MD. However, there were no increases in the incidence of tumors in either tissue.

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Toxicokinetics: LCI699 blood plasma levels were determined in toxicokinetic mice (2/sex at 1 and 3 hr post-dose) to confirm exposure to the test article during weeks 4 and 26. Systemic drug levels increased in a greater than dose-proportional manner at both timepoints evaluated. Systemic drug levels between males and females did not differ. Data are summarized in the sponsor table below:

Table 51: Mean Drug Concentrations - Mice

Time (h)		1	3
Week	Dose (mg/kg/day)	Male LCI699 concentration (ng/mL)	
4	3	563	172
	10	2650	1560
	30	13400	8730
26	3	640	159
	10	3080	1600
	30	12100	8080
		Female LCI699 concentration (ng/mL)	
4	3	542	115
	10	2270	985
	30	9680	7500
26	3	504	181
	10	2890	1780
	30	9410	8190
n = 2			

Sponsor table

Concentrations at the 1 hour timepoint most closely approximated C_{max} and were compared to C_{max} concentrations at doses of 10 and 30 mg/kg from the 2-week and 13-week mouse toxicology studies. Mean concentrations at 1 hour post-dose during Week 26 were approximately 12% to 20% lower at 10 mg/kg, 4% to 14% lower at 30 mg/kg in males, and ~30% lower at 30 mg/kg in females than drug exposure levels from the previous toxicology studies in mice. Although less than ideal, the slightly lower exposure values are unlikely to have a significant effect on dose margin calculations. Overall, the drug exposure levels in the 2-year study are reasonably comparable to the C_{max} data in the general toxicology studies and adequate drug exposure levels were attained.

Dosing Solution Analysis: Dosing formulations were within 10% of claim and considered acceptable.

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8.2 A 104-Week Oral (Gavage) Carcinogenicity Study in Rats

Study no.:	1270061
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	LCI699, lot # 1212007, 100%
CAC concurrence:	(b) (4)

Methods

Doses:	0, 3, 10, 30 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Ultra-pure water
Basis of dose selection:	Clinical signs (hypersensitivity, increased activity levels, etc) and microscopic alterations in ovary, liver, uterus, and adrenal cortex were observed that indicated 50 mg/kg exceeded the MTD.
Species/Strain:	Rat / Wistar Hannover Crl:WI (Han)
Number/Sex/Group:	50
Age:	6 weeks
Weight:	♂ 89 – 158g; ♀ 96 – 145g
Satellite groups:	20/sex/group for tk
Unique study design:	No
Deviation from study protocol:	None that effected study integrity

Key Study Findings

Neoplastic NOAEL: The neoplastic NOAEL was 3 mg/kg for males based on a statistically significant increases in the incidence of hepatocellular adenomas at ≥ 10 mg/kg and an increased incidence of thyroid follicular cell adenomas and combined adenomas/carcinomas at 30 mg/kg. The neoplastic NOAEL for females was 10 mg/kg based on increased incidences of hepatocellular adenomas, carcinomas, and combined adenomas/carcinomas at 30 mg/kg. Exposure multiples are summarized in the reviewer table below:

Table 52: Exposure Multiples to Neoplastic Findings in Wistar Rats

Wistar Hannover Rat Neoplastic Findings			Exposure Multiple [b]
Sex	NOAEL	$AUC_{0\text{-}24h}$ (ng*h/mL)[a]	AUC
♂	3 mg/kg/day	8,038	5X
♀	10 mg/kg/day	35,6712	21X

[a]$AUC_{0\text{-}24h}$ for rats were extrapolated based on data from Study #: 0870522, a 26 week repeat dose study (0.2 to 20 mg/kg/day, Day 149)

[b]MRHD = 60 mg/day or 30 mg twice daily ($AUC_{0\text{-}24h}$ = 1,680 ng*h/mL from CLCI699C2108

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Non-neoplastic NOAEL: The non-neoplastic NOAEL for males was considered 3 mg/kg based on an increased incidence of polyarteritis in the testes and pancreas at dosages ≥ 10 mg/kg. In females, the non-neoplastic NOAEL was considered 30 mg/kg based on the absence of adverse findings at this dosage.

Table 53: Exposure Multiples to Non-neoplastic Findings in Wistar Rats

Wistar Hannover Rat Non-Neoplastic Findings			Exposure Multiple
Sex	NOAEL (AUC)	$AUC_{0\text{-}24h}$ (ng*h/mL)[a]	AUC[b]
♂	3 mg/kg	8,038	5X
♀	30 mg/kg/day	109,554	65X

[a]$AUC_{0\text{-}24h}$ for rats were extrapolated based on data from Study #: 0870522, a 26 week repeat dose study (0.2 to 20 mg/kg/day)
[b]MRHD = 60 mg/day or 30 mg twice daily ($AUC_{0\text{-}24h}$ = 1,680 ng*h/mL and C_{max} = 306 ng/mL from CLCI699C2108).

Adequacy of Carcinogenicity Study: The study design was considered adequate for evaluating the potential for LCI699 to induce carcinogenicity.

Appropriateness of Test Model: The rat was considered an acceptable test species.

Evaluation of Tumor Findings: There was an increase in liver tumors in males at dosages ≥ 10 mg/kg and in females at 30 mg/kg. A numerical increase in thyroid tumors was also seen in males at ≥ 10 mg/kg, but the incidence was statistically increased only at 30 mg/kg.

Observations and Results

Mortality: There was no effect on survival rate or mortality considered drug-related. A comparable number of animals died or were terminated early between groups for each sex. The number of animals surviving to various timepoints is summarized in the sponsor table below:

Reference ID: 4532599

Table 54: Survival Rates at Various Timepoints - Rats

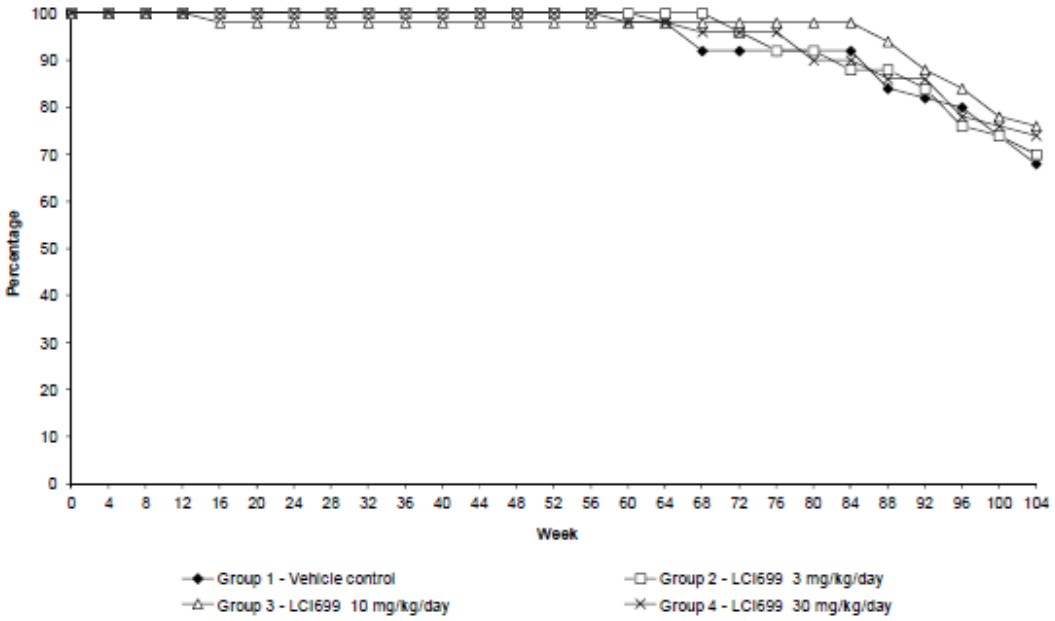
Study week	Males				Females			
	Dose (mg/kg/day)				Dose (mg/kg/day)			
	0	10	30	90	0	3	10	30
16	50 (100%)	50 (100%)	49 (98%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 100%)
28	50 (100%)	50 (100%)	49 (98%)	50 (100%)	49 (98%)	49 (98%)	50 (100%)	49 (98%)
56	50 (100%)	50 (100%)	49 (98%)	50 (100%)	49 (98%)	47 (94%)	50 (100%)	47 (94%)
80	46 (92%)	46 (92%)	49 (98%)	45 (90%)	48 (96%)	45 (90%)	43 (86%)	44 (88%)
96	40 (80%)	38 (76%)	42 (84%)	39 (78%)	43 (86%)	44 (88%)	41 (82%)	39 (78%)
100	37 (74%)	37 (74%)	39 (78%)	38 (76%)	43 (86%)	43 (86%)	39 (72%)	36 (72%)
104	34 (68%)	35 (70%)	38 (76%)	37 (74%)	38 (76%)	43 (86%)	36 (72%)	35 (70%)

Note: n = 50 for each dose group.

Sponsor table

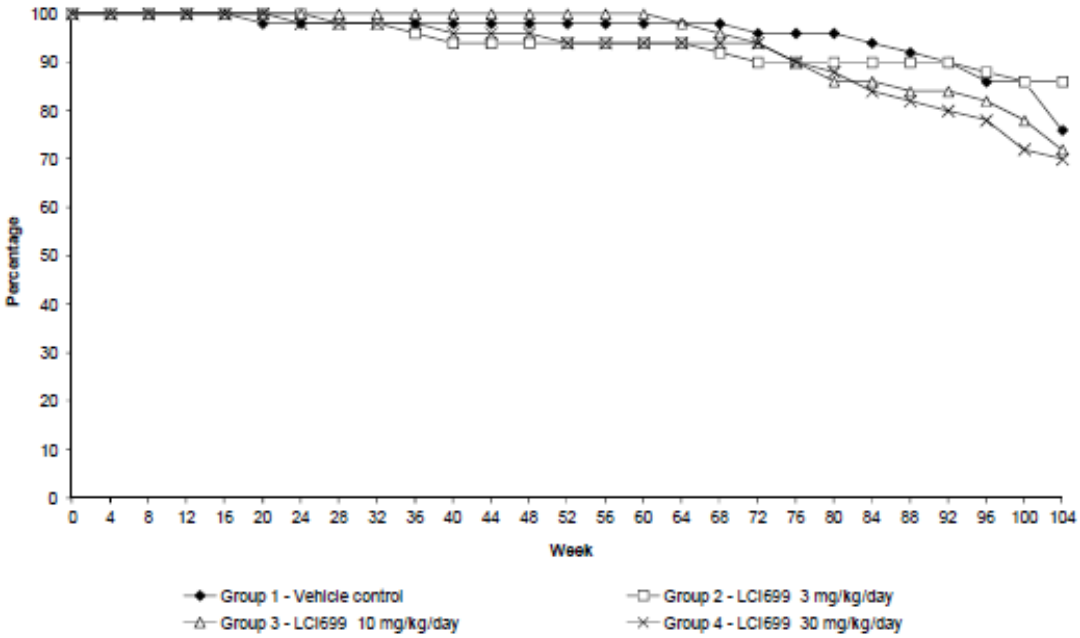
Survival curves for both sexes are shown in the sponsor figures below:

Figure 19: Survival Curve - Male Rat



Sponsor figure

Figure 20: Survival Curve Female Rat



Sponsor figure

The cause of death/moribundity for each preterminal decedent was determined where possible. The most common neoplastic cause of death in control and drug-treated rats were pituitary adenomas, hemolymphoreticular neoplasms, or other neoplasms, but there were no statistically-significant or dose-dependent differences detected. Pituitary adenomas and malignant lymphomas are common spontaneous tumors observed in rats. The most common non-neoplastic cause of death in controls and LCI699-treated rats involved inflammation of various organs (nasal cavity, prostate, skin, urinary tract, and pancreas). These data indicate that there were no drug-related effects on mortality rates in male or female rats.

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Table 55: Cause of Death in Rats

Table 4-3 Causes of death/moribundity in preterminal decedent animals

Sex	Males				Females			
Dose (mg/kg/day)	0	3	10	30	0	3	10	30
No. of animals[a]	16	15	12	13	12	7	14	15
Undetermined	1	0	0	1	0	2	0	3
Accidental (gavage accident)	0	0	0	0	0	0	0	1
Neoplastic:								
Pituitary tumors[b]	4	9	6	9	6	2	8	9
Hemolymphoreticular neoplasms[c]	2	2	1	1	0	0	0	0
Neoplasms, other	7	4	2	1	5	2	6	2
Non-Neoplastic:								
Inflammation[d]	2	0	3	1	1	1	0	0

a Total number of found dead plus preterminally euthanized animals.
b Including adenoma and carcinoma.
c Including lymphoma and histiocytic sarcoma.
d Nasal cavity, prostate, skin, urinary tract, pancreas.

Sponsor table

Clinical Signs: There were no adverse clinical signs noted. LCI699-treated rats had a higher incidence of wet fur (≥ MD in males and HD in females) and salivation (HD in males and ≥ MD in females). LCI699 treatment did not appear to influence the number of palpable masses observed. The incidence of notable clinical signs is summarized in the reviewer table below:

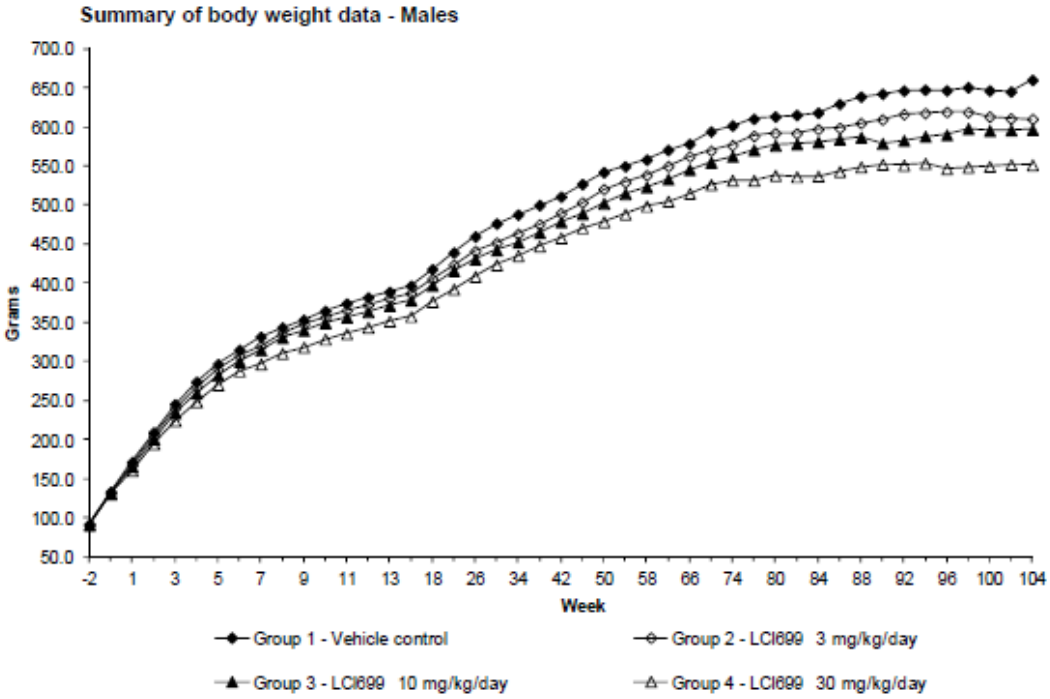
Table 56: Clinical Signs in Rats

		Male				Female			
# of animals		50	50	50	50	50	50	50	50
mg/kg/day		0	3	10	30	0	3	10	30
Fur wet	# Observations	6	1	4	25	.	1	.	35
	# animals	3	1	3	12	.	1	.	10
	Days from – to	682-716	736-736	66-645	52-669	.	571-571	.	25-494
Salivation	# Observations	.	.	2	31	.	.	9	173
	# animals	.	.	1	15	.	.	4	25
	Days from – to	.	.	472-572	52-570	.	.	172-354	11-592

Body Weights: In males, drug- and dose-related reductions (approximately 5% to 9%) in absolute body weights were evident in the MD and HD groups beginning week 4, with the reductions reaching statistical significance. By the end of the study, absolute weights were approximately 10% and 16% lower (statistically significant) in these two groups. Weight gain over the study period was approximately 11% and 20% lower than controls at the MD and HD, respectively. The growth curve is shown in the sponsor figure below:

Reference ID: 4532599

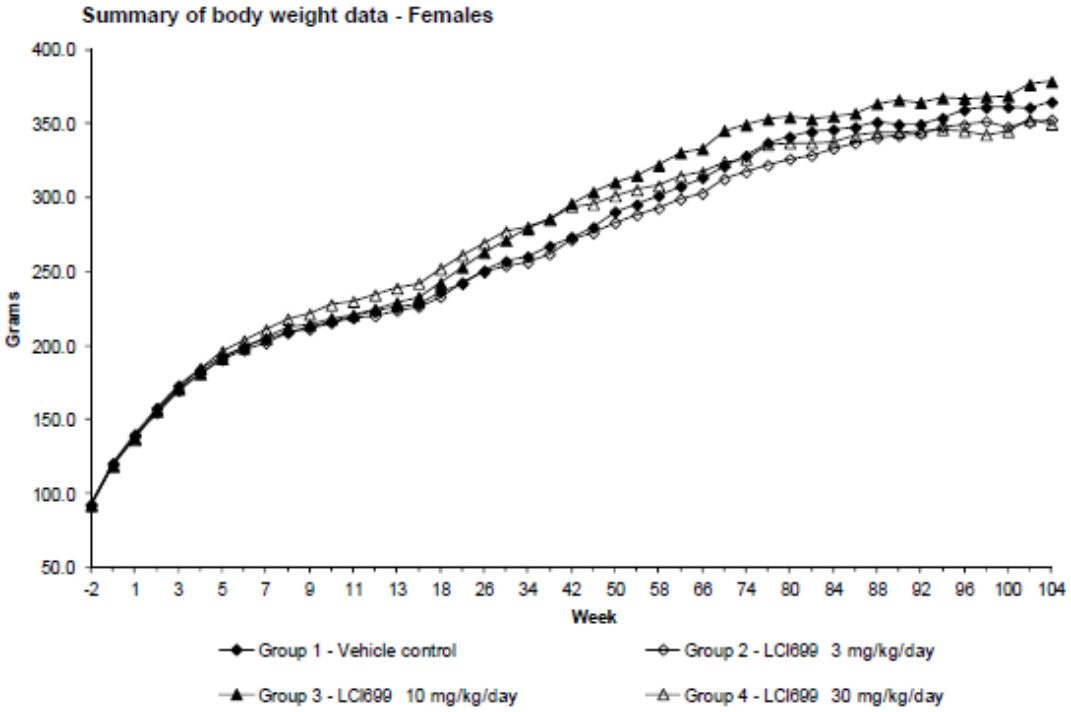
Figure 21: Body Weight in Male Rats



Sponsor figure

In females, fluctuations in body weight were seen (including some intervals in which statsitically significant increases were observed) but there was no consistent pattern. At the end of the study, HD females weighed 4% less than controls. Data are summarized in the sponsor figure below:

Figure 22: Body Weight in Female Rats



Sponsor figure

Feed Consumption: No effect was noted.

Gross Pathology: Dose-dependent increased incidences of findings in the liver, thyroid, and testis were observed in males while increases in liver findings were observed in females. In the liver, males and females had a higher incidence of pale/raised foci at dosages ≥ MD. Females also had a higher incidence of masses at the HD. Raised foci and masses generally correlated with hepatocellular adenoma and carcinoma and pale foci generally correlated with hepatocellular vacuolation or focus of cellular alteration. Male rats also had higher incidences of liver enlargement (≥MD) and pale discoloration (HD) that generally correlated with hepatocellular vacuolation and hypertrophy.

In the thyroid gland, males had an increased incidence of enlargement (≥ MD), masses (≥ MD) and dark/raised foci (HD) that generally correlated with follicular adenoma or carcinoma.

LCI699-treated male rats also had a drug-related increase in the incidence of enlarged testes or pale fluid that accumulated in the testes at all doses examined and pale foci at the HD. Testes enlargement usually correlated with interstitial edema, pale focus formation, and polyarteritis in some cases.

The incidences of these observations are summarized in the sponsor table below:

Reference ID: 4532599

Table 57: Noteworthy Gross Postmortem Observations - Rats

Tissue/Finding Sex	Males				Females			
Dose (mg/kg/day)	0	3	10	30	0	3	10	30
No. of animals examined	50	50	50	50	50	50	50	50
Liver								
Enlargement	5	3	**7**	**11**	0	0	2	3
Focus, pale	20	20	**24**	**30**	13	13	**22**	**26**
Focus, raised	1	2	**7**	**11**	0	3	**7**	**15**
Discoloration, pale	**4**	**4**	1	**8**	2	1	2	2
Mass	1	2	1	1	0	3	1	**12**
Thyroid gland								
Enlargement	6	**4**	**8**	**15**	2	1	2	3
Mass	1	1	**3**	**4**	1	0	0	0
Focus, dark	1	1	1	**4**	0	0	1	0
Focus, raised	0	0	0	**3**	1	0	0	1
Testis								
Enlargement	1	**10**	**11**	**10**	-	-	-	-
Fluid accumulation, pale	3	**9**	**13**	**12**	-	-	-	-
Focus, pale	1	1	2	**6**	-	-	-	-

Bolded values are used for test item-related findings.

Histopathology
Peer Review: Yes

Neoplastic: Statistically significant increases in the incidences of neoplastic findings were observed in the liver (males and females) and thyroid gland (males).

In the liver, there was a statistically significant (pairwise comparison) increase in the incidence of hepatocellular adenomas at ≥ MD in males while in females there were statistically significant increases in the incidence of hepatocellular adenomas and carcinomas at the HD. The adenomas were observed in 14% and 10% of the males at the MD and HD, respectively. These incidences exceeded the historical mean (8.59%) although the range of historical controls for this facility reaches a maximum of 17.5%. The incidence of hepatocellular adenomas did not show a statistically significant increase when trend-wise comparisons were performed.

In HD females, the incidences of adenomas and carcinomas were 32% and 12%, respectively. The incidence of hepatocellular adenomas exceeded the historical average (4.3%) and range (maximum of 9.17%) while hepatocellular carcinomas have not been reported in female rats of this strain at the test facility. When hepatocellular adenomas and carcinomas in females were combined, statistically significant increases were observed by trend test and pair-wise test between the HD group and controls.

Additional LCI699 proliferative changes in the liver included increased incidences of foci of cellular alteration of both mixed cells and eosinophilic cells. The increases were seen in all

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LCI699 treated groups of males while the mixed cell and eosinophilic cell effects were seen in females at dosages ≥ MD and at the HD, respectively. The observations in the liver are summarized in the sponsor table below:

Table 58: Proliferative/Neoplastic Changes in the Liver - Rats

Tissue/Finding	Sex	Males				Females			
	Dose (mg/kg/day)	0	3	10	30	0	3	10	30
Liver	No. examined	50	50	50	50	50	50	50	50
Hepatocellular adenoma									
	Total no. affected	0	1	**7***	**5**	3	0	0	**16***
Hepatocellular carcinoma									
	Total no. affected	0	0	0	0	0	1	0	**6***
Focus of cellular alteration, mixed cell									
	Total no. affected	10	**35**	**30**	**30**	12	16	**31**	**30**
	Minimal	10	31	26	28	12	16	24	15
	Mild	0	4	4	2	0	0	7	13
	Moderate	0	0	0	0	0	0	0	2
Focus of cellular alteration, eosinophilic									
	Total no. affected	2	**11**	**13**	**29**	9	5	5	**18**
	Minimal	2	11	13	22	8	5	5	14
	Mild	0	0	0	6	1	0	0	3
	Moderate	0	0	0	1	0	0	0	1

Bold values indicate test item related findings.

* - Statistically significant increase (pairwise), when compared to control values

Sponsor table

In the thyroid gland, a statistically significant increase (pair-wise comparison) in follicular cell adenomas was observed in HD males (32%) as compared to the incidence in controls (8%) and the historical range observed for this facility (1.33-11.67%). A low incidence of follicular cell carcinomas was also seen, contributing to a statistically significant increase (by pair-wise and trend analyses) when follicular cell adenomas and carcinomas were combined. In females, there was no increase in the incidence of follicular cell adenomas or carcinomas, but there was an increase in hyperplasia at the LD and HD. Data are summarized in the sponsor table below:

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Table 59: Proliferative/Neoplastic Changes in the Thyroid - Rat

Tissue/Finding	Sex	Males				Females			
	Dose (mg/kg/day)	0	3	10	30	0	3	10	30
Thyroid gland	No. examined	50	50	50	50	50	50	50	50
Follicular cell adenoma									
	Total no. affected	4	2	9	**16***	0	1	2	2
Follicular cell carcinoma									
	Total no. affected	0	0	3	**2**	0	1	0	0
Hyperplasia, follicular cell									
	Total no. affected	6	3	6	3	1	5	2	8
	Minimal	3	3	2	1	0	2	1	5
	Mild	2	0	3	2	1	3	1	3
	Moderate	1	0	1	0	0	0	0	0

Bold values indicate test item related findings.
Sponsor table

Statistical analysis by the FDA revealed similar results. In males, thyroid follicular cell adenomas and combined adenomas + carcinomas were increased by both pairwise and trend analysis at the HD. In the liver of males, analyses revealed statistically significant increases (pairwise comparison) in the incidence of hepatocellular adenomas at dosages ≥ MD. In females, there were statistically significant increases (pairwise and trend analyses) in hepatocellular adenomas and carcinomas at the HD, both alone and when combined. The FDA statistical results are shown below:

Table 60: FDA Statistical Analysis of Tumor Types - Rats

Sex	Organ Name	Tumor Name	0 mg Veh. Cont (N=50) P - Trend	3 mg Low (N=50) P -VC vs. L	10 mg Med (N=50) P -VC vs. M	30 mg High (N=50) P -VC vs. H
Male	Gland, Thyroid	Follicular Cell Adenoma	4/50 (44) <0.0001*	2/50 (44) 0.8988	9/50 (46) 0.1326	16/50 (45) 0.0026*
		Follicular Cell Carcinoma	0/50 (44) 0.1124	0/50 (44) NC	3/50 (46) 0.1292	2/50 (45) 0.2528
		Follicular Cell Adenoma/Carcinoma	4/50 (44) <0.0001*	2/50 (44) 0.8988	12/50 (46) 0.0321@	18/50 (45) 0.0007*
	Liver	Hepatocellular Adenoma	0/50 (44) 0.0285@	1/50 (44) 0.5000	7/50 (46) 0.0072*	5/50 (45) 0.0294*

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Sex	Organ Name	Tumor Name	0 mg Veh. Cont (N=50) P - Trend	3 mg Low (N=50) P -VC vs. L	10 mg Med (N=50) P -VC vs. M	30 mg High (N=50) P -VC vs. H
Female	Liver	Hepatocellular Adenoma	3/50 (46) <0.0001*	0/50 (45) 1.0000	0/50 (44) 1.0000	16/50 (44) 0.0005*
		Hepatocellular Carcinoma	0/50 (46) 0.0006*	1/50 (45) 0.4945	0/50 (44) NC	6/50 (43) 0.0105*
		Hepatocellular Adenoma/Carcinoma	3/50 (46) <0.0001*	1/50 (45) 0.9389	0/50 (44) 1.0000	16/50 (44) 0.0005*

& 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 0.005 and 0.025 for common and rare tumors, respectively in dose response relationship (trend) tests and significance levels of 0.01 and 0.05 for common and rare tumors, respectively in pairwise comparisons.
@: not statistically significant at 0.025 for rare tumor in dose response relationship (trend) tests nor at 0.01 for common tumors in pairwise comparisons.

FDA table

There were no other neoplastic changes considered drug-related.

Non-Neoplastic: Dose-dependent but non-adverse increases in non-neoplastic microscopic findings were observed in the liver, thyroid gland, testes, pancreas, and kidney.

In the liver, an increased incidence of hepatocellular vacuolation, hypertrophy, and cystic degeneration were evident in all drug-treated male groups. In females, increased incidences of hepatocellular vacuolation were observed at ≥ MD and hepatocellular hypertrophy was seen at all dosages; no cystic degeneration was noted. The vacuolations typically consisted of single large cytoplasmic vacuoles and the hypertrophy was typically observed in centrilobular to midzonal hepatocytes that extended to periportal areas in some cases.

In the thyroid gland, follicular cysts occurred at an increased incidence in males administered the HD while follicular hypertrophy was increased at all dosage levels. Thyroid observations were not increased in females.

Data for the liver and thyroid are summarized in the sponsor table below:

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Table 61: Non-neoplastic Changes in the Liver and Thyroid - Rats

Tissue/Finding	Sex	Males				Females			
	Dose (mg/kg/day)	0	3	10	30	0	3	10	30
Liver	No. examined	50	50	50	50	50	50	50	50
Hepatocellular vacuolation									
	Total no. affected	7	**28**	**45**	**49**	3	2	**38**	**49**
	Minimal	4	22	16	5	3	2	24	16
	Mild	2	4	22	19	0	0	11	18
	Moderate	1	2	7	19	0	0	3	15
	Marked	0	0	0	6	0	0	0	0
Hepatocellular hypertrophy									
	Total no. affected	0	**15**	**44**	**49**	0	**7**	**33**	**46**
	Minimal	0	12	29	12	0	7	28	13
	Mild	0	3	14	30	0	0	5	31
	Moderate	0	0	1	7	0	0	0	2
Cystic degeneration									
	Total no. affected	0	**4**	**10**	**12**	0	0	0	0
	Minimal	0	3	6	6	0	0	0	0
	Mild	0	1	3	5	0	0	0	0
	Moderate	0	0	1	1	0	0	0	0
Thyroid gland	No. examined	50	50	50	50	50	50	50	50
Cyst, follicular									
	Total no. affected	3	4	3	**11**	1	1	3	2
	Minimal	3	4	3	9	1	1	2	1
	Mild	0	0	0	2	0	0	1	1
Hypertrophy, follicular									
	Total no. affected	0	**5**	**3**	**7**	0	0	0	0
	Minimal	0	5	3	7	0	0	0	0

Bold values indicate test item related findings.
Sponsor table

Drug-related non-neoplastic observations in the testis, pancreas, and kidney were all sex specific. In the testes there were increases in tubular degeneration/atrophy (typically unilateral) that was often associated with interstitial edema at all doses while polyarteritis was increased at dosages ≥ MD. Polyarteritis was also noted in the pancreas of males at dosages ≥ MD. The only female-specific finding included a dose-dependent increase in the incidence and severity of minimal to marked chronic progressive nephropathy. These data are summarized in the sponsor table below:

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Table 62: Non-neoplastic Changes in Testis, Pancreas, and Kidney - Rat

Tissue/Finding	Sex	Males				Females			
	Dose (mg/kg/day)	0	3	10	30	0	3	10	30
Testis	No. examined	50	50	50	50	0	0	0	0
Degeneration/atrophy, tubular									
	Total no. affected	4	**10**	**15**	**17**	-	-	-	-
	Minimal	2	7	9	10	-	-	-	-
	Mild	0	1	4	4	-	-	-	-
	Moderate	0	1	1	1	-	-	-	-
	Marked	2	1	1	2	-	-	-	-
Edema, interstitial									
	Total no. affected	12	**16**	**22**	**21**	-	-	-	-
	Minimal	6	6	7	8	-	-	-	-
	Mild	3	6	5	3	-	-	-	-
	Moderate	3	1	7	8	-	-	-	-
	Marked	0	2	3	2	-	-	-	-
	Severe	0	1	0	0	-	-	-	-
Polyarteritis									
	Total no. affected	1	0	**7**	**9**	-	-	-	-
	Minimal	0	0	3	4	-	-	-	-
	Mild	1	0	2	3	-	-	-	-
	Moderate	0	0	2	2	-	-	-	-
Pancreas	No. examined	50	50	50	50	50	50	50	50
Polyarteritis									
	Total no. affected	0	0	**3**	**8**	0	1	0	0
	Minimal	0	0	2	1	0	1	0	0
	Mild	0	0	0	0	0	0	0	0
	Moderate	0	0	0	2	0	0	0	0
	Marked	0	0	0	5	0	0	0	0
	Severe	0	0	1	0	0	0	0	0
Kidney	No. examined	50	50	50	50	50	50	50	50
Chronic progressive nephropathy									
	Total no. affected	33	29	33	37	14	18	**24**	**31**
	Minimal	26	24	25	28	14	17	20	27
	Mild	3	4	6	5	0	1	4	3
	Moderate	3	1	2	2	0	0	0	1
	Marked	1	0	0	2	0	0	0	0

Bold values indicate test item related findings.
Sponsor table

No other non-neoplastic changes observed in this study were considered drug-related.

Toxicokinetics: Systemic drug levels LCI699 increased in a dose-proportional manner (as measured by mean plasma concentrations). LCI699 concentrations did not differ remarkably between males and females, and levels at week 26 were comparable to those measured at week 4. Data are summarized in the sponsor table below:

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Table 63: LCI699 Concentrations in Rat

Mean LCI699 concentrations(ng/mL) in rat plasma			
Time (h)		1	3
Week	Dose (mg/kg/day)	Male LCI699 concentration (ng/mL)	
4	3	986	761
	10	3360	2860
	30	7220	7740
26	3	1290	1050
	10	4180	4310
	30	8070	9170
		Female LCI699 concentration (ng/mL)	
4	3	1420	889
	10	3290	3640
	30	7630	7250
26	3	1790	1160
	10	3900	3390
	30	9070	8940

n = 2
Sponsor table

Since t_{max} for LCI699 is achieved by 1 hour post-dosing, concentrations determined at the 1 hour timepoint approximated C_{max} and were compared to concentration estimates at doses of 3, 10, and 30 mg/kg that were extrapolated from linear regression analysis of empirical C_{max} data at doses of 0.2, 2, and 10 mg/kg obtained from Day 149 of the 26-week rat toxicology study. Although mean concentrations were only obtained from 2 rats/sex/group/timepoint, they were slightly higher and/or within ±10% of estimated concentrations. Thus, drug exposure levels were considered to be consistent with and comparable to those achieved in the pivotal 26-week toxicology study.

Dosing Solution Analysis: Dosing formulations were within 10% of claim and considered acceptable.

9 Reproductive and Developmental Toxicology

Brief summaries of the fertility and developmental toxicity studies are provided below as these studies have previously been reviewed in the IND. The pre- and postnatal toxicity and juvenile toxicity study are reviewed in detail.

9.1 Fertility and Early Embryonic Development

9.1.1 An Oral (Gavage) Fertility and Early Embryonic Development Study in Rats

Dosages of 0.5, 5, and 50 mg/kg were administered in this fertility study (Study 0770785). Male rats were treated for 28 days prior to cohabitation with treated female rats, through mating, and until termination at 71 days. Males were also mated to untreated females two weeks following the first mating. Treated females were administered drug for 14 days prior to mating and through mating until gestation day (GD) 6.

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Transient decreases in male body weight, weight gain, and food consumption occurred during the first week at 50 mg/kg. In females administered 50 mg/kg, increased body weight, weight gain, and food consumption was observed during the premating period, but weight gain was reduced during gestation. The higher weight gain pre-mating was countered by lower weight gain during gestation such that body weight at termination was comparable between groups. The only postmortem observation of note was decreased epididymal weights (-8%) at 50 mg/kg. There were no effects on male fertility.

In females administered 50 mg/kg, prolonged estrous cycles (in 88% of females), increased precoital interval, decreased fertility, decreased corpora lutea (↓ 21%), decreased implantation sites (↓ 78%), increased preimplantation loss (↑ 6-fold), increased post-implantation loss manifested by increased early resorptions (↑ 3-fold), and decreased viable fetuses (↓ 81%); the majority of these effects attained statistical significance. No effects occurred at lower doses or in untreated females.

The key observation from the study are summarized in the sponsor table below:

Table 64: Cesarean Data from Rat Fertility Study

Summary of Cesarean Data

	DOSAGE	0 MG/ KG/DAY	0.5 MG/ KG/DAY	5 MG/ KG/DAY	50 MG/ KG/DAY
Females Mated	N	25	25	25	25
Pregnant	N	22	21	24	6**
	%	88.0	84.0	96.0	24.0
Aborted	N	0	0	0	0
Premature Births	N	0	0	0	0
Female Mortality	N	1	0	0	0
Pregnant at C-section	N	21	21	24	6
Dams with Viable Fetuses	N	21	21	24	5
Dams with all Resorptions	N	0	0	0	1
Corpora Lutea	N	280	284	341	63
	MEAN	13.3	13.5	14.2	10.5**
	S.D.	2.0	1.4	2.1	3.4
Implantation Sites	N	246	237	290	16
	MEAN	11.7	11.3	12.1	2.7**
	S.D.	1.8	2.5	3.3	2.3
Preimplantation Loss	%	12.1	16.5	15.0	74.6
Postimplantation Loss	%	7.3	6.3	9.3	25.0
Dead Fetuses	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Resorptions, total	N	18	15	27	4*
	%	7.3	6.3	9.3	25.0
	MEAN	0.9	0.7	1.1	0.7
	S.D.	1.7	1.1	1.1	0.8
Early Resorptions	N	18	15	27	4*
	%	7.3	6.3	9.3	25.0
	MEAN	0.9	0.7	1.1	0.7
	S.D.	1.7	1.1	1.1	0.8
Late Resorptions	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
	MEAN	0.0	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0
Viable Fetuses	N	228	222	263	12*
	%	92.7	93.7	90.7	75.0
	MEAN	10.9	10.6	11.0	2.0**
	S.D.	2.3	2.7	3.7	2.1

Statistical key: * = p<0.05 ** = p<0.01

Sponsor table

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Reviewer Summary: Effects on fertility were evident in females at 50 mg/kg. Observations included abnormal estrous cycles, increased time to mating, decreased mating and fertility indices, decreased pregnancy rate, decreased corpora lutea, and decreased implantations and viable embryos. As there were no effects on these parameters when treated males were cohabited with untreated females, the effects were considered female mediated. As a result, the NOAEL for effects on female fertility was the MD of 5 mg/kg. In males, there was a slight reduction in epididymal weights but no functional correlates. The NOAEL for male fertility was the HD of 50 mg/kg.

9.2 Embryonic Fetal Development

9.2.1 An Oral (Gavage) Embryo-fetal Embryonic Development Study in Rats

LCI699 dosages of 0.5, 5, and 50 mg/kg were administered on gestation days (GD) 6 – 17 to presumed gravid rats in this developmental toxicity study (Study 0770784).

In maternal animals, signs of toxicity were evident at 50 mg/kg. Observations in maternal animals at this dose included clinical signs of red vaginal discharge & red pan stains associated with resorptions. Decreased body weight gain over entire study (↓24%) resulting in terminal weights that were 8% lower, and reduced carcass and adjusted body weights were also observed. At lower dosages the only effects seen were transient reductions in weight gain/food consumption at 5 mg/kg that were not considered adverse.

Excessive amniotic fluid in the uterus was observed in 52% of females at 50 mg/kg. The percentage of dams with viable fetuses was reduced at 50 mg/kg, with 25% (6/24) of dams having resorptions of the entire litter. Dose-dependent increases in the incidence of resorptions occurred with the incidence at 50 mg/kg being 54% greater than controls (statistically significant) reflecting increases in early (↑19%) and late (↑35%) resorptions. As a result, the post implantation loss rate reached 60% at HD which was 10-fold higher than that of controls. Significant increases in resorptions also paralleled significant decreases in the total and mean number of viable fetuses at 50 mg/kg. There were no effects on fetal sex ratios. Key observations are summarized in the sponsor table below:

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Table 65: Cesarean Data from Embryo-fetal Development Study in the Rat

Summary of Cesarean Data [PCDSU]

DOSAGE		0 MG/KG/DAY	0.5 MG/KG/DAY	5 MG/KG/DAY	50 MG/KG/DAY
Females Mated	N	25	25	25	25
Pregnant	N	24	25	24	24
	%	96.0	100.0	96.0	96.0
Aborted	N	0	0	0	0
Premature Births	N	0	1	0	0
Female Mortality	N	0	0	0	0
Pregnant at C-section	N	24	24	24	24
Dams with Viable Fetuses	N	24	24	24	18
Dams with all Resorptions	N	0	0	0	6
Corpora Lutea	N	278	274	261	273
	MEAN	11.6	11.4	10.9	11.4
	S.D.	1.7	1.8	1.9	1.6
Implantation Sites	N	241	254	213	235
	MEAN	10.0	10.6	8.9	9.8
	S.D.	2.4	2.1	2.8	2.3
Preimplantation Loss	%	13.3	7.3	18.4	13.9
Postimplantation Loss	%	6.2	5.5	9.4	60.0
Dead Fetuses	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Resorptions, total	N	15	14	20	141**
	%	6.2	5.5	9.4	60.0
	MEAN	0.6	0.6	0.8	5.9**
	S.D.	0.8	0.8	1.1	3.7
Early Resorptions	N	15	14	17	59**
	%	6.2	5.5	8.0	25.1
	MEAN	0.6	0.6	0.7	2.5**
	S.D.	0.8	0.8	0.9	2.8
Late Resorptions	N	0	0	3	82**
	%	0.0	0.0	1.4	34.9
	MEAN	0.0	0.0	0.1	3.4**
	S.D.	0.0	0.0	0.3	2.8
Viable Fetuses	N	226	240	193	94**
	%	93.8	94.5	90.6	40.0
	MEAN	9.4	10.0	8.0	3.9**
	S.D.	2.4	2.4	3.2	3.3

Statistical key: ** = p<0.01

Sponsor table

Effects on the embryo/fetus at 50 mg/kg included decreased fetal weight (↓16-17%) and potentially drug-related external malformations (shortened snout, protruding tongue, and malrotated limbs), visceral variations (irregular lung surface), and skeletal variations (incomplete ossification, and misshapen, fused, or supernumerary ribs or sternebra).

Reviewer Summary: Maternal toxicity in the form of clinical signs, decreased body weight, and decreased food consumption were evident at 50 mg/kg. Excessive amniotic fluid and dose-dependent increase in resorptions (early & late, statistically significant at the 50 mg/kg) were observed resulting in an increased incidence of post-implantation loss at 50 mg/kg. Fetal effects at 50 mg/kg included a marked reduction in viability, decreased fetal weight, possible drug-related external malformations, increased visceral variations, and increased skeletal variations. Based on the increased resorptions, decreased number of viable fetuses, and effects on fetal development at 50 mg/kg, the NOAEL for the study is considered to be the MD of 5 mg/kg. At the NOAEL, the exposure (AUC_{0-24}) to LCI699 at the end of treatment was 12800 ng·hr/mL.

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9.2.2 LCI699/LCI698: An Oral (Gavage) Embryo-Fetal Development Study in Rats

An embryo-fetal developmental toxicity study was performed in rats in to evaluate the potential role that the enantiomer impurity LCI698 has in inducing embryo-fetal toxicity (Study 1170415). Rats were administered LCI698 at concentrations of 0.006% or 0.07% with LCI699 at 50 mg/kg or LCI698 alone at a dose of 0.03 mg/kg on GD 6 – 17 to compare the effects on embryofetal development between LCI699 with various levels of LCI698 to those seen with LCI698 alone.

Mortality occurred in groups administered LCI699 with both levels of the impurity LCI698. In the group administered LCI699 with 0.07% impurity, 3 animals were found dead on GD's 19 or 21. Gross observations (antemortem or of carcass) were limited to pale appearance while dark fluid in the uterus/vagina was noted in the gross postmortem examination. A single animal administered LCI699 with 0.006% impurity was found dead on GD 17 with red liquid vaginal discharge. Clinical signs, reduced weight gain in late gestation, and reduced food consumption were observed in both LCI699 with impurity groups. The effects on weight gain in the LCI699 with 0.07% impurity resulted in a statistically significant lower body weight at the end of the study.

Increased post-implantation loss (up to 12x) resulting from increases in early (up to 5x) and late resorptions (up to 46x) with resulting reductions in the number of viable fetuses (↓ up to 66%) in both LCI699 with impurity groups. These included 3 dams in the 0.07% impurity group and 5 animals in the 0.006% impurity group that had total litter resorptions. A slight increase in early resorptions was also seen in the LCI698 alone treated group, but this was the only effect in this group. Increased incidences of postmortem observations in the maternal carcass, adrenal, spleen, uterus, and vagina were observed in both LCI699 groups with the impurity. The key study findings are summarized in the reviewer table below:

Table 66: Summary of LCI699/LCI698 Cesarean Data in the Rat

Ovarian/Uterine Observations (% of control)				
Parameter	Treatment Group			
	0	LCI699 50 mg/kg LCI698 at 0.07%	LCI699 50 mg/kg LCI698 at 0.006%	LCI698 at 0.03 mg/kg
Number dying/euthanized	0	3	1	0
Number with total resorptions	0	3	5	0
Number not pregnant	0	0	1	0
Implantation Sites/Litter	11.6	10.1	12.7	11.8
Live Fetuses/Litter	11.0	3.8*** (34%)	5.6*** (51%)	9.9
Early Resorptions/Litter	0.5	2.7** (5x)	2.5*** (5x)	1.5** (3x)
Late Resorptions/Litter	0.1	3.6*** (36x)	4.6*** (46x)	0.5
Post Implantation Loss[a]	5.3%	64.6%*** (12x)	56.5%*** (11x)	19.0
Gravid Uterine Weight (g)	77.5	54.9	78.4	75.7
*p<0.05%, **p<0.01, ***p<0.001 a) Values are rounded by reviewer				

Fetal weights were reduced 17% in both combination-treated groups. In the fetuses, there was a statistically significant increase in the fetal (12.5%) and litter (29.4%) incidence of misshapen heart (cardiomegaly). Also observed were single occurrences of cleft palate, ventricular septal defect, supernumerary digits, and abnormal hindlimb flexure at the HD;

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abnormal hind paw flexure was noted in single fetuses of both combination treatment groups. Similar effects were not observed in fetuses exposed to LCI698 alone.

Reviewer Summary: At the administered dosage of LCI699 (50 mg/kg) with either level of the impurity LCI698 (0.006% or 0.07%), but not with LCI698 administered directly, drug-related mortality occurred at the end of gestation after dose administration had been completed. Additional effects in maternal animals administered LCI699 with both levels of the impurity included clinical signs and reduced weight gains and food consumption. Similar effects were not seen in the group administered LCI698. In both LCI699 with impurity groups, embryofetal survival was reduced, fetal weights were decreased, and there were increased incidences of malformations and ossification delays. Similar effects were not seen in the group administered LCI698 alone, with the exception of early resorptions. Consequently, the observed maternal and fetal effects were attributed to LCI699 rather than to the impurity LCI698.

9.2.3 An Oral (Gavage) Embryo-fetal Embryonic Development Study in Rabbits

Dosages of 3, 10, or 30 mg/kg of LCI699 was administered to presumed gravid rabbits on GD 7 – 20 in this developmental toxicity study (Study 0670281). Dosages were based on a ranging study (Study 0670245) in which increased numbers of resorptions, decreased numbers of viable fetuses, and increased post-implantation loss were observed at doses ≥ 25 mg/kg.

Maternal animals administered the 30 mg/kg dose exhibited weight loss during the treatment period which resulted in body weights that were 10% lower than controls. There were no abortions, but total litter loss occurred in 2 and 6 females at 10 and 30 mg/kg, respectively. The numbers of corpora lutea and implantation sites were comparable between groups. An increase in post-implantation loss was evident at ≥ 10 mg/kg even though the incidences did not reach statistical significance. However, statistically significant increases in both early and total resorptions occurred at ≥ 10 mg/kg which resulted in a decrease in the number of viable fetuses in these groups. As a result of the fetal loss at 30 mg/kg, gravid uterine weights in this group were also lower. These data are summarized in the sponsor table below (table modified by reviewer to show significant parameters):

Reference ID: 4532599

Table 67: Cesarean Data from Embryo-Fetal Development Study in the Rabbit

Summary of Cesarean Data [PCDSU]

DOSAGE		0 MG/KG/DAY	3 MG/KG/DAY	10 MG/KG/DAY	30 MG/KG/DAY
Females Mated	N	20	20	20	20
Pregnant	N	20	20	20	20
	%	100.0	100.0	100.0	100.0
Aborted	N	0	0	0	0
Premature Births	N	0	0	0	0
Female Mortality	N	0	0	0	0
Pregnant at C-section	N	20	20	20	20
Dams with Viable Fetuses	N	20	20	18	14
Dams with all Resorptions	N	0	0	2	6
Corpora Lutea	N	205	207	197	198
	MEAN	10.3	10.4	9.9	9.9
	S.D.	1.7	2.1	1.9	3.2
Implantation Sites	N	187	181	166	168
	MEAN	9.4	9.1	8.3	8.4
	S.D.	2.0	2.2	2.2	2.6
Preimplantation Loss	%	8.8	12.6	15.7	15.2
Postimplantation Loss	%	2.1	5.0	10.8	50.0
Dead Fetuses	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Resorptions, total	N	4	9	18**	84**
	%	2.1	5.0	10.8	50.0
	MEAN	0.2	0.4	0.9	4.2**
	S.D.	0.4	0.6	2.4	4.3
Early Resorptions	N	2	5	17**	83**
	%	1.1	2.8	10.2	49.4
	MEAN	0.1	0.3	0.9	4.2**
	S.D.	0.3	0.4	2.4	4.3
Late Resorptions	N	2	4	1	1
	%	1.1	2.2	0.6	0.6
	MEAN	0.1	0.2	0.1	0.1
	S.D.	0.3	0.4	0.2	0.2
Viable Fetuses	N	183	172	148**	84**
	%	97.9	95.0	89.2	50.0
	MEAN	9.1	8.6	7.4	4.2**
	S.D.	2.0	2.0	3.2	4.2

Statistical key: ** = p<0.01

Sponsor table

There were no drug-related fetal external or visceral variations or malformations or skeletal malformations. However, there were increased incidences of several skeletal variations at dosages ≥ 10 mg/kg that included incomplete ossification or unossified metacarpals and forepaw phalanx. At 30 mg/kg there were increases in the incidences of bipartite sternebra, fused sternebra, unossified sternebra, and supernumerary ribs (full or short). These effects were statistically significant on a fetus basis but not on a litter basis.

Reviewer Summary: Drug-related effects on maternal animals included decreased stool and red stains in the cage pan at doses ≥ 10 mg/kg and weight loss with associated reductions in absolute weights (-10%)at 30 mg/kg. Embryo-fetal toxicity (increased resorptions, decreased viable fetuses, total litter loss) occurred at ≥ 10 mg/kg. There also were increases in the incidences of various skeletal variations, particularly at 30 mg/kg. Based on these data, the maternal and developmental NOAEL for the study was considered the LD of 3 mg/kg. At the NOAEL, exposure to LCI699 (AUC_{0-24}) at the end of treatment was 850 ng·hr/mL.

Reference ID: 4532599

9.3 Prenatal and Postnatal Development

9.3.1 An Oral (Gavage) Pre and Postnatal Developmental Toxicity Study in the Rat

Study no.:	R1470797
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	LCI699, batch 1212007, 100%

Methods

Doses:	0, 1, 5, 20 mg/kg
Frequency of dosing:	Once daily
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Ultrapure water
Species/Strain:	Rat / Wistar Hannover
Number/Sex/Group:	24 females
Study design:	Standard design. Dosing was post coitum (PC) day 6 through lactation day 20
Deviation from study protocol:	None affecting study integrity

Key Study Findings: The 20 mg/kg dose was not tolerated by pregnant females. Reduced weight gain and food consumption along with prolonged gestation/dystocia and associated clinical signs led to the early termination of this group; multiple postmortem findings were also evident. Growth and development of the F_1 generation was not affected by *in utero* and/or lactational exposure to the drug at dosages $\leq$ 5 mg/kg leading to identification of 5 mg/kg as the NOAEL.

Observations and Results

F0 Dams

Mortality/Clinical Observations: Drug-related mortality occurred at the HD. One animal was euthanized on post coitum (PC) 21 and 3 were euthanized on PC 22 due to dystocia and clinical signs (red/black discharge from vagina/vulva, prostration, decreased activity, coldness to touch, suspected dehydration, skin pallor/discoloration, hunched posture, fur discoloration, partially closed eyes, etc). An additional female was euthanized on the day of delivery as there were no surviving pups.

Delays in parturition up to PC 24 were seen in the remaining HD dams along with the above-mentioned clinical signs. As a result, all dams in this group were terminated between PC day 21 and 24 or on the day of parturition.

Body Weight: Reduced weight gain occurred at the HD, primarily between PC 6 – 9 and PC 18 – 21 while increased gain was seen from PC 12-15. Over the treatment period that occurred during gestation, weight gain was approximately 9% lower in this group. Regardless of the effects on weight gain, there were no resultant differences on absolute

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weights during gestation. No effects occurred on body weight parameters during the lactation period. Data are summarized for various intervals in the reviewer-created table below:

Table 64: F0 Body Weight Summary - Rat

Absolute Body Weight/Weight Gain in grams[a] (% of control)				
Day or Interval	Dosage (mg/kg/week)			
	0	1	5	20
Gestation				
6	213	213	214	214
9	224	224	224	222
12	237	237	237	237
15	250	250	252	254
18	280	281	286	286
21	311	314	320	304
6-9	11.3	10.3	9.9	7.6** (67%)
12-15	12.8	13.0	15.5	17.6*** (1.4x)
18-21	30.1	33.0	34.3	18.0* (60%)
6-21	97.5	100.4	106.8	89.1 (91%)
Lactation				
0	237	236	235	-
7	255	254	254	-
14	278	277	277	-
21	279	276	275	-
0-21	41.8	39.4	40.7	-

*p<0.05%, **p<0.01, ***p<0.001
a) Values are rounded by reviewer
b) Indicated weight gain intervals were calculated by reviewer so no statistical analyses performed

Food consumption: Reduced food consumption occurred in HD females, particularly after PC 18 when consumption was 21% to 23% lower than controls. Although the reductions did not reach statistical significance, they were consistent with a period of significantly lower weight gain.

Maternal Reproductive Performance: At the HD, dystocia and delays in parturition were evident with a mean of 23.3 days as compared to 21.5 days in Controls. Litters with multiple stillborn pups and pups found dead were also evident in this group which resulted in a reduction in the live birth index. There were single incidences of stillborn pups found in 1 (LD) or 2 (MD) litters but no effect on the mean number of live pups/litter in these groups. No effects were seen on gestation length, implant scars, or sex ratio in these groups.

Postmortem Examination: Multiple postmortem observations were made on the uterus, vagina, liver, and carcass of HD animals. In the uterus, placentae were enlarged with pale or mottled discoloration; dark fluid and material accumulation was observed in both the uterus and vagina at the HD. Pale discoloration of the liver and carcass were also common observations at the HD. The incidences of these findings are summarized in the sponsor table below:

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Table 65: F0 Postmortem Observations - Rat

Table 4-1 Incidence of LCI699-related macroscopic findings in terminal and preterminal F_0 dams

Tissue/Finding	Sex	Females			
	Dose (mg/kg/day)	0	1	5	20
	Number of animals examined	24	24	24	24
Uterus					
Enlargement[a]		-	-	-	17
Discoloration; mottled[a]		-	-	-	17
Discoloration; pale[a]		-	-	-	4
Focus, pale[a]		-	-	-	1
Material accumulation, dark[b]		-	-	-	3
Fluid accumulation, dark[b]		-	-	-	6
Vagina					
Fluid accumulation; dark		-	-	-	4
Material accumulation; dark		-	-	-	7
Liver					
Discoloration, pale		-	-	-	6
Whole animal					
Discoloration, pale		-	-	-	6

a Findings noted in placentas.
b Findings noted in the uterus.

Sponsor table

F1 Generation

The HD group was terminated on the day of delivery. As a result, minimal F1 generation data were collected for this group.

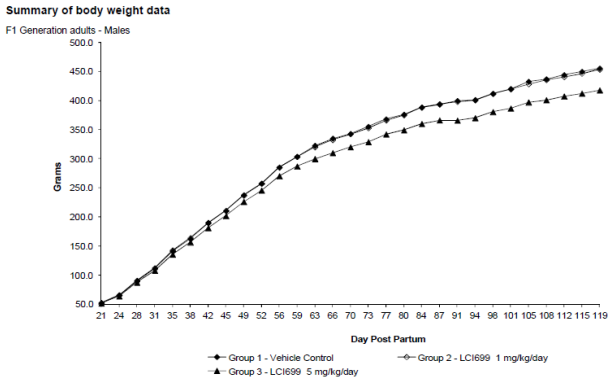
Mortality: The number of pups surviving (97.7%) to day 4 was statistically reduced at the MD as a result of there being single pups in 5 litters that did not survive. This reduction was not considered drug-related as the number surviving was within the historical range (94.4% to 100%). There was no mortality thereafter.

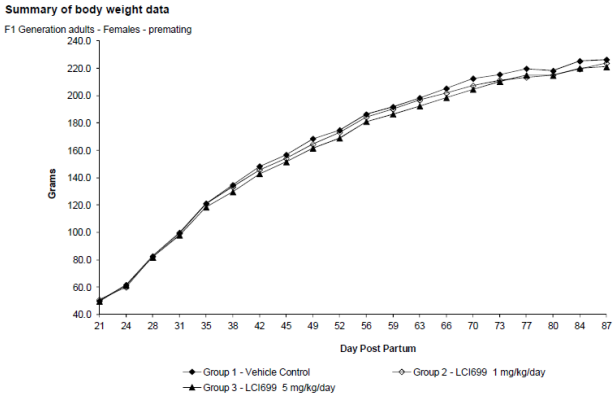
Clinical Observations: No drug-related effects were observed during either the pre- or post-weaning periods.

Body Weight: No effects were noted on pup growth during the pre-weaning period. In male pups selected for retention into adulthood, statistically significant reduced weight gains (10% to 30% lower than C) were observed at the MD between days 31 and 63. Resultant body weights were 5% to 8% lower (statistically significant) beginning at day 42 and continuing through the premating period. Similar effects were not seen in females. These results are summarized in the sponsor figures below:

Reference ID: 4532599

Table 66: F1 Body Weights - Rat





Sponsor figures

There were no effects on female body weights during gestation.

Food Consumption: No effects were seen on food consumption in animals retained at weaning.

Physical/Reflex Development: There were no effects on the mean day of eye-opening, pinna unfolding, development of the righting reflex, or on the auricular startle response during the preweaning period. Following weaning, no effects were observed on visual placing and pupillary responses or on startle response parameters (startle at start, maximum startle, time to maximum startle, average startle).

Sexual Maturation: The mean age preputial separation occurred at was comparable between groups even though the mean body weight at which this event was attained was reduced 5% (statistically significant) at the MD. There was no effect on the age vaginal patency occurred at or on the corresponding body weights.

Motor Activity: The mean number of ambulations and fine movements were comparable between drug-treated and control groups.

Learning/Memory: No effects were noted on swimming ability or the number of errors or trial durations using the Cincinnati water maze.

Estrous Cyclicity: The number of days in estrus, number of cycles, and average cycle length was comparable between groups.

Mating/Fertility: The number of animals mating, resulting number of pregnancies, and number of females with live embryos were comparable between groups. The mean number of days to mating was statistically lower in the LD group, but this was considered an incidental finding.

Caesarian Observations: There was a dose-related reduction in the numbers of corpora lutea with a corresponding reduction in the number of implantation sites (statistically significant) and live embryos (statistically significant). The reduced number of implant sites

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and resulting number of live embryos at the MD was primarily attributed to two dams that had minimal implants (1 or 2). The numbers of live embryos, resorptions, and pre- and post-implantation loss were comparable between groups. Although the pre-implantation loss rate was higher than controls at the MD, this is primarily a reflection of the small number of implants in the two females mentioned above. Data are summarized in the sponsor table below:

Table 67: F1 Generation Cesarean Observations - Rat

Summary of ovarian and uterine finding data

F1 Generation adults

Group 1 - Vehicle Control
Group 2 - LCI699 1 mg/kg/day
Group 3 - LCI699 5 mg/kg/day

Group	Summary Information	Total Number of Corpora Lutea	Total Number of Implantation Sites	Live Embryos	Dead Embryos
1	Mean	14.3	13.5	12.8	0.0
	SD	1.5	1.3	1.5	0.0
	N	22	22	22	22
2	Mean	13.3	12.4 D	11.3 D	0.0
	SD	1.6	1.4	1.8	0.0
	N	23	23	23	23
	% Diff (G1)	-6	-8	-11	
3	Mean	12.6	11.3 E	10.9 D	0.0
	SD	3.0	3.5	3.5	0.2
	N	21	21	21	21
	% Diff (G1)	-12	-16	-15	

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Summary of ovarian and uterine finding data

F1 Generation adults

Group 1 - Vehicle Control
Group 2 - LCI699 1 mg/kg/day
Group 3 - LCI699 5 mg/kg/day

Group	Summary Information	Early Resorptions	Sum of Early Resorptions and Dead Embryos	Preimplantation Loss (%)	Post Implantation Loss (%)
1	Mean	0.7	0.7	5.16	5.45
	SD	0.8	0.8	4.95	5.51
	N	22	22	22	22
2	Mean	1.1	1.1	6.71	8.63
	SD	1.4	1.4	8.01	11.05
	N	23	23	23	23
	% Diff (G1)	49	49	30	58
3	Mean	0.4	0.5	12.98	5.63
	SD	0.7	0.7	20.32	11.32
	N	21	21	21	21
	% Diff (G1)	-41	-35	151	3

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Sponsor tables

Postmortem Examination: Abnormalities were observed in pups found dead or euthanized on the day of birth. At the HD, a split tongue and abnormal hindlimb flexure was noted in individual pups from separate litters; a vascular variation was also observed in a

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pup found dead. The significance of these observations in this group is uncertain given the limited numbers evaluated.

At the MD, edema was observed in two pups from separate litters. Given the low incidence, a relationship to treatment is uncertain.

There were no gross postmortem observations in pups terminated at weaning (day 21 postpartum) that were considered drug-related.

In animals terminated as adults, no drug-related findings were observed.

9.4 Juvenile Animal Studies

9.4.1 A 4-Week Oral Gavage Study in the Juvenile Rat with a 6-Week Recovery Period

Study no.:	1570248
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes
QA Statement:	Yes
Drug, lot #, and % purity:	LCI699, batch 1010005673, 100.0%. Dosing formulations ranged from 100% to 103% of claim.

Methods

Doses:	1, 5, 50 mg/kg
Frequency of dosing:	Once daily from day 28 through 55
Number/Sex/Group:	20 (main) + 12 (pathology) + 10 or 15 (tk)
Dose volume:	5
Formulation/Vehicle:	In ultrapure water
Route of administration:	ORAL GAVAGE
Species:	rat
Strain:	Wistar Han
Age at start of experiment:	28 days
Period of development studied:	postpartum
Comment on Study Design:	Standard design; includes a 6-week recovery period

The design of this study was partially based on the results of a dose-ranging study (study 1570247) in which juvenile rats were administered 0, 20, 50, or 150 mg/kg of LCI699 from days 28 to 55 postpartum. Drug-related effects included, but were not limited to, salivation at ≥ MD, reduced weight gain and sporadically reduced food consumption (primarily at the HD), clinical pathology alterations indicative of inflammation (increased lymphocyte, neutrophil, and monocyte counts) and effects on liver enzymes (increased ALT/ALP) at the HD, liver discoloration at the HD, and increased adrenal gland, liver, and kidney weights at

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the HD. The effects on the adrenal gland and liver were considered related to the pharmacologic activity of the drug. Although the effects at doses ≥MD were drug-related, none were considered adverse. Results from the 4-week toxicity study (study 06700141) and the fertility study (study 0770785) were also considered for dose selection.

Key Study Findings: Dosages of 1, 5, or 50 mg/kg administered. Notable observations included excessive salivation and delays in the attainment of markers of sexual maturation at 50 mg/kg. Although there were no effects on mean estradiol or testosterone levels, the number of males with low testosterone levels increased in a dose-related manner. There were no effects on mating or fertility parameters, although increased ovarian follicle degeneration with multiple large corpora lutea, increased ovarian weights, and vaginal/cervical mucification were evident at the HD (all reversible). Clinical pathology alterations at 50 mg/kg included reversible increases in various white cell populations, increases in CO_2 and magnesium, and decreases in chloride and potassium. Effects on organ weights with microscopic correlates were observed at 50 mg/kg, particularly for the adrenal glands, liver, and female reproductive tract, all of which were reversible. Based on these observations, the NOAEL was considered 5 mg/kg.

Observations and Results

Mortality: There was no drug-related mortality. Two control animals were electively euthanized on study. A male was killed on day 90 due to poor clinical condition and a female was killed on day 43 as a result of trauma-related injuries (caught in cage).

Clinical Signs: Wetness around the jaw/salivation occurred at an increased incidence in both sexes at the HD. The other effects seen either occurred at low incidences, did not follow a dose-response pattern, and/or were seen at a comparable incidence in controls.

Body Weight: In males, reduced weight gains occurred in all drug-treated groups during the treatment period which resulted in dose-related reductions in body weight (<10% lower) beginning approximately 10 days into the treatment period. Although weight gains were increased for a short period of time at the beginning of the recovery period, absolute weights remained lower than controls for a few weeks. However, weight gain over the entire recovery period was comparable between groups. Data are summarized in the reviewer-created table and sponsor figure below:

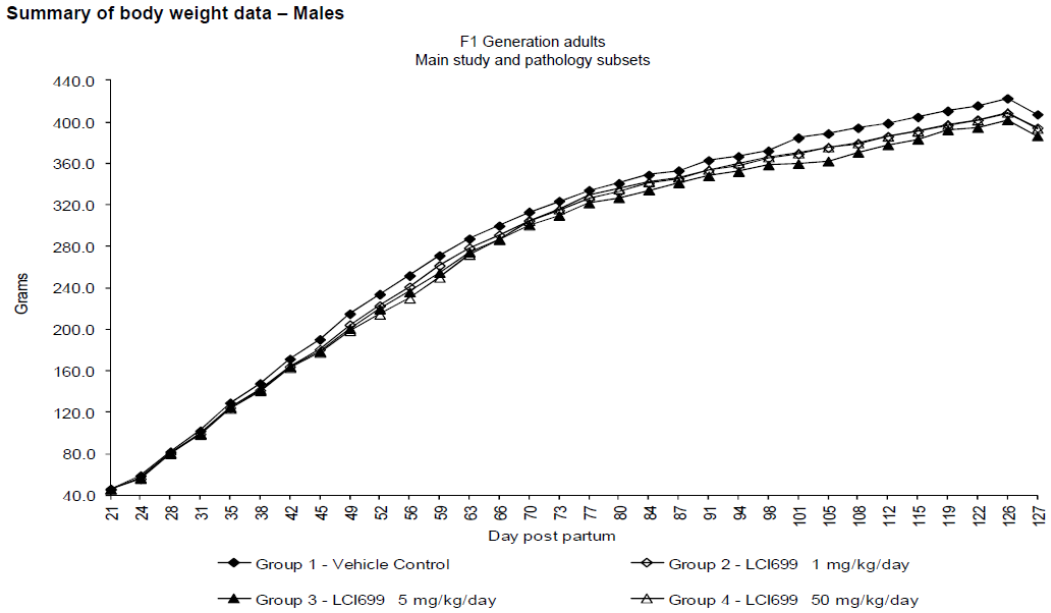
Table 68: Juvenile Rat Body Weights (Male)

Absolute Body Weight/Weight Gain in grams[a] (% of control) - Male				
Day or Interval	Dosage (mg/kg/week)			
	0	1	5	50
Dosing Period				
28	82	80	80	81
35	129	124	125	124
42	171	164** (96%)	164** (96%)	163** (95%)
49	215	204** (95%)	201*** (93%)	199*** (93%)
56	252	241* (96%)	236*** (94%)	230*** (91%)
28 – 56	170	161* (95%)	156*** (92%)	149*** (88%)
Recovery Period				
59	272	262	255*** (99%)	250*** (92%)

Reference ID: 4532599

66	300	291	286* (95%)	287* (96%)
73	324	315	310	316
101	385	369	360	370
127	407	394	387	394
56 – 66[b]	8	50 (6.3x)	50 (6.3x)	57 (7.1x)
59 – 127[b]	135	132	132	144
*p<0.05%, **p<0.01, ***p<0.001 a) Values are rounded by reviewer b) Indicated weight gain intervals were calculated by reviewer so no statistical analyses performed				

Figure 23: Juvenile Body Weight (Male) - Rat



Sponsor figure

In females, increased weight gains resulting in increased body weights were evident at the HD. Increased weights continued to be seen during the post-treatment phase for approximately 4 weeks before controls caught up. In females used for the reproductive assessment, no effects were seen on weight during gestation. Data are summarized in the reviewer created table and sponsor figure below:

Table 69: Juvenile Rat Body Weights (Female)

Absolute Body Weight/Weight Gain in grams[a] (% of control) - Female				
Day or Interval	Dosage (mg/kg/week)			
	0	1	5	50
Dosing Period				
28	75	73	73	74
35	110	108	108	109
42	132	131	131	138* (105%)
49	150	149	149	161** (107%)
56	165	162	164	179*** (108%)
28 – 56	90	90	91	104*** (116%)

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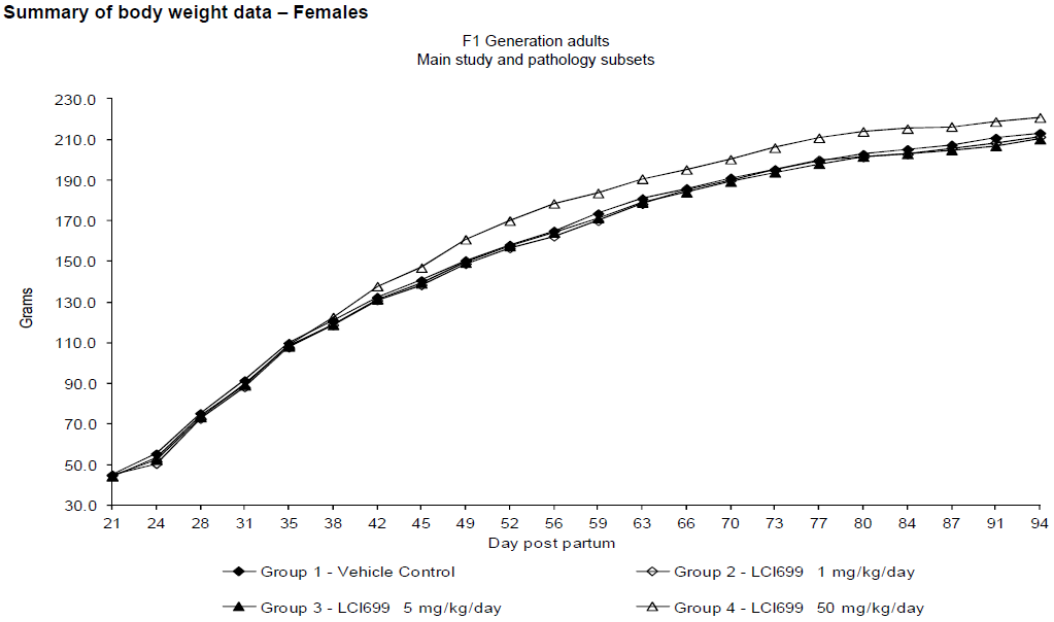
Recovery Period				
59	174	170	171	184** (106%)
66	186	185	184	195** (105%)
73	195	195	194	206** (106%)
94	213	211	210	221
59 – 94	40	42	39	37
Gestation				
0	220	213	218	224
7	240	231	239	244
13	262	253	263	266
0 – 13[b]	42	40	45	42

*p<0.05%, **p<0.01, ***p<0.001
a) Values are rounded by reviewer
b) Indicated weight gain intervals were calculated by reviewer so no statistical analyses performed

Figure 24: Juvenile Body Weight (Female) - Rat



Sponsor figure

Feed Consumption: Food consumption was increased by approximately 9% over controls in HD females during the treatment period, correlating with the increased weight gains during this interval. There were no effects in any other groups.

Long Bone Growth: The length of the right tibia was measured coincident with body weight measurements in a subset of animals. No effects were noted.

Hematology: At the end of treatment, minor changes (<30% difference from control) were noted in males. In female, alterations exceeding 30% were seen for white blood cells (+31%), lymphocytes (+35%), leukocytes (+51%), and reticulocytes (+41%), but only the increase in reticulocytes reached statistical significance. No differences were noted in males following recovery. In females following recovery, eosinophil counts were 54% higher

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at the LD and 46% higher at the HD, and leukocyte counts were 47% lower at the HD; none of these effects were statistically significant.

Coagulation parameters were not affected in either sex by treatment with LCI699.

Clinical Chemistry: In males, reductions in total bilirubin (-74% and -45% at the LD and HD, respectively) and creatinine kinase (-38% and statistically significant) were observed at the end of the treatment period. Following recovery, creatinine kinase levels were reduced (-37%) at the LD; no other alterations in excess of 30% were noted. In females, effects seen at the end of treatment included exceeding 30% included a reduction in creatinine kinase (-31%) at the LD, total bilirubin at the HD (-50%). Although of minimal magnitude, increases in total CO_2 (+17%) and magnesium (+15%), and reductions in total protein (-5%), albumin (-5%), and chloride (-3%) in HD females reached statistical significance. Following recovery, total bilirubin was still lower in HD females (-38%); this effect did not reach statistical significance

Urinalysis: No effects were noted on urinalysis parameters.

Sexual Maturation: The mean day for preputial separation and vaginal opening occurred later for HD males and females. These effects were statistically significant. For males, the ages were outside of the historical range (41.2 – 43.7 days) of the test facility while the ages for females were still within the historical range (31.1 – 33.7 days). Data are summarized in the reviewer table below:

Table 70: Sexual Maturation Summary

Mean Age of Sexual Maturation (in days)				
Day or Interval	Dosage (mg/kg/week)			
	0	1	5	50
Preputial Separation	42.6	42.6	43.4	47.2***
Vaginal Opening	31.1	31.0	30.7	33.2***
***p<0.001				

Hormone Levels: There were no effects on estradiol levels at either the end of treatment or recovery, although many animals in the drug-treated groups had levels BLQ. Mean testosterone levels were also comparable between groups at the end of treatment and recovery. However, the number of animals with low testosterone levels (<1 ng/mL) increased in a dose-related manner (3/18, 8/19, 7/20, and 12/20 at C, LD, MD, and HD, respectively).

Motor Activity: There were no effects on activity (ambulations, fine movements) levels.

Auditory Startle: Auditory startle parameters (startle at start, maximum response, time to maximum response average startle) were not affected by treatment.

Learning/Memory Assessment: No effects were evident on learning or memory based on the use of the Cincinnati water maze (number of trials and number of errors with either pathway or on swimming performance).

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Estrous Cyclicity: There were no effects on estrous cycle parameters (number of days in estrous, number of cycles, cycle length) in females used for evaluating reproductive capability.

Mating/Fertility: There were no effects on the time to mate, number of animals mating, or the resulting number of pregnancies.

Caesarian Observations: The number of corpora lutea, implants, pre- and post-implantation loss, live and dead embryos, and early resorptions were comparable between groups.

Gross Pathology: Adrenal gland enlargement was noted in a single HD female that correlated with cortical hypertrophy seen microscopically. Adrenal gland enlargement was also seen in a single HD female at the end of recovery, but there was no correlate seen microscopically.

Organ Weights: In males, liver weights (absolute, body weight adjusted, brain weight adjusted) were increased and correlated with centrilobular hypertrophy and vacuolation. In males, prostate weights were reduced in HD males without microscopic correlates. Data are summarized in the sponsor table below:

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Table 71: Organ Weight Summary (Male) - Rat

Table 4-3 LCI699-related differences in organ weights[a] compared to the vehicle control group - males, pathology subset

Sex	Males			
Dose (mg/kg/day)	0	1	5	50
Number examined	12	12	12	12
Body weight mean (g)	232.9	221.1	225.4	209.6
(% diff)	(–)	-5	-3	-10
Brain weight mean (g)	1.8533	1.8316	1.8648	1.8498
(% diff)	(–)	-1	1	0
Liver				
Absolute mean (g)	7.9672	7.4711	7.7987	8.7246
(% diff)	(–)	-6	-2	10
Rel.[b] to body weight (%)	3.3908	3.3559	3.4336	4.1320[c]
(% diff)	(–)	-1	1	22
Rel. to brain weight	429.4832	407.5040	417.6042	470.5855
(% diff)	(–)	-5	-3	10
Prostate gland				
Absolute mean (g)	0.3698	0.3722	0.3925	0.2563[c]
(% diff)	(–)	1	6	-31
Rel. to body weight (%)	0.1597	0.1687	0.1755	0.1233[c]
(% diff)	(–)	6	10	-23
Rel. to brain weight	19.9348	20.3253	21.0335	13.8648[c]
(% diff)	(–)	2	6	-30

[a] Expressed as percent difference of group means (% diff).
[b] Relative.
[c] Based upon statistical analysis of group means, values are significantly different from control group at $P \leq 0.05$; refer to data tables for actual significance levels and tests used.

Sponsor table

In females administered the HD, observations included increased liver weights correlating with centrilobular hypertrophy and vacuolation, increased adrenal gland weights correlated with cortical hypertrophy, decreased uterine weights that correlated with uterine atrophy, increased ovary/oviduct weights that correlated with follicle degeneration and multiple large corpora lutea, and increased kidney weights without a microscopic correlate. Data are summarized in the sponsor table below:

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Table 72: Organ Weight Summary (Female) - Rat

Table 4-4 LCI699-related differences in organ weights[a] compared to the vehicle control group - females, pathology subset

Sex	Females			
Dose (mg/kg/day)	0	1	5	50
Number examined	12	12	12	12
Body weight mean (g)	160.6	155.8	157.0	165.8
(% diff)	(–)	-3	-2	3
Brain weight mean (g)	1.7648	1.7426	1.7433	1.7736
(% diff)	(–)	-1	-1	0
Liver				
Absolute mean (g)	5.1223	4.9275	5.1314	7.0457[c]
(% diff)	(–)	-4	0	38
Rel.[b] to body weight (%)	3.1646	3.1571	3.2504	4.2429[c]
(% diff)	(–)	0	3	34
Rel. to brain weight	289.7883	283.0579	294.5772	398.0734[c]
(% diff)	(–)	-2	2	37
Adrenal glands				
Absolute mean (g)	0.06237	0.06153	0.06460	0.08743[c]
(% diff)	(–)	-1	4	40
Rel. to body weight (%)	0.03893	0.03938	0.04127	0.05298[c]
(% diff)	(–)	1	6	36
Rel. to brain weight	3.53341	3.52664	3.70875	4.93399[c]
(% diff)	(–)	0	5	40
Uterus				
Absolute mean (g)	0.5244	0.3999	0.4823	0.3169[c]
(% diff)	(–)	-24	-8	-40
Rel. to body weight (%)	0.3318	0.2553	0.3071	0.1921[c]
(% diff)	(–)	-23	-7	-42
Rel. to brain weight	29.8311	22.8674	27.6023	17.8767[c]
(% diff)	(–)	-23	-7	-40
Ovary/Oviduct				
Absolute mean (g)	0.1099	0.1078	0.1168	0.1364[c]
(% diff)	(–)	-2	6	24
Rel. to body weight (%)	0.0690	0.0693	0.0747	0.0827[c]
(% diff)	(–)	0	8	20
Rel. to brain weight	6.2574	6.1950	6.7029	7.6868[c]
(% diff)	(–)	-1	7	23

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Kidney				
Absolute mean (g)	1.2691	1.2524	1.3020	1.4820[c]
(% diff)	(–)	-1	3	17
Rel. to body weight (%)	0.7919	0.8034	0.8298	0.8938[c]
(% diff)	(–)	1	5	13
Rel. to brain weight	71.8722	71.9025	74.7464	83.6037[c]
(% diff)	(–)	0	4	16

[a] Expressed as percent difference of group means (% diff).
[b] Relative.
[c] Based upon statistical analysis of group means, values are significantly different from control group at $P \leq 0.05$; refer to data tables for actual significance levels and tests used.

Sponsor table

Histopathology: Drug-related effects were observed in the adrenal gland (hypertrophy in HD males and females and cortical vacuolation in HD females), liver (centrilobular hepatocellular hypertrophy and vacuolation at the HD of both sexes), ovary (follicle degeneration), and uterus (atrophy). The incidences and severities are summarized in the sponsor table below (revised by reviewer to present effected organs).

Table 73: Microscopic Observations - Rat

Removal Reason: TERMINAL EUTHANASIA	Male				Female			
	0 mg/kg /day Group 1	1 mg/kg /day Group 2	5 mg/kg /day Group 3	50 mg/kg /day Group 4	0 mg/kg /day Group 1	1 mg/kg /day Group 2	5 mg/kg /day Group 3	50 mg/kg /day Group 4
Number of Animals:	12	12	12	12	12	12	12	12
GLAND, ADRENAL								
Examined	12	12	12	12	12	12	12	12
No Visible Lesions	10	12	12	4	12	12	12	4
Hypertrophy; cortical	0	0	0	0	0	0	0	8
.... minimal	0	0	0	0	0	0	0	8
Vacuolation; cortical	2	0	0	8	0	0	0	3
.... minimal	2	0	0	7	0	0	0	3
.... mild	0	0	0	1	0	0	0	0
LIVER								
Examined	12	12	12	12	12	12	12	12
No Visible Lesions	10	7	12	0	4	4	3	0
Vacuolation; centrilobular, hepatocellular	0	0	0	8	0	0	0	12
.... minimal	0	0	0	8	0	0	0	10
.... mild	0	0	0	0	0	0	0	2
Hypertrophy; centrilobular, hepatocellular	0	0	0	12	0	0	0	12
.... minimal	0	0	0	12	0	0	0	12
OVARY								
Examined	.	.	.	.	12	12	12	12
No Visible Lesions	.	.	.	.	11	12	12	1
Degeneration; increased, follicle	.	.	.	.	0	0	0	11
.... minimal	.	.	.	.	0	0	0	11
UTERUS								
Examined	.	.	.	.	12	12	12	12
No Visible Lesions	.	.	.	.	12	12	11	10
Atrophy	.	.	.	.	0	0	0	2
.... minimal	.	.	.	.	0	0	0	2

Sponsor table

Following recovery, minimal hepatocellular vacuolation was observed in a single HD male. All other findings after recovery occurred at incidences/severities comparable to controls.

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Toxicokinetics: Exposure to LCI699 increased in a proportional (C_{max}) to greater than proportional (AUC) manner with dose. Peak plasma levels were generally attained at 0.5 to 3 hours post-dosing, although the t_{max} was reported as 7 hours on the first day of dosing for males at the HD. There was no consistent evidence of accumulation and there was no gender effect. Data are summarized in the sponsor table below:

Table 74: Exposure Summary - Rat

Table 4-8 Summary mean LCI699 toxicokinetic parameters in juvenile rat plasma on days 28 and 55 pp

Dose (mg/kg/day)	Sex	Study day (pp)	T_{max} (hr)	C_{max} (ng/mL)	C_{max}/D (ng/mL/ (mg/kg/day))	$AUC_{(0-24)}$ (hr*ng/mL)	$AUC_{(0-24)}$/D (hr*ng/mL/ (mg/kg/day))	R_{AUC}[a]
1	Male	28	0.5	210	210	589	589	NA
		55	0.5	272	272	1100	1100	1.86
	Female	28	1	252	252	637	637	NA
		55	1	352	352	1530	1530	2.40
5	Male	28	1	1400	281	6000	1200	NA
		55	1	1670	334	8220	1640	1.37
	Female	28	1	1540	308	9960	1990	NA
		55	1	2110	423	12500	2510	1.26
50	Male	28	7	12300	246	184000	3680	NA
		55	3	11800	237	119000	2380	0.649
	Female	28	3	14500	289	197000	3950	NA
		55	1	13200	265	153000	3060	0.774

NA = Not Applicable.

[a] R_{AUC} = Day 55 pp $AUC_{(0-24)}$/Day 28 pp $AUC_{(0-24)}$.

Sponsor table

For the chiral impurity LCI698, the levels were (b) (4)% that of the parent, when detected.

10 Special Toxicology Studies

10.1 Primary Skin Irritation/Corrosion Study with LCI699-D11(=AZA) in the Rabbit

Study no.: R0506123
Study report (Electronic) location: EDR
GLP Compliance: Yes (OECD)
QA Statement: Yes

Skin irritation was evaluated in three rabbits by exposing them to 0.5g of LCI699 for 4 hours using a semi-occlusive dressing.

No skin irritation was evident.

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10.2 Assessment of Contact Sensitizing Potential with the Murine Local Lymph Node Assay (LLNA TIER 1)

Study no.: R0506124
Study report (Electronic) location: EDR
GLP Compliance: Yes (EU)
QA Statement: Yes

The purpose of this study was to evaluate the potential contact sensitivity following epidermal exposure to mice. Test or control articles were placed on the dorsum of both ears of 6 female Balb/C mice once daily for three days, and auricular lymph nodes were taken 24 hours after the last administration. Visual examination of the ears, ear weight, weight of draining lymph nodes, and cell counts were recorded. LCI699 concentrations of 0.25%, 2.5%, and 25% were evaluated.

Treatment with LCI699 induced dose-related increases in ear weight, lymph node weight, and cell counts, with many of the effects attaining statistical significance. Data are shown in the sponsor table below:

Table 75: Tissue Weight Changes in LLNA

Table 4-1 Summary of results

Group	Item	b.w. gain [g] (mean ± SD)	Ear weight index	LN weight index	Cell count index
1	Dimethyl sulphoxide	0.2 ± 0.4	1.00 [25.04]	1.00 [4.43]	1.00 [4.58]
2	0.5% DNCB	-0.2 ± 1.0	1.26**	1.81**	2.68**
3	0.25% LCI699-D11(=AZA)	0.3 ± 0.8	0.99	1.20	1.55*
4	2.5% LCI699-D11(=AZA)	0.5 ± 0.5	1.00	1.45*	2.30**
5	25% LCI699-D11(=AZA)	0.0 ± 0.6	1.06*	1.67**	2.79**
	Threshold conc. (%)	n.a.	22.7	n.a.	n.a.

Sponsor table

Based on these results, LCI699 was considered a strong sensitizer.

10.3 Impurities

A series of *in vitro* and *in vivo* studies were performed to evaluate to genotoxicity of potential or actual impurities.

10.3.1 In Silico Studies

The Derek Nexus, Case Ultra, and Sarah systems were used to predict the potential mutagenicity of the main degradation products (b) (4) (b) (4) and (b) (4) (Reports 1419395 and 1419328). Based on these models, the three degradation products were

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considered to have a low likelihood of being mutagenic. The degradants (b) (4) and (b) (4) were also evaluated in an Ames assay. In this study (Report 1470352), LCI699 spiked with 3.21% of (b) (4) and 3.5% of (b) (4) were evaluated at concentrations up to 5000 µg/plate. No increase in mutations were seen in the presence or absence of S9 metabolic activation.

10.3.2 In Vivo Studies

A 4-week toxicity study (Report 1470353) in the rat with a micronucleus assessment was performed with LCI699 spiked with 3.21% of (b) (4) and 3.5% of (b) (4) (b) (4) and (b) (4) are both degradation products. Groups included a vehicle control, groups dosed with 5 or 50 mg/kg of LCI699 spiked with impurities, and a group administered LCI699 alone without the impurities. Key study observations are summarized below:

- No mortality occurred.
- Salivation was reported in LCI699 groups at 50 mg/kg with or without impurities
- Reduced weight gain occurred in 50 mg/kg males with/without impurities during week 1. Increased weight gain occurred in 50 mg/kg females with/without impurities during weeks 3 and 4. No effects on food consumption.
- Slight increases (≤ +26%) in fibrinogen were evident in 50 mg/kg males/females with/without impurities, and in neutrophil counts (+41%) in males at 50 mg/kg with/without impurities. There were no effects on clinical chemistry of urinalysis parameters.
- Liver weights (absolute and adjusted) were increased 40% to 49% in males/females at 50 mg/kg with/without impurities. Increases in the weights (absolute and adjusted) of kidneys (18% to 24%) and adrenal gland (31% to 41%), and decreases in uterus weights (-41% to -49%), were seen in females administered 50 mg/kg LCI699 with/without impurities.
- Increased incidences of centrilobular hypertrophy correlated with the increased liver weights in groups administered 50 mg/kg LCI699 with/without impurities.
- Cortical vacuolation and hypertrophy of the adrenal glands occurred at a comparable incidence in groups administered 50 mg/kg LCI699 with/without impurities; these effects correlated with increased weights in females.
- Renal tubular degeneration and tubular basophilia, ovarian follicular degeneration, uterine atrophy, and vaginal mucification occurred at comparable incidences in females administered 50 mg/kg with/without impurities. The ovarian, uterine, and vaginal effects were also seen in females administered LCI699 with impurities at 5 gm/kg, but at a lower incidence than that seen at 50 mg/kg with impurities.
- Exposure increased approximately dose proportionally. Exposures were comparable between groups administered LCI699 at 50 mg/kg with/without impurities.
- There were no differences in the frequency of micronuclei between the various LCI699-treated groups and negative controls.

It was concluded that the findings in animals administered LCI699 with impurities were analogous to those in animals administered LCI699 without impurities. Furthermore, there was no evidence of mutagenicity based on the absence of micronuclei formation.

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A similar study (Report 1470796) was performed to qualify the degradation product (b) (4) In this 4-week toxicity study in the rat with a micronucleus assessment, the groups included a vehicle control, groups dosed with 5 or 50 mg/kg of LCI699 spiked with 3.1% of the impurity, and a group administered LCI699 alone without the impurity. The key study observations are summarized below:

- No mortality occurred.
- Salivation was seen in the groups administered 50 mg/kg LCI699 with/without the impurity.
- Reduced weight gain occurred in 50 mg/kg males with/without impurity during week 1. Increased weight gain occurred in 50 mg/kg females with/without impurity during weeks 1 and 3. Although weight gain was nearly twice that of controls, absolute weights were only increased approximately 7%. There were no effects on food consumption.
- Slight increases ($\leq$ +35%) in reticulocyte counts were reported in 50 mg/kg males/females with/without impurity. Fibrinogen was increased (10% to 25%) and APTT decreased (7% to 13%) in 50 mg/kg males/females with/without the impurity.
- Increases (<2-fold) in AP, creatinine, and CO_2 of comparable magnitudes were seen in the LCI699 groups administered 50 mg/kg with/without the impurity. Decreases of comparable magnitudes ($\leq$ 66%) for bilirubin, protein, ALB, Glob, K, and Cl were seen in the LCI699 groups administered 50 mg/kg with/without impurity. No effects noted for urinalysis parameters.
- Increased liver (32% to 56%) and adrenal gland (7% to 22%) weights (absolute/adjusted) occurred in groups administered 50 mg/kg LCI699 with/without impurity. Increases in the weights of the kidneys (11% to 20%) and decreases in uterine weights (-16% to -31%) were seen in females administered 50 mg/kg LCI699 with/without impurity.
- Hepatocellular vacuolation and hypertrophy of the liver, and cortical vacuolation and hypertrophy of the adrenal gland, occurred at comparable incidence and magnitude in males/females administered 50 mg/kg LCI699 with/without impurity. In females, follicular dilatation of the ovary, atrophy of the uterus, and mucification of the vagina occurred at similar incidences between the groups administered 50 mg/kg LCI699 with/without impurity. Lower incidences of the ovarian and vaginal observations occurred in the group administered 5 mg/kg of LCI699 with the impurity.
- Exposure increased with dose in an approximately proportional manner. Exposures were considered similar between the groups administered 50 mg/kg LCI699 with/without impurity.
- The frequency of micronuclei was comparable between all LCI699-treated groups and controls.

The toxicity profile was not altered by the degradant (b) (4) and there was no evidence of mutagenicity.

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10.4 Gene Expression Studies

10.4.1 Evaluation of Published Rodent Liver Gene Expression Signatures for CAR-mediated Mode of Action and Comparison with LCI699 Liver Gene Expression Data and Historical Data from a 4-week Phenobarbital Study

Study no.: 1520459a
Study report (Electronic) location: EDR
GLP Compliance: No
QA Statement: No

The purpose of this study was to characterize LCI699 mediated activation of constitutive androstane receptor (CAR) as a possible rodent specific mechanism for the induction of the liver and thyroid tumors observed in the rodent carcinogenicity studies.

Liver gene expression profiles were obtained from samples collected in the 4-week toxicity study with LCI699. Relevant findings from the 4-week study included minimal to marked centrilobular hepatocellular cytoplasmic vacuolation and hypertrophy, increases in liver weight, and increased thyroid weights at 50 mg/kg (see section 6.2).

The gene profiles obtained from this mechanistic study, along with historical liver gene expression data from phenobarbital treated rats, were compared to published rodent gene expression markers as summarized in the sponsor table below:

Table 76: Liver gene expression biomarkers

Table 2-1 Rodent liver gene expression biomarkers for evaluating nuclear receptor-mediated modes of action

Mode of action	Gene expression biomarkers (Peffer et al 2018)	Gene expression biomarkers (Rooney et al 2018)
CAR/PXR	Show liver induction (CYP2B>CYP3B) *in vivo* = signature of CAR/PXR activators	
CAR	Cyp2b10 (= Cyp2b1 in rats) and Cyp3a11 in mice, Gadd45b and/or Ki67	Cyp2b1, Ugt2b1, Ces2c
AhR	Large induction of Cyp1a and Cyp1b isoforms	Cyp1a1, Cyp1a2, Aldh3a1
PPAR alpha	Large induction of Cyp4a	Cyp4a1, Cpt1b, Lpl
ER	-	Ctr9, Gdf15, Lifr
Cell proliferation	Ki67	Gadd45a, Rhob, Mdm2

Sponsor table

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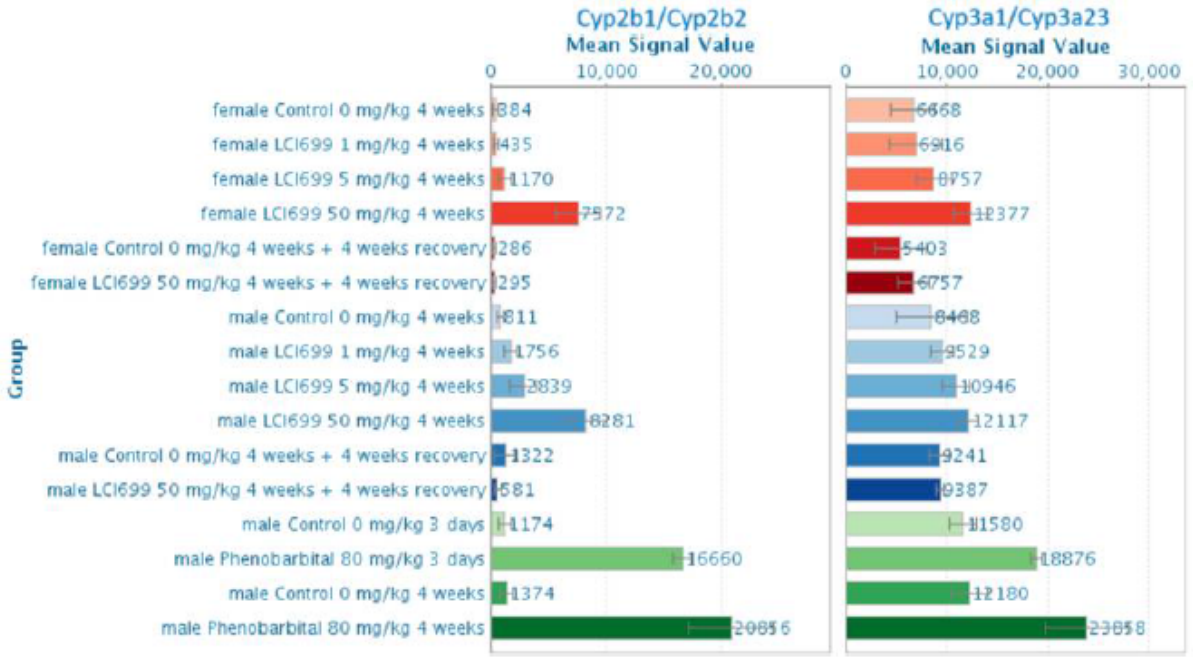
CAR activators alter gene expression of cytochrome P450 metabolizing enzymes by predominantly inducing expression of Cyp 2b isoforms while Cyp3a isoforms are induced to a lesser extent.

In comparison to the biomarkers propagated by Peffer et al (2018), treatment with LCI699 for 30 days resulted in a dose-dependent increase in Cyp2b1/Cyp2b2 expression that ranged between 1- and 20-fold in females and 2- and 10-fold in males. LCI699 treatment induced expression of Cyp3a1/3a32 to a lesser extent (1- to 2-fold in females and 1- to 1.4-fold in males). Expression of all examined Cyp enzymes returned to levels similar to control rats after the recovery period. Historical liver gene expression data from male rats treated with 80 mg/kg phenobarbital (a model CAR activator) for 4 weeks produced a 15-fold induction of Cyp 2b1/2b2 and a 2-fold induction of Cyp 3a1/3a23. When a series of genes that represent a signature of CAR-mediated activation were examined in liver tissue from LCI699-treated rats (Cyp2b1, Ugt2b1, and Ces2c), dose-dependent increases in induction were observed that ranged between 1- and 5-fold in females and 2- and 9-fold in males (data not provided). Again, evaluation of historical samples from phenobarbital-treated rats (the model CAR activator) showed increased expression of CAR-signature genes both after 3 days or 4 weeks of exposure (increases in expression ranged between 8- and 10-fold). Results are summarized in the sponsor figure below:

Figure 25: CYP2B and CYP3A Family mRNA Levels

Figure 3-1 Comparison of CAR- versus PXR-mediated activation gene expression signatures

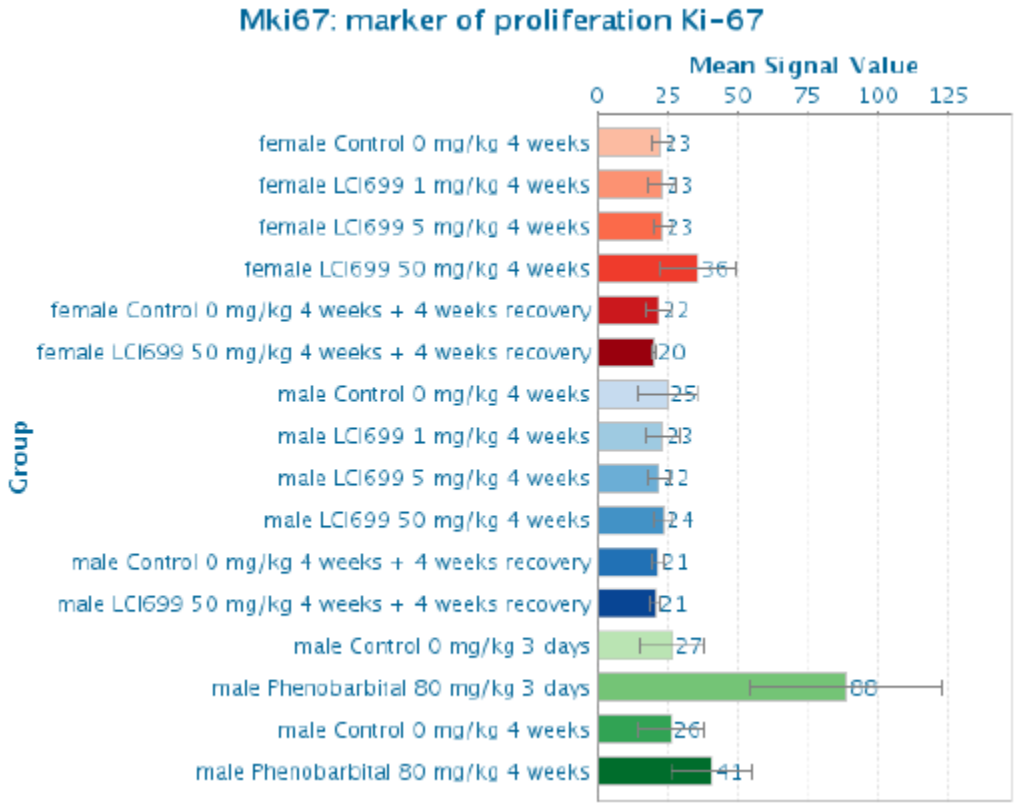


Sponsor figure

Exposure to most CAR activators result in cell proliferation in the whole liver during the first 1-3 weeks after initial exposure subsequently followed by a return to levels similar to

controls during sustained exposure to the CAR activator. This hallmark of CAR-mediated tumorigenesis was evaluated in rats treated with phenobarbital. In historical liver gene expression data, three days after exposure to 80 mg/kg phenobarbital, Ki-67, a marker for cell proliferation, increased 3-fold. However, after 4 weeks of continuous exposure to phenobarbital, Ki-67 levels had decreased and were similar to controls. Like phenobarbital, exposure to LCI699 for 4 weeks in female and male rats did not have a remarkable effect on Ki-67 expression. However, since an earlier time point shortly after treatment was initiated was not examined, it was not possible to determine if LCI699 induced a similar pattern of acute, reversible increases in levels of the Ki-67 biomarker (Figure 30). Further support for the hypothesis that LCI699 caused hepatocyte proliferation in rodents through a CAR-dependent mechanism was observed in the 13-week mouse toxicity study where increased mitoses (refer to Table 26 above) were observed in hepatocytes of the mice that were found dead or were terminated after being treated with 200 mg/kg of LCI699.

Figure 26: Ki-67 mRNA Levels



Sponsor figure

To determine if LCI699-treatment influenced alternative mechanisms of liver tumorigenesis independent of CAR activation, the Sponsor also evaluated gene expression signatures for the aryl hydrocarbon receptor (AhR), peroxisome proliferator-activated receptor (PPARα), and estrogen receptor (ER).

When examining AhR-signature genes (e.g. Cyp1a1, Cyp1a2 and Aldh3a1), an increase in Cyp1a1 gene expression was seen in male and female rats administered 50 mg/kg LCI699

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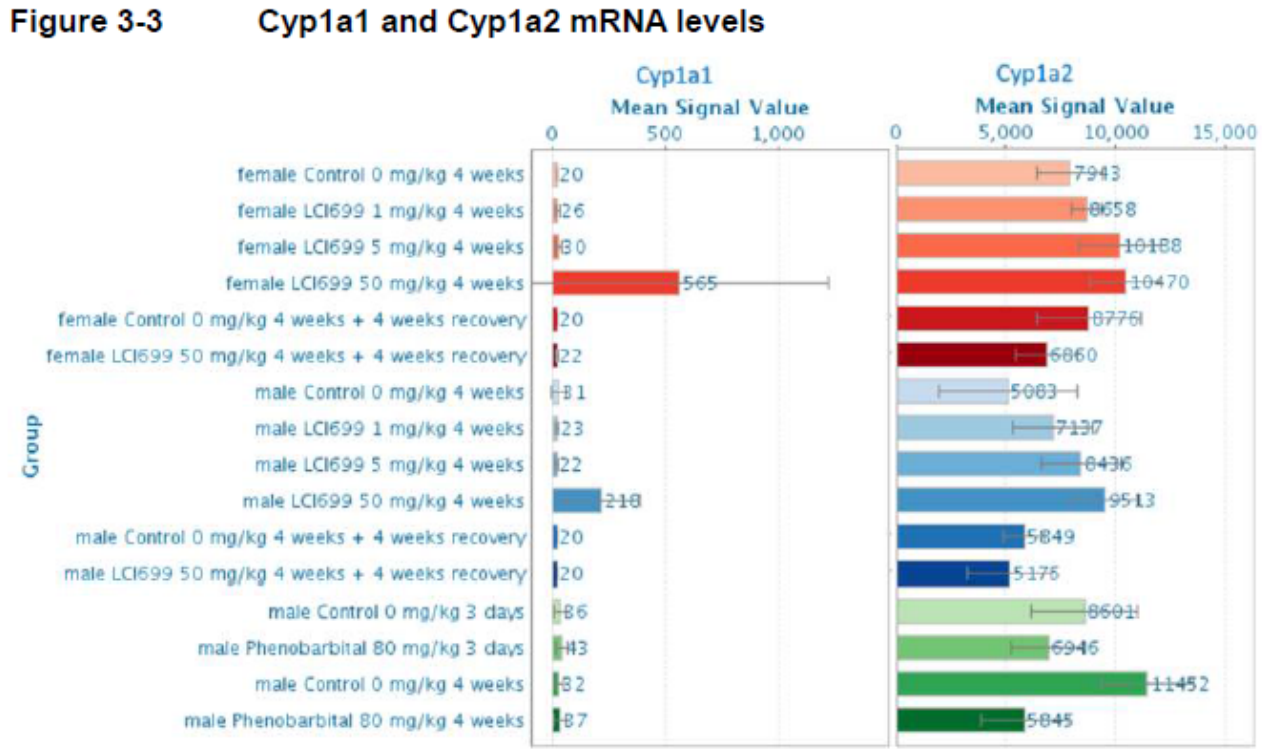
(77x and 118x the MRHD, respectively in male and female rats from 4-week rat study) that is consistent with AhR activation and provides some evidence that LCI699 could promote tumorigenesis through an AhR-dependent mechanism at high multiples to clinical exposure (Figure 31). In contrast, the model CAR-activator phenobarbital did not cause activation of AhR signature genes at either time point examined (3 days or 4 weeks of exposure). The Sponsor believed the CYP1a1 induction was a reflection of the large variation between individual animals and a large induction in a single female. Additionally, the Sponsor argued that the low magnitude of induction relative to CAR responsive genes together with an absence of upregulation of the AhR activator response gene Aldh3a1 is not consistent with a gene signature pattern of an AhR activator mode of action.

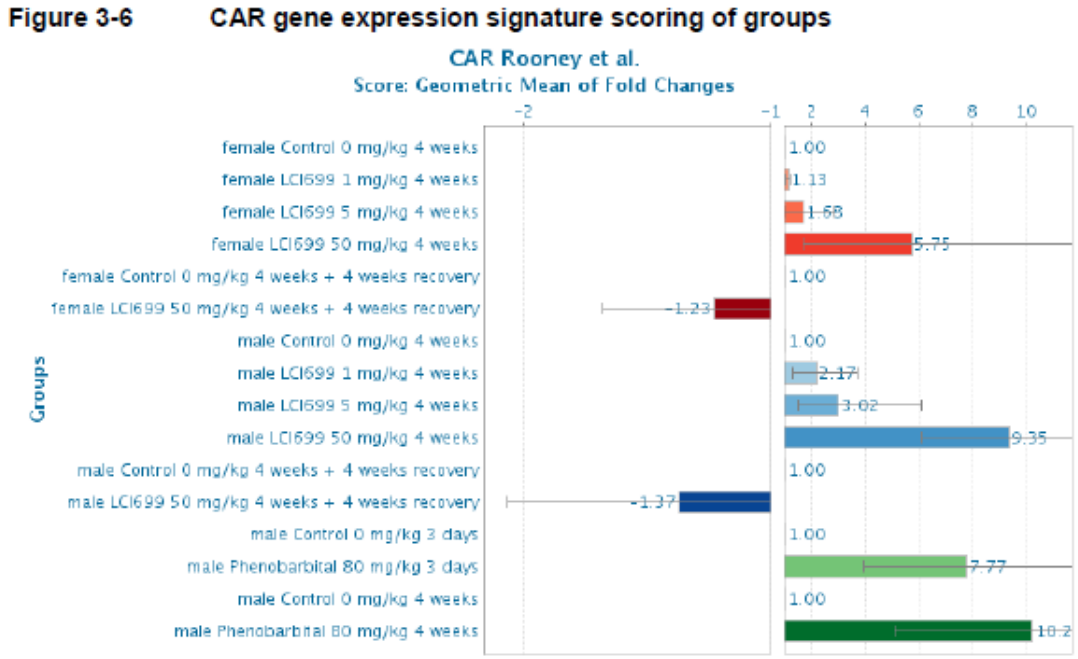
Figure 27: CYP1A Family mRNA Levels



Sponsor figure

In comparison to the biomarkers propagated by Rooney et al (2018), treatment with LCI699 induced gene expression of CAR biomarkers (CYP2B1, Ugt2B1, and Ces2C mRNA levels) similar to that seen with phenobarbital. No meaningful effects were seen following a reversal period. Data are summarized in the sponsor figure below:

Figure 28: CAR Gene Expression Markers



Sponsor figure

For AhR gene expression biomarkers (CYP1A1, CYP1A2, and Aldh3A1 nRNA levels), a slight increase was seen at the highest dose of LCI699, but not with phenobarbital. Data are summarized in the sponsor figure below:

Figure 29: AhR Gene Expression Markers

Sponsor figure

LCI699 did not induce increases in the expression of PPARα or ER gene expression markers nor did it result in increase in gene expression of cell proliferation markers (Gadd45a, Rhob, and Mdm2 mRNA).

In summary, treatment with LCI699 resulted in induction of CAR-responsive genes in a pattern similar to the CAR-inducer phenobarbital, although the magnitude of the LCI699 response was not necessarily as strong as that seen with phenobarbital. An induction of AhR-responsive genes was also seen, but the magnitude was less than that seen towards the CAR-responsive genes. However, the comparisons were based on published data and not generated concurrent with the LCI699 data. These results are supportive of a CAR-based mechanism of tumorigenesis, but the possibility that LCI699 could promote liver tumorigenesis in humans at high multiples (≥77x MRHD) through an AhR-dependent mechanism could not be completely ruled out.

10.4.2 Comparative Computational Analyses of LCI699 Liver Gene Expression Data with Historical Data for Transcriptomic Responses to Established AhR and CAR Activators

Study no.:	1520459b
Study report (Electronic) location:	EDR
GLP Compliance:	No
QA Statement:	No

The purpose of this study was to compare liver gene expression profiles from LCI699 treated rats to historical liver gene expression profiles from rats treated with compounds known to be AhR or CAR activators using the Collaborative Toxicogenomics web application.

The LCI699 liver gene expression profiles were obtained from the 4-week toxicity study in rats (section 6.2). The activation of AhR markers CYP1A1 and -1A2 by TCDD from 58 published experiments were used to derive an AhR score and identify three thresholds that correspond to an increased risk of observing AhR-induced tumors in a rat 2-year study (Qin et al, 2019):

- a level corresponding to 2x the standard deviation of the score calculated for vehicles (0.65),
- a level of low concern (1.0), and
- a level of high concern (1.33)

The AhR score derived for LCI699 and other compounds, including AhR inducers hexachlorobenzene (HB) and omeprazole (OMEP) and the CAR activator phenobarbital (PB), were compared to the scores for TCDD. TCDD inductions exceeded the “high” concern threshold at concentrations $\geq$ 0.3 µg/kg, HB showed a dose-response that approached a “high” concern but did not reach that level, and OMEP approached “high” concern threshold on day 1, but not at later timepoints. In contrast, LCI699 was within the range of two standard deviations of controls at the dosages used in the 4-week study when

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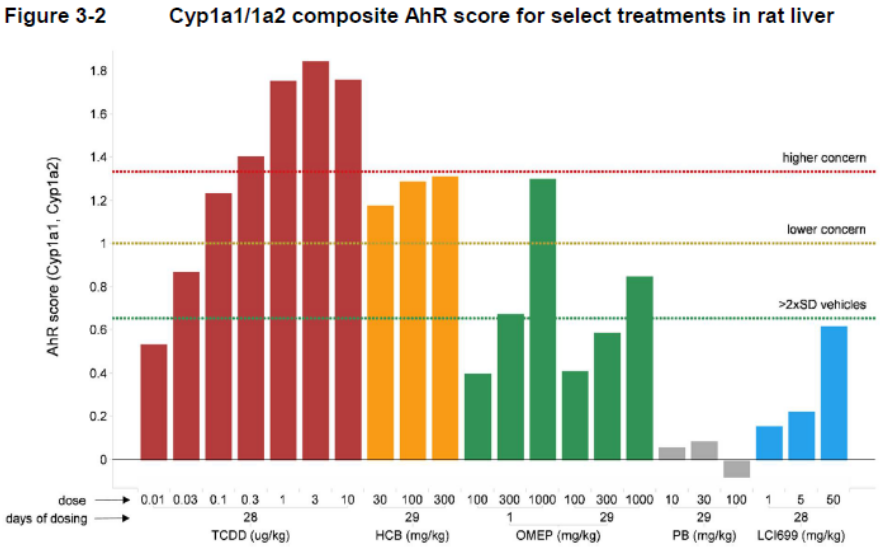
male and female data were pooled (the scores were 0.56 and 0.67 for females and males, respectively). Data are summarized in the sponsor figure below:

Figure 30: AhR Activation Scores



Sponsor figure

The co-occurrence of CAR and AhR responses for these compounds were compared. PB and TCDD were CAR and AhR inducers, respectively, while OMEP was a modest inducer of both CAR and AhR. LCI699 was more similar to PB than it was to TCDD or OMEP. At the highest dose tested in the 4-week study (50 mg/kg), both males and females exceeded the 95th percentile CAR response; males also exceeded the 95th percentile AhR response. Data are summarized in the sponsor figure below:

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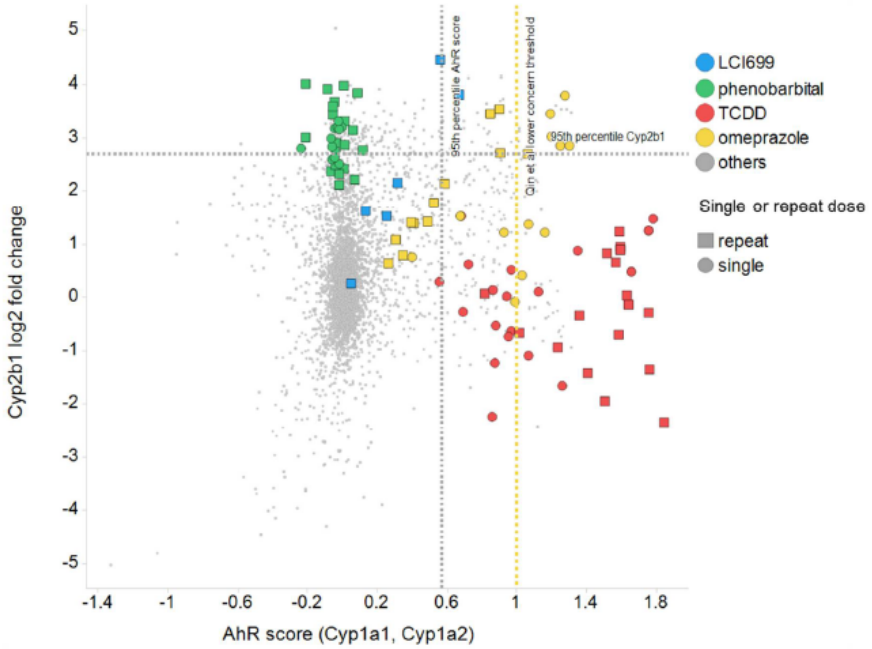
Figure 31: Comparison of CAR Versus AhR Responses

Figure 3-4 Comparison of CAR response, measured via Cyp2b1 and AhR score across 4230 rodent liver experiments



TCDD experiments from transgenic mice, and those for doses ≤0.03 µg/kg are excluded; also excluded are 4 TCDD experiments from GSE31411 (<1 day exposure) on RAE230A microarrays where the CTox application did not provide Cyp2b1 results (methods); dashed gray lines denote 95th percentile for Cyp2b1 log2 fold change (2.71) and AhR score (0.57), calculated on the combined DM and TG repositories

Sponsor figure

11 Integrated Summary and Safety Evaluation

Novartis seeks to market osilodrostat for the treatment of Cushing's disease which is characterized by chronic overproduction of cortisol. It reduces cortisol levels by inhibiting 11β-hydroxylase (CYP11B1), the enzyme involved in the final step in the synthesis of cortisol. It also inhibits the synthesis of aldosterone via inhibition of aldosterone synthase (CYP11B2) to which it is 95% identical in coding region and 93% identical in amino acid sequence. . At the MRHD of 30 mg bid, the C_{max} is 306 ng/mL (~ 1.3 µM) and the AUC_{tau} is 1680 ng·h/mL (Study 2108).

LCI699 effects on cortisol levels were initially identified in clinical studies. As a result, nonclinical investigations to demonstrate this activity were limited. An *in vitro* study showed inhibition of cortisol production in cell lines overexpressing human CYP11B1 and corticosterone levels were lowered in the *in vivo* rat models, but only minimal effects on cortisol levels occurred in a NHP model.

LCI699 is a slightly weaker inhibitor of CYP11B1 (IC_{50} 2.5 nm) as compared to CYP11B2 (IC_{50} 0.7 nm). Both enzymes are expected to be inhibited at clinically relevant concentrations. Since both enzymes are also inhibited in nonclinical species, it is not possible to ascribe nonclinical study findings to inhibition of a particular enzyme. Furthermore, aldosterone and cortisol levels were not measured in the dog so it is unknown

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if there was pharmacologic activity in this species, or if study observations in this species were related to pharmacology or represent off-target effects. LCI699 also inhibits aromatase (IC_{50} = 1.7 µM), a member of the cytochrome P450 superfamily that catalyzes the conversion of androstenedione to estrone and testosterone to estradiol. Clinical levels of LCI699 total drug and free drug plasma concentrations of are likely to achieve 1.3 µM and 0.8 µM at C_{max} suggesting a potential for inhibition of aromatase as well. The impurity LCI698, the (+) enantiomer of LCI699, inhibits human aromatase IC_{50} = 9 nM. However, plasma levels of LCI698 were below the LOQ (0.5 ng/mL) in all subjects treated with 200 mg osilodrostat (Study A2101). Based on the observed C_{max}, LCI698 represents <0.03% the level of LCI699. Thus, some degree of LCI699-mediated off-target inhibition of aromatase may be anticipated at clinically relevant exposures, but LCI698-mediated inhibition is unlikely.

There were no adverse effects on respiratory or neurological functioning noted in safety pharmacology study in rats at doses up to 5 mg/kg. However, CNS effects were seen in toxicology or cardiovascular studies in mice (≥ 30 mg/kg; 44 times the exposure (C_{max}) at the MRHD), dog (≥ 15 mg/kg; 29-times the exposure at the MRHD) and/or NHP (100 mg/kg; 26-times the exposure at the MRHD). CNS effects observed in the various animal species included dilated pupils, disorientation, ataxia, ptosis, altered locomotor activity, head shaking, muscle twitches, tremors, and/or aggression, some of which may share similarities to clinical CNS effects associated with adrenal crisis seen in the pivotal Phase III clinical trial with LCI699. No significant CNS-related toxicities were observed in the juvenile rat study. In the rat whole body tissue distribution study, LCI699 concentrations were approximately 65% higher in the brain than blood plasma levels at C_{max}, indicating that clinical CNS exposures may be higher than plasma levels. Although the molecular mechanisms for LCI699-mediated CNS effects are unknown, they were associated with sufficient margins of safety at the NOAEL.

Multiple studies were performed to evaluate cardiovascular safety. Inhibition of the hERG current (IC_{50} of 54 µM), prolongation of the APD_{60} indicating a torsadogenic potential at 10 µM, and a concentration-related prolongation of the QT interval and T_{p-e} (but not QRS) durations were observed in various *in vitro* studies (the C_{max} at the MRHD equates to approximately 1.3 µM). In dogs, increased QRS, QT, and QTc intervals were evident at dosages ≥ 50 mg/kg administered intravenously (approximately 11-fold the mean of both sexes C_{max} at the MRHD), but not at dosages up to 7.5 mg/kg administered orally (approximately 14-fold the C_{max} at the MRHD). In NHPs, prolonged QTc intervals were seen at doses ≥ 30 mg/kg (≥15-fold the C_{max} at the MRHD); Torsades de pointes and non-sustained ventricular tachycardia were observed in a single male at 100 mg/kg (approximately 100-fold the male C_{max} at the MRHD). Effects on the cardiac system were also evident in clinical trials that included a prolongation of the QTc interval at a dose of 150 mg and a correlation between osilodrostat concentration and QTcF prolongation in a thorough QT study.

Pharmacokinetic/toxicokinetic studies demonstrated that exposure to LCI699 increases with dose in a proportional to greater than proportional manner with no apparent gender effect or accumulation upon repeated dosing. The t_{max} is reached by 1 hour in plasma and most

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tissues. Plasma $t_{1/2}$ in the rat and dog was approximately 2 hours, slightly shorter than that recorded for humans (approximately 4 hours). In juvenile rats, exposures were lower than those seen at the same dose administered to adult rats at the end of 4-weeks of treatment. LCI699 is rapidly and widely distributed following oral administration. Tissue distribution studies revealed high levels (and high tissue to plasma ratios) in the uveal tract, kidney, skin, eye, glandular stomach, liver, pancreas, bile, spleen, pituitary, and adrenal gland of the pigmented and/or albino rat. The levels in the uveal tract and skin in pigmented rats were higher than those recorded in albino rats indicating an affinity for melanin. LCI699 concentrations were 70% higher in the brain than blood plasma levels at C_{max}, indicating significant penetrance into the CNS at exposures higher than blood plasma levels. Protein binding was comparable between species, ranging from 26% to 36%.

Metabolic profiles were determined in the mouse, rat, dog, and/or human both *in vitro* (hepatocytes) and *in vivo*. The primary pathways in these species were predominantly oxidation and glucuronidation. All metabolites formed in humans were formed in one or both of the nonclinical species, with the most abundant human metabolites being M34.5 (up to 67%), M24.9 (up to 20%), and M16.5 (up to 11%). The pharmacologic activity of these three human metabolites was assessed. Metabolite M24.9 is an inhibitor of both CYP11B1 (IC50 = 40 nM) and CYP11B2 (IC50 = 7 nM) with potency 12-fold and 4-fold, respectively, lower than LCI699. M34.5 and M16.5 were sufficiently qualified in nonclinical evaluations with sufficient margins of safety and are not associated with any safety concerns. Urinary excretion is the primary route of elimination in nonclinical species and humans, accounting for 80% - 90% of radioactivity. Most of the radioactivity is in the form of metabolites as <10% of radioactivity was parent compound in the various species.

The potential for osilodrostat and the primary human metabolite M34.5 to inhibit or induce various transporters/enzymes was evaluated. For LCI699, the IC_{50} values for OCT2 (8.6 µM) and MATE1 (5.6 µM) are <10-fold the LCI699 C_{max} (306 ng/mL, 1.3 µM) at the MRHD. With the M34.5 metabolite, an IC_{50} value of 3.23 µM was calculated for OAT3 inhibition indicating a slight potential for interaction based on an estimated C_{max} of 148 ng/mL (0.6 µM) for M34.5 (based on a reviewer extrapolation from a C_{max} of 222 ng/mL (0.9 µM) following a single 50 mg dose (DMPK R1800128)). LCI699 was found to inhibit CYPs 1A2, 2C19, 2D6, and 2E1 ($IC_{50's}$ of 0.8, 4.6, 2, and 0.5 µM, respectively) within 5-fold of the C_{max} exposure indicating a potential for osilodrostat to interfere with drugs metabolized by these CYP enzymes. Weak inhibition was seen for CYPs 3A4/5, 2B6, and 2C9 ($IC_{50's}$ of 6.5, 20, and 40 µM, respectively). No inhibition of the CYP enzymes was seen with the M34.5 metabolite. LCI699 and its' primary metabolite inhibited UCG1A1 (IC_{50} of 30 and 0.7 µM, respectively) indicating a potential interaction with UGT1A1 substrates by the metabolite.

LCI699 was shown to induce CYPs 1A2 and 2B6 while the metabolite M34.5 induced CYPs 2B6 and 3A4. However, induction was only seen at multiples in excess of 100-fold the predicted concentrations of parent and M34.5.

Plasma levels of the enantiomer (impurity) LCI698 were determined in the pivotal toxicology studies. The levels of LCI698 measured in rat and dog plasma were generally $\leq$ 0.1% at the NOAEL doses and remained ≤1% of LCI699 concentrations at the highest doses evaluated

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in nonclinical studies. Furthermore, LCI698 and LCI699 have different pharmacological profiles and do not undergo chiral inversion *in vivo*. Therefore, LCI698 is unlikely to enhance affects associated with LCI699 administration and it is not expected to present a significant safety concern.

The primary species used to evaluate the toxicity of LCI699 were the rat and dog, including a study in juvenile rats. The mouse and rat were used to evaluate the carcinogenic potential while effects on embryofetal development were evaluated in the rat and rabbit.

Across nonclinical species and studies, the number and magnitude of effects tended to increase with dosage and duration of treatment. The majority of effects were reversible or partially reversible after a recovery period. As study duration increased, the dosages at which various effects were noted also tended to be lower. Drug-related mortality (either deaths or elective euthanasia based on clinical signs) was only evident in rodents and occurred at a dosage of 200 mg/kg (greater than 216-fold exposure at the MRHD). The primary target organs of toxicity were the adrenal glands, female reproductive tract, and liver as summarized below.

Adrenal Glands

Drug-related effects on the adrenal glands were consistently observed across studies in rats and dogs. Effects on the adrenal glands are not surprising given that the pharmacologic targets (11β-hydroxylase produced in the zona fasciculata, aldosterone synthase produced in zona glomerulosa) are within the adrenal gland. In rats, increased adrenal weights and hypertrophy were evident in females at ≥ 20 mg/kg (43-fold the exposure at the MRHD). In shorter term studies previously reviewed, hypertrophy was seen in males at ≥ 50 mg/kg (77-fold the exposure at the MRHD) and cytoplasmic vacuolation was observed in the zona fasciculata of both sexes at dosages ≥ 50 mg/kg (90-fold the exposure at the MRHD. Increased weights along with cortical hypertrophy and vacuolations were also seen in the juvenile female rats, but not males, at 50 mg/kg (81-fold the exposure at the MRHD). In dogs, increased adrenal gland weights, atrophy, and/or vacuolation of zona glomerulosa of adrenal cortex (neither microscopic effect considered adverse) occurred at dosages ≥ 0.5 mg/kg (0.2-fold the exposure at the MRHD). Across species and studies, the observed effects were not associated with alterations in clinical pathology endpoints and were reversible.

The observed effects are likely related to the pharmacologic activity of osilodrostat and may be reflective of adaptive responses, such as increased ACTH stimulation. The presence of vacuoles in the adrenal cortex is likely due to accumulation of precursor lipids secondary to pharmacological inhibition of cortisol synthase. Although there were no signs of adrenal dysfunction in non-clinical toxicology studies, HPA-axis hormonal effects, including clinically adverse events of hypocortisolism and/or hypoaldosteronism, were observed at multiple daily doses of ≥1 mg in human subjects with normal adrenal function. Nevertheless, drug-related decreases in adrenal gland function and adrenal insufficiency (Addison's disease) would be less of a concern in Cushing's disease patients. Although the effects on the adrenals occurred at exposures that were above the clinical exposure, signs of adrenal dysfunction will be monitored for clinically.

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Female Reproductive Track
In rats, dosages ≥ 5 mg/kg (from an earlier study; 7-fold the exposure at the MRHD) were associated with effects on the ovary (increased weights, follicular degeneration, prominent corpora lutea) while higher dosages affected the uterus (increased/decreased weight, atrophy) and vagina (mucification); these effects were also generally reversible. In the juvenile female rat, there were reversible effects on the ovary (increased weights, follicular degeneration, large corpora lutea) and uterus (decreased weights, atrophy) at 50 mg/kg (79-fold the exposure at the MRHD). In dogs, the only effects seen were reversible alterations in ovarian and uterine weights occurring at dosages ≥ 1 mg/kg (0.3-fold the exposure at the MRHD), but the effects were not consistent in directionality across studies and there were no corresponding microscopic observations.

LCI699 and its' enantiomer LCI698 are inhibitors of aromatase (located in estrogen-producing cells in the adrenal glands, placenta, ovaries, testes, and brain) which is involved in the conversion of testosterone to estradiol. Based on the IC_{50} values for aromatase, off-target inhibition would be expected at the doses administered in the reproductive and developmental toxicology studies. This off-target pharmacologic activity may be the basis for the observed effects on female reproductive organs, and possibly the effects on some reproductive parameters (see below).

Liver
Effects on the liver (increased weights, hypertrophy, and vacuolation), were observed in rodent but not dogs. One or more of these effects were seen in rats at dosages ≥ 5 mg/kg (from an earlier study; 7-fold the exposure at the MRHD) and in juvenile rats at 50 mg/kg (81-fold the exposure at the MRHD). These effects were evident at lower dosages as dosing duration increased in adult animals. Correlative alterations in liver enzyme levels were only seen in mice (studies reviewed under IND) at dosages ≥ 100 mg/kg (172-fold the exposure at the MRHD) where there were 2- to 4-fold increases in ALT levels (no effects on bilirubin levels). These effects may be adaptive responses to the induction of metabolic enzymes by LCI699 based on patterns of gene expression. The possibility also exists that these effects are either rodent-specific (since they did not occur in dogs) or are related to the presence of metabolite M34.5 which is found in higher levels than LCI699 in rodents (up to 10-fold higher) and humans (up to 2.4-fold higher), but not dogs. Thus, potential liver toxicities mediated by the metabolite M34.5 cannot be ruled out in humans. For this reason, rodents are the more relevant species for determination of human safety margins for metabolite M34.5. Levels of M34.5 were considered to be sufficiently present in rodent toxicology studies and reproductive & development studies for toxicological analysis. Neoplastic and non-neoplastic lesions (see discussion below) in the liver were also observed in the rodent carcinogenicity studies.

Other systems/organs
The CNS was also a target as described previously. Effects on other organs were observed, but the effects were not consistent across studies or species. These included reductions in prostate weights of adult rats at 20 mg/kg (4-fold the exposure at the

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MRHD) and in juvenile rats at 50 mg/kg (5-fold the exposure at the MRHD), and altered thymus weights in mice (reviewed previously; increased in males at 100 mg/kg (165-fold the exposure at the MRHD) and decreased in females at ≥ 30 mg/kg (43-fold the exposure at the MRHD)), dosages that were at or above the NOAEL in the studies they were observed in. There were no microscopic observations in these systems/organs.

LCI699 was evaluated in Ames, chromosome aberration (HPBL cells), and micronucleus (TK cells) assays for genotoxocity. Although the Ames and micronucleus assays were negative for the induction of genetic toxicity, there was an increase in structural abnormalities in the chromosome aberration assay. The increases were seen both the presence and absence of S9 activation at concentrations ≥ 2000 µg/mL (mitotic inhibition < 45%). The concentrations where an effect was observed are greater than 6000-fold the C_{max} at the MRHD. The relevance of this finding was further evaluated *in vivo* with a Comet assay and in a rat micronucleus test. Both of these assays were negative for genotoxicity at the highest dosages administered. These results, in association with the negative *in vitro* assays, support the interpretation that osilodrostat does not pose a genotoxic risk to humans.

Carcinogenicity studies revealed an increased risk of liver and thyroid tumors. Dosages of 3, 10, and 30 mg/kg were administered to both mice and rats for 2 years. Exposure multiple projections are based on data generated in the 13-week mouse and 26-week rat studies.

In male mice, dosages ≥ 10 mg/kg (6-fold the exposure at the MRHD) were associated with an increased incidence of hepatocellular adenomas, carcinomas, and/or combined adenomas and carcinomas. There also was a corresponding increase in hepatocellular hypertrophy at ≥ 3 mg/kg in males (≥ 1.8-fold the exposure at the MRHD) and in females at 30 mg/kg (31-fold the exposure at the MRHD). Bile duct hyperplasia also occurred at an increased incidence at ≥ 10 mg/kg (6-fold the exposure at the MRHD) in males and at 30 mg/kg in females. There was no increase in the incidence of tumors in females. The NOAEL for tumorigenesis was considered 3 mg/kg in males (1.8-fold the exposure at the MRHD) and 30 mg/kg in females (31-fold the exposure at the MRHD).

In rats, a numerical increase in the incidence of hepatocellular adenomas was evident in males at ≥ 10 mg/kg (18-fold the exposure at the MRHD) while in females there were increased incidences of hepatocellular adenomas and carcinomas at 30 mg/k (65-fold the exposure at the MRHD) alone and when the tumor types were combined. Corresponding increases in proliferative changes in the liver (mixed cell and eosinophilic focus of cellular alteration) was seen at all dosages (≥ 3 mg/kg; 6-fold the exposure at the MRHD) in males and at ≥ 10 mg/kg (22-fold the exposure at the MRHD) in females. There was also an increased incidence of non-neoplastic observations in the livers that included increased incidence of hepatocellular hypertrophy (both sexes), vacuolation (both sexes), and cystic degeneration (males only). Also observed in males at 30 mg/kg (55-fold the exposure at the MRHD) was an increased incidence of follicular cell adenomas in the thyroid gland which resulted in an increased incidence of combined thyroid gland adenomas and carcinomas. Increases in the incidences of follicular cysts and/or hypertrophy were also evident at dosages ≥ 3 mg/kg in males. The NOAEL for tumor formation in male rats was 3 mg/kg (6-

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fold the exposure at the MRHD) and in female rats was 10 mg/kg (22-fold the exposure at the MRHD).

The increase in thyroid tumors seen in male rats at ≥ 30 mg/kg may also be related to the hepatocellular events. The sponsor has proposed that the thyroid follicular tumorigenesis occurred due to sustained hepatic enzyme induction leading to increased clearance of thyroid hormones. As a result, a compensatory increase in TSH secretion occurs that can lead to development of thyroid follicular hypertrophy, hyperplasia, and progression to neoplasia (Ennulat et al 2010). This hypothesis was supported by the finding that Ugt 2b1, which is responsible for accelerated biliary excretion of thyroxine (T_4) and tri-iodothyronine (T_3), was induced after exposure to LCI699. However, measurement of T_3, T_4, and TSH levels were not performed. Regardless, due to species specific differences in sensitivity to fluctuations in thyroid hormone homeostasis and thyroid hormone metabolism (Alison et al 1994; Capen, 1997), these events are unlikely to occur in humans.

The liver tumors observed in mice and rats were consistent with a CAR-related mechanism that does not translate to a clinical concern due to species differences (Elcombe et al 2014; La Vecchia and Negri 2014). In rats and mice, activation of CAR can alter expression of genes and promote a proliferative and anti-apoptotic environment. In the liver, a possible outcome of altered gene expression is increased hepatocellular proliferation and hepatocyte hypertrophy that can eventually lead to hepatocellular adenomas and carcinomas. In the thyroid, increased expression of metabolizing enzymes can result in increased elimination of thyroid hormones, increased secretion of thyroid stimulating hormone, and increased thyroid follicular cell proliferation that can promote a neoplastic transformation.

In an attempt to confirm a CAR-based mechanism, gene expression profiling was performed by isolating mRNA from the liver of rats that had been treated daily with LCI699 for 4 weeks. Treatment with LCI699 was found to induce genes associated with a CAR-based mechanism (e.g., Cyp2b1, Cyp2b2, Ugt2b1, and Ces2c). Slight induction of genes associated with an AhR-based mechanism (e.g., Cyp1a1, CYP1a2 but not Aldh3a1) which is relevant to humans also occurred, but only at the highest dose administered (50 mg/kg, >77-fold the exposure at the MRHD). The effects on the genes associated with a CAR-based mechanism were comparable in magnitude to those induced by phenobarbital, a prototypical CAR activator. The sponsor also looked at cell proliferation in the liver. With CAR activators, cell proliferation is seen after initial exposure but not after multiple weeks of treatment. No evidence of proliferation was seen after 4-weeks of treatment with LCI699, but it was not possible to determine if the typical CAR profile occurred since proliferation was not evaluated at an early time point. Gene expression signatures for mechanisms other than CAR were also evaluated. LCI699 did not induce PXR, PPARα, or ER signature genes. However, the gene expression comparisons were made to published data and not based on concurrently generated data using positive controls such as known AhR-activating carcinogens (e.g., TCDD) or AhR-activating non-carcinogens (e.g., omeprazole) which would have provided strong and concurrent data for interpreting the strength of the observed AhR activation.

In a subsequent evaluation, the sponsor compared the gene expression data to much larger gene expression datasets generated for CAR and AhR gene activation. These analyses

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revealed that LCI699 has a profile similar to that of phenobarbital and that even at the highest dose used in the 4-week study, the gene expression profile was comparable to that of omeprazole but not to that of TCDD. These data add further support to the interpretation that the likely mechanism of tumorigenesis seen in the rodent carcinogenicity studies is likely CAR-mediated and not a clinical concern.

The potential for osilodrostat to affect fertility, embryo-fetal development, and postnatal growth and maturation were evaluated in a series of reproductive and developmental toxicity studies. The rat was the primary species evaluated, with the rabbit used as the second species for assessing embryo-fetal risk.

Adverse drug-related effects were evident on reproductive endpoints at the higher dosages of LCI699 administered to female animals. In the fertility study, effects on maternal animals included prolonged estrous cycles, increased time to mating, decreased mating and fertility indices, decreased corpora lutea and implantation sites, increased pre- and post-implantation loss/resorptions, and decreased viable fetuses at 50 mg/kg (118-fold the exposure at the MRHD). The NOAEL was considered 5 mg/kg (8-fold the exposure at the MRHD). Similar effects were not seen in untreated females mated to drug-treated males, indicating the effects are female mediated.

Embryofetal development is adversely affected by LCI699. In the rat embryofetal development study, increases in resorptions (early & late) with associated clinical signs in maternal animals (e.g., red vaginal discharge) were observed at dosages $\geq$ 5 mg/kg that were considered adverse at 50 mg/kg (118-fold the exposure at the MRHD). Effects on fetal viability, weight, and morphological development (increased malformations and visceral/skeletal variations) also occurred at 50 mg/kg. Maternal toxicity, in the form of significant reductions in weight gain during the post-dosing period in late gestation was inter-related with the observed embryofetal toxicity. The NOAEL for the study was considered 5 mg/kg (8-fold the exposure at the MRHD). Similarly, effects in the maternal rabbit included red staining in the drop pan at dosages $\geq$ 10 mg/kg (7-fold the exposure at the MRHD) that was associated with effects on body weight and embryo-fetal toxicity (increased resorptions, decreased viable fetuses, total litter loss). Based on these data, the maternal and developmental NOAEL was 3 mg/kg (0.5-fold the exposure at the MRHD) in the rabbit.

In the pre- and postnatal toxicity study in rats, effects on maternal weight gain and food consumption, prolonged gestation/dystocia (with associated clinical signs), and increased numbers of stillborn pups led to the early termination of the group administered 20 mg/kg (37-fold the exposure at the MRHD). There were no adverse effects on maternal animals or on growth and development (including reproductive capacity) of the offspring at dosages $\leq$ the MD (5 mg/kg) which is considered the NOAEL (8-fold the exposure at the MRHD).

The observations in the reproductive studies, in conjunction with the effects observed on female reproductive organs in the repeat dose studies, suggest the female reproductive system is sensitive to perturbations induced by LCI699. Although the mechanism(s) by which these effects were induced is unknown, they could be related to the inhibition of CY11B1/cortisol, CYP11B2/aldosterone, or aromatase either alone or in combination. The

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CYP11B1 null mouse is generally infertile (Mullins, L et al 2009) so it's not surprising that reduced fertility would occur in rats with reduced corticosterone/cortisol levels. The antifungal ketoconazole, which acts on 11β-hydroxylase as one of its pharmacologic actions, was also found to induce post-implantation loss and effects on fetal development (Amaral, V and Nunes, G 2009). The aldosterone antagonist spironolactone induced altered estrous cycles, reduced fertility, decreased number of implants, increased resorptions (rabbits), and delayed puberty in rodents (Nagi S and Virgo, B 1982). These effects were analogous to those seen in reproductive toxicity and juvenile animal studies conducted with LCI699. Alternatively, these effects may be related to the inhibition of aromatase as similar effects on reproduction and embryofetal development have been reported with aromatase inhibitors such as letrozole and fadrozole (Tiboni GM, et al 2008; Tamada H. et al 2003). The enantiomer LCI698 is also an aromatase inhibitor. In a study in which the enantiomer at various concentrations was administered with LCI699, increased post-implantation loss, decreased fetal viability, and altered fetal morphological development occurred, but effects on embryofetal development were not seen in the group administered the enantiomer alone, suggesting the effects are related to LCI699 treatment. Although the specific mechanism by which these compounds exert their effects on fertility/embryofetal development are not known, the hormonal environment is altered in all cases which may be responsible for the observed effects. These potential effects on reproductive and embryofetal development can be addressed by labelling.

Potential impurities and degradation products were qualified in various nonclinical studies, including studies in mice in which formulations containing increased levels of specific impurities were administered. Based on evaluations of drug lots used across the drug development program, potential and actual impurities identified in the drug product have been adequately qualified in nonclinical toxicology studies and/or are controlled to an acceptable specification limit. The overall weight of evidence indicates that the potential impurities in osilodrostat are unlikely to present a potential risk for genotoxicity, mutagenicity, carcinogenicity, or organ toxicity.

LCI699 did not induce reactions in a skin irritation study. However, it was determined to be a potent contact sensitizer (Category 1A) based on a local lymph node assay.

In summary, the nonclinical data support the marketing authorization for LCI699. The Sponsor has conducted a full battery of nonclinical safety assessments. The nonclinical toxicology studies revealed that osilodrostat has the potential to induce adverse effects on the adrenal glands, liver, female reproductive tissues and on female reproductive performance and embryofetal development that are likely related to the pharmacologic activity of LCI699 or were secondary pharmacological effects on the HPA-axis. Carcinogenicity studies revealed drug-related tumors in the liver and thyroid gland of rodents that are most likely CAR-mediated and not relevant to humans. The neoplastic and reproductive toxicity findings will be addressed in the label. The notable observations from the pivotal repeat-dose, carcinogenicity, and reproductive and developmental toxicity studies with corresponding projected safety margins are summarized in the reviewer table below:

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Table 77: Summary of Nonclinical Safety Margins for LCI699-Related Observations

Study Duration / Study #	Dosages	NOAEL, Exposure, and Safety Margin	Notable Observations
Rat			
26-week Study 0870522	0, 0.2, 2, 20 mg/kg	♂ 2 mg/kg 6170 ng·hr/mL 4x ♀ 2 mg/kg 5150 ng·hr/mL 3x	20 mg/kg (♂ 37x; ♀ 43x) : Adverse: ↑ liver, adrenal, ovarian weights, ↓ prostate weights, hepatocellular vacuolation and hypertrophy, adrenal hypertrophy in ♀, Non-adverse: ↑ body weight/food consumption in ♀ ≤ 2 mg/kg: No notable observations
Dog			
39-week Study 0870521	0, 0.1, 1, 10 mg/kg	♂ 10 mg/kg 22200 ng·hr/mL 13x ♀ 10 mg/kg 20300 ng·hr/mL 12x	10 mg/kg: Non-adverse: ↑ pituitary cysts in ♂, ↓ food consumption, adrenal vacuolation and atrophy in zona glomerulosa in ♀ 1 mg/kg: adrenal vacuolation and atrophy in zona glomerulosa in ♂, ↑ ovarian and uterine weights ≥ 0.1 mg/kg: ↑ pituitary cysts in ♀
Mouse Carcinogenicity			
104-week Study 1270062	0, 3, 10, 30 mg/kg	NOAEL for tumors ♂ 3 mg/kg 3122 ng· hr/mL 1.8x ♀ >30 mg/kg >52000 ng·hr/mL 31x Exposure multiple estimates based on data from 13-week study	30 mg/kg: Tumor: ↑ hepatocellular carcinomas in ♂ (37x) Non-tumor: ↑ hypersensitivity/hyper-reactivity, ↑ shallow breathing/respiratory rate in ♀, ↑ hepatocellular hypertrophy and bile duct hyperplasia in ♀ ≥ 10 mg/kg: Tumor: ↑ Hepatocellular adenoma and/or adenoma + carcinoma in ♂ (6x) Non-Tumor: ↑ shallow breathing/respiratory rate in ♂, ↑ convulsions, ↑ bile duct hyperplasia in ♂ ≥ 3 mg/kg: ↑ tremors in ♂, ↑ hepatocellular hypertrophy in ♂

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Rat Carcinogenicity			
104-week Study 1270061	0, 3, 10, 30 mg/kg	NOAEL for tumors ♂ 3 mg/kg 9255 ng· hr/mL 6x (1.5x exposure at 2 mg/kg from 26 wk study) ♀ 10 mg/kg 36350 ng·hr/mL 22x Exposure multiple estimates based on data from 26-week study	30 mg/kg: Tumor: ↑ thyroid follicular cell adenoma in ♂ (55x), ↑ hepatocellular adenomas, carcinomas, and adenoma + carcinoma in ♀ (65x) Non-tumor: ↑ thyroid cysts in ♂ ≥ 10 mg/kg: Tumor: ↑ hepatocellular adenomas in ♂ (18x), ↑ thyroid follicular cell adenoma + carcinoma in ♂ Non-Tumor: ↓ body weight in ♂, ↑ hepatocellular vacuolation in ♀ ≥ 3 mg/kg: ↑ foci of cellular alteration of mixed and eosinophilic cells, hepatocellular vacuolation, hypertrophy, and cystic degeneration in liver of ♂; ↑ foci of cellular alteration of mixed cells and hepatocellular hypertrophy in liver of ♂, and ↑ thyroid hyperplasia in ♀
Reproductive and Developmental Toxicity			
Fertility and early embryonic development Study 077085	0, 0.5, 5, 50 mg/kg	♂ 50 mg/kg 130000 ng·hr/mL 77x ♀ 5 mg/kg 13100 ng·hr/mL 8x Exposure multiple estimates based on data from 4-week study	50 mg/kg: Adverse: In ♀ (43x), prolonged estrous cycles, ↓ weight gain during gestation, ↓ fertility, ↓ corpora lutea, ↓ implantation sites, ↓ fetal viability, ↑ resorptions and ↑ post-implantation loss Non-adverse: transient ↓ in body weight gain and ↓ epididymal weight in ♂ ≤ 5 mg/kg: No notable observations
Embryofetal development in rats Study 077084	0, 0.5, 5, 50 mg/kg	5 mg/kg 12800 ng·hr/mL 8x	50 mg/kg (70x): Adverse: Clinical signs associated with resorptions, ↓ body weight and food consumption, ↑ resorptions and ↑ post-implantation loss with associated ↓ in viable fetuses, ↓ fetal weights, ↑ fetal malformations and variations 5 mg/kg: transient ↓ body weight 0.5 mg/kg: No effects
Embryofetal development in rabbits Study 0670281	0, 3, 10, 30 mg/kg	3 mg/kg 850 ng·hr/mL 0.5x	30 mg/kg (46x): Adverse: ↓ body weight/weight gain 10 mg/kg (7x): Adverse: Clinical signs associated with fetal resorptions, ↓ food consumption, ↑ does with total litter loss, ↑ resorptions/post-implantation loss, ↓ viable fetuses

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			Non-adverse: ↑ fetal skeletal variations 3 mg/kg: No effects
Pre/postnatal in rats Study 1470797	0, 1, 5, 20	5 mg/kg 13100 ng·hr/mL 8x Exposure multiple based on data from 26-week study	20 mg/kg (43x): Adverse: dystocia/delayed parturition, poor condition (prostration, coldness to touch, ↓ activity, discharge from vulva, etc) 5 mg/kg: Non-adverse: ↓ postweaning body weight and food consumption in ♂

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TODD M BOURCIER
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I concur