CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

207620Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 207620
Submission date: 10/29/14 (date of submission of nonclinical module)
Drug: sacubitril/valsartan
Applicant: Novartis

Indication: reducing the risk of cardiovascular mortality and (b) (4) hospitalization in patients with chronic heart failure

Reviewing Division: Division of Cardiovascular and Renal Products, Division of Hematology Products

Discussion:
The primary reviewer and supervisor found the nonclinical information adequate to support the approval of sacubitril/valsartan for the indication listed above. Sacubitril is a new molecular entity whereas valsartan is in several approved drug products.

The carcinogenicity of valsartan has been previously assessed. The carcinogenicity of sacubitril was assessed in 2-year rat and mouse studies. These studies were found to be acceptable by the executive carcinogenicity assessment committee and the committee concluded that there were no drug-related neoplasms in either species.

The applicant provided embryofetal studies in rats and rabbits with sacubitril and with the combination of sacubitril/valsartan. These studies showed some adverse effects such as embryo-fetal lethality and hydrocephaly that appear to be attributable to angiotensin receptor antagonism. Interaction with the renin-angiotensin system in general is considered to have the potential to induce adverse fetal effects.

A pre/postnatal study in rats with sacubitril showed some slight body weight effects in pups. Adverse effects in pre/postnatal studies have also been observed with valsartan.

An acceptable established pharmacologic class for sacubitril could be “neprilysin inhibitor”. Valsartan is an angiotensin II receptor blocker.

Conclusions: I agree that this NDA can be approved from a pharm/tox perspective for the indication listed above. I have provided comments on labeling separately.

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/s/

PAUL C BROWN
07/02/2015

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:	207620
Supporting document/s:	SDN 001
Applicant's letter date:	10/29/14
CDER stamp date:	10/29/14
Product:	LCZ696 (sacubitril/valsartan) tablets
Indication:	Heart failure
Applicant:	Novartis
Review Division:	Div. Cardio-Renal Products
Reviewer:	William T. Link, Ph.D.
Supervisor/Team Leader:	Albert De Felice, Ph. D.
Division Director:	Norman Stockbridge, M.D., Ph.D.
Project Manager:	Alexis Childers

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

LCZ696 (sacubitril/valsartan) is a combination angiotensin receptor/neprilysin (neutral endopeptidase 24.11; NEP) inhibitor (ARNI) intended as an oral treatment for heart failure (b) (4) The target dose is 200 mg twice daily (BID).

LCZ696 is a salt complex comprising sacubitril (AHU377, a new-molecular entity) and valsartan, sodium cations, and water molecules in the molar ratio of 1:1:3:2.5 (ratio of 6:6:18:15 in the asymmetric unit cell of the solid-state crystal). Each 200-mg dose of LCZ696 contains approximately 97 mg of AHU377 and 103 mg of valsartan. Dosage forms of 50 mg LCZ696 and 100 mg LCZ696 are also available for initiation of treatment and/or down-titration.

Following oral administration, LCZ696 dissociates into valsartan and the pro-drug AHU377, which is further metabolized to the NEP inhibitor LBQ657. Exposures to both LBQ657 and valsartan are dose-proportional and predictable. LCZ696 doses of 50, 100, and 200 mg deliver valsartan exposures which are similar to those delivered from the Diovan® dose strengths of 40, 80, and 160 mg, respectively.

The pharmacodynamic activity and selectivity of LCZ696 was characterized in a number of *in vitro* and *in vivo* pharmacological studies conducted with LCZ696, AHU377, LBQ657, or valsartan. The animal studies were conducted in cardiorenal disease models and demonstrate a beneficial effect of LCZ696 on cardiac, renal, and vascular function and organ protection.

Pharmacology

The pro-drug AHU377 is a poor inhibitor of human recombinant NEP enzyme activity *in vitro* (IC_{50} = 16,700 ± 2,300 nM), whereas its metabolite LBQ657 is a potent inhibitor (IC_{50} = 2.3 ± 0.4 nM). Both AHU377 and LBQ657 are poor inhibitors of human recombinant NEP-2 enzyme activity. Valsartan does not inhibit NEP or NEP-2 activity at concentrations up to 100,000 nM.

Valsartan blocks angiotensin II binding to AT_1 receptors in rat aortic smooth muscle cell membranes with a K_i of 2.4 nM and is more than 30,000-fold selective relative to the AT_2 receptor subtype. Neither AHU377 nor LBQ657 inhibit binding to the human AT_1 receptor at concentrations of 30 µM.

AHU377, LBQ657, and valsartan show no meaningful inhibition (>2000-fold selective) of a set of ten proteases that are related to NEP or enzymes associated with NP/RAAS pathways. AHU377 and LBQ657 were assessed for their off-target activities on a panel of GPCRs, transporters, ion channels, nuclear receptors, and enzymes. No significant inhibitory effects were found for AHU377 at 30 µM or for LBQ657 at ≥10 µM for any of the targets tested.

LCZ696 lowered blood pressure in rat models of hypertension with divergent etiologies. A single administration of LCZ696 (60 mg/kg) reduced MAP by 73 mmHg in double-transgenic rats overexpressing human renin and angiotensinogen (dTGR), a high-renin model of hypertension. LCZ696 (68 mg/kg/day) also reduced arterial pressure for the duration of the two-week treatment period in a normal-renin, salt-insensitive model of hypertension (SHR). Likewise, LCZ696 (68 mg/kg/day) blunted or prevented the gradual rise in arterial pressure in two respective studies in a low-renin volume-dependent model of hypertension (DSS rat on high salt diet).

In cell culture, concomitant exposure to LBQ657 and valsartan reduced angiotensin II-mediated rat cardiomyocyte hypertrophy and rat cardiac fibroblast collagen synthesis to a greater extent than valsartan alone. In a rat post-myocardial infarction model, LCZ696 reduced the hypertrophy that occurs in response to injury. In stroke-prone spontaneously hypertensive rats (SHRSP), combined AHU377 and valsartan was more effective than valsartan alone in improving cardiac and vascular fibrosis and vascular remodeling.

Safety pharmacology

At the maximum feasible concentrations (3 mM for LCZ696; 1 mM for AHU377), no meaningful hERG inhibition was observed. Therefore, the risk for QT prolongation at anticipated exposures associated with the proposed clinical dose of 200 mg BID is perceived to be low.

AHU377 up to 250 mg/kg had no effects on blood pressure, heart rate or ECG parameters in normal, conscious dogs. Slight reductions in blood pressure (systolic, diastolic and mean arterial blood pressures) were observed in cynomolgus monkeys at a dose of 100 mg/kg LCZ696, consistent with the greater blood pressure lowering effect of the ARNI compared to single-acting therapy.

LCZ696 andAHU377 had no adverse effects on respiratory or CNS endpoints following single oral administration in rodents at doses which provided valsartan exposure multiples of ~1X, and LBQ657 exposure multiples in excess of 8X those associated with a 200 mg BID clinical dose.

Pharmacokinetics (ADME)

LCZ696 or AHU377 was well absorbed in all animal species (65-100%) after p.o. dose. In human, absorption was estimated to be at least 61%. The absorption was relatively rapid in rate and onset in all species (mouse, rat, dog, rabbit, monkey, and human) with Tmax ranging from 0.25 - 2 h.

Following an oral dose, the terminal half-life for AHU377 was short (1.3-3.2 h) in all species. The terminal half-life of LBQ657 or valsartan was relatively short in mice, rats and dogs (1.1-3.1 h), but was long in monkeys (~6 h) and humans (12-21 h). Bioavailability of LBQ657 was moderate to high (41-100%) in animals and was estimated to be greater than 50% in human. Dose-normalized plasma or blood

exposure values (AUC) for LCZ696-associated components (AHU377 or LBQ657) in monkey were higher than other species (rank order: monkey >dog >rat ≈mouse). The lower dose-normalized exposure seen in mouse, rat, and dog was associated with its high clearance (as high as hepatic blood flow).

Plasma exposure (AHU377, LBQ657 and valsartan) is generally proportional to the dose in all tested animal species (mice, rats, rabbits, and monkeys)

Uptake of AHU377, LBQ657, and valsartan into blood cells was not significant and therefore drug concentrations in plasma were higher than in blood. Both AHU377 and LBQ657 were moderately to highly bound to plasma proteins with some species differences (90%, 80%, 93% and 97% in rat, dog, monkey, and human, respectively for LBQ657 and 91% and 97% in monkey and human, respectively, for AHU377). Similarly, valsartan was bound to serum proteins with some species differences (82-97%). In human, serum albumin was found to be the primary binding protein for AHU377, LBQ657 and valsartan.

In rats, drug-related radioactivity was widely and rapidly distributed to most tissues following a single i.v. or p.o. dose of radiolabelled AHU377. The highest tissue radioactivity (2-4 fold higher than blood) was found in kidney and liver 5 min after i.v. dosing. The lowest radioactivity levels were observed in brain, eye, seminal vesicles and spinal cord. Based on AHU377-derived radioactivity, brain and testis penetration is minimal (tissue: blood ratios were 0.02 and 0.05, respectively) and affinity to melanin-rich tissues (pigmented skin and uveal tract) is low.

Uptake of AHU377, LBQ657, and valsartan into blood cells was not significant and therefore drug concentrations in plasma were higher than in blood. Both AHU377 and LBQ657 were moderately to highly bound to plasma proteins with some species differences (90%, 80%, 93% and 97% in rat, dog, monkey, and human, respectively for LBQ657 and 91% and 97% in monkey and human, respectively, for AHU377). Similarly, valsartan was bound to serum proteins with some species differences (82-97%). In human, serum albumin was found to be the primary binding protein for AHU377, LBQ657 and valsartan.

In pregnant rats dosed with [^{14}C]LCZ696, the extent of the transfer of AHU377-derived radioactivity from maternal blood into the embryo-fetal compartment was moderate (fetus-to maternal blood ratio at fetus Tmax: 0.509 on day 12 and 0.246 on day 17). In pregnant rabbits dosed with LCZ696, the fetal exposure relative to maternal plasma was low (~0.06 - 0.21 for LBQ657 and ~0.01 for valsartan), indicating that LCZ696 related materials was poorly transferred into the fetus.

Following an oral dose (30 mg/kg) of [^{14}C]LCZ696 to lactating rats, transfer of LBQ657 into milk was observed. The overall milk:plasma concentration ratio of total radioactivity was 0.91 based on AUC0-∞ values.

AHU377 primarily underwent ethyl ester hydrolysis to form LBQ657 in all species. The rate of conversion from AHU377 to LBQ657 was high in mouse, rat and human, and the major compound detected in plasma/blood was LBQ657 (mouse: ~73% of AUC, rat: ~80% AUC, human: 93-98% of AUC comparing to AHU377). In contrast, the rate of conversion was moderate in dog and monkey where both AHU377 and LBQ657 were present in plasma/blood (AHU377:LBQ657 = ~34%:~46% of AUC in dogs, AHU377:LBQ657 = ~37%:~62% of AUC in monkeys).

AHU377 and LBQ657 undergo further hydroxylation, glucuronidation, sulfation, and glycine/taurine conjugation to generate several minor metabolites in plasma/blood of various species. LBQ657 was the major component recovered in excreta from all species studied, accounting for ~70-100% of the oral dose. Unchanged AHU377 recovered in excreta was minimal in all species studied, accounting for <1-14% of the oral dose. The metabolic profiling studies did not reveal any major unique human metabolite.

In mice, rats and dogs, AHU377-derived radioactivity was predominantly excreted in the feces (~74-98% of the dose). In monkeys and humans, however, AHU377-derived radioactivity had higher excretion into urine (~42-65% of the dose).

LCZ696 was eliminated primarily as LBQ657 and valsartan with minimal AHU377 (~3% of dose, except for ~13% in dog) and some minor metabolites in both animals and humans.

CYP Inhibition/Induction

AHU377 showed little or no inhibition (IC_{50} >100 μM) in *in vitro* assays using human CYP enzymes 1A2, 2C9, 2D6, 2E1, 3A4/5, and only a weak inhibition against CYP2C8 (IC_{50} ~15 μM) and 2C19 (IC_{50} ~20 μM).

LBQ657 demonstrated weak inhibition of CYP2C9 (IC_{50} ~40 μM) in *in vitro* assays. The potential inhibition of CYP2C9 was investigated in the clinical trial [CLCZ696B2112] in which no interaction occurred between LCZ696 and warfarin, a drug metabolized by CYP2C9. Since LBQ657 is mainly eliminated unchanged, its elimination is unlikely to be influenced by CYP enzyme inhibitors. Therefore, the potential for a DDI between LBQ657 and concomitantly administered drugs is considered to be low.

AHU377, LBQ657 and valsartan at concentrations up to 100 μM did not induce the expression and/or catalytic activities of CYP1A2, CYP2B6, CYP2C9, or CYP3A in primary human hepatocytes.

Transporters The *in vitro* inhibition studies suggested likely involvement of P-glycoprotein (P-gp) in AHU377 transport; however, this is not anticipated to have a significant effect on its oral absorption because the affinity of AHU377 for P-gp was low (>100 μM) and a high absorption (65-100%) was observed in animals. These data also imply that potential inhibition of AHU377 absorption by other P-gp inhibitors is expected to be low.

AHU377, up to a concentration of 50 µM, did not inhibit multi-drug resistance protein (MRP2) or P-gp transport activity and only very weakly inhibited breast cancer resistance protein (BCRP)-mediated transport activity. These data suggest that AHU377 is unlikely to significantly inhibit BCRP, P-gp or MRP2. Similarly, LBQ657 was found not to be an inhibitor of P-gp and BCRP activities, suggesting a low likelihood of pharmacokinetic interactions when co-administered with P-gp and BCRP substrates. The *in vitro* results suggest that AHU377 or LBQ657 are weak inhibitors of multidrug and toxin extrusion transporter 1 (MATE1) and 2-K (MATE2-K) but these effects are unlikely to be clinically relevant.

The *in vitro* results suggest that active transport by hepatic organic anion-transporting polypeptide 1B1 (OATP1B1) and 1B3 (OATP1B3) contributes to the systemic clearance of LBQ657 which may be altered when LBQ657 is co-administered with drugs that inhibit these transporters such as cyclosporine and a number of protease inhibitors.. LBQ657 was shown to be an *in vitro* inhibitor of OATP1B1 (IC_{50} = 126 ± 26 µM) but not of OATP1B3. Based on the Cmax of LBQ657 (16531 ng/mL = 43 µM) observed therapeutically, it is unlikely that LBQ657 will increase the systemic exposure of OATP1B1 substrates. AHU377, LBQ657, and valsartan did not inhibit the hepatic organic cation transporters 1 (OCT1) or renal OCT2 in the *in vitro* assays

Toxicokinetics

In the toxicokinetic studies, with respect to AHU377, LBQ657 and valsartan concentrations, the AUC values in all tested species (mouse, rat, rabbit, and monkey including pregnant rat and rabbit) were generally proportional to dose. No clear evidence of accumulation in rats and monkeys was observed following multiple daily dosing for 26 or 39 weeks. There was no evidence of gender differences. Specific exposure and exposure multiples are discussed with the toxicology studies.

1.2 Brief Discussion of Nonclinical Findings

LCZ696 has been tested in a range of species including mice, rats, rabbits, and cynomolgus monkeys. The cynomolgus monkey was chosen as the principal non-rodent species for toxicity assessment of LCZ696 based on homology of NEP and NEP substrates to human, but AHU377 has also been evaluated in dogs and marmosets. Toxicology studies in the marmoset with AHU377 provided a basis for comparison to previous valsartan marmoset studies and helped define the specific toxicologic effects of these two LCZ696 components.

In repeated dose general toxicity studies up to 39 weeks in duration, the NOAEL for LCZ696 was established at 50 mg/kg/day in mice, and 30 mg/kg/day in rats and cynomolgus monkeys.

Targets organs for LCZ696 and/or AHU377 were kidney, red blood cells, heart, and the GI tract. Each is discussed in greater detail in the Integrated Summary at the end of this review. None of these findings are considered an impediment to safe use of LCZ696, as

all represent adaptive responses or result from exaggerated pharmacodynamic responses to high doses.

Genotoxicity assays of LCZ696, AHU377 and LBQ657 were uniformly negative. These studies, which were compliant with (ICH S2 (R1)), included *in vivo* testing and *in vitro* assays in bacterial and mammalian systems, with and without S9 metabolic activation. The S9 fraction enables conversion of AHU377 to LBQ657 thereby ensuring *in vitro* characterization of this *in vivo* active metabolite.

There was no evidence of carcinogenicity when AHU377 was administered by oral gavage for a minimum of 104 weeks to mice at doses up to 1200 mg/kg/day (LBQ657 exposure multiples 14x (males) and 46X (females), and to rats at doses up to 400 mg/kg/day (LBQ657 exposure multiples 1.6x (males) and 3.6 (females), based on Cmax relative to 200 mg BID clinical dose).

The carcinogenicity studies were evaluated with Executive CAC concurrence as to conduct and interpretation.

LCZ696 had no effect on fertility in rats. In embryo-fetal development studies LCZ696 treatment was associated with increased embryo lethality in both rats and rabbits. LCZ696 was teratogenic in rabbits at doses of 10 mg/kg and higher based on a low but dose-dependent increase in hydrocephaly which occurred at maternally toxic doses. Both AHU377 and valsartan have been associated with fetal toxicity and embryo-fetal lethality in rabbits.

The NOAEL for embryo-fetal toxicity in studies with LCZ696 was 30 mg/kg/day in rats and 3 mg/kg in rabbits. As for any drug that acts directly on the RAAS, LCZ696 is contraindicated during pregnancy.

Results of pre and postnatal development studies with AHU377 and valsartan suggest that LCZ696 exposure during these periods may impair fetal development and survival.

Results of neonatal and juvenile toxicology studies with AHU377 and valsartan suggest that LCZ696 treatment may impact bone growth and mineralization and impair kidney development, however these effects were minor and show recovery.

A theoretical risk associated with NEP inhibition relates to effects on β-amyloid (Aβ) metabolism, and the potential accumulation of Aβ in the brain. Elevated levels of Aβ were present in CSF and plasma but not brain samples of cynomolgus monkeys treated with LCZ696 for two weeks at a clinically relevant dose of 50 mg/kg/day, suggesting that the newly synthesized Aβ was eliminated from brain tissue by other (non-NEP) clearance pathways. The relevance to human patients is not clear.

All of the above findings are discussed in the product labeling.

1.3 Recommendations

1.3.1 Approvability

The preclinical toxicology program was well conducted and thorough. The studies were well planned and employed sufficient numbers of dose groups and animals to allow proper interpretation and review.

This reviewer agrees with the sponsor's interpretation of the data, as presented, and recommends approval of LCZ696 for the indication sought.

1.3.2 Additional Non Clinical Recommendations

none

1.3.3 Labeling

Labeling edits were submitted independently through SharePoint.

2 Drug Information

2.1 Drug

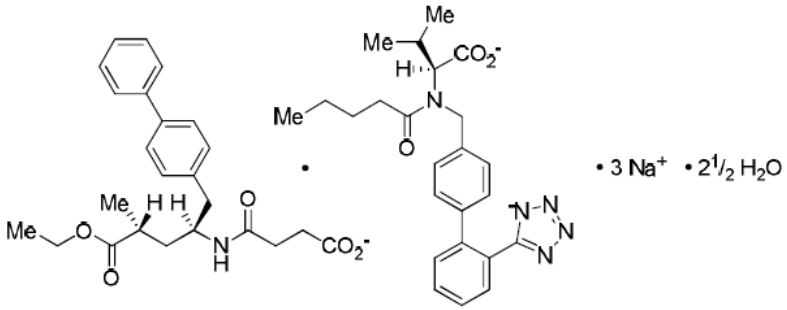
Generic Name: sacubitril (AHU377)/ valsartan

Code Name: LCZ696

Molecular Formula/Molecular Weight

NEPi moiety, sacubitril: $C_{24}H_{29}NO_{5,\ MW}$ 411.49
ARB moiety, valsartan: $C_{24}H_{29}N_5O_{3,\ MW}$ 435.52

Structure or Biochemical Description



sacubitril (AHU377) valsartan

Pharmacologic Class: 2 components

sacubitril – neutral endopeptidase (NEP) inhibitor
valsartan – AT1 receptor blocker (ARB)

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND (b) (4), IND (b) (4), IND 104628

2.3 Drug Formulation

Composition of one LCZ696 (sacubitril/valsartan) 50 mg, 100 mg, 200 mg film-coated tablet (mg/ tablet)

Ingredients	Amount (mg) per tablet			Function	Reference to standards
	50 mg[1]	100 mg[2]	200 mg[3]		
Tablet core					
(b) (4)	(b) (4) (50 mg)	(100 mg)	(200 mg)	Delivers the active substances	Novartis monograph
Microcrystalline cellulose / Cellulose, microcrystalline	(b) (4)				NF/ Ph. Eur.
Low Substituted Hydroxypropylcellulose					NF
Crospovidone					NF/ Ph. Eur.
Magnesium stearate[5]					NF/ Ph. Eur.
Talc					USP/ Ph. Eur.
Colloidal silicon dioxide/ Silica, colloidal (b) (4)					NF/ Ph. Eur.
Total core tablet weight					

2.4 Comments on Novel Excipients

none

2.5 Comments on Impurities/Degradants of Concern

none

2.6 Proposed Clinical Population and Dosing Regimen

LCZ696 (sacubitril/valsartan) is an angiotensin receptor neprilysin (neutral endopeptidase 24.11; NEP) inhibitor (ARNI) intended as an oral treatment for heart failur (b) (4) The target dose is 200 mg twice daily (BID).

3 Studies Submitted

3.1 Studies Reviewed

The Pharmacology and Pharmacokinetics sections are presented, mostly verbatim, from the sponsor. General toxicology, genetic toxicology, carcinogenicity and reproductive toxicology studies for LCZ696 and AHU377 were reviewed.

3.2 Studies Not Reviewed

Dose-.ranging and non-GLP studies were examined, but not formally reviewed.

4 Pharmacology

4.1 Primary Pharmacology

LCZ696 is a novel combination angiotensin receptor neprilysin (neutral endopeptidase 24.11; NEP) inhibitor (ARNI) intended as an oral treatment for heart failure (b) (4) [redacted]. LCZ696 is a salt complex comprising sacubitril (AHU377, a new-molecular entity) and valsartan, sodium cations, and water molecules in the molar ratio of 1:1:3:2.5 (ratio of 6:6:18:15 in the asymmetric unit cell of the solid-state crystal). Following oral administration, LCZ696 dissociates into valsartan and the pro-drug AHU377, which is further metabolized to the NEP inhibitor LBQ657.

LCZ696 exhibits a dual mechanism of action of an ARNI by simultaneously inhibiting NEP via LBQ657and by blocking the angiotensin II type-1 (AT_1) receptor via valsartan. The resulting increase in natriuretic peptide (NP) activity due to NEP inhibition and renin – angiotensin - aldosterone system (RAAS) inhibition through AT_1 receptor blockade have complementary cardiovascular and renal effects that are considered beneficial in heart failure.

In rats and dogs, oral administration of AHU377 delivers LBQ657 systemically, which resulted in concentration-dependent inhibition of NEP activity, increases in circulating levels of the NEP substrate atrial natriuretic peptide (ANP), and consequent elevations in renal sodium excretion and urine volume. LCZ696 also dose-dependently increased circulating ANP levels in rats.

In dogs, oral administration of LCZ696 elevated plasma and urinary cyclic guanosine monophosphate (cGMP), indicating NP receptor activation. In dogs fed a low-salt diet to activate the RAAS, plasma renin activity was increased less and plasma aldosterone concentrations were suppressed more by LCZ696 than by valsartan alone, which reflects the convergence of these two pathways by the dual activities of LCZ696.

The *in vitro* binding and inhibitor potencies of AHU377, LBQ657, and valsartan were evaluated for the NEP enzyme and AT_1 receptor. Additional data for valsartan is provided in the [Diovan® NDA 20-665] and the (Diovan® - US Prescribing Information).

LCZ696
LCZ696 was not tested *in vitro* because it readily dissociates into AHU377 and valsartan in aqueous media. Therefore, these compounds and the NEPi LBQ657 were tested individually.

AHU377 and LBQ657
The Table below summarizes the *in vitro* pharmacological profiles for AHU377, LBQ657, and valsartan as well as comparator reference compounds on NEP and NEP-2 protease activity. The pro-drug AHU377 was a weak inhibitor of NEP enzyme activity

whereas LBQ657 was a potent NEP inhibitor. Both AHU377 and LBQ657 were weak inhibitors of human recombinant NEP-2 enzyme activity.

The reference NEP inhibitors thiorphan and omapatrilat inhibited human NEP activity at concentrations that were >10-fold higher than that of LBQ657. Both agents also inhibited NEP-2, exhibiting less selectivity (<40-fold) than LBQ657 (37,000-fold).

***In vitro* potency and selectivity of AHU377, LBQ657, valsartan, and reference compounds on human recombinant NEP and NEP-2 enzyme activity**

Compound	Human NEP IC_{50} (nM)	Human NEP-2 IC_{50} (nM)	Selectivity NEP-2:NEP
AHU377	16,700 ± 2,300	>100,000	NC
LBQ657	2.3 ± 0.4	84,700 ± 2,800	36,826
Valsartan	>100,000	>100,000	NC
Thiorphan	30 ± 4	1,100 ± 300	37
Omapatrilat	110 ± 20	700 ± 300	6

Human recombinant forms of NEP and NEP-2 protein were used to measure enzyme activity with Cys(PT14)-Arg-Arg-Leu-Trp-OH as a substrate. Results [RD-2013-00079] are the mean ± SD of n ≥ 4 determinations. NC; not calculated due to NEP IC_{50} being greater than 15,000 nM.

LBQ657 was also tested in microsome preparations isolated from rat and human renal cortex tissue. LBQ657 inhibited NEP activity from rat renal cortex with an IC_{50} of 1.4 ± 0.02 nM and from human renal cortex with an IC_{50} of 7.3 ± 0.8 nM [RD-2005-50314]. NEP activity present in human plasma or human cerebrospinal fluid was inhibited by LBQ657 with an IC_{50} of 2,500 ± 1,100 nM [RD-2013-00079] and 50 ± 20 nM [RD-2014-00338], respectively. The increase of IC_{50} in human plasma was in part due to a reduction of the free fraction of LBQ657 due to high protein binding (97%). Neither AHU377 nor LBQ657 blocked the human AT_1 receptor at concentrations of 30 µM [RD-2012-50571], [RD-2012-50566], indicating that these two compounds have no valsartan-like activity.

Cellular activity

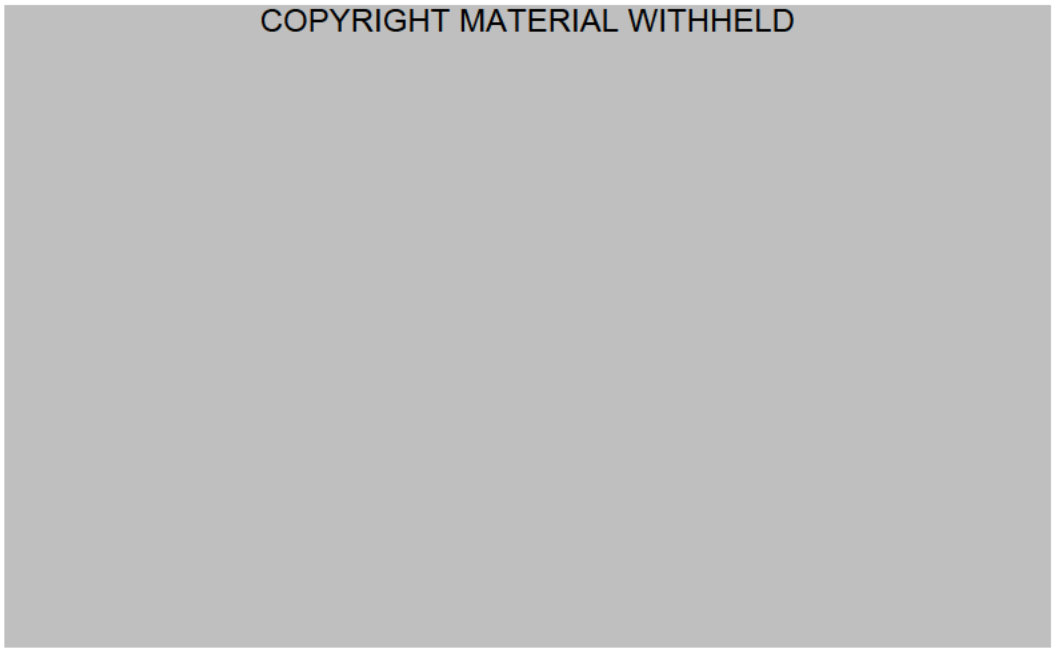
LCZ696 was not tested in cell culture models; however, the pharmacologically active LBQ657 and valsartan that are delivered by LCZ696 were evaluated. Studies were conducted to assess the interaction of NEP inhibition by LBQ657 and AT_1-receptor blockade by valsartan on mechanisms associated with fibrosis and cardiac hypertrophy. The model was based on treatment of neonatal rat cardiac fibroblasts or cardiomyocytes with angiotensin II, which elicited increased collagen synthesis or hypertrophy in these two cell types, respectively.

Suppressed collagen production with LBQ657 and valsartan in rat cardiac fibroblasts

LBQ657 and valsartan were evaluated for anti-fibrotic effects in a cell model of angiotensin II-induced collagen synthesis measured with ^{3}H-proline incorporation. To stimulate fibroblast collagen synthesis, neonatal rat cardiac fibroblasts were cultured with 100 nM angiotensin II. The effects of NEP inhibition and AT_1-receptor blockade were assessed by co-treating cells with increasing concentrations of valsartan (30 nM to 1 µM) in the presence or absence of 10 µM LBQ657 (Figure below). LBQ657 alone had

no effect on collagen production. In contrast, valsartan alone exhibited a concentration-dependent decrease in angiotensin II-mediated collagen production. The valsartan effect was significantly enhanced in the presence of 10 µM LBQ657. These results showed that concomitant inhibition of NEP in the presence of AT_1-receptor blockade provided greater reductions of rat cardiac fibroblast collagen synthesis than would be expected from the additive effects of NEP inhibition and AT_1-receptor blockade alone (von Lueder et al 2013).

Effects of LBQ657 and valsartan on rat cardiac fibroblast collagen production

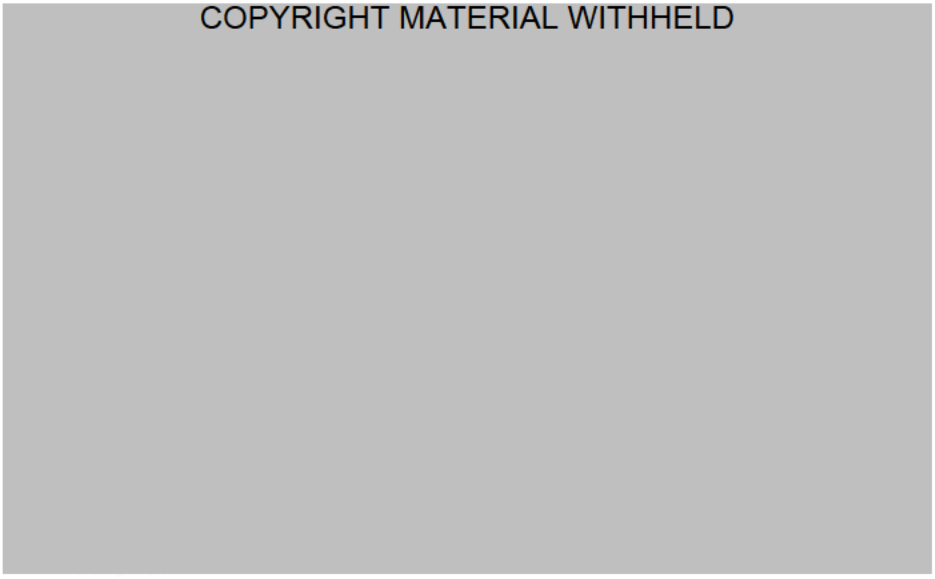


Effects of LBQ657 and valsartan were assessed on angiotensin II-stimulated neonatal rat cardiac fibroblast collagen synthesis as determined by ^{3}H-proline incorporation. Data (mean ± SEM) are shown as a percentage of unstimulated control (100%; dashed line). Unstimulated (negative) and angiotensin II–stimulated (positive) controls are the first open and solid columns, respectively, from the left. ***P<0.001 vs angiotensin II–stimulated control, ##P<0.01 valsartan (Val) + LBQ657 (10 µM; NEPi) vs valsartan for equal concentrations, unpaired t test.

Anti-hypertrophic effects of LBQ657 and valsartan in rat cardiomyocytes

LBQ657 and valsartan were evaluated for anti-hypertrophic effects of angiotensin II-induced cardiomyocyte hypertrophy using ^{3}H-leucine incorporation in cultured neonatal rat cardiomyocytes. To stimulate cardiomyocyte hypertrophy, cells were cultured with 100 nM angiotensin II. The effects of NEP inhibition and AT_1-receptor blockade were assessed by co-treating cells with increasing concentrations of valsartan (30 nM to 1 µM) in the presence or absence of 10 µM LBQ657 (Figure below). LBQ657 alone reduced cardiomyocyte hypertrophy by approximately 50%. In comparison, valsartan alone concentration-dependently decreased angiotensin II-mediated cardiomyocyte hypertrophy by approximately 60% at 1 µM. The valsartan effects at both 30 nM and 1 µM were significantly enhanced in the presence of 10 µM LBQ657. These results showed that concomitant inhibition of NEP in the presence of AT_1-receptor blockade provided greater reductions of rat cardiomyocyte hypertrophy than either agent alone, such that at the highest concentration tested the effect of angiotensin II was completely prevented (von Lueder et al 2013).

Effects of LBQ657 and valsartan on rat cardiomyocyte hypertrophy



Effects of LBQ657 and valsartan were assessed on angiotensin II-stimulated neonatal rat cardiomyocyte hypertrophy as determined by ^{3}H-leucine incorporation. Data (mean ± SEM) are shown as a percentage of unstimulated control (100%; dashed line). Unstimulated (negative) and angiotensin II–stimulated (positive) controls are the first open and solid columns, respectively, from the left. ***P<0.001 vs angiotensin II–stimulated control, ##P<0.01 valsartan (Val) + LBQ657 (10 µM; NEPi) vs valsartan for equal concentrations, unpaired t test.

In vivo pharmacology

Ex vivo effect on tissue NEP activity and AT_1-receptor blockade

A study was performed to assess the pharmacologic effect on tissue NEP activity of oral dosing with AHU377, valsartan, or the two agents together.

LCZ696

LCZ696 was not tested in studies to assess *ex vivo* effects on targets in tissues. Instead, AHU377 and valsartan were tested independently to demonstrate effects on targets and pathways since it was considered more relevant to evaluate these individual components of LCZ696.

AHU377

NEP activity in male Sprague Dawley rat renal cortex was measured *ex vivo* 60 minutes after an oral administration of AHU377 at either 30 or 100 mg/kg. NEP enzyme activity in the renal cortex homogenate was inhibited by 73 and 84%, respectively [RD-2005-50315], demonstrating that the active metabolite of AHU377 (LBQ657) reaches target tissue at concentrations sufficient to inhibit a majority of the NEP activity.

Because AHU377 did not block the AT_1 receptor at pharmacologically relevant concentrations *in vitro*, studies to assess effect of AHU377 on AT_1 receptor *ex vivo* were not performed.

Valsartan
NEP activity in male Sprague Dawley rat renal cortex was measured *ex vivo* 60 minutes after oral administration of valsartan at 30 mg/kg. Valsartan (30 mg/kg p.o.) had no effect on tissue NEP activity demonstrating that it did not block or activate this enzyme. Valsartan (30 mg/kg p.o.) co-administration with AHU377 (100 mg/kg p.o.) also had no effect on tissue NEP inhibitory activity compared to AHU377 alone [RD-2005-50315], indicating that valsartan did not interfere with the NEP inhibition by dosing AHU377.

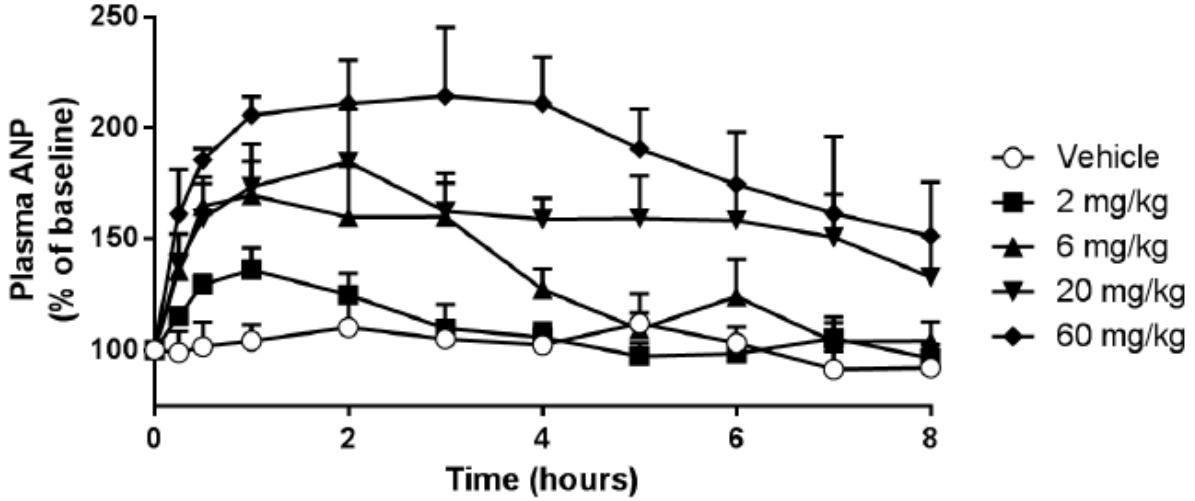
Effects of valsartan on tissue AT_1 blockade were previously described in the Diovan NDA 20-665.

NEP inhibition increases circulating ANP levels

ANP is a hormone released by the heart primarily in response to increased atrial distension to cause, among other effects, vasodilation, diuresis, and natriuresis. Under normal physiologic conditions, ANP is cleared by both receptor-mediated (NP receptor-C, NPR-C) and enzymatic (NEP) pathways. In preclinical models, infusion of ANP to saturate the receptor-mediated clearance pathway enables evaluation of the role of the NEP enzymatic pathway on ANP clearance. These methods were used to demonstrate the dose-dependent effect on ANP potentiation in rats and dogs.

LCZ696
When ANP was infused at a constant rate (450 ng/kg/min i.v.) in conscious male Sprague Dawley rats, acute administration of LCZ696 (2, 6, 20, and 60 mg/kg p.o.) resulted in a time- and dose-dependent potentiation of plasma ANP levels (Figure and Table below). Elevated plasma ANP was evident as early as 15 minutes after dosing and reached a peak between 1 and 3 hours. A dose of 2 mg/kg produced a small and brief elevation of ANP. Increasing the LCZ696 dose to 6 and 20 mg/kg increased the peak levels of ANP, but most significantly increased the duration of ANP potentiation through at least 4 hours. At the highest LCZ696 dose tested (60 mg/kg) peak ANP levels were increased by 132% (232% of baseline) with elevations maintained through 8 hours. The ANP levels returned to background at all doses by 24 hours [RD-2006-51044].

Plasma ANP potentiation after LCZ696 administration to rats



Rat ANP was infused intravenously in conscious rats at 450 ng/kg/min for 9 hours. LCZ696 was administered orally in mini-capsules one hour after the start of the ANP infusion. ANP concentrations (mean ± SEM) are expressed as a percent of baseline (post-ANP infusion) ANP levels (n=4/group).

Regression analysis of the dose response relationships for peak ANP levels identified LCZ696 doses needed to increase ANP by 20%, 50% and 100% to be 0.9, 2.8 and 16.7 mg/kg, respectively.

Dose-dependent effects of LCZ696 on ANP potentiation in rats

Dose	Peak ANP	TWA 0-4 hours	TWA 0-8 hours
Vehicle	126 ± 9%	105 ± 10%	103 ± 8%
2 mg/kg	137 ± 9%	120 ± 6%	110 ± 6%
6 mg/kg	176 ± 15%	155 ± 16%	134 ± 12%
20 mg/kg	207 ± 17%	166 ± 16%	160 ± 9%
60 mg/kg	232 ± 26%	202 ± 16%	189 ± 20%

Mean ± SEM as a percent of baseline of peak, time-weighted average (TWA) 4 hour and 8 hour ANP elevation (n=4/group).

AHU377

Rat

In male Sprague Dawley rats, AHU377 (1, 3, 10, and 30 mg/kg p.o.) dose-dependently increased time-weighted average (TWA; 0-4 hours) ANP concentrations from baseline in rats by 34 – 5, 35 – 3, 66 – 21 and 80 – 14% (mean – SEM), respectively. The increase in ANP at the two highest doses was significant ($p < 0.05$), demonstrating that the NEP inhibition after AHU377 administration is sufficient to potentiate circulating ANP levels in rats [RD-2002-50641].

Dog

In anesthetized mongrel dogs, AHU377 (3, 10, and 30 mg/kg i.d.) dose-dependently increased plasma TWA (0-5 hours) ANP concentrations by 16 – 6, 48 – 10 and 101 – 16%, respectively. The increase in ANP at the two highest doses was significant (p <

0.05), demonstrating that NEP inhibition after AHU377 administration is sufficient to potentiate circulating ANP levels in dogs [RD-2002-50641].

Valsartan
Valsartan was not tested for effect on ANP potentiation *in vivo* since it did not inhibit NEP enzyme activity, nor was AT_1-receptor blockade expected to affect the clearance of ANP.

Mechanistic model systems

Effect on diuresis and natriuresis

LCZ696-induced-pressure natriuresis in DSS rats
LCZ696 was assessed for effect on urine volume and sodium excretion in DSS rats on a high salt diet. DSS rats have a defect in renal sodium clearance requiring increased blood pressure to promote sodium excretion (rightward shift in pressure-natriuresis relationship). The effect of LCZ696 on renal sodium excretion and subsequent pressure natriuresis in DSS rats was compared to losartan, HCTZ, or losartan plus HCTZ. DSS rats were fed a normal-salt diet (0.49% NaCl) or a high-salt diet (4% NaCl) for three weeks prior to initiating treatment. Rats on a high-salt diet then received either vehicle, LCZ696 (68 mg/kg), losartan (30 mg/kg), HCTZ (9.7 mg/kg), or the losartan/HCTZ combination for 4 weeks.

DSS rats fed a high-salt diet had elevated water intake, urine volume, and urinary sodium excretion relative to the normal-salt-fed rats (Table below). At the end of four weeks of treatment, none of the compounds had a significant effect on water intake, urine volume, or urinary sodium excretion, except losartan/HCTZ, which reduced urine volume at week 4. Urinary sodium excretion was elevated approximately 6-fold in the high-salt-fed rats, and remained elevated in all of the treatment groups throughout the treatment period.

Water intake, urine volume, and sodium excretion after 4 weeks of treatment in DSS rats

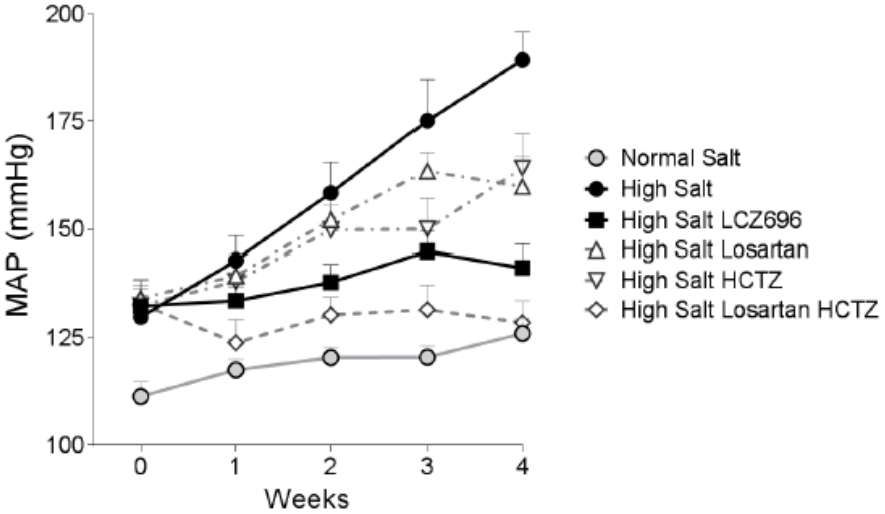
Treatment (n= 11-16/group)	Water intake (mL/day)	Urine volume (mL/day)	Urinary sodium excretion (mmol/day)
Normal salt	30.4 ± 1.1	13.7 ± 1.2	1.4 ± 0.1
High salt	53.0 ± 6.4 *	39.6 ± 3.5 *	7.4 ± 0.7 *
High salt LCZ696	54.3 ± 3.8 *	36.9 ± 1.7 *	7.4 ± 0.7 *
High salt losartan	54.2 ± 5.3 *	42.4 ± 4.2 *	8.3 ± 0.6 *
High salt HCTZ	46.3 ± 4.3 *	32.7 ± 3.8 *	7.7 ± 0.4 *
High salt losartan/HCTZ	42.3 ± 2.7 *	28.9 ± 1.8 *#	8.1 ± 0.4 *

Results are mean ± SEM (n=16/group). *p<0.05 vs normal salt, # p<0.05 vs high salt, two-way ANOVA, Newman-Keuls post hoc analysis.

Over the 3 weeks prior to the start of treatments, MAP increased by ~23 mmHg in DSS rats on high-salt diet relative to those on normal chow. Figure 2-5 shows the time profile of MAP over the 4-week treatment period. MAP continued to increase by 60 mmHg in the high-salt diet group during this time. In contrast, LCZ696 prevented the salt-induced

hypertension with a change in MAP (9 ± 5 mmHg) that was similar to the normal-salt controls (15 ± 3 mmHg) [RD-2013-50369].

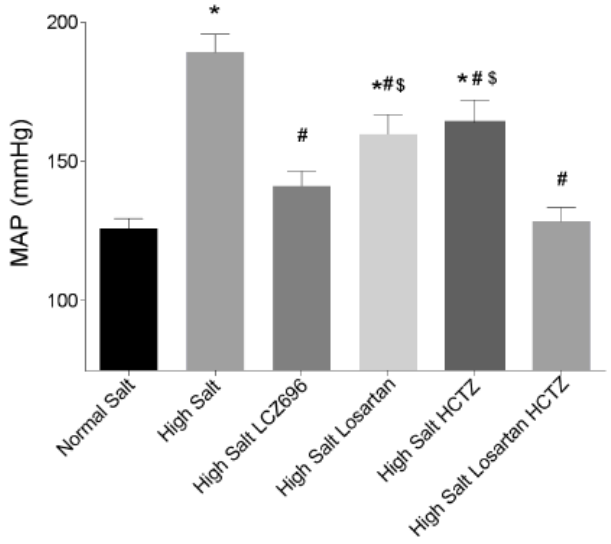
Time course of MAP over 4 weeks of treatment in DSS rats



DSS rats were maintained on normal-salt diet (0.49%) or switched to high-salt diet (4%) starting at week -3. At week 0, drug treatment was initiated with daily (q.d.) oral administration of compounds for 4 weeks. Mean arterial pressure (MAP; mean ± SEM) was measured by tail cuff; n=11-16/ group.

After 4 weeks, all treatments significantly reduced MAP compared to high-salt vehicle (Figure below). Only LCZ696 and losartan/HCTZ prevented salt-induced hypertension (nonsignificant vs. normal-salt diet), and both had significantly lower MAP compared to either the losartan or HCTZ monotherapy groups. There was no significant difference between LCZ696 and losartan/HCTZ. These data demonstrate that LCZ696 maintained elevated levels of urinary sodium excretion while also reducing blood pressure in these DSS rats on high-salt diet. Neither AHU377 nor valsartan were evaluated in this study.

MAP at the end of 4 weeks of treatment in DSS rats



DSS rats were placed on a normal-salt (0.49%) or high-salt (4%) diet for seven weeks and treated the last four weeks with drug or vehicle (p.o., q.d.). MAP (mean ± SEM) was measured by tail cuff. n = 11-16/group; * $p<0.05$ vs normal salt; # $p<0.05$ vs high salt; $ $p<0.05$ vs high salt LCZ696, two way ANOVA, Newman-Keuls post hoc analysis.

AHU377 and LBQ657 effects on ANP potentiation of diuresis and natriuresis in rats and dogs

Rats - Male Sprague Dawley rats were anesthetized and catheters were inserted in a femoral artery and vein and the urinary bladder to measure arterial pressure, administer ANP, and collect urine, respectively. A continuous infusion of normal saline (33 µL/min) was maintained throughout the experiment to promote diuresis and sodium excretion. There were no significant baseline differences in MAP, urine volume or urinary sodium excretion (UNaV). In vehicle-treated (i.d.) rats, ANP increased UNaV 4.5-fold from 0.72 ± 0.25 to 3.26 ± 0.63 µEq/kg/min. This response was potentiated in AHU377-treated (30 mg/kg, i.d.) animals in which ANP increased UNaV 14-fold from 0.63 ± 0.22 to 8.84 ± 2.13 µEq/kg/min ($p < 0.05$ vs. vehicle group). There was a trend toward a greater ANP-induced diuresis in rats that received AHU377; however, the effect was not statistically significant [RD 2002 50823].

Dogs - Male mongrel dogs were anesthetized and the right femoral vein was cannulated for vehicle and drug administration. The ureter of the left kidney was cannulated for the collection of urine, and a catheter was inserted into the left renal artery for intrarenal arterial infusions of ANP or saline. During the study, urine was collected in 20-minute intervals to measure urine volume and sodium concentration. Vehicle or LBQ657 (10 mg/kg) was administered i.v. immediately after the start of the ANP infusion. In vehicle-treated dogs, ANP administration increased UNaV from 17.3 ± 3.6 to 199.5 ± 18.4 µEq/kg/min. This effect was potentiated significantly in animals that received LBQ657, with a peak UNaV increase from 20.8 ± 4.2 to 289.2 ± 28.8 µEq/kg/min. Urinary volume was also increased with ANP administration in vehicle-treated dogs from 0.09 ± 0.01 to 1.07 ± 0.09 mL, and LBQ657 potentiated ANP's effect

from 0.10 ± 0.01 to 1.59 ± 0.21 mL (Ksander et al 1995). These results demonstrated that LBQ657 enhanced the ANP-mediated natriuresis and diuresis in dogs.

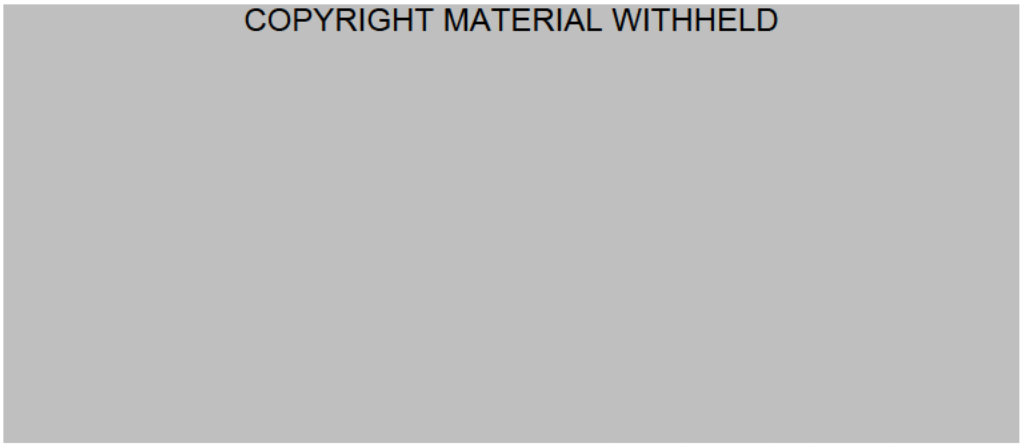
Relevant disease model systems

The effects of LCZ696, AHU377, and valsartan were evaluated for effects on the cardiovascular system in three different rodent disease models. First, SHRSP is a rat model that has a high mortality rate from stroke and exhibits progressive vascular and cardiac pathophysiology. Second, DSS rats develop a progressive cardiovascular and renal pathology when placed on a high-salt diet (8.0% NaCl), but not when maintained on a normal-salt diet. Third, an acute myocardial infarction (MI) rat model mimics the progression to heart failure, including cardiac remodeling and tissue pathology, which is similar to the situation in patients who sustain a large MI.

AHU377, valsartan, and their combination effect on vascular remodeling and cardiac fibrosis in SHRSP

Ten-week-old SHRSP were treated for 10 weeks with vehicle, valsartan (10 mg/kg/day), valsartan plus AHU377 (100 mg/kg/day), or AHU377 alone (100 mg/kg/day) as food admixtures. Wistar-Kyoto rats (WKY) served as normotensive controls. Pretreatment systolic blood pressures (tail-cuff measurements) were ~200 mmHg. The Figure below shows the diastolic blood pressure (radio-telemetry) in SHRSP during the last 4 weeks of treatment. Valsartan plus AHU377 decreased diastolic blood pressure significantly, whereas valsartan or AHU377 alone had little effect.

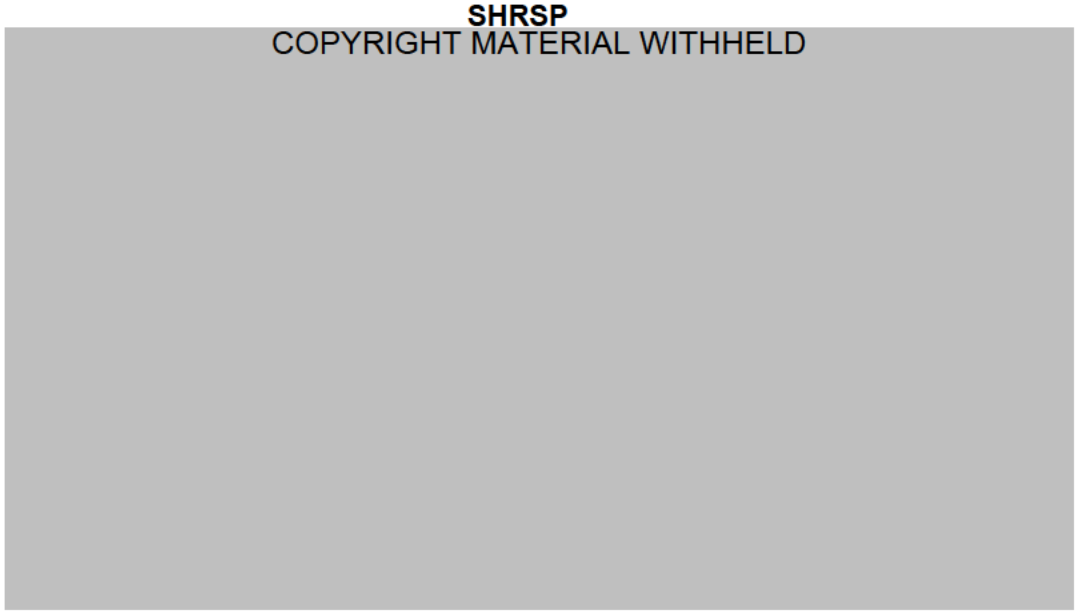
Effect of valsartan plus AHU377 on diastolic blood pressure in SHRSP



Diastolic blood pressure was assessed from weeks 6–10 by radio-telemetry in untreated control (normal chow) SHRSP and SHRSP treated with valsartan, AHU377, or valsartan+AHU377 as chow admixtures. Results are mean ± SEM. * P<0.01 vs SHRSP. (Figure reproduced from Pu et al 2008)

Intramyocardial coronary arteries of SHRSP exhibited increased media/lumen ratio and increased perivascular interstitial collagen density (Figure below). Ten-week treatment with AHU377 alone had no effect on these parameters, and valsartan alone significantly decreased both media/lumen ratio and collagen density. The combination of valsartan plus AHU377 further reduced both intramyocardial media/lumen ratio and perivascular collagen density.

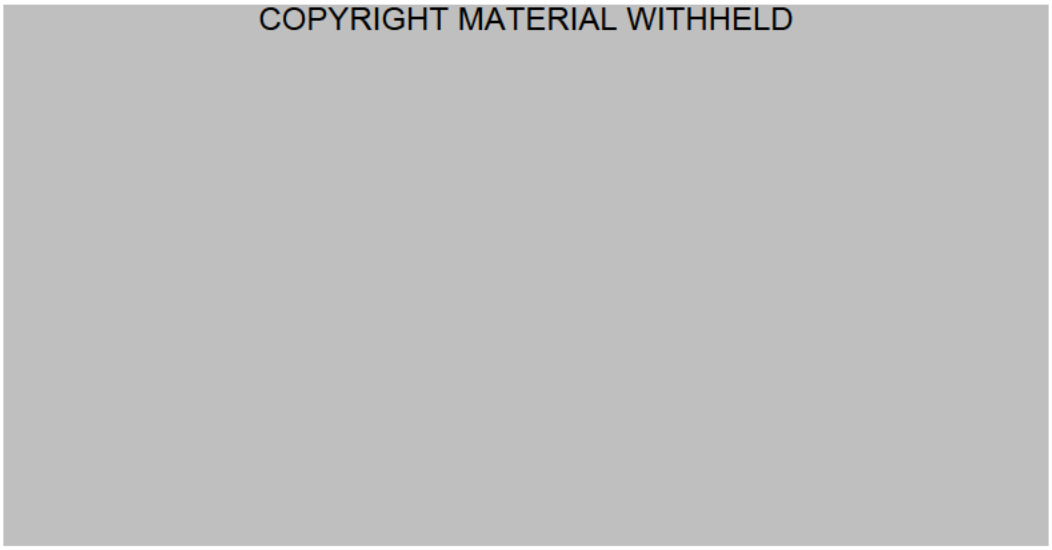
Effect of valsartan plus AHU377 on cardiac arteriole size and collagen deposition in SHRSP



Media-to-lumen ratio (left panel) and collagen density (right panel) of intramyocardial coronary arteries were measured in normotensive Wistar–Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSP) treated with vehicle, AHU377, valsartan, or AHU377 + valsartan. Results are mean ± SEM. * P<0.05 vs WKY vehicle; # P<0.05 vs SHRSP vehicle. (Figure reproduced from Pu et al 2008)

Valsartan plus AHU377 also increased vascular matrix metalloproteinase-2 (MMP-2) activity, and decreased tissue inhibitor of metalloproteinases-2 (TIMP-2) activity whereas the individual treatments had no effect (Figure below) (Pu et al 2008). Macrophage infiltration was decreased by all treatments with valsartan plus AHU377 being more effective than valsartan or AHU377 alone (data not shown). These results demonstrated that NEP inhibition plus AT_1-receptor blockade was an effective treatment for improving cardiac fibrosis and vascular fibrosis, remodeling, and inflammation in SHRSP.

Effect of valsartan plus AHU377 on aortic MMP-2 activity and TIMP-2 binding in SHRSP



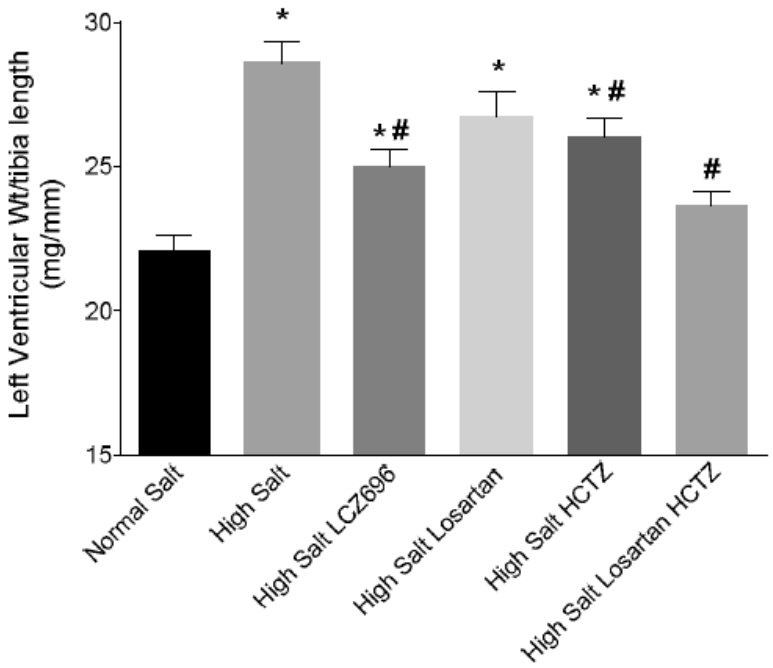
Gelatin zymography measurement of matrix metalloproteinase-2 (MMP-2) activity (left panel) and reverse gelatin zymography showing the differences in the binding capacity of aortic TIMP-2 (right panel). Wistar–Kyoto (WKY) rats, stroke-prone spontaneously hypertensive rats (SHRSP) treated with vehicle, AHU377, valsartan or AHU377 + valsartan together. Results are mean ± SEM. * P<0.05 vs SHRSP vehicle; # P<0.01 vs SHRSP vehicle; $ P<0.01 vs SHRSP vehicle. (Figure reproduced from Pu et al 2008)

Cardiac stress in DSS rats

LCZ696

DSS rats fed a high-salt (4%) diet for seven weeks exhibited a 29% increase in left-ventricular weight compared to rats on a normal-salt diet (Figure below). Four weeks of treatment (weeks 3 to 7) with LCZ696, HCTZ, or losartan/HCTZ significantly reduced left-ventricular weight compared to high-salt vehicle treatment. In contrast, losartan treatment did not significantly reduce left-ventricular weight compared to high-salt vehicle controls. Consistent with enlargement of the left ventricle, N-terminal pro-ANP (NT-proANP) levels were increased 3- fold in high-salt vehicle animals (51.4 ng/mL) compared to normal-salt control animals (17.2 ng/mL, $p < 0.05$). Both LCZ696 and losartan/HCTZ prevented the salt-induced increase (32.8 and 24.6 ng/mL; not significant vs normal-salt control), whereas losartan and HCTZ monotherapies both had significantly elevated NT-proANP (45.1 and 43.1 ng/mL, respectively; $p < 0.05$ vs normal-salt control) [RD-2013-50369]. Based on these findings, LCZ696 exhibited greater cardioprotection than an ARB alone in DSS rats on a high-salt diet.

Effect of LCZ696 on left-ventricular weight in DSS rats



Left-ventricular weights (normalized to tibial lengths) were measured in DSS rats after seven weeks on either a normal-salt diet or a 4% salt diet and treatment with vehicle, LCZ696, losartan, HCTZ or losartan plus HCTZ during the last four weeks. Results are mean ± SEM (n = 14 - 16/ group). * $p<0.05$ vs normal salt; # $p<0.05$ vs high salt, one-way ANOVA, Newman-Keuls post hoc analysis.

Valsartan

In DSS rats switched to 8% NaCl diet at 6 weeks of age, valsartan treatment concurrent with the high-salt diet reduced (3 mg/kg) or prevented (10 mg/kg/day) left-ventricular hypertrophy (Yoshida et al 2007). In contrast, left-ventricular hypertrophy was only attenuated in DSS rats fed 8% NaCl diet and treated with valsartan (30 mg/kg/day) from 7 weeks of age (Kim et al 2001).

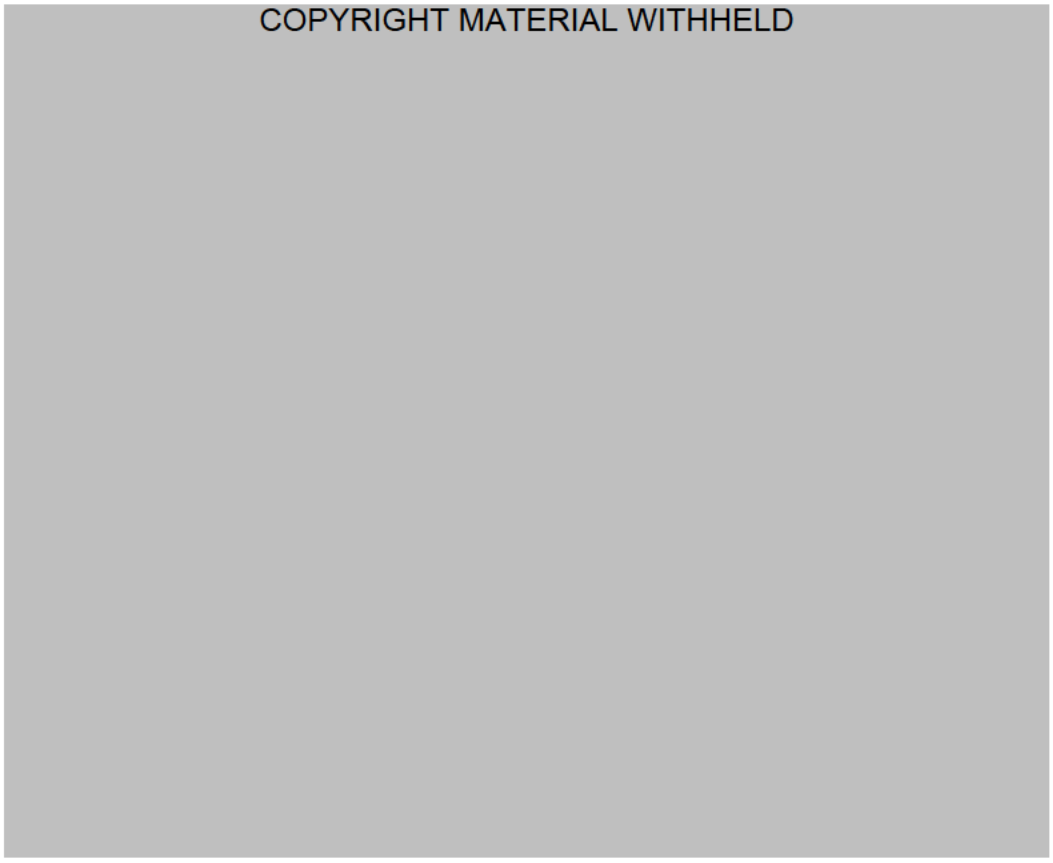
Cardiac remodeling in a rat MI model

MI induced by coronary artery ligation in the rat is a widely used preclinical model of heart failure. In the rat, the infarcted heart undergoes remodeling that includes hypertrophy and chamber dilation with impaired function. This model is often used to profile the effects of therapeutic agents for heart failure.

LCZ696

LCZ696 was tested to assess the impact on cardiac remodeling after MI in Sprague Dawley rats. One week after induction of MI, animals were randomized to 4 weeks of LCZ696 (68 mg/kg p.o.) or vehicle. LCZ696-treated rats exhibited significantly reduced cardiac hypertrophy at the end of the study (Figure below), which is indicative of effects that are considered beneficial for the failing heart (von Lueder et al 2013).

Effect of LCZ696 on cardiac remodeling after MI in Sprague Dawley rats



One week after surgical ligation of the left anterior descending coronary artery (MI) in male Sprague Dawley rats, animals were randomized to treatment with LCZ696 (68 mg/kg p.o.; n = 11), or vehicle (n = 6). After 4 weeks of treatment, LCZ696-treated rats exhibited significantly smaller weights of all cardiac chambers (mean ± SEM), consistent with reduced cardiac hypertrophy (von Lueder et al 2013).

AHU377 or valsartan

Neither AHU377 nor valsartan were tested in the rat MI model shown above.

Effects of LCZ696, AHU377, or valsartan on blood pressure in rat models of hypertension

Effects of LCZ696 were assessed in several models that have different physiologic and pathologic bases for elevated blood pressure. The dTGR is a model of high-renin activity that develops fulminant hypertension with premature morbidity and mortality. SHR is a moderate renin model that develops age-dependent hypertension and cardio-renal pathology. DSS rat is a low-renin model with a defect of renal sodium excretion and compensatory hypertension to maintain natriuresis. The Table below summarizes the effects of LCZ696, AHU377, or valsartan on blood pressure in these different models.

Summary of effects of LCZ696, AHU377, and valsartan in rat hypertension models

Model	Compound	Study	Key findings
Double-transgenic rat	LCZ696 (2, 6, 20 and 60 mg/kg p.o.)	[RD-2006-51202]	Male dTGRs had a baseline MAP in the range of 170-180 mmHg. A single administration of LCZ696 as neat drug substance in a capsule resulted in a dose- and time-dependent reduction of blood pressure. The peak changes in MAP from baseline were -21 ± 6, -31 ± 8, -52 ± 8*, -57 ± 4*, and -73 ± 5* mmHg for LCZ696 at 0, 2, 6, 20, and 60 mg/kg, respectively (*p < 0.05 vs vehicle; one-way ANOVA and Tukey HSD test). An antihypertensive effect was still observed for more than 24 hours after dosing with LCZ696 at 60 mg/kg.
DOCA rat	AHU377 (30 mg/kg/day p.o.)	[RD-2002-50881]	In male Sprague Dawley rats made hypertensive with deoxycorticosterone acetate (DOCA)-salt, AHU377 produced a modest reduction in MAP (maximum decrease of 13 mmHg as compared to the vehicle-treated group), which was not statistically significant.
DSS rat	LCZ696 (68 mg/kg/day p.o.) Valsartan (*31 mg/kg/day p.o.)	[RD-2011-50290]	Male DSS rats at 7-8 weeks of age were instrumented with radio-transmitters for blood pressure recording and were placed on an 8% salt diet. After eight days, MAP increased by ~6 mmHg and treatments were initiated. Over the ensuing 2 weeks, the 24-hour average MAP gradually increased by an additional 35±5 and 25±4 mmHg in the vehicle- and valsartan-treated rats, respectively, whereas LCZ696 limited this rise to 15±2 mmHg. Furthermore, vehicle (2±3) and valsartan (-2±3) had no effect on the peak MAP response in the first 7 hours after each daily dosing whereas LCZ696 lowered MAP (-13±2 mmHg). These results demonstrated that LCZ696 was more effective than valsartan as an antihypertensive in this low-renin, volume-dependent model of hypertension.
SHR	LCZ696 (68 mg/kg/day) Valsartan (*31 mg/kg/day)	[RD-2011-50289]	Adult male SHR were surgically instrumented with radio-transmitters for blood pressure recording. LCZ696 or valsartan similarly reduced MAP over the first 5 days of dosing and thereafter for the two week dosing period. These results demonstrated that LCZ696 and valsartan were equieffective antihypertensive agents in this normal-renin, salt-insensitive model of hypertension.

*An equimolar dose to the valsartan delivered by LCZ696

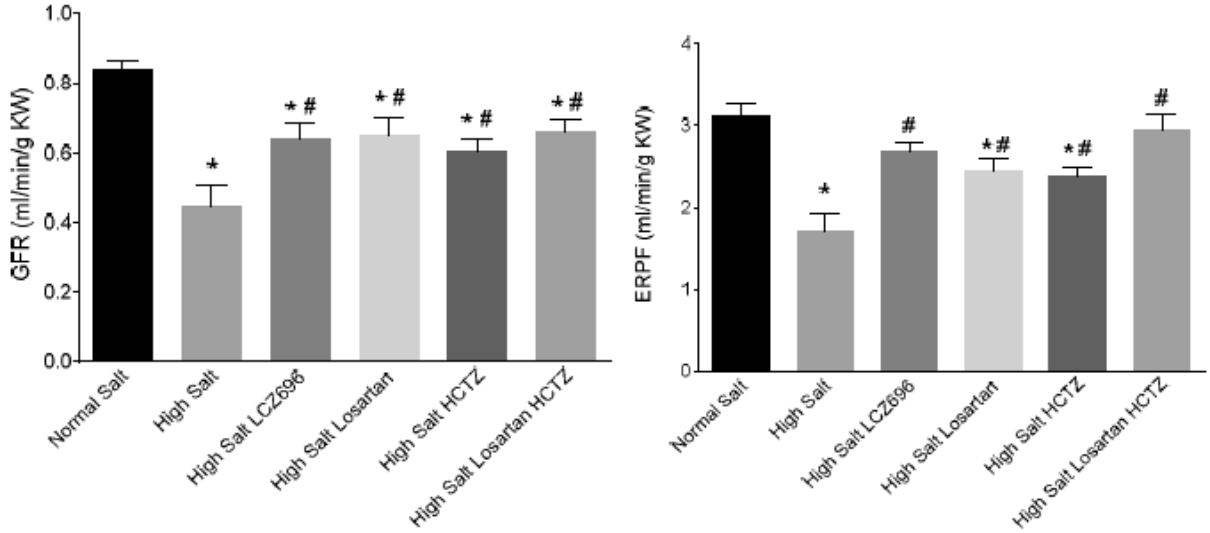
LCZ696 effect on renal function in DSS rats

In DSS rats, renal function and plasma flow were evaluated after 4 weeks of treatment using the plasma clearance technique with ^{3}H-inulin and ^{14}C-para-aminohippurate (PAH) as respective tracers. On the day of assessment, rats received vehicle or test article (LCZ696, losartan, HCTZ, or losartan/HCTZ combination) prior to tracer infusion [RD-2013-50369].

GFR was decreased 47% by the high-salt diet relative to normal-salt diet (Figure below). The high-salt-related reduction in GFR was ameliorated approximately 50% by all test articles including LCZ696, with no significant difference between test article treatment groups.

ERPF was decreased 45% by the high-salt diet relative to normal-salt diet (Figure below). All test articles significantly suppressed the salt-induced decrease of ERPF. LCZ696 treatment and losartan/HCTZ combination treatment prevented the salt-induced decrease of ERPF (- 14% and -6%, respectively, both non-significant relative to normal salt). In contrast, losartan and HCTZ treatment partially protected against the salt-induced reduction of ERPF (-22% and -24%, respectively, $p < 0.05$ vs normal salt).

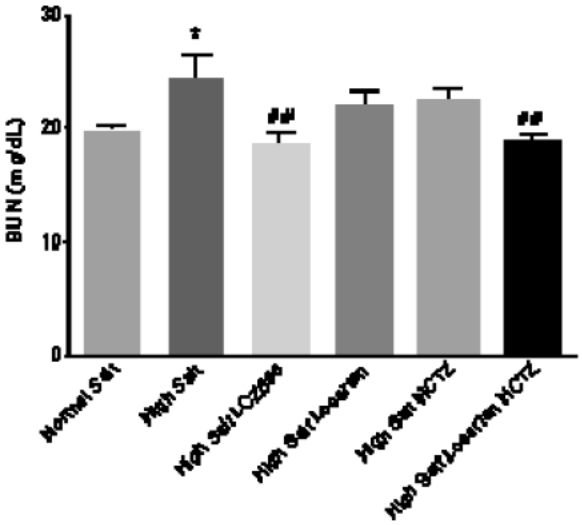
Glomerular filtration rate and effective renal plasma flow after 4 weeks of treatment in DSS rats



After four weeks of treatment, renal function and renal plasma flow were assessed using ^{3}H-inulin and ^{14}C-PAH as respective tracers with the plasma clearance technique. Test articles were administered prior to infusion of the tracers. Results are mean ± SEM (n = 7 or 8/ group). * $p<0.05$ vs normal salt; # $p<0.05$ vs high salt, one-way ANOVA, Newman-Keuls post hoc analysis.

High-salt diet impaired renal blood flow (data not shown) by 50% and doubled renal vascular resistance ($p<0.05$ vs normal salt). All treatments except HCTZ prevented this impairment of renal blood flow and increase of renal vascular resistance (all non-significant vs normal salt) and none of the treatments were significantly different from each other. High-salt diet increased blood urea nitrogen (BUN) 24% over the last two weeks of the study ($p<0.05$ vs normal salt; Figure below). Both LCZ696 and the losartan/HCTZ combination significantly reduced BUN relative to high-salt vehicle over that time period ($p < 0.05$ vs high salt vehicle). Losartan and HCTZ monotherapy had no effect on BUN.

BUN after 3 and 4 weeks of treatment in DSS rats



BUN was measured at the end of weeks 3 and 4 of the study. Data are expressed as the average of weeks 3 and 4 and are the mean ±SEM (n=13 - 16/group). * p<0.05 vs normal salt; ## p<0.05 vs high salt, one-way ANOVA, Newman-Keuls post hoc analysis.

Markers of renal injury were measured in urine after four weeks of treatment (Table below). Kidney injury molecule-1 (KIM-1) is a transmembrane protein that is up-regulated in proximal tubular epithelial cells after renal damage or ischemia. High-salt diet increased KIM-1 protein levels in the urine 5.6-fold. All four treatments, including LCZ696, significantly decreased salt-induced urinary KIM-1 levels by >30%. Only losartan/ HCTZ prevented the salt-induced increase of KIM-1 (nonsignificant vs normal salt). Neutrophil gelatinase-associated lipocalin (NGAL) is a marker of acute kidney injury and of kidney disease. High-salt diet increased urinary NGAL levels 4.3-fold. All four treatments significantly reduced the level of NGAL in the urine to levels that were not significantly different from normal-salt controls. Osteopontin is a secreted glycoprotein that is expressed in both tubular and glomerular cells and is up-regulated in response to injury. High-salt diet increased urinary osteopontin protein levels by approximately 6.8-fold. Only losartan/HCTZ significantly reduced osteopontin levels by half relative to high-salt-fed vehicle-treated animals.

Renal injury urinary biomarkers after 4 weeks of treatment in DSS rats

Treatment	KIM-1	NGAL	Osteopontin
Normal salt	18 ± 2 #	23 ± 2 #	15 ± 1 #
High salt vehicle	100 ± 13 *	100 ± 20 *	100 ± 18 *
High salt LCZ696	57 ± 7 * #	51 ± 6 #	60 ± 15
High salt Losartan	58 ± 8 * #	54 ± 11 #	92 ± 22 *
High salt HCTZ	59 ± 10 * #	53 ± 16 #	71 ± 19 *
High salt Losartan/HCTZ	32 ± 5 #	25 ± 5 #	35 ± 5 #

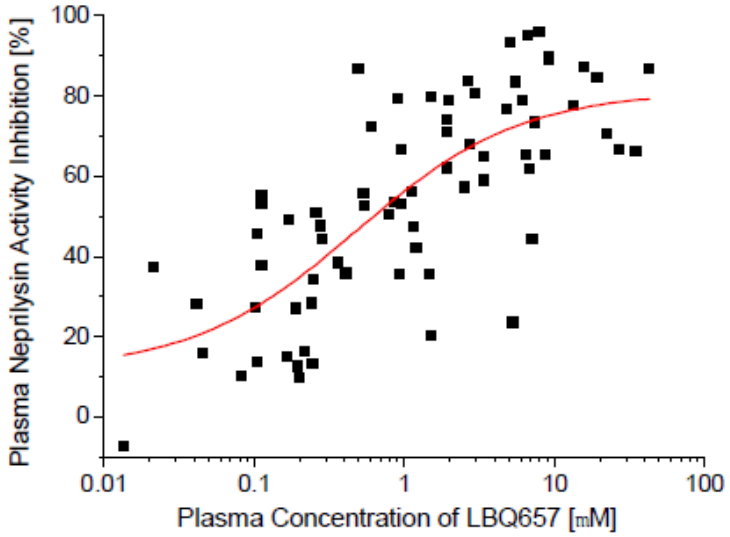
Results are expressed as % of high-salt-diet vehicle-treated mean value. Kidney injury molecule-1 (KIM-1); neutrophil gelatinase-associated lipocalin (NGAL). Results are mean ± SEM (n 12 - 16/group). * p<0.05 vs normal salt, # p < 0.05 vs high-salt vehicle, one-way ANOVA, Newman-Keuls post hoc analysis.

Other pharmacological studies

Pharmacokinetic/pharmacodynamic relationship

Male cynomolgus monkeys were administered AHU377 (3, 10, and 30 mg/kg p.o.). Blood samples were collected for plasma analysis of AHU377 and LBQ657 and NEP enzyme activity over a 24-hour period post-dosing. After administration of AHU377, plasma exposure to LBQ657 increased dose- and time-dependently reaching a maximum after ~1.2 to 2 h with Cmax values of 6.1 µM, 9.6 µM and 62.2 µM for the 3 respective doses. At the 10 and 30 mg/kg doses of AHU377, 79 to 85% inhibition of plasma NEP activity was achieved and lasted up to 5 hours for the highest dose. The concentration-dependent inhibition of plasma NEP activity predicted a functional IC_{50} value of 0.5 ± 0.3 µM for LBQ657 (Figure below, [RD-2013-00171]).

Plasma NEP enzyme inhibition versus systemic LBQ657 exposure in cynomolgus monkeys treated with AHU377



The % inhibition of plasma NEP activities relative to pretreatment values (0% inhibition) measured at various times after AHU377 (3, 10, 30 mg/kg p.o.) were plotted against the corresponding plasma LBQ657 concentrations. Non-linear logistic fit provided an IC_{50} value of 0.5 ± 0.3 µM and a Hill coefficient of 0.79 ± 0.41.

Plasma concentrations of AHU377 or LBQ657 were not measured in the rat pharmacology studies.

Effects in combination with other agents

LCZ696 was not tested in preclinical pharmacology models in combination with other agents.

Surrogate markers

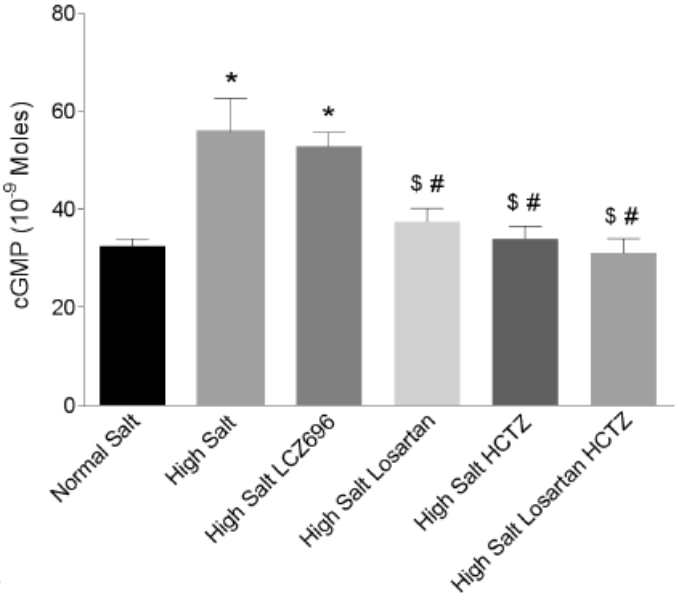
The pharmacology of LCZ696 is evident through evaluation of changes of markers of both the RAAS and the NP system. Below are results from rat and dog studies that measured effects of LCZ696 on markers of these pathways.

LCZ696 effect on urinary cGMP in DSS rats

In response to increased blood volume and atrial stretch, salt-overloaded DSS rats exhibit increased ANP levels. ANP stimulates cGMP production by the binding to NP receptor-A (NPR-A), leading to cardiac, vascular, and renal biological responses including elevated urinary ANP and cGMP excretion.

Urinary cGMP responses to LCZ696 and other agents were measured in the previously described DSS rat study. After the first dose of test articles, urine was collected over a 24-hour period. High-salt diet significantly increased urinary cGMP by 52% compared to normal-salt vehicle animals (Figure below). Treatment with LCZ696 did not alter the elevated cGMP (non-significant from high-salt vehicle). In contrast, administration of losartan, HCTZ or the combination losartan/HCTZ all significantly reduced urinary cGMP excretion, effects that were significantly different from LCZ696 [RD-2013-50369].

Urinary cGMP excretion after the first dose of LCZ696 in DSS rats



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Urine was collected over a 24-hour period after the first dose of test agents. cGMP concentration was measured and normalized for urine volume and expressed as moles excreted per 24 hours. Results are mean ± SEM (n = 15 - 16/ group). * p<0.01 vs normal salt; # p<0.01 vs high salt; $ p<0.01 vs high salt LCZ696, one-way ANOVA, Newman-Keuls post hoc analysis.

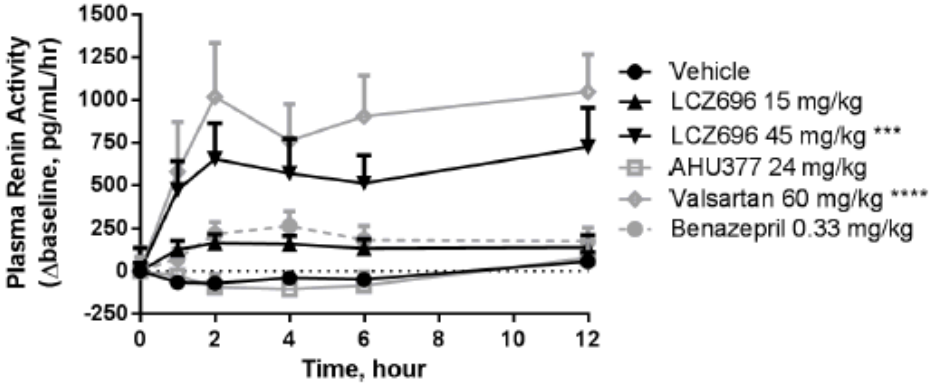
LCZ696 effect on plasma RAAS markers and cGMP in dogs

Eighteen beagle dogs (4-5 years of age) were placed on a low-salt diet during the study period to activate the RAAS. The effect of 10 days of treatment (p.o., q.d.) with LCZ696 (15 mg/kg/day, n=6; 45 mg/kg/day, n=12) was compared to vehicle (n=12), valsartan (60 mg/kg/day, n=12), AHU377 (24 mg/kg/day, n=6), and benazepril (0.33 mg/kg/day, n=6) using an incomplete 3-way cross-over design. Plasma samples were collected over 12 hours on days 1, 5, and 10 of treatment and analyzed for biomarkers of the RAAS and NEP inhibitor effect. Dogs were dosed after the time 0 blood sample was collected, and results from the three collection days for all of the dogs/group were combined for the analyses of plasma renin activity (Figure A below), plasma angiotensin

II concentrations (Figure B), plasma aldosterone concentrations (Figure C), and plasma cGMP concentrations (Figure D). Urine samples were collected on day 5.

Plasma renin activity (pg/mL/hr of angiotensin I formation from angiotensinogen) over the 12-hour post-dosing was unchanged after dosing vehicle or AHU377 (Figure A). Valsartan and LCZ696 (45 mg/kg) significantly increased plasma renin activity ($p<0.001$). The lower dose of LCZ696 (15 mg/kg) and benazepril modestly elevated plasma renin activity, although the effect was not statistically significant. Similar results were obtained for plasma angiotensin I levels (data not shown). These results demonstrated that LCZ696 blocked angiotensin II signaling through the AT_1 receptor, causing the known compensatory upregulation of plasma renin and angiotensin I.

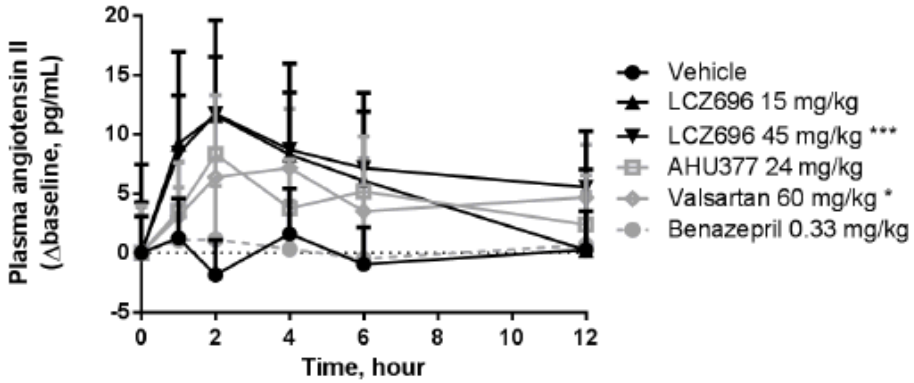
Figure A: Plasma renin activity changes after treatments in conscious dogs



Beagle dogs were dosed daily for 10 days, and plasma renin activity (pg/mL/hr of angiotensin I formation) was measured on days 1, 5, and 10. Dogs were dosed after the time 0 sample was collected on each day. Mean ± SEM change from time 0 baseline (n = 12/group for vehicle, LCZ696 45 mg/kg and valsartan; n = 6/group for LCZ696 15 mg/kg, AHU377 and benazepril times 3 days). *** $p < 0.001$, **** $p < 0.0001$ vs vehicle, one-way ANOVA on 0- to 12-hour AUC values.

Increases in plasma angiotensin II levels were similar to the changes observed for plasma renin activity and angiotensin I for all treatments except for AHU377 and benazepril (Figure B). While AHU377 had no effect on plasma renin activity or angiotensin I levels, there was a significant increase of angiotensin II levels at two hours after dosing ($p < 0.05$). This was consistent with LBQ657 being an inhibitor of NEP, an enzyme known to also degrade angiotensin II. In contrast, benazepril, which slightly increased both plasma renin activity and plasma angiotensin I levels, had no effect on plasma angiotensin II levels. This was consistent with benazepril being an angiotensin converting enzyme inhibitor that partially blocked the production of angiotensin II at the utilized dose.

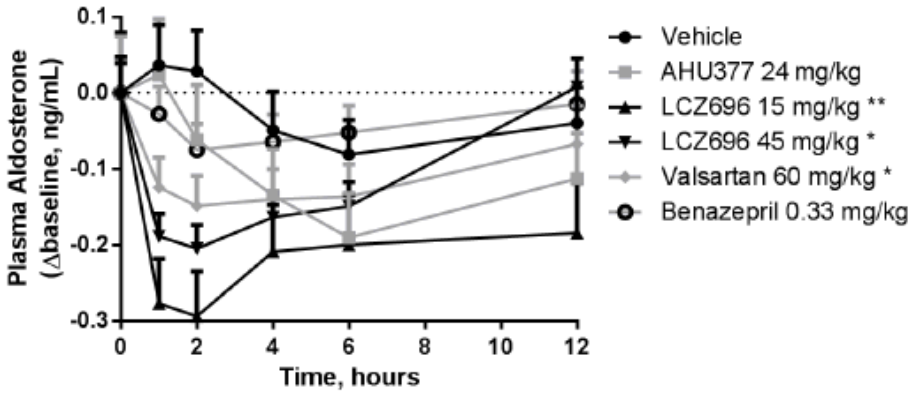
Figure B: Plasma angiotensin II concentration changes after treatments in conscious dogs



Beagle dogs were dosed daily for 10 days, and plasma angiotensin II concentrations (pg/mL) were measured on days 1, 5 and 10. Dogs were dosed after the time 0 sample was collected on each day. Results are mean ± SEM change from time 0 baseline (n = 12/group for vehicle, LCZ696 45 mg/kg and valsartan; n = 6/group for LCZ696 15 mg/kg, AHU377 and benazepril times 3 days). * $p < 0.05$, *** $p < 0.001$ vs vehicle, one-way ANOVA on 0- to 12-hour AUC values.

Plasma aldosterone (ng/mL) levels were measured for 12 hours on days 1, 5 and 10 of dosing and analyzed as described above (Figure C). Vehicle had no significant effect on aldosterone levels integrated over the 12-hour period after dosing (-0.006 ng•hr/mL). AHU377 (-0.090 ng•hr/mL) and benazepril (-0.079 ng•hr/mL) resulted in modest trends ($p < 0.08$) to reduce plasma aldosterone over the 12-hour period. In contrast, LCZ696 15 mg/kg, LCZ696 45 mg/kg, and valsartan all significantly decreased plasma aldosterone levels (-0.228, -0.142, and -0.126 ng•hr/mL, respectively). The greatest reductions were observed in the LCZ696-treated groups within the first four hours after dosing, where LCZ696 15 mg/kg at 2 hours reduced aldosterone 2-fold more than valsartan ($p < 0.05$).

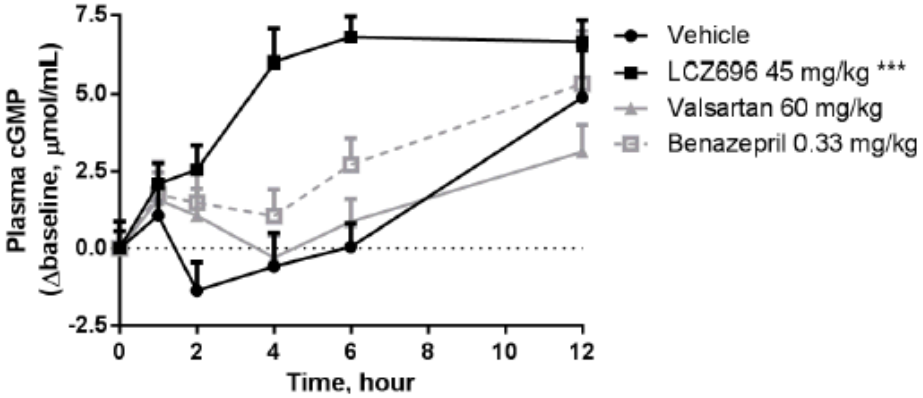
Figure C: Plasma aldosterone concentration changes after treatments in conscious dogs



Beagle dogs were dosed daily for 10 days, and plasma aldosterone concentrations (ng/mL) were measured on days 1, 5 and 10. Dogs were dosed after the time 0 sample was collected on each day. Results are mean ± SEM change from time 0 baseline (n = 12/group for vehicle, LCZ696 45 mg/kg and valsartan; n = 6/group for LCZ696 15 mg/kg, AHU377 and benazepril times 3 days). * $p < 0.05$, ** $p < 0.01$ vs vehicle, one-way ANOVA on 0- to 12-hour AUC values.

LCZ696 on treatment days 1, 5 and 10 and analyzed as described above (Figure D). Plasma cGMP levels significantly increased from baseline in all groups ($p<0.05$ vs baseline), indicating a diurnal increase of plasma cGMP over the 12-hour sampling period. When compared to vehicle, administration of LCZ696 at 45 mg/kg resulted in a significant ~180% increase in the average plasma cGMP ($p<0.0001$). In contrast, neither valsartan nor benazepril changed cGMP relative to vehicle control.

Figure D: Plasma cGMP concentration changes after treatments in conscious dogs



Beagle dogs were dosed daily for 10 days, and plasma cGMP concentrations (µmol/mL) were measured on days 1, 5 and 10. Dogs were dosed after the time 0 sample was collected on each day. Results are mean ± SEM change from time 0 baseline (n = 12/group for LCZ696 45 mg/kg and valsartan; n = 6/group for vehicle and benazepril times 3 days). *** $p < 0.0001$ vs vehicle, one-way ANOVA on 0- to 12-hour AUC values.

Average plasma cortisol levels did not differ by day, time of day, or treatment (not significant when analyzed by random-effect repeated-measures ANOVA).

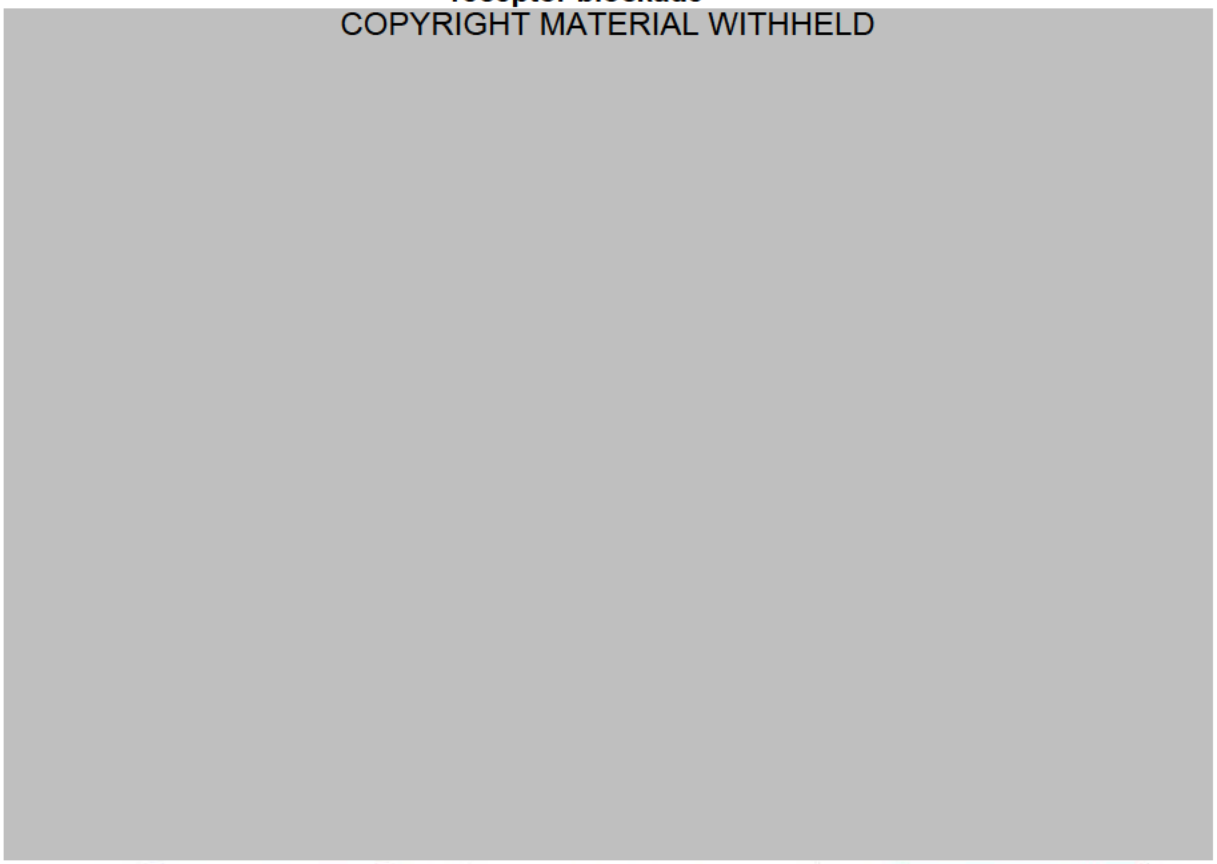
Mechanism of action

The effects of LCZ696 are attributed to the enhancement of beneficial effects of NPs and potentially other NEP targets, and the inhibition of deleterious cardiovascular and renal effects of angiotensin II and its effectors. The NP system is an important endocrine system that, together with the RAAS and the sympathetic nervous system regulates vascular tone, fluid and electrolyte balance and cardiovascular homeostasis. NPs represent a family of peptide hormones, including ANP, brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). ANP and BNP are mainly synthesized and released into the circulation by the heart in response to myocardial stress, whereas CNP is synthesized by endothelial and renal epithelial cells and acts in a paracrine manner. NPs are cleared from the circulation by NEP-dependent proteolytic degradation and through a clearance receptor (NPR-C). LCZ696 potentiates the physiological effects of NPs by inhibiting NEP-dependent degradation resulting in increased levels of NPs.

NPs exert their effects by activating membrane-bound guanylyl cyclase-coupled receptors (NPR-A and -B), resulting in increased concentrations of the second messenger cGMP that can be used as a biomarker indicative of NEP inhibition. NPs have been associated with a wide range of beneficial cardiovascular and renal effects, including reduction of blood pressure, vasodilation, natriuresis and diuresis, increased

glomerular filtration and renal blood flow, inhibition of renin and aldosterone release, reduction of sympathetic activity, and antihypertrophic and anti-fibrotic effects (Kishimoto et al 2011, de Bold 2011, Potter et al 2009, Kuhn 2009, Porzionato et al 2010). The inhibition of NEP by LBQ657 is believed to exert its effect in heart failure patients by slowing the degradation of NPs. Thus, concentrations of NPs are increased by LCZ696, which increases and prolongs the action of these peptide hormones (Figure below).

LCZ696 modulates the natriuretic peptide system while providing simultaneous AT_1-receptor blockade



LCZ696 modulates two counter-regulatory neurohormonal systems in heart failure: the NP system and the RAAS. ANG, angiotensin; (Figure adapted from Langenickel and Dole 2012)

Angiotensin II and aldosterone are the principal effector hormones of the RAAS, with effects that include vasoconstriction and renal sodium and fluid retention. These effects result in increased blood pressure and blood volume, as well as activation of cellular growth and proliferation of vascular and cardiac cells that lead to adverse remodeling of cardiovascular tissues (Mehta and Griendling 2007, Weir and Dzau 1999). LCZ696 delivers valsartan, which inhibits the RAAS by selective and competitive blockade of the binding of angiotensin II to the AT_1-receptor in many tissues, such as the vasculature, heart, kidney and the adrenal gland. LCZ696 also inhibits the RAAS via the inhibitory effects of NPs on renin and aldosterone release. In addition, AT_1-receptor blockade simultaneously to NEP inhibition mitigates harmful effects associated with reduced NEP-dependent degradation of angiotensin II.

In summary, the beneficial effects of LCZ696 in patients with heart failure result from enhancement of protective endogenous systems such as the NP system and the simultaneous inhibition of organ injury driven by activation of the RAAS.

4.2 Secondary Pharmacology

The secondary pharmacology of AHU377, LBQ657, and valsartan is summarized from *in vitro* studies that evaluate the selectivity and broad *in vitro* pharmacology profile of the compounds.

In vitro pharmacology

AHU377, LBQ657, valsartan, and several other compounds were assessed for selectivity and potency against ten proteases that are closely related to NEP or are associated with the NP/RAAS pathways (Table below). NEP is a member of the M13 zinc metalloproteinase family, where NEP-2, endothelin converting enzyme (ECE)-1, and ECE-2 are related enzymes. While LBQ657 was a potent inhibitor of human NEP enzyme (IC_{50} = 2.3 nM), it was a much weaker inhibitor of NEP-2 (IC_{50} = 84,700 nM, 36,826-fold less potent), ECE-2 (IC_{50} = 5,500 nM; 2391-fold less potent), and ECE-1 (IC_{50} >100,000 nM). LBQ657 showed no inhibitory effect on insulin degrading enzyme (IDE), aminopeptidase N (APN), aminopeptidase P (APP- 2), meprin-α/β or dipeptidylpeptidase (DPP)-4 at concentrations up to 100 µM. Both AHU377 and valsartan showed no effect on any of the enzymes tested at concentrations up to 100 µM. LBQ657 was >2000-fold selective for NEP over all others enzymes that were tested.

Three reference compounds were evaluated under the same assay conditions. Enalaprilat (active metabolite of enalapril) was a potent and selective angiotensin converting enzyme (ACE)-1 inhibitor with an IC_{50} of 0.3 ± 0.03 nM and had no effect on the other enzymes tested at concentrations up to 100 µM. Thiorphan was a modestly potent inhibitor of NEP (30 nM) with inhibitory effects on NEP-2 and ACE-1 at concentrations 37-fold (IC_{50} 1.1 ± 0.3 µM) and 117-fold (IC_{50} 3.5 ± 0.8 µM) higher, respectively. Omapatrilat was a potent ACE-1 inhibitor (IC_{50} of 1.4 ± 0.5 nM) with modest inhibitory effect on NEP (IC_{50} 110 ± 20 nM), NEP-2 (IC_{50} 700 ± 300 nM), and APP-2 (IC_{50} 600 ± 100 nM) [RD-2013-00079], [RD-2005-50314], [RD-2005-50318].

***In vitro* IC_{50} values of LBQ657, AHU377, valsartan, and reference compounds on human enzyme activities**

Enzyme	LBQ657	AHU377	Valsartan	Enalaprilat	Thiorphan	Omapatrilat
NEP	0.0023 ± 0.0004	16.7 ± 2.3	>100	>100	0.03 ± 0.004	0.11 ± 0.02
NEP-2	84.7 ± 2.8	>100	>100	>100	1.1 ± 0.3	0.7 ± 0.3
ACE-1	>100	>100	>100	0.0003 ± 0.00003	3.5 ± 0.8	0.0014 ± 0.0005
ECE-1	>100	>100	>100	>100	>100	>100
ECE-2	5.5 ± 0.6	>100	>100	>100	>100	>100
IDE	>100	>100	>100	>100	>100	>100
APN	>100	>100	>100	>100	>100	>100
APP-2	>100	>100	>100	>100	>100	0.6 ± 0.1
Meprin-α	>100	>100	>100	>100	>100	>100
Meprin-β	>100	>100	>100	>100	>100	>100
DPP-4	>100	>100	>100	>100	>100	>100

IC_{50} values are µM. Results [RD-2013-00079] are expressed as mean ± SD (n≥4 determinations)

AHU377 was assessed for its off-target activity on 57 G protein-coupled receptors (GPCRs), transporters, ion channels, nuclear receptors, and enzymes. A greater than 50% inhibition or activation at 30 µM (the highest concentration tested on all targets) was only found in the cholecystokinin CCKa receptor assay (IC_{50} = 15.5 µM, n=2). In a functional follow-up cell-based assay, it was determined that AHU377 inhibited the CCKa receptor by 21% at 30 µM [RD-2012-50571]. AHU377 was also tested against a panel of 62 kinases at a concentration of 30 µM. No significant inhibitory effects (greater than 50% inhibition at 30 µM) were observed in any assay [RD-2013-00245].

LBQ657 was assessed for its off-target activity on 129 GPCRs, transporters, ion channels, nuclear receptors and enzymes. These assays were run at concentrations up to 30 µM or at the single concentration of 10 µM. No relevant effect (greater than 50% inhibition or activation at 30 µM or 10 µM, respectively) was found in any assay [RD-2012-50566]. LBQ657 was also tested against a panel of 62 kinases at a concentration of 30 µM. A greater than 50% inhibition at 30 µM was observed in only one of the 62 assays; LBQ657 weakly inhibited mitogen-activated protein kinase kinase kinase kinase 4 (MAP4K4) with an IC_{50} of > 22 µM [RD-2013-00246].

Valsartan was assessed for its off-target activity on 81 GPCRs, transporters, ion channels, nuclear receptors and enzymes. No significant effect (greater than 50% inhibition or activation at 10 µM) was found in any assay except for the AT_1 receptor (K_i = 0.0023 µM), which is the primary target of this compound.

In vivo pharmacology

Evaluation of effect on Bradykinin (BK) action

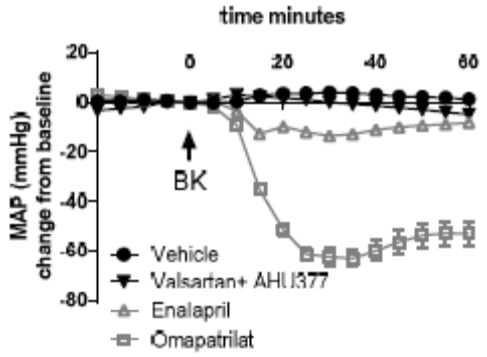
BK is a peptide hormone that can cause vasodilation and natriuresis, and an increase in vascular permeability. Overactivation of BK is associated with angioedema and may be an important factor in angioedema induced by omapatrilat (Sulpizio et al 2004, Knecht

et al 2014). NEP cleaves BK_{1-9} between proline-7 and phenylalanine-8. Several other enzymes are also known to cleave BK_{1-9} including ACE (kininase II), APP, DPP-4, and kininase I. Studies were conducted in two animal models to evaluate the potential of AHU377 alone, or in the presence of valsartan, to potentiate the biologic effects of BK as a consequence of inhibiting BK inactivation. Results from these studies showed no potentiation by AHU377 in the presence of valsartan to potentiate BK action. In contrast, ACE inhibitors or omapatrilat potentiated the effects of BK in these models.

Effect on BK-induced paw edema in rats
The effects of AHU377 (100 mg/kg, p.o.), valsartan (30 mg/kg, p.o.), and the combination were studied in the BK-induced paw edema model in conscious SHR or Sprague Dawley rats. Neither the individual treatments nor their concomitant use potentiated the BK-induced paw edema beyond that of the vehicle-treated animals. Accordingly, there were no significant effects of treatment on the change in paw volume over time (repeated-measures two-way ANOVA), on the peak response (one-way ANOVA), or on the time-weighted average response [RD-2005-50363]. In contrast, the comparator ACE inhibitor enalapril and omapatrilat potentiated the BK response in this model [RD-2005-50307].

Effect on BK-induced hypotension in rats
Male Sprague Dawley rats were anesthetized with thiobutabarbital (Inactin). Catheters were inserted into a femoral artery for recording mean arterial pressure and into a femoral vein for infusion of BK. The Figure below shows that concomitant administration of AHU377 (100 mg/kg i.d.) and valsartan (30 mg/kg i.d.) did not cause any change in the hemodynamic response to a 60-minute i.v. infusion of BK (1 µg/kg/min at 20 µL/min) relative to vehicle controls. Likewise, neither compound on its own affected the BK blood pressure response. In contrast, pre-treatment with enalapril or omapatrilat caused a significant depressor effect during BK infusion [RD-2005-50365], [RD-2005-50366]. Enhanced BK activity by the ACE inhibitor enalapril and further potentiation of its effects by the ACE/NEP/APP inhibitor omapatrilat are consistent with the rank order of clinical angioedema liability with these respective agents. The lack of effect on BK metabolism by valsartan plus AHU377 suggests that this combination has low potential for inducing angioedema in humans.

BK potentiation of blood pressure lowering by treatments in anesthetized Sprague Dawley rats



Blood pressure was measured in anesthetized rats that received drug at time -90 minutes. BK was infused starting at time 0 and the blood pressure response was measured for 60 min. Drugs were administered i.d.; valsartan 30 mg/kg, AHU377 100 mg/kg, enalapril 30 mg/kg, and omapatrilat 30 mg/kg. The AUC reduction for valsartan + AHU377 was not different from vehicle, whereas enalapril and omapatrilat were significantly different from vehicle ($p < 0.05$; ANOVA followed by Tukey's HSD multiple comparisons). (Figure adapted from [RD-2005-50365] and [RD-2005-50366])

4.3 Safety Pharmacology

Safety pharmacology studies were conducted to assess the effects of LCZ696 on vital organ systems. Studies with LCZ696 included an *in vitro* electrophysiological study, a cardiovascular telemetry study in monkeys, and assessments of the respiratory and central nervous system in rats. Safety pharmacology studies were also conducted with AHU377 and included an *in vitro* electrophysiological study, a cardiovascular study in telemeterized dogs, an assessment of the respiratory system in rats, and an assessment of the central nervous system in mice. Most of the studies were conducted in accordance with ICH guidelines S7A and S7B; those that were not were also conducted with scientific rigor, applying standards that are consistent with good laboratory practice (GLP) and thus are considered adequate to support registration. The *in vivo* central nervous system (CNS), respiratory, and cardiovascular telemetry studies were performed in accordance with GLP guidelines. Safety pharmacology studies have not been conducted with valsartan, which was approved in the US prior to the issuance of ICH Safety Pharmacology Guidance (ICH S7A 2001), (ICH S7B 2005). Clinical studies with Diovan have not identified adverse effects on cardiovascular, respiratory or central nervous system function.

Cardiovascular

Cardiovascular safety pharmacology studies with LCZ696
In an in vitro electrophysiology study [Study 0670356] the effect of LCZ696 (batch no. 0651002) on hERG channel current (IKr, the rapidly activating, delayed rectifier cardiac potassium current), expressed in mammalian cells (HEK293) was evaluated using the patch-clamp technique. In this non-GLP study, four concentrations of LCZ696 (10, 100, 787 and 3,000 µM) were evaluated in order to determine the concentration-

response relationship. At least three cells (n≥3) were exposed to each of these concentrations at physiological conditions (35 ± 2ºC).

LCZ696 inhibited hERG current (mean ± SEM) by 1.8 ± 0.6% at 10 µM (n=3), 1.7 ± 0.2% at 100 µM (n=3), 2.9 ± 0.3% at 787 µM (n=3) and by 32.4 ± 0.7% at 3,000 µM (n=4), vs 0.7 ± 0.2% in control (n=4). The IC_{50} for the inhibitory effect of LCZ696 on hERG current was not determined but estimated to be higher than 3,000 µM. Assuming 3000 µM LCZ696 dissociates into approximately 3,000 µM valsartan and 3,000 µM AHU377, this concentration is greater than 2700X the clinical exposure to valsartan and AHU377 (unbound Cmax) associated with a 200 mg LCZ696 BID dose. Under identical conditions, the positive control terfenadine produced a 78.8 ± 2.6% (mean ±SD) blockade of the hERG current at 60 nM when tested on two cells (n=2).

In an *in vivo* telemeterized cynomolgus monkey study [Study 0670360], the pharmacological effects of LCZ696 on hemodynamic and electrocardiographic parameters were evaluated. In this GLP study, LCZ696 (batch no. 0650001) was administered to 3 animals as single oral (gavage) doses of 0, 25 or 100 mg/kg (dose volume of 5 mL/kg) using a cross-over dosing paradigm with a 7-day washout period between each dose. Due to animal replacement, three monkeys received a single oral (gavage) dose of vehicle, 25 and 100 mg/kg of test article; one monkey received a single dose of 25 mg/kg of test article, and another monkey received a single dose of vehicle and 100 mg/kg to complete a 3x4 cross-over design.

The following parameters were obtained in all animals: clinical signs, arterial blood pressures (mean, systolic and diastolic blood pressure, pulse pressure), heart rate, electrocardiographic intervals (PR, RR, QRS, QT, QTc and ST evaluation), and qualitative evaluation of the electrocardiographic waveforms. Endpoints were evaluated twice prior to each dose (at least 30 minutes apart) and at approximately 30 minutes, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 hours post-dose for each dose level.

No deaths or test-article-related clinical signs were observed following exposure to the test article. LCZ696 at 25 mg/kg had no effect on hemodynamic and electrocardiographic parameters for up to 24 hours following administration. LCZ696 at 100 mg/kg had no adverse effects on the heart rate, pulse pressure, and ECG intervals (PR, QRS, QT and QTc) for up to 24 hours post dose. A qualitative review of ECGs showed no treatment-related waveform abnormalities or arrhythmias.

Slight decreases in the mean arterial (14 mmHg; 17.9% from baseline), systolic (13 mmHg; 14.5% from baseline) and diastolic blood pressures (14 mmHg; 22% from baseline) were noted starting 6 hours following the oral administration of 100 mg/kg of LCZ696, when compared both to baseline and the same time points in the control animals, with all values returning to baseline level by 18 hours post dose.

A 100 mg/kg dose of LCZ696 delivered Cmax exposure of 6,250 ng/mL for valsartan, 8300 ng/mL for AHU377 and 53,900 ng/mL for LBQ657 (as extrapolated from the day 1 exposure at 100 mg/kg in [Study 0670282]. The human Cmax for a 200 mg BID dose

was 6,044 ng/mL for valsartan, 2,408 ng/mL for AHU377 and 16,345 ng/mL for LBQ657). This 100 mg/kg dose in primates provided valsartan Cmax exposures similar to those achieved at the 200 mg BID clinical dose; exposures to AHU377 and LBQ657 were approximately 10X and 8X the clinical Cmax, when corrected for differences in protein bindings (AHU377 is 91% protein bound/9% unbound in monkey and 97% bound/3% unbound in human; LBQ657 is 93% bound/7% unbound in monkey and 97% bound/3% unbound in human).

In conclusion, a single oral administration of LCZ696 at doses of 25 and 100 mg/kg resulted in no test-article-related changes on the electrocardiographic parameters of the ECG. LCZ696 at 25 mg/kg did not show effects on hemodynamic parameters in the cynomolgus monkey. LCZ696 at 100 mg/kg showed a slight, reversible decrease in the systolic, diastolic and mean arterial blood pressures beginning 6 hours post dose.

Cardiovascular safety pharmacology studies with AHU377

In an *in vitro* electrophysiology study [Study 0359201] the effect of AHU377 (batch no. 1) on hERG channel current IKr expressed in mammalian cells (HEK293) was evaluated using the patch-clamp technique in the whole-cell configuration at 36 ± 1ºC. Cells were exposed to each concentration for approximately 10 minutes. In this non-GLP study, cells (n=5) were exposed to AHU377 at 1 mM (383.5 µg/mL), and AHU377 was found not to inhibit hERG tail current. As higher concentrations could not be evaluated due to insolubility of AHU377, the concentration-response curve and the frequency-dependency of the AHU377 hERG tail current inhibition could not be determined. The highest soluble concentration of AHU377 tested (1 mM or 383.5 µg/mL) did not change hERG current amplitude relative to vehicle control. This concentration was more than 5,000 times the unbound Cmax concentration of AHU377 at the 200 mg BID dose (total Cmax of 2,408 ng/mL; unbound Cmax of 72.24 ng/mL or 0.18 µM based on 97% bound/3% unbound). Under identical conditions, the positive control E-4031 produced a 92.8% (mean ±SD) blockade of the hERG current at 100 nM when tested on two cells (n=2), an effect consistent with its known activity.

In conclusion, AHU377 did not inhibit hERG channels stably expressed in HEK293 cells at its highest soluble concentration of 1 mM. This concentration was greater than 5000X the clinical exposure to AHU377 (unbound Cmax) associated with a 200 mg BID dose.

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In an *in vivo* telemeteterized beagle dog study [Study 0470026], AHU377 (batch no. 0457001) was administered orally via gavage as a suspension in 0.5% (w/v) sodium carboxymethylcellulose, Type 7HF, aqueous solution (0.5% CMC) to four telemeterized female beagle dogs. On day 1, these animals were dosed with vehicle (0.5% CMC); on day 4 with AHU377 at 50/52.3 mg/kg (base/salt), and on day 8 with AHU377 at 250/261.5 mg/kg (base/salt). An additional group consisting of one female dog was dosed on days 1, 4 and 8 with Purified Water, USP at an equivalent dosing volume of 12 mL/kg and served as a system control. Cardiovascular data (mean arterial, systolic and diastolic blood pressure, heart rate and electrocardiographic) and body temperature were collected using the implanted transmitters. Measurements were recorded for approximately one minute every five minutes on a continuous basis for the duration of the study. The following data points were analyzed at baseline and after each dose and reported: 1 minute of data at approximately 0.25, 0.5, 1, 2 and 4 hours post dose.

There were no test-article-related alterations in blood pressure, heart rate or body temperature. In addition, there were no electrocardiographic changes associated with the administration of the compound.

In conclusion, oral administration of AHU377 at single doses up to 250 mg/kg to female beagle dogs had no adverse effect on cardiovascular function as measured by telemetry. The resulting Cmax exposure to LBQ657 (2,686 ng/mL total; 537 ng/mL unbound; assuming 80% bound and 20% unbound; as extrapolated from a single dose of 24 mg/kg dose in [CRA-11-014 Main Report]) was approximately 11-fold higher than the Cmax observed following dosing of LCZ696 200 mg BID to patients with heart failure.

Respiratory

Respiratory safety pharmacology studies with LCZ696
In an *in vivo* Wistar Hannover rat study [Study 0670393], the effects of a single oral dose of LCZ696 on the respiratory system were evaluated by “head out” plethysmography. In this GLP study, LCZ696 (batch no. 0650001) suspensions in 0.5% CMC were administered to male rats (n=6/group) at dose levels of 0, 200, or 600 mg/kg (dose volume of 5 mL/kg). Animals were placed in head out plethysmographs and,

following a *ca.* 15-minute settling period, ventilatory parameters (tidal volume, respiratory rate and derived minute volume) were measured for 15 minute periods at the following time points: pre-dose and at approximately 1, 2, 4 and 24 hours post dose. No deaths or treatment-related clinical signs were recorded following exposure to LCZ696. No adverse changes in respiratory function were noted following administration of LCZ696. In conclusion, oral administration of LCZ696 to male rats, at doses of 200 and 600 mg/kg, did not produce any adverse effects on respiratory function.

The highest evaluated LCZ696 dose (600 mg/kg) resulted in unbound Cmax (from day 1 [Study 0670220]) exposure multiples of 1.2-fold (valsartan), 0.24-fold (AHU377), and 2.4-fold (LBQ657) at LCZ696 200 mg BID in humans.

Respiratory safety pharmacology studies with AHU377

In an *in vivo* Wistar Hannover rat study [Study 0480095], the effects of a single oral dose of AHU377 on the respiratory system were evaluated by “head out” plethysmography. In this GLP study, AHU377 (batch no. 0457001) suspensions in 0.5% CMC were administered to male rats (n=6/group) at dose levels of 0, 250, 1,000, or 2,000 mg/kg (base) (dose volume of 10 or 20 mL/kg). Animals were placed in head out plethysmographs and ventilatory parameters (tidal volume, respiratory rate and derived minute volume) were measured for 15-minute periods at the following time points: pre-dose and at approximately 1, 2, 6 and 24 hours post dose. No deaths or test article-related clinical signs were observed following administration of AHU377. No adverse compound-related effects on respiratory function were observed. In conclusion, oral administration of AHU377 to male rats at doses up to 2,000 mg/kg produced no adverse effects on respiratory function, and all parameters were within normal limits for healthy, untreated animals.

The highest evaluated AHU377 dose (2,000 mg/kg) resulted in an unbound LBQ696 Cmax (from day 1 [Study 0670107]) exposure multiple of 8-fold at LCZ696 200 mg BID in humans.

CNS safety pharmacology studies

CNS safety pharmacology studies with LCZ696

In an *in vivo* Wistar Hannover rat study [Study 0670464], the neuropharmacological profile of LCZ696 was evaluated following a single oral dose. In this GLP study, LCZ696 (batch no. 0651002) suspensions in 0.5% CMC were administered to male rats (n=10/group) at dose levels of 0, 200, or 600 mg/kg (dose volume of 5 mL/kg). The rats were observed for signs of neuropharmacological or toxicological activity at approximately 15, 30, and 45 minutes and 1 2, 3, 4 and 24 hours post dose. Body temperatures were taken on all animals using a rectal thermometer at 60 minutes post dose. There were no apparent neuropharmacological or toxicological signs observed in any animals from 15 minutes through 3 hours or at 24 hours post dose. A decrease in fecal excretions was noted in the 600 mg/kg dose group at 4 hours post dose. No biologically relevant compound-related changes in body temperature were noted.

The highest evaluated LCZ696 dose (600 mg/kg) resulted in unbound Cmax (from day 1 [Study 0670220]) exposure multiples of 1.2-fold (valsartan), 0.24-fold (AHU377), and 0.42-fold (LBQ657) at LCZ696 200 mg BID in humans.

Observations of increased locomotor activity and hypersensitivity to touch were observed sporadically in some repeated-dose rodent studies with LCZ696 in mice at doses ≥50 mg/kg/day [Study 0670524] and rats at doses ≥30 mg/kg/day [Study 0670283] but not in primates; given the sporadic nature of these findings they were not deemed to be of toxicological significance.

CNS safety pharmacology studies with AHU377
In an *in vivo* study in CD-1 mice [Study 0470089], the general and neurobehavioral activity of AHU377 was evaluated following a single oral dose. In this GLP study, vehicle (n=5/group) or AHU377 (batch no. 0457001) at 2000 mg/kg (n=10/group) as a suspension in 0.5% CMC was administered to male mice at a volume of 20 mL/kg. The mice were observed for signs of neurobehavioral activity at 15 minutes and 1 2, 6 and 24 hours post dose. Body temperatures were taken on all animals in pretest and a 1 hour post dose. There were no test-article-related effects on neurobehavioral activities or body temperature and no test-article-related clinical signs or gross lesions were observed at 2000 mg/kg. As a result, lower doses were not examined. In conclusion, no effects of AHU377 on general or neurobehavioral activities in male mice were observed at a single dose of 2000 mg/kg.

The highest evaluated AHU377 dose (2000 mg/kg) resulted in an unbound LBQ696 Cmax (extrapolated from day 1 at 1,200 mg/kg [Study 0770782]) exposure multiple of 120-fold at LCZ696 200 mg BID in humans.

Pharmacodynamic Drug Interactions

No studies were performed with LCZ696 to evaluate pharmacodynamic drug interactions.

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5 Pharmacokinetics/ADME/Toxicokinetics

5.1 ADME

Summary

This section summarizes all studies which investigated the absorption, distribution, metabolism, and excretion (ADME) of AHU377, LBQ657 and valsartan in preclinical species. The *in vivo* ADME studies using ^{14}C-labeled drug substance were performed with i.v. and p.o. dosing. The first section, "Methods of Analysis", describes the radiolabeled drug used, the animal studies and the methods used for characterization and analysis of AHU377, LBQ657, valsartan and their metabolites in biological samples. The remaining sections provide comparative ADME information, summarizing the results obtained in animals and comparing them with the corresponding data in humans.

CD-1 mice ((b) (4)), HanWistar rats ((b) (4)), beagle dogs ((b) (4)) and cynomolgus monkeys ((b) (4)) were housed in appropriate metabolism cages and were provided with food and water *ad libitum*. The animals received a designated range of oral doses or an intravenous reference dose as shown in the Table below. The majority of these doses was within the dose ranges and formulated in the same manner as used in the LCZ696, AHU377, and valsartan toxicity studies. Results from a relevant study in humans, which are also described in [Summary of Clinical Pharmacology] are included here for comparison purposes.

After dosing, serial blood or plasma samples and quantitative urine and feces were collected from each species. Milk samples were collected from a selected group of rats. Distribution of radioactivity in pigmented rats and pregnant rats was investigated quantitatively by whole-body autoradiography. The binding of AHU377, LBQ657, and valsartan to plasma proteins of mouse, rat, dog, monkey, and human was determined *in vitro* by the ultracentrifugation or equilibrium dialysis method.

LCZ696, AHU377 or LBQ657 ADME and selected TK and clinical studies

Species	Radiotracer	Dose (mg/kg)	Dosage Form*	Dose Route	Dose Duration [a]	Reference
Mouse	^{14}C	30 (LBQ)	solution	i.v.	S	R1200673-01
	^{14}C	150 (AHU)	suspension	p.o.	S	R1200673-01
	- [b]	400/day (AHU)	suspension	p.o.	M	R0770896
	-	800/day (AHU)	suspension	p.o.	M	R0770896
	-	1200/day (AHU)	suspension	p.o.	M	R0770896
Rat	^{14}C	15 (AHU)	solution	i.v.	S	R0300247-1
	^{14}C	45 (AHU)	solution	p.o.	S	R0300247-1
	^{14}C	15 [c] (AHU)	solution	i.v.	S	R0300247-2
	^{14}C	45 [c] (AHU)	solution	p.o.	S	R0300247-2
	^{14}C	25 [d] (LCZ)	solution	p.o.	S	R1200674
	^{14}C	30 [e] (LCZ)	solution	p.o.	S	R1200622
	-	10/day(LCZ)	suspension	p.o.	M	R0670283
	-	30/day (LCZ)	suspension	p.o.	M	R0670283
	-	100/day (LCZ))	suspension	p.o.	M	R0670283
	-	400/day (AHU)	suspension	p.o.	M	R0770711-01
	-	800/day (AHU)	suspension	p.o.	M	R0770711-01
	-	1200/day (AHU)	suspension	p.o.	M	R0770711-01
	-	30/day (LCZ)	suspension	p.o.	M	R0670390
	-	100/day (LCZ)	suspension	p.o.	M	R0670390
	-	200/day (LCZ)	suspension	p.o.	M	R0670390
Rabbit	-	3/day (LCZ)	suspension	p.o.	M	R0670280
	-	10/day (LCZ)	suspension	p.o.	M	R0670280
	-	30/day (LCZ)	suspension	p.o.	M	R0670280
Dog	^{14}C	3 (LBQ)	solution	i.v.	S	R0301244
	^{14}C	15 (AHU)	solution	p.o.	S	R0301244
Monkey	^{14}C	10 (LBQ)	solution	i.v.	S	R1200729-01
	^{14}C	30 (LCZ)	solution	p.o.	S	R1200729-01
	-	30/day (LCZ)	suspension	p.o.	M	R0670621
	-	100/day (LCZ)	suspension	p.o.	M	R0670621
	-	300/day (LCZ)	suspension	p.o.	M	R0670621]
Human	^{14}C	200 mg/subject (LCZ)	(b) (4)	p.o.	S	CLCZ696B2105 CLCZ696B2105AM1

LBQ: LBQ657, AHU: AHU377, LCZ: LCZ696 (for [^{14}C]LCZ696, the labeled position is on AHU377).
*Based on the solubility of LCZ696 in water (>100 mg/mL), the majority of suspensions containing 0.5% CMC will be a solution.
[a] S = single dose; M = multiple daily dose
[b] Non-radiolabeled
[c] Tissue distribution in pigmented rat, whole body autoradiography
[d] Tissue distribution in pregnant rat, whole body autoradiography
[e] Passage into milk study
Source [Table 2.6.5.3A-R1200673-01], [Table 2.6.5.4A-R0770896], [Table 2.6.5.3B-R0300247-1], [Table 2.6.5.5- R0300247-2], [Table 2.6.5.8-R1200674], [Table 2.6.5.9I-R1200622], [Table 2.6.5.4B-R0670283], [Table 2.6.5.4B-R0770711], [Table 2.6.5.7A-R0670390], [Table 2.6.5.7B-R0670280], [Table 2.6.5.3A-R0301244], [Table 2.6.5.3A-R1200729-01], [Table 2.6.5.4C-R0670621], [CLCZ696B2105 and CLCZ696B2105AM1]

Absorption and Pharmacokinetics

In general, LCZ696 was well absorbed in all animal species (65-100%) after p.o. dosing. In human, absorption was estimated to be at least 61%. The absorption was relatively rapid in rate and onset in all species (mouse, rat, dog, rabbit, monkey, and human) with Tmax ranging from 0.25 - 2 h. Bioavailability of LBQ657 was moderate to high (41-100%) in animals after p.o. administrations and was estimated to be greater than 50% in human. The apparent terminal half-life of LBQ657 and/or valsartan after a p.o. dose ranged from 1 - 6 h in the preclinical species and 12 - 21 h in humans. In the

toxicokinetic (TK) studies, the AUC values of AHU377, LBQ657, and valsartan were generally proportional to the dose in all animal species (mice, rats, rabbits, and monkeys). There was no evidence of gender differences in the exposure. No evidence of accumulation after multiple doses for rats and monkeys was observed.

Distribution

AHU377, LBQ657, and valsartan uptake into blood cells was not significant and therefore drug concentrations in plasma were higher than in blood. Both AHU377 and LBQ657 were moderately to highly bound to plasma proteins with some species differences (80-97%). In human, serum albumin was found to be the primary binding protein for AHU377 and LBQ657 as compared to α_1-acid glycoprotein. Similarly, valsartan was bound to serum proteins with some species differences (82-97%). In Human, valsartan is mainly bound to serum albumin.

AHU377-related radioactivity (AHU377 and its metabolites) was widely and rapidly distributed to most rat tissues following a single i.v. or p.o. dose of AHU377. The highest tissue radioactivity (2-4 fold higher than blood) was found in kidney and liver after i.v. dose. The lowest radioactivity levels were observed in brain, eye, seminal vesicles and spinal cord. AHU377-related radioactivity showed minimal brain and testis penetration. The drug-related radioactivity also exhibited low affinity to melanin (pigmented skin and uveal tract). Tissue distribution patterns of the drug-related radioactivity following the p.o. dose were similar to those following the i.v. dose. The tissue peak concentration of the radioactivity was reached at 1 h for most tissues, with the highest concentrations observed in the kidney and liver. After 24 h postdose, radioactivity in the tissues was below the limit of quantification (LOQ) except for the kidneys.

After an i.v. dose of valsartan, relatively high concentrations of radioactivity were found in blood, plasma, liver and kidney. Valsartan-related radioactivity showed minimal brain and testis penetration. After p.o. dosing, the pattern of distribution was similar to that following the i.v. dose. By either route of administration, residual radioactivity after 7 days was at or below the limits of detection in all investigated tissues except liver.

In pregnant rats, the AHU377-related radioactivity showed a low distribution to fetal organs with a fetus-to-maternal blood ratio less than 0.51 at fetus Tmax. In pregnant rabbits the relative exposure of fetus to maternal plasma was low (less than 0.21) indicating that LCZ696 was poorly absorbed into the fetus.

Metabolism

[^{14}C]LCZ696 delivers AHU377 and valsartan. AHU377 was predominantly converted to LBQ657 via esterase catalyzed hydrolysis in liver slices from rat, dog and human. LBQ657 was not significantly further metabolized in liver slices. *In vitro* biotransformation of valsartan was investigated with the post-mitochondrial liver fraction (S12) of mouse, rat, rabbit, dog, marmoset and human. The oxidative metabolism was slow and minimal. Cytochrome P450 (CYP) 2C9 is the enzyme responsible for the formation of 4- hydroxyvaleryl metabolite in human microsomes.

Following oral administration of LCZ696 to rabbit, monkey and human, AHU377, LBQ657 and valsartan were identified in plasma. Similarly, AHU377 and LBQ657 were identified in plasma/blood after oral dosing of AHU377 to mouse, rat and dog. AHU377 primarily underwent ethyl ester hydrolysis to form LBQ657 in all species. The rate of conversion from AHU377 to LBQ657 was rapid in mouse, rat and human and LBQ657 was the major component in plasma. The rate of conversion was moderate in dog and monkey with both AHU377 and LBQ657 being prominent components in plasma. Several minor metabolites were also identified in plasma from various species. LBQ657 was the major component recovered in excreta from all species studied, accounting for ~70-100% of the oral dose. Unchanged AHU377 recovered in excreta was minimal in all species studied, accounting for <1-14% of the oral dose.

Excretion

LCZ696 was eliminated primarily as LBQ657 and valsartan with minimal AHU377 and some minor metabolites in both animals and humans. In mice, rats and dogs, the excretion of AHU377-related radioactivity was predominantly in the feces. In monkeys and humans, however, AHU377-related radioactivity was excreted slightly more into urine (~42-65% of the dose). Valsartan was excreted mainly via biliary-fecal route in mice, rats, dogs, marmosets, and human. In human, 9% and 86% of the valsartan dose was excreted in urine and feces, respectively.

Drug-drug interactions

Enzyme inhibition

AHU377 showed little or no inhibition (IC_{50} >100 μM) in *in vitro* assays using human cytochrome P450 (CYP) enzymes 1A2, 2C9, 2D6, 2E1, 3A4/5, and only a weak inhibition potential against CYP2C8 (IC_{50} ~15 μM) and 2C19 (IC_{50} ~20 μM). Based on the Cmax of AHU377 (5.9 □M) observed in heart failure patients treated with LCZ696 200 mg BID, no drug-drug interactions (DDI) between AHU377 and co-medications that are CYP substrates are expected. LBQ657 demonstrated weak inhibition potential against CYP2C9 (IC_{50} ~40 μM) in *in vitro* assays. The potential inhibition of CYP2C9 was investigated in clinical trial [CLCZ696B2112] which showed that no interaction exists between LCZ696 and warfarin, a drug metabolized by CYP2C9. LBQ657 elimination is not anticipated to be influenced by CYP enzyme inhibitors, since it is mainly eliminated unchanged. Therefore, the potential for a DDI between LBQ657 and concomitantly administered drugs is considered low.

Valsartan did not inhibit CYP enzymes 1A2, 2A6, 2C19, 2D6, 2E1, or 3A4/5 to any significant extent. It marginally inhibited CYP2C9 with a relatively high Ki value (135 μM). No interaction was observed in a clinical drug interaction study with warfarin. CYP2C9 is the enzyme responsible for the formation of 4-hydroxyvaleryl metabolite of valsartan in human microsomes. As valsartan was predominately excreted unchanged in urine and feces. CYP-mediated DDIs between valsartan and co-administered drugs are negligible.

Enzyme induction
AHU377 and LBQ657 (up to 100 μM) did not induce the expression and/or catalytic activities of CYP1A2, CYP2B6, CYP2C9, or CYP3A in primary human hepatocytes. Valsartan did not induce the expression and/or catalytic activities of CYP1A2, CYP2B6, CYP2C9, or CYP3A in primary human hepatocytes.

Transporters
Inhibition studies indicated the likely involvement of P-glycoprotein (P-gp) in AHU377 transport; however, this is not predicted to have a significant effect on its oral absorption because a high absorption (> 65%) was observed in animals. AHU377 concentrations up to 50 μM did not inhibit multi-drug resistance protein (MRP2) or P-gp transport activity and only very weakly inhibited breast cancer resistance protein (BCRP)-mediated transport activity. Similarly, LBQ657 was found not to be an inhibitor of P-gp and BCRP activities, suggesting the low likelihood of pharmacokinetic interactions when co-administered with P-gp and BCRP substrates. The *in vitro* results suggested that AHU377 and LBQ657 are weak inhibitors of multidrug and toxin extrusion transporter 1 (MATE1) and 2-K (MATE2-K). This interaction is likely not clinically relevant.

The *in vitro* results suggested that active transport by hepatic organic anion-transporting polypeptide (OATP)1B1 and OATP1B3 contributes to the systemic clearance of LBQ657. LBQ657 clearance may be altered when co-administer with drugs that inhibit such transport. LBQ657 was shown to be an *in vitro* inhibitor of OATP1B1 (IC_{50} = 126 ± 26 μM) but not OATP1B3. Based on the Cmax of LBQ657 (43 μM) observed therapeutically, it is unlikely that LBQ657 will increase the systemic exposure of OATP1B1 substrates. AHU377, LBQ657, and valsartan did not inhibit hepatic organic cation transporters 1 (OCT1) or renal OCT2 in the *in vitro* assays. AHU377 has an *in vitro* inhibitory effect on OATP1B1, OATP1B3, and OAT3 with IC_{50} values of 1.91 ± 0.56 μM, 3.81 ± 2.2 μM, and 0.795 ± 0.058 μM, respectively. As a result, AHU377 may increase the systemic exposure of OATP1B substrates *in vivo*. However since LBQ657 is formed following the hydrolysis of AHU377, at steady state, circulating plasma LBQ657 concentrations have already been adjusted accordingly and are not altered to any further extent. *In vivo* inhibition of renal OAT3 transport activity is unlikely due to insufficiently high enough unbound concentrations at the site of expression. Valsartan was found to be both an inhibitor and substrate of OAT1 and OAT3 *in vitro* but it was transported poorly by OAT1. LBQ657 was found to be an *in vitro* inhibitor and substrate of OAT3 but not OAT1.

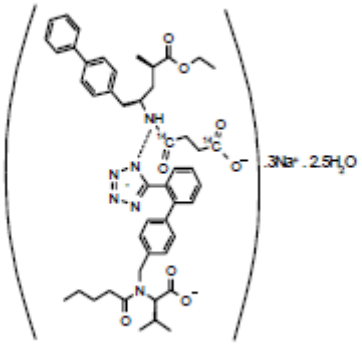
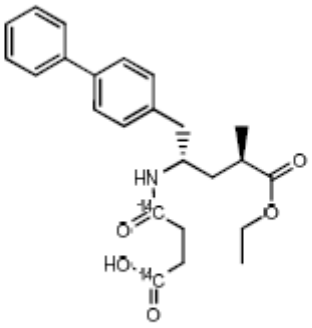
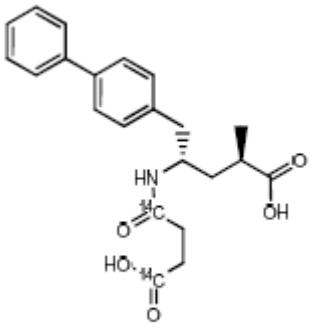
Valsartan was found to be an *in vitro* substrate of OATP1B1, OATP1B3 and MRP2 in the test system of human hepatocytes and doubly transfected (OATP1B1/MRP2) Madin-Darby Canine Kidney II (MDCKII) cells. Based on this information, although the clinical relevance has not been proved yet, co-administration of the inhibitors of these uptake or efflux transporters may increase the systemic exposure to valsartan. The *in vitro* results suggested that valsartan poorly inhibited the transport activity of MATE1 and MATE2-K and this is unlikely to be clinically relevant.

Methods of Analysis

Isotopically labeled LCZ696, AHU377, and LBQ657

Radiolabeled LCZ696, AHU377, and LBQ657 were synthesized by the Isotope Laboratory, Novartis Pharma (East Hanover, USA). All *in vivo* studies using radiolabeled LCZ696, AHU377, and LBQ657 were conducted with ^{14}C -labeled drug substance. The ^{14}C-labeled materials were synthesized and prepared for use in the plasma protein binding assessments, in vitro biotransformation, in vitro DDI assessment, rat QWBA (quantitative whole-body autoradiography), mouse, rat, dog, monkey, and human ADME studies with the position of the labels as shown in below.

LCZ696, AHU377, LBQ657 labeled with ^{14}C

LCZ696	AHU377	LBQ657
		
[R0900365]	[R0600259]	[R1200840A]

The chemical identity of the ^{14}C-labeled materials was confirmed by comparison of HPLC retention time, ultraviolet spectroscopy (UV) and mass spectrometry (MS) and/or NMR with the non-labeled reference standard. The radiochemical purity was typically ≥98%, as determined by HPLC with radioactivity detection.

Analysis of radioactivity

Radioactivity was measured by liquid scintillation counting. The samples were assayed in an appropriate scintillant either by counting an aliquot directly or after homogenization, and solubilization. For some metabolite profiling experiments, radioactivity was measured using a microplate scintillation counter. The tissue distribution of drug-related radioactivity was measured by QWBA.

Analysis of AHU377, LBQ657 and valsartan

Plasma and tissue concentrations of AHU377, LBQ657 and valsartan were determined by LCMS. The TK reports for the toxicity studies also describe the analytical method used. The performance characteristics of these assays were adequate for the purpose of the studies with limit of quantification of 1-10 ng/mL for plasma and urine samples, and 300 ng/g for fetal tissue samples. Drug concentrations in animal and human excreta after a dose of radiolabeled drug were determined by HPLC with radioactivity detection.

Characterization and structure elucidation of metabolites

Metabolites were characterized and quantified by HPLC with radioactivity detection. Metabolites were identified by mass spectrometry (LC-MS/MS) when possible.

Absorption

The pharmacokinetic parameters in all species are shown in the following three Tables (1-3) for total radioactivity and for AHU377, LBQ657, and valsartan.

Absorption and bioavailability

As shown in Tables 1 and 2, the absorption of orally administered LCZ696 or AHU377 ((b) (4) was relatively rapid in both onset and rate in all species, including human. In general, time to peak concentrations of drug-related radioactivity, AHU377, LBQ657 and valsartan ranged from 0.25 to 2 h after oral dosing. The rate and onset of absorption appeared to be independent of dose and frequency of administration (single versus repeated) in TK studies. In all animal species, the absorption of LCZ696 or AHU377 was high (65-100%) with a good bioavailability for LBQ657 (41%-complete) after oral dosing indicating minimal first pass effect. In the human ADME study with LCZ696, absorption was determined to be >61%, based on drug-related radioactivity in urine, with an estimated bioavailability >50% for LBQ657. Evaluation of AHU377 permeability in the *in vitro* Caco-2 cell monolayer system showed that permeability of AHU377 was moderate to high , consistent with the good absorption observed in all species. The absorption of valsartan ranged from approximately 20-70% in animal species. Exposures of valsartan from LCZ696 in dogs were three times higher than those following administration of physically combined AHU377 and valsartan, indicating enhanced absorption of valsartan from LCZ696. In addition, pharmacokinetic variability of valsartan delivered by LCZ696 administration appeared to be improved.

Pharmacokinetics and toxicokinetics

Following a single p.o. dose, the decline of total blood or plasma radioactivity in animals and humans was associated with apparent terminal half-lives ranging from 1.2 to 26 h (Table 1). The terminal half-life for AHU377 was short (1.3-3.2 h) in all species (Table 2). The terminal half-lives of LBQ657 or valsartan were relatively short in mice, rats and dog (1.1-3.1 h), but was longer in monkeys (~6 h) and humans (12-21 h). Among the animal species, dose-normalized plasma or blood exposure values (AUC) for AHU377 or LBQ657 in monkey were higher than other species (rank order: monkey >dog >rat ≈mouse) when dosed with either AHU377 or LCZ696. The lower exposure seen in mouse, rat, and dog was associated with its high clearance (as high as hepatic blood flow) (Table 3).

Table 1: Pharmacokinetic parameters of radioactivity in blood and plasma after a single oral dose ^{14}C-LCZ696 or AHU377 in various species

PK parameters	Mouse	Rat [a]	Dog [a]	Monkey	Human
p.o. dose (mg/kg or subject)	150 (AHU)	45 (AHU)	15 (AHU)	30 (LCZ)	200 mg (LCZ) (2.55 mg/kg)
Blood					
Tmax (h)	0.25	0.33	0.70	0.83	2.0
Cmax (nM)	46700	5600	13800	24700	12700
AUCinf (nM·h)	50900	20700	36500	57600	108000
Apparent terminal T1/2 (h)	1.3	2.5	26	5.2	na
Plasma					
Tmax (h)	0.25	na	na	0.83	2.0
Cmax (nM)	57900	na	na	49700	26400
AUCinf (nM·h)	66900	na	na	114000	228000
Apparent terminal T1/2 (h)	1.2	na	na	7.2	na

na = not available; All data were rounded to 2 or 3 significant figures; AHU = AHU377; LCZ = LCZ696

[a] Blood matrix was measured.

Source [Table 2.6.5.3A-R1200673-01], [Table 2.6.5.3A-R0300247-1], [Table 2.6.5.3A-R0301244], [Table 2.6.5.3A-R1200729-01], [Table 2.6.5.3A-CLCZ696B2105 and CLCZ696B2105AM1]

Table 2: Pharmacokinetic parameters of AHU377, LBQ657, and valsartan in plasma or blood after a single oral dose in various species

PK parameters	Mouse	Rat [a]	Dog [a]	Monkey	Human
p.o. dose (mg/kg or subject)	150 (AHU)	45 (AHU)	15 (AHU)	30 (LCZ)	200 mg (LCZ) (2.55 mg/kg)
AHU377					
Tmax (h)	0.25	0.60	0.60	0.75	0.5
Cmax (nM)	6250	128	7740	6500	5140
Cmax/dose (nM)/(µmol/kg)	17.1	1.17	212	184	1710
AUCinf (nM·h)	3140	238	5980	8750	6220
AUCinf/ dose (nM·h)/(µmol/kg)	8.61	2.18	164	247	2070
Apparent terminal T1/2 (h)	1.6	1.4	2.8	3.2	1.3
Cl/F (L/h)	na	na	na	na	49.4 ± 21.3
Vz/F (L)	na	na	na	na	82.7 ± 23.0
Absorption (%)	100	>70	90-complete	~40-65	>61 [b]
LBQ657					
Tmax (h)	0.25	0.5	0.7	0.83	2.0
Cmax (nM)	51400	6340	7230	32200	22500
Cmax/dose (nM)/(µmol/kg)	141	58.0	198	909	7470
AUCinf (nM·h)	55800	17400	9600	72000	186000
AUCinf/ dose (nM·h)/(µmol/kg)	153	159	263	2030	61900
Apparent terminal T1/2 (h)	1.1	2.7	3.1	5.9	12
Bioavailability (%)	Complete (119)	72	77 (range 60-110)	41	>50 [c]
Valsartan					
Tmax (h)	na	na	na	1.1	1.5
Cmax (nM)	na	na	na	1150	12300
Cmax/dose (nM)/(µmol/kg)	na	na	na	32.5	4090
AUCinf (nM·h)	na	na	na	6300	61800
AUCinf/ dose (nM·h)/(µmol/kg)	na	na	na	178	20500
Apparent terminal T1/2 (h)	na	na	na	6.5	21
Cl/F (L/h)	na	na	na	na	4.22 ± 1.92
Vz/F (L)	na	na	na	na	101 ± 77.4
Absorption (%)	na	na	na	na	na
Bioavailability (%)	na	na	na	na	na

na = not calculated or not applicable; All data were rounded to 2 or 3 significant figures; AHU = AHU377, LCZ = LCZ696

[a] Blood matrix was measured.

[b] ~61% of dose was recovered in human urine sample. A significant amount of radioactivity after i.v. dosing in animals (23-98%), depending on species, was excreted in feces, Therefore, it was considered that the absorption in human should be higher than 61%.

[c] ~50% of dose was recovered as LBQ657 in the 0-24 h human urine sample. A significant amount of LBQ657 after i.v. dosing in animals (20-96%) , depending on species, was excreted in feces, Therefore, it was considered that bioavailability of LBQ657 in human should be higher than 50%.

Source [Table 2.6.5.3B-R1200673-01], [Table 2.6.5.3B-R0300247-1], [Table 2.6.5.3B-R0301244], [Table 2.6.5.3B-R1200729-01], [Table 2.6.5.3B-CLCZ696B2105 and CLCZ696B2105AM1]

In the TK studies, the AUC values for AHU377, LBQ657 and valsartan were generally proportional to dose in all species (mouse, rat, rabbit, and monkey, including pregnant rat and rabbit). There was no apparent evidence of gender differences. No apparent evidence of accumulation in rats and monkeys was observed following multiple daily dosing for 13 or 39 weeks. In humans [CLCZ696A2101], [CLCZ696A2102], [CLCZ696A1101], Cmax and AUC values of AHU377 and LBQ657 are also approximately dose proportional with minimal accumulation up to 600 mg, although

valsartan is slightly less than dose-proportional at high dose. In juvenile rats, no consistent difference in exposure to either LBQ657 or AHU377 was observed between male and female rats. The total exposure (AHU377 plus LBQ657) was, in general, proportional with increasing dose on day 7, although it appeared slightly over-proportional (~2 fold) on day 64, especially at the highest dose. Juvenile rats (day 7 after initial dosing) showed a 4-11 fold higher total exposure as compared to older rats (day 64 after initial dosing). As shown in Table 3, after an intravenous dose the clearance of LBQ657 was relatively high in mouse (7.8 L/h/kg), rat (4.3 L/h/kg) and dog (2.9 L/h/kg), and moderate to low in monkey (0.21 L/h/kg) compared to hepatic blood flow. Steady-state volume of distribution of LBQ657 was lower in monkey (0.28 L/kg) than in mouse, rat and dog (1.3-2.5 L/kg). The terminal half-life of LBQ657 was relatively short in mouse (0.17 h), moderate in rat (5.7 h) and dog (3.2 h), and long in monkey (14 h).

Table 3: Pharmacokinetic parameters of LBQ657 after a single intravenous dose in various species

PK parameters	Mouse	Rat [a]	Dog [a]	Monkey	Human
i.v. dose (mg/kg or subject)	30	15	3.0	10	na
AUCinf (nM·h)	10100	8030	2810	130000	na
AUCinf/dose (nM·h)/(µmol/kg)	129	232	359	4980	na
CL (L/h/kg)	7.8	4.3	2.9	0.21	na
Vss (L/kg)	1.3	2.0	2.5	0.28	na
T1/2 (h)	0.17	5.7	3.2	14	na

na = not available; All data were rounded to 2 or 3 significant figures

[a] Blood matrix was measured.

Source [Table 2.6.5.3B-R1200673-01], [Table 2.6.5.3B-R0300247-1], [Table 2.6.5.3B-R0301244], [Table 2.6.5.3B-R1200729-01], [Table 2.6.5.3A-CLCZ696B2105 and CLCZ696B2105AM1]

Distribution

Protein binding and distribution in blood

In vitro studies in rat, dog, monkey and human indicate that AHU377 and LBQ657 uptake into blood cells was not significant with the drug concentrations in plasma being higher than in blood. The blood to plasma ratios, of AHU377 or LBQ657, ranged from 0.5 to 0.6, and were concentration-independent over a 0.02-100 µg/mL concentration range. Similar values were observed *in vivo* for the blood-to-plasma ratios of radioactivity in mouse, rat, monkey, dog and human after single oral doses], suggesting that the major circulating component was LBQ657 or AHU377.

In vitro protein binding of [^{14}C]AHU377 and [^{14}C]LBQ657 was determined in rat, dog, monkey and human plasma at concentrations of 0.02 to 100 µg/mL using the ultracentrifugation method. At 37°C, both AHU377 and LBQ657 were moderately to highly bound to plasma proteins with some species differences (90%, 80%, 93% and 97% in rat, dog, monkey and human, respectively for LBQ657 and 91% and 97% in monkey and human, respectively, for AHU377). The binding appeared to be independent of concentration in all species. An *in vitro* study using physiological concentrations of human serum albumin (HSA) and α_1-acid glycoprotein (AGP) indicated that both AHU377 and LBQ657 were highly bound to HSA (99%) but less

extensively to AGP (13-30%). The variation of serum albumin concentration between 60 - 600 µM did affect the binding of both AHU377 and LBQ657 to plasma proteins. The results indicate that the protein binding of AHU377 and LBQ657 may decrease if plasma protein concentration levels decrease significantly. The protein binding of [^{14}C]valsartan was measured by equilibrium dialysis method. Valsartan was 93-97% bound to serum proteins (mainly albumin) in rat, dog, rabbit, marmoset and man. Binding to serum proteins in the mouse was lower (82%).

Tissue and organ distribution

Tissue and organ distribution studies were conducted in the pigmented (Long Evans Hooded) and non-pigmented (HanWistar) rat, and pregnant rat using quantitative whole body autoradiography.

Drug-related radioactivity was widely and rapidly distributed to most rat tissues following a single intravenous (15 mg/kg) or oral (45 mg/kg) dose of [^{14}C]AHU377. Peak concentration of the radioactivity was reached at 5 min for all tissues observed following the intravenous dose. The highest tissue radioactivity (2-4 fold higher than blood) was found in kidney and liver. The lowest radioactivity levels were observed in brain, eye, seminal vesicles and spinal cord. The maximum radioactivity concentration for brain and testis was observed at 5 min postdose and the tissue:blood ratios were 0.02 and 0.05, respectively, indicating minimal passage across the blood:brain and blood:testis barriers. From 24 h through 168 h postdose, radioactivity in the tissues was below the LOQ (4.61 ngEq/g of tissue) except for the small intestine and kidney. Drug-related radioactivity had low affinity to melanin indicated by lack of the radioactivity in pigmented skin and uveal tract. Tissue distribution patterns of the drug-related radioactivity following the oral dose were similar to those following the intravenous dose. The tissue peak concentration of the radioactivity was reached at 1 h for most tissues, with the highest concentrations observed in kidney and liver. From 24 h through 168 h postdose, tissue radioactivity was below the LOQ except for the GI and kidneys.

After intravenous administration of 1 mg/kg [^{14}C]valsartan, relatively high concentrations of radioactivity were found in blood, plasma, liver and kidney; with low levels in some 20 other tissues that were investigated. Concentrations of radioactivity in the brain and cerebrospinal fluid were below the limit of detection, indicating that penetration of valsartan across the blood-brain barrier was very limited. After oral dosing, the pattern of distribution at 15 min was qualitatively similar to that obtained shortly after intravenous dosing, but the absolute quantities in the tissues were about half. By either route of administration, residual radioactivity after 7 days was at or below the limits of detection in all investigated tissues except liver. In liver, levels were very low (0.12 to 0.20 nmol/g).

Placental transfer

Following a single 25 mg/kg oral dose of [^{14}C]LCZ696 to gestational Day 12 and Day17 rats, the drug-related radioactivity in blood and tissues including the fetus reached the peak concentration between 1 and 3 h postdose except for kidney cortex of Day 17 rat (6 h postdose). The highest tissue-to-maternal blood concentration ratio at peak tissue

concentration was observed in the maternal liver (~3 - 8) and kidney (~2 - 34). The distribution of drug-related radioactivity to the central nervous system (maternal brain) was minimal (brain-to-blood concentration ratio = 0.014 - 0.037). At 24 h post dose, the radioactivity in blood and most tissues was too low to be distinguishable from background except for the kidney cortex, kidney medulla, and kidney pelvis. The drug-related radioactivity showed a moderate distribution to fetal organs with a fetus-to-maternal blood ratio of 0.246 - 0.509 at fetus Tmax.

In pregnant rabbits after daily oral LCZ696 doses of 3, 10 or 30 mg/kg, a dose-proportional increase in AUC of AHU377, LBQ657 and valsartan was observed. There was a proportional increase in exposure with increasing dose of LBQ657 and valsartan observed in fetal tissue. The mean concentration of LBQ657 and valsartan at 24 h postdose in fetuses was 1.35 and 8.54 ng/g tissue, respectively, in the 3 mg/kg group, 3.68 and 30.7 ng/g tissue, respectively, in the 10 mg/kg group, and 25.3 and 130 ng/g tissue, respectively, in the 30 mg/kg group. The relative exposure of fetus to dam (fetus-to- maternal plasma ratio) was estimated to be low (~0.06-0.21 for LBQ657 and ~0.01 for valsartan) at 24 h after the last dose, suggesting that LCZ696 related materials were minimally absorbed into the fetus.

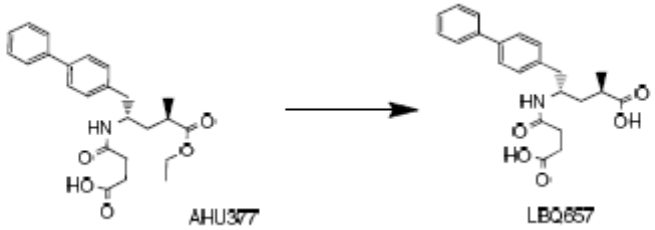
Metabolism (inter-species comparison) of LCZ696

LCZ696 delivers systemic exposure to AHU377, LBQ657 and valsartan. The metabolism of AHU377 and valsartan are described below separately.

In vitro metabolism of AHU377

In incubations of [^{14}C]AHU377 with slices from rat, dog, and human livers, AHU377 was converted predominantly to LBQ657 (Figure below).The metabolic profiles of AHU377 in rat and dog liver slices were similar to that of human and all contained one major metabolite, LBQ657, which was not significantly further metabolized. However, AHU377 was metabolized at a faster rate in rat liver slices than in dog and human liver slices.

Conversion of AHU377 to LBQ657



In vitro metabolism of valsartan

In vitro biotransformation of valsartan was investigated with the post-mitochondrial liver fraction (S12) of mouse, rat, rabbit, dog, marmoset and human. The oxidative metabolism was slow and minimal. The in vivo oxidative metabolism of valsartan was also limited. CYP2C9 is the enzyme responsible for the formation of 4-hydroxyvaleryl metabolite in human microsomes.

In vivo metabolism of LCZ696

Following oral administration of LCZ696 to mouse, rat, rabbit, dog, monkey and human, the following three components were identified in plasma/blood: valsartan, LBQ657, and AHU377. As expected, AHU377 primarily underwent ethyl ester hydrolysis to form LBQ657 in all species based on the findings from in vitro liver slices. The exposure of AHU377 and LBQ657 across species is summarized in the Table below. The rate of conversion from AHU377 to LBQ657 was high in mouse and rat and the major circulating component was LBQ657 in mouse plasma (~73% of AUC) and in rat blood (~80% AUC). However, the rate of conversion was moderate in dog and monkey and both AHU377 and LBQ657 were prominent components in dog blood (AHU377: LBQ657 = ~34%:~46% of AUC) and monkey plasma (AHU377: LBQ657 = ~37%:~62% of AUC). The rate of conversion was high in human (n=4) and the major circulating component was LBQ657 (AHU377: LBQ657 = ~2-6%:~93-98% of AUC). Several minor metabolites were also identified in plasma/blood from various species.

Exposure of [^{14}C]AHU377 and [^{14}C]LBQ657 in plasma from nonclinical species and human

Species	Mouse	Rat*	Dog*	Monkey	Human
Dose (µmol/kg)	365	109	36.5	35.4	3.01
Metabolite	Dose normalized AUC0-last (nM.h)/(µmol/kg)				
AHU377	9.24	6.71	115	1129	2691
LBQ657	129	71.4	153	1915	63173

* Blood samples were analyzed

AHU377 was primarily hydrolyzed to LBQ657, which was subsequently excreted either in urine (Table below) or feces (following Table). Unchanged AHU377 recovered in excreta was minimal in all species studied (<1% of the dose in mouse, rat, and human feces; ~3% of the dose in monkey feces; ~13% of the dose in dog feces; and <1.5% in urine from all species studied). LBQ657 was primarily excreted unchanged in feces (~96% of the dose in mouse; ~87% of the dose in rat; ~71% of the dose in dog). However, LBQ657 was excreted partially in feces (~41% of the dose in monkey and ~36% of the dose in human) and partially in urine (~29% of the dose in monkey and ~50% of the dose in human). Several minor metabolites were also identified in feces, each accounting for <1% of the dose.

Excretion of AHU377 and LBQ657 in urine from nonclinical and human following oral dosing of [^{14}C]LCZ696 or [^{14}C]AHU377

AHU377 and LBQ657 recovered in urine as percentage of AHU377 dose					
Species	Mouse	Rat	Dog	Monkey	Human
Dose	150 mg/kg	45 mg/kg	15 mg/kg	30 mg/kg	200 mg
AHU377	0	0	0.30	1.0	1.49
LBQ657	8.04	7.37	6.73	29.0	49.6

Excretion of AHU377 and LBQ657 in feces from nonclinical and human following oral dosing of [^{14}C]LCZ696

AHU377 and LBQ657 recovered in feces as percentage of AHU377 dose					
Species	Mouse	Rat	Dog	Monkey	Human
Dose	150 mg/kg	45 mg/kg	15 mg/kg	30 mg/kg	200 mg
AHU377	0.48	0.33	13.2	3.22	0.573
LBQ657	96.1	87.0	71.1	40.6	35.9

Metabolic pathways of LCZ696

LCZ696 delivers systemic exposure to AHU377, its active metabolite (LBQ657) and valsartan. AHU377 primarily undergoes ester hydrolysis to generate LBQ657, the active metabolite. LBQ657 was the major circulating component in plasma as well as the major radiolabeled component excreted in feces and/or urine in all species studied. In addition, AHU377 and LBQ657 undergo further hydroxylation, glucuronidation, sulfation, glycine and taurine conjugation to generate several minor metabolites. The metabolites detected in various matrices from all species studied are summarized in the Table below. The proposed metabolic pathways of LCZ696 are shown in the following Figure.

Table 5-4 Metabolites of LCZ696 after oral doing to various species

Metabolites (compound)	Mouse (AHU377)	Rat (ADME) (AHU377)	Rat (milk study) (LCZ696)	Dog (AHU377)	Monkey (LCZ696)	Human (LCZ696)
M13.4	U	--	--	--	--	--
M13.8	F	--	--	--	--	--
M14.2	U	--	--	--	--	--
M14.4	F	--	--	--	--	--
M16.2	--	--	--	--	F	--
M16.8	F	--	--	--	U, F	--
M17.4	F	--	--	--	F	--
M17.8	F	--	--	--	F	--
M18.2	F	--	--	--	F	--
M18.7	--	--	--	--	F	--
M19.4	F	--	--	--	F	--
M19.5	F	--	--	--	F	--
M20.5	--	--	--	--	F	--
M21.5	--	--	--	--	F	--
P21.5	--	--	--	--	--	U
P21.5A	--	--	--	U	--	--
P21.5B	--	--	--	U	--	--
P22	--	--	--	U(t)	--	--
P22.1A	--	--	--	U	--	--
P22.1B	--	--	--	U	--	--
P22.5	--	F	--	--	--	P, U, F
M22.8	F	--	P	--	F	--
P23	--	U(t), F	--	U, F	--	P, U, F
P24	--	F(t)	--	--	--	U
P24.7	--	--	--	--	--	U
M25	--	--	--	F	--	--
P25/P25.2	--	--	--	U	--	--
P25.1	--	F	--	--	--	--
P25.5	--	--	--	--	--	U
P25.8	--	--	--	--	--	U, F
P26	--	F(t)	--	P, U, F	--	U
M26.8	--	P	--	--	--	--
LBQ657	P, U, F	B, U, F	P, M	P, U, F	P, U, F	P, U, F
AHU377	P, U, F	B, U(t)	P	P, U, F	P, U, F	P, U, F
valsartan	Not measured in the excreta of these studies; valsartan is in plasma for dosed with LCZ696					

Abbreviations B, P, U, F, M and t represent blood, plasma, urine, feces, milk and trace abundance, respectively.

Metabolites were assigned the label P followed by their approximate HPLC retention time from legacy reports [Table 2.6.5.9C-R0300247-1], [Table 2.6.5.9D-R0300247-1], [Table 2.6.5.9E-R0301244], [Table 2.6.5.9F-R0301244], [Table 2.6.5.9J-CLCZ696B2105 and CLCZ696B2105AM1], [Table 2.6.5.9K-CLCZ696B2105 and CLCZ696B2105AM1] and metabolites were assigned the label M followed by their approximate HPLC retention time from recent reports [Table 2.6.5.9A-R1200673-01], [Table 2.6.5.9B-R1200673-01], [Table 2.6.5.9G-R1200729-01], [Table 2.6.5.9H-R1200729-01] and [Table 2.6.5.9I-R1200622].

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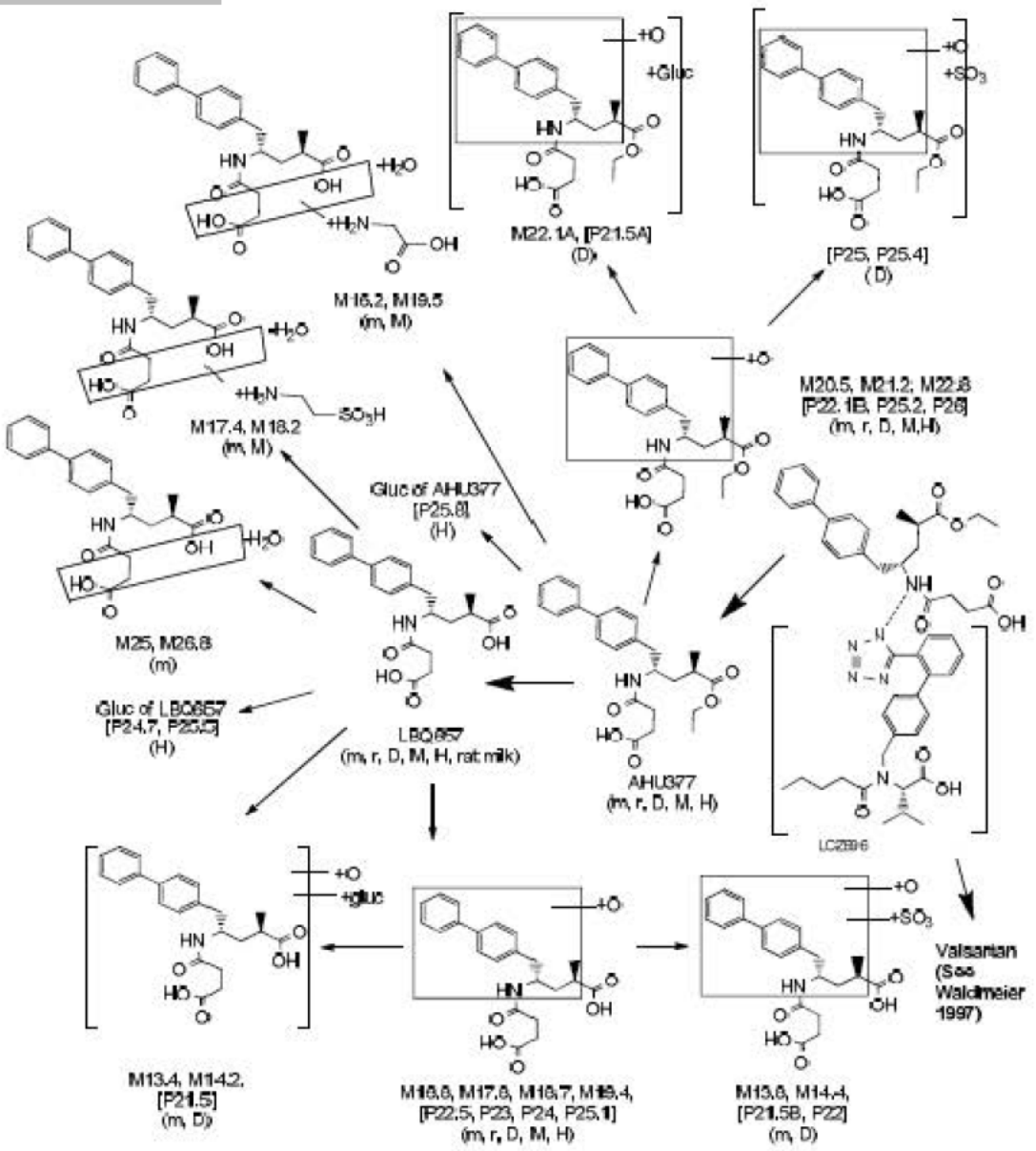
Proposed metabolic pathways of LCZ696



Abbreviations m, r, D, M, H represent mouse, rat, dog, monkey and human, respectively.

Metabolites were assigned the label P followed by their approximate HPLC retention time from legacy reports [Table 2.6.5.9C -R0300247-1], [Table 2.6.5.9D-R0300247-1], [Table 2.6.5.9E -R0301244], [Table 2.6.5.9F-R0301244], [Table 2.6.5.9J-CLCZ696B2105 and CLCZ696B2105AM1], [Table 2.6.5.9K-CLCZ696B2105 and CLCZ696B2105AM1] and metabolites were assigned the label M followed by their approximate HPLC retention time from recent reports [Table 2.6.5.9A-R1200673-01], [Table 2.6.5.9B-R1200673-01], [Table 2.6.5.9G -R1200729-01], [Table 2.6.5.9H-R1200729-01] and [Table 2.6.5.9I-R1200622].

Excretion

Urinary and fecal excretion data following a single dose of radiolabeled LCZ696 or AHU377 are summarized in the following Table.

In mice, rats and dogs, the excretion of AHU377-related radioactivity was predominantly in the feces (~74-98% of the dose) and urinary excretion was minor. In monkeys and humans, however, AHU377-related radioactivity had higher excretion into urine (~42-65% of the dose). AHU377 was eliminated primarily by LBQ657 excretion with minimal intact AHU377 (~3% of dose, except ~13% in dog) in both urine and feces. There was no indication of enterohepatic recycling based on the plasma pharmacokinetics. In general, the excretion was complete within 7 days.

Valsartan was excreted mainly via biliary-fecal route in mice, rats, dogs, marmosets, and human. In human, 9% and 86% of the dose was excreted in urine and feces, respectively. Valsartan was mainly excreted unchanged in all investigated species.

Excretion of major metabolites and total radioactivity following a single dose of radiolabeled LCZ696, AHU377, or LBQ657

			Amount Excreted (% of Dose)					
			Urine			Feces		
			Radioactivity		LBQ657	Radioactivity		LBQ657
Species	Dose mg/kg	Dose Route	0-24 h	0-168 h	0-24 h	0-48 h	0-168 h	0-48 h
Mouse [a]	30 (LBQ)	i.v.	4.57	4.90	4.48	97.4	98.2	95.8
	150 (AHU)	p.o.	8.30	8.77	7.89#	103 (92.2)**	103 (92.2)**	95.9
Rat [b]	15 (AHU)	i.v.	23.0	23.7	23.0	71.6	74.4	69.6
	45 (AHU)	p.o.	7.37	8.75	7.40	90.3	91.4	87.0
Dog [c]	3 (LBQ)	i.v.	10.5	11.0	10.5	79.9	88.8	86.2
	15 (AHU)	p.o.	8.61	9.94	6.73	83.3	89.2	71.1 (AHU377:13.2)
Monkey [d]	10 (LBQ)	i.v.	50.3	56.1 (65.2)*	55.1#	21.1	23.0	21.1
	30 (LCZ)	p.o.	26.1	31.1 (42.1)*	29.0#	46.4	47.9	40.6
Human [e]	2.55 (LCZ)	p.o.	54.8	60.7	44.0- 53.8	22.4	41.8	31.6 - 41.5[f]

* including cage wash; ** corrected for the total recovery of 112% of dose; # 0-48 h sample; AHU = AHU377, LBQ = LBQ657, LCZ = LCZ696

[a] [Table 2.6.5.13A-R1200673-01]
[b] [Table 2.6.5.13B-R0300247-1]
[c] [Table 2.6.5.13D-R0301244]
[d] [Table 2.6.5.13E-R1200729-01]
[e] [Table 2.6.5.13F-CLCZ696B2105 and CLCZ696B2105AM1]; 2.55 mg/kg oral dose (200 mg LCZ696 in human ADME study (average weight 78.53 kg)
[f] Due to different frequency in producing stool, sample pooling time points (to get at least 90% of the total radioactivity) varied between subjects and the longest pooling time was 0-120 h.

After a single 30 mg/kg [^{14}C]LCZ696 dose to lactating rats, transfer of LBQ657 (active metabolite) into milk was observed. The overall milk:plasma (M/P) concentration ratio of total radioactivity was 0.76 and 0.91, respectively, based on AUC0-24h and AUCinf values. Projecting the rat data to humans, it is estimated that the maximum amount of AHU377-related materials including LBQ657, that a breast-fed infant could be exposed

to by ingesting 1 L of milk daily, is 0.889% of a 400 mg (or 200 mg BID) LCZ696 adult dose**.** LBQ657 was the major drug-related component in rat milk. After a single oral 3 mg/kg [^{14}C]valsartan dose to lactating rats, the transfer of valsartan into milk was observed as noted in the Diovan® prescribing information.

Pharmacokinetic Drug Interactions

Cytochrome P450 inhibition

The potential of AHU377 and its active metabolite LBQ657 to inhibit the activity of cytochrome P450 enzymes *in vitro* was investigated using pooled human liver microsomes. This potential was determined by testing the effect of increasing concentrations of AHU377 and LBQ657 on the *in vitro* metabolism of P450 enzyme-selective probe substrates (phenacetin, paclitaxel, diclofenac, S-mephenytoin, bufuralol, chlorzoxazone, midazolam, and testosterone). The probe substrate concentrations used for these determinations were less than or equal to their reported K_m values. AHU377 showed little or no inhibition (IC_{50} >100 µM) in *in vitro* assays using human cytochrome P450 (CYP) enzymes 1A2, 2C9, 2D6, 2E1, 3A4/5, and only a weak inhibition potential against CYP2C8 (IC_{50} of ~15 µM) and CYP2C19 (IC_{50} of ~20 µM). The active metabolite LBQ657 showed little or no inhibition (IC_{50} >100 µM) against the same set of CYPs, and only a weak inhibition potential against CYP2C9 (IC_{50} of ~ 40 µM). Based on these *in vitro* inhibition results, it is concluded that AHU377 and LBQ657 following administration of LCZ696 at therapeutic doses are unlikely to inhibit the metabolic clearance of concomitantly administered medications metabolized by CYP enzymes 1A2, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4/5.

Valsartan did not inhibit CYP enzymes 1A2, 2A6, 2C19, 2D6, 2E1, or 3A4 to any significant extent. It marginally inhibited CYP2C9 with a relatively high Ki value (135 µM). No interaction was observed for warfarin (CYP2C9-mediated metabolism) when co-administered with LCZ696 200 mg BID. [CLCZ696B2112].

Cytochrome P450 phenotyping

CYP2C9 is the enzyme responsible for the formation of 4-hydroxyvaleryl metabolite of valsartan in human microsomes. However, valsartan was predominately excreted unchanged in urine and feces. CYP-mediated DDIs between valsartan and co-administered drugs are negligible.

Enzyme induction

The potential for AHU377 and valsartan to induce CYP enzymes mRNA and activities *in vitro* was evaluated in human hepatocytes prepared from three separate donor livers after 48 h of treatment. Induction of mRNA, relative to vehicle control, was determined by real-time PCR and evaluation of changes in enzyme activities were assessed after the induction period by quantitative LC-MS/MS analysis of enzyme-selective probe substrate metabolism. Treatment of human hepatocytes with AHU377 (10-100 µM) or valsartan (1-100 µM) did not result in induction of CYP1A2, CYP2B6, CYP2C9, or CYP3A4 mRNA or activity (induction levels were < 2-fold and/or < 40% of the positive control response) in the three donor hepatocytes (except one donor outlier for valsartan

on CYP1A2 activity but no induction at mRNA level). AHU377 was converted to its active metabolite, LBQ657 by > 50% at 4 h incubation time point and by 100% at 24 h incubation time point. Based upon these *in vitro* studies, AHU377, LBQ657 and valsartan are unlikely to induce the expression and/or catalytic activities of CYP1A2, CYP2B6, CYP2C9, or CYP3A *in vivo*.

In vitro permeability assessments and transporter interactions

The apparent permeability of AHU377 and LBQ657, across confluent Caco-2 cell monolayers was examined using [^{14}C AHU377 and [^{14}C]LBQ657]. The bidirectional transport experiments (apical-to-basolateral and basolateral-to-apical) were performed under steady-state conditions at two [^{14}C]AHU377 and [^{14}C]LBQ657 concentrations (~5.0 and ~23 µM). Additionally, the effect of P-glycoprotein (P-gp) and multi-resistance protein 2 (MRP2) inhibitors PSC833 (1.0 µM) and indomethacin (50 µM) on the rate of transport was investigated. The permeability values for [^{14}C]AHU377 in the apical to basolateral and the basolateral to apical directions were (23-28) × 10^{-5} cm/min and (56-60) × 10^{-5} cm/min, respectively. The permeability values for [^{14}C]LBQ657 in the apical to basolateral and the basolateral to apical directions were (0.67-0.75) × 10^{-5} cm/min and (2.7- 4.2) × 10^{-5} cm/min, respectively. The rate of AHU377 movement across Caco-2 monolayers was affected by PSC833, but not indomethacin. PSC833 increased the apical to basolateral permeability of [^{14}C]AHU377 by ~2 fold, concomitantly the basolateral to apical permeability decreased ~2.7 fold. These results suggest that AHU377 may be a substrate for P-gp. However, the K_m value associated with AHU377 interaction with P-gp was determined to be in excess of 100 µM, indicating a low affinity interaction. In conclusion, the estimated *in vitro* permeability values classify AHU377 as a moderately high permeable drug substance, whereas LBQ657 was a poorly permeable substance. AHU377 exhibited P-gp–mediated efflux. However, the interaction combined with the moderately high permeability is predicted not to have a significant effect on the net absorption of AHU377.

The potential of the organic anion transporting polypeptide 1B1 (OATP1B1) or organic anion transporting polypeptide 1B3 (OATP1B3) to transport LBQ657 was examined using human embryonic kidney (HEK293) cells stably expressing OATP1B1 or OATP1B3. The accumulation of the positive control substrate [^{3}H]$E_2$17ßG (1.6 µM) into OATP1B1 and OATP1B3 cells was 6.6-fold and 2.6-fold higher than that into control cells, respectively, indicating the adequate expression of the OATP transporters. Moreover, the accumulation of the positive control OATP substrates in the OATP-expressing cells was reduced to background levels (i.e. control cells) in the presence of the model OATP inhibitor rifamycin SV (25 µM). The OATP1B1- and OATP1B3-mediated accumulation of [^{14}C]LBQ657 was observed in the range of tested concentrations (12 µM to 490 µM). The K_m value of OATP1B3 transport activity was estimated to be 174 ± 49.8 µM whereas the K_m of OATP1B1 transport was not calculated due to insufficient saturation of the activity. These *in vitro* results suggested that active transport by OATP1B1 and OATP1B3 contributes to the systemic clearance of LBQ657 which could potentially be altered when LBQ657 is co-administered with drugs that inhibit these transporters such as cyclosporine and a number of protease inhibitors.

The potential for LBQ657 to be a substrate of the human orthologs of OAT1 and OAT3 stably expressed in human embryonic kidney (HEK) cells was examined. Compared to HEK parental cells, the cellular accumulation of [^{14}C]LBQ657 (10.8 µM) was stimulated 14.5-fold by the expression of OAT3, but only 2-fold by the expression of OAT1. In kinetic studies, the maximal rate of transport (J_{max}) and Michaelis constant (K_m) of OAT3 for LBQ657 were 77.1 ± 3.69 pmol·mg protein^{-1}·min^{-1} and 10.6 ± 2.02 µM, respectively. In conclusion, LBQ657 was found to be a substrate of OAT3 *in vitro*. However, this is not of clinical relevance at the therapeutic doses.

The potential for valsartan to be a substrate of the human orthologs of OAT1 and OAT3 stably expressed in human embryonic kidney (HEK) cells was examined The cellular accumulation of [^{14}C]valsartan compared to the parental cells was stimulated 1.5-fold and 3.2-fold by the expression of OAT1 and OAT3, respectively. The J_{max} and K_m values for OAT3-mediated valsartan transport were 19.9 ± 1.86 pmol·mg protein^{-1}·min^{-1} and 1.93 ± 0.77 µM, respectively. In conclusion, valsartan was found to be a substrate of OAT1 and OAT3 *in vitro* but it was transported poorly by OAT1. However, the potential effect of the OAT1 or OAT3 inhibition on the distribution and elimination of valsartan *in vivo* is not clinically relevant.

Valsartan was found to be an *in vitro* substrate of OATP1B1, OATP1B3 (hepatic uptake transporter) and MRP2 (efflux transporter) in the test system of human hepatocytes and double transfected (OATP1B1/MRP2) MDCKII cells. While co-administration of the inhibitors of these uptake or efflux transporters may increase exposure to valsartan, in clinical practice no relevant interactions have been identified to date.

In vitro transporter inhibition

The potential of AHU377 to inhibit the activity of P-gp, BCRP, or MRP2 mediated efflux was investigated in BCRP (T8 cells), P-gp (MDA435 T0.3 cells) and MRP2 (MDCKII cells) overexpressed mammalian cells. Flow cytometry assays were used to assess the potential for AHU377 to inhibit the efflux of fluorescent substrates Bodipy FL prazosin (BDP) and Rhodamine 123 (Rho123) by BCRP and P-gp, respectively. ^{14}C]Valsartan was used as a probe substrate of MRP2. At 50 µM AHU377, BDP levels were elevated 1.9-fold (I_{max}), but this level of inhibition was only 6.7% ($\%_{max}$) of that observed with the positive control inhibitor of BCRP, fumitremorgin C (10 µM). Rho123 efflux from P-gp-expressing MDA435 T0.3 cells and [^{14}C]valsartan efflux from MRP2-expressing MDCKII cells was not inhibited by AHU377 up to a concentration of 50 µM. These data suggest that AHU377 up to a concentration of 50 µM is unlikely to significantly inhibit BCRP, P-gp or MRP2. The inhibition potential of LBQ657 towards P-gp and BCRP was examined using inside-out membrane vesicles containing expressed P-gp or BCRP. The choice of this test system was dictated by the poor membrane permeability properties of LBQ657. LBQ657 at concentrations up to 50 µM was shown to be a poor *in vitro* inhibitor P-gp ([^{3}H]*N*-methylquinidine transport) or BCRP ([^{3}H]estrone-3-sulfate transport) suggesting the low likelihood of a clinical interaction with co-administered P-gp and BCRP substrates.

The potential of LBQ657 and AHU377 to inhibit the transport activities of OATP1B1 or OATP1B3 was examined using human embryonic kidney (HEK) cells stably expressing either transporter. The inhibition of the transport activity was evaluated by assessing the effect of increasing concentrations of LBQ657 (up to 500 µM for OATP1B1 and up to 250 µM for OATP1B3) and AHU377 (up to 50 µM) on the accumulation of model OATP substrate [^{3}H]estradiol-17ßglucuronide ([^{3}H]E$_2$17ßG) in transporter-expressing cells and in control cells. LBQ657 at the highest concentration tested (500 µM), inhibited [^{3}H]E$_2$17ßG uptake into OATP1B1 cells by 82.8% with an IC$_{50}$ value of 126 ± 26 µM. In comparison, the transport activity of OATP1B3 was not inhibited by LBQ657 at concentrations up to 250 µM and therefore an IC$_{50}$ value was not estimated. AHU377 at the highest concentration tested (50 µM), inhibited [^{3}H]E$_2$17ßG uptake into OATP1B1 and OATP1B3 cells by 98.3% and 89.9%, respectively. The estimated IC$_{50}$ values for OATP1B1 and OATP1B3 inhibition were 1.91 ± 0.56 µM and 3.81 ± 2.2 µM, respectively. These *in vitro* findings indicated that AHU377 and LBQ657 may result in an increase in systemic exposure of co-medications whose clearance is significantly mediated by OATP1B1 and OATP1B3 (not for LBQ657) transport activity. Based on the Cmax of LBQ657 (16531 ng/mL = 43 µM) observed in patients at LCZ696 200 mg BID (Summary of Clinical Pharmacology Section 3.1.9.1), a DDI between LBQ657 and OATP1B1 or OATP1B3 substrates is not expected while the potential for DDI with AHU377 is possible at therapeutic concentrations (Cmax 2421 ng/mL = 5.9 µM for 200 mg dose) and when considering the site of interaction at the liver inlet which is exposed to higher concentrations following oral absorption.

The potential of AHU377, LBQ657 and valsartan to inhibit the transport activities of the organic cation transporters 1 (OCT1) and 2 (OCT2) was examined using human embryonic kidney (HEK) cells stably expressing the human orthologs of OCT1 or OCT2]. Neither AHU377, LBQ657 or valsartan inhibited OCT1-mediated [^{3}H]methyl-4-phenylpyridinium ([^{3}H]MPP$_+$) transport or OCT2-mediated [^{14}C]metformin transport within the concentration ranges tested (1.0 - 50 µM for AHU377, 5.0- 500 µM for LBQ657, and 1 - 100 µM for valsartan). The uptake of [^{3}H]MPP$_+$ into OCT1 cells and [^{14}C]metformin into OCT2 cells was much greater than uptake into parental HEK cells (control), and the uptake activity was abolished by the known OCT inhibitor, decynium 22 (20 µM). These data indicate that the cell lines used were appropriate for assessing the inhibitory effect of AHU377, LBQ657 or valsartan toward OCT1 and OCT2. In summary, LCZ696 (AHU377, LBQ657 and valsartan) did not inhibit hepatic OCT1 or renal OCT2 in these *in vitro* assays.

The potential of LBQ657 and AHU377 to inhibit the human orthologs of OAT1 and OAT3 stably expressed in human embryonic kidney (HEK) cells was examined. LBQ657 maximally inhibited OAT3-mediated transport of 6-carboxyfluorescein (6CF) by 2.8-fold and 6.8-fold at the highest concentrations examined (100 and 200 µM, respectively), but had little effect on OAT1. The IC$_{50}$ for LBQ657 inhibition of OAT3-mediated 6CF transport was 15.2 ± 5.60 µM. Similarly, AHU377 maximally inhibited OAT1 and OAT3 activities by 36.6% and 91.1% at the highest concentration tested in either system. The IC$_{50}$ values for OAT1 and OAT3 inhibition were greater than 50 µM and 0.795 ± 0.058 µM, respectively. In conclusion, LBQ657 and AHU377 were found to

be inhibitors of OAT3 *in vitro*. However, this is not clinically relevant at therapeutic doses where the plasma unbound Cmax is ~0.18 µM (estimated based on Cmax 5.9 µM and 97% protein binding) for AHU377.

The potential of AHU377 or LBQ657 to inhibit the human multidrug and toxin extrusion transporter 1 (MATE1) and human multidrug and toxin extrusion transporter 2-K (MATE2-K) was examined in human embryonic kidney (HEK) cells stably expressing these transport proteins. AHU377 and LBQ657 were shown to be weak inhibitors of MATE1 and MATE2-K *in vitro*. They are unlikely to increase the systemic exposure to co-medications whose clearance is significantly mediated by MATE1 and MATE2-K *in vivo*.

The potential of valsartan to inhibit the human orthologs of OAT1 and OAT3 stably expressed in human embryonic kidney (HEK) cells was examined. The uptake of 6-carboxyfluorescein by OAT1 and OAT3 was reduced by 96.4% and 100% at the highest concentrations of valsartan tested. The IC_{50} for valsartan inhibition of OAT1- and OAT3-mediated 6-carboxyfluorescein transport was 14.8 ± 5.42 µM and 1.11 ± 0.33 µM, respectively. Based on these data, valsartan was found to be an inhibitor of OAT1 and OAT3 *in vitro*. However, this is not clinically relevant at therapeutically doses.

The potential of valsartan to inhibit the human MATE1 and MATE2-K was examined in human embryonic kidney (HEK) cells stably expressing these transport proteins. Valsartan poorly inhibited the transport activity of MATE1 and MATE2-K in the *in vitro* assays and the inhibition is unlikely to be clinically relevant.

5.2 Toxicokinetics

Toxicokinetics data are presented and discussed in the individual toxicology study reviews.

6 General Toxicology

6.1 Single-Dose Toxicity

Single dose toxicity studies, no longer required by ICH M3 (R2) Guidelines on Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals, were not performed with LCZ696. Single oral doses up to 600 mg/kg were tested and tolerated in respiratory and CNS safety pharmacology studies conducted with LCZ696 in rats.

An acute oral (gavage) toxicity study with AHU377 in mice
In this acute study **[**0480024, (GLP)], single oral doses of AHU377 (batch no. 0457001) were administered to CD-1 mice at doses of 50, 250, and 1000 mg/kg (3/sex/group) or a dose of 2000 mg/kg (5/sex). One additional group of mice (3/sex) was administered the vehicle control (0.5% CMC) at an equivalent dosing volume of 20 mL/kg. Animals were observed for clinical signs and mortality for 14 days; a gross necropsy was performed on all animals on day 15.

No clinical signs were observed in any animals during the study and there were no biologically significant differences in body weight. No gross necropsy findings were present. The no-effect level was identified at 2000 mg/kg.

An acute oral (gavage) toxicity study with AHU377 in rats
In this acute study [0480025, (GLP)], single oral doses of AHU377 (batch no. 0457001) were administered to Han Wistar rats at doses of 50, 250, and 1000 mg/kg (3/sex/group) or a dose of 2000 mg/kg (5/sex). One additional group of rats (3/sex) was administered the vehicle control (0.5% CMC) at an equivalent dosing volume of 20 mL/kg. Animals were observed for clinical signs and mortality for 14 days; a gross necropsy was performed on all animals on day 15.

No clinical signs with the exception of soft feces were observed in any animals receiving the vehicle formulation or AHU377 at 50, 250, 1000 and 2000 mg/kg. There were no biologically significant differences in body weight. No gross necropsy findings were present. In conclusion, the single oral administration of AHU377 at 50, 250, 1000 and 2000 mg/kg revealed no clinical signs with an absence of mortality. The no-effect level was identified at 2000 mg/kg.

6.2 Repeat-Dose Toxicity

6.2.1 Studies with LCZ696

Study title: 13-week oral (gavage) toxicity study in rats with a 4-week recovery period

Study no.:	0670283
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover, New Jersey
Date of study initiation:	8/16/2006
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0650001, 95.2%

Key Study Findings

Thirteen weeks of oral (gavage) administration of LCZ696 to rats was tolerated at doses up to 100 mg/kg/day. However, test article-related effects on food consumption, mean body weight, body weight gain, and organ weight (heart) as well as microscopic findings in the stomachs were noted at 100 mg/kg/day in both sexes. Based on these findings, a NOAEL of 30 mg/kg/day was established.

Methods

Doses:	10, 30, 100 mg/kg
Frequency of dosing:	daily
Route of administration:	oral
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% CMC
Species/Strain:	IGS Wistar Hannover Rat; Crl: WI(Glx/BRL/Han)IGS BR
Number/Sex/Group:	10
Age:	8 weeks
Weight:	222.8 to 268.8 grams (males) 152.6 to 192.2 grams (females)
Satellite groups:	6/group for control and high dose recovery
Unique study design:	4 week recovery
Deviation from study protocol:	deviations had no impact on the overall interpretation of the study.

Observations and Results

Mortality

Twice daily (AM and PM) on weekdays and at least once daily on weekends and holidays.

There was no mortality in this study.

Clinical Signs

Twice daily (predose and approximately 2 hours postdose)

All clinical signs observed during the study were considered to be incidental and unrelated to treatment.

Body Weights

Measured twice weekly in week one, once weekly in subsequent weeks.

There were no toxicologically significant changes to mean body weight at doses ≤ 30 mg/kg/day. Test article-related effects on mean body weight were observed at 100 mg/kg/day. In the males, statistically significant decreases (5.4 % to 8.7 %) were first observed at day 15 and continued throughout the study with the greatest decrease at day 78 (8.7 %). In the females, statistically significant decreases (5.1 % to 6.2 %) were more sporadic (days 29, 43, 71, 78) with the greatest decreases at day 78 (6.2 %). There was a trend toward recovery during the 4 weeks after the completion of dosing.

There were no toxicologically significant changes to absolute weight gain at doses ≤ 30 mg/kg/day. Test article-related effects on absolute weight gain were observed at 100 mg/kg/day. In the males, statistically significant decreases (16.4 % to 34.1 %) were first observed at day 4 and continued throughout the study with the greatest decrease at day 8 (34.1 %). In the females, statistically significant decreases (20.7 % to 34.3 %) were first observed at day 15 and continued throughout the study with the greatest decrease at day 15 (34.3 %). There was a trend toward recovery during the 4 weeks after the completion of dosing.

Feed Consumption

Calculated from feeder weights collected once weekly.

There were no toxicologically significant changes to food consumption at doses ≤ 30 mg/kg/day. There was a test article-related decrease in food consumption observed at the 100 mg/kg/day. In the males, statistically significant decreases (7.2 % to 12.1 %) were most consistent at the end of dosing (days 22, 36, 57, 71, 78, 85, 92) with the greatest decrease at day 78 (12.1 %). The statistically significant decreases in the female animals (7.6 % to 9.7 %) were more sporadic (days 8, 22, 29, 57) and were greatest at day 8 (9.7 %).

Ophthalmoscopy

All surviving control and high dose animals

Ophthalmoscopic examinations performed during the course of the study did not reveal any ocular changes attributable to treatment with LCZ696.

Hematology

Parameters: erythrocytes, Wintrobe indices, white blood cell, count, hematocrit, red cell distribution width (RDW), white blood cell differential, hemoglobin, reticulocytes, and platelets

There were no test article-related changes in hematology.

Clinical Chemistry

alanine aminotransferase	globulins (G)	chloride
alkaline phosphatase	glucose	calcium
aspartate aminotransferase	blood urea nitrogen	inorganic phosphorus
total bilirubin	creatinine	triglycerides
total protein	sodium	cholesterol
albumin (A)	potassium	A/G ratio
creatine kinase	magnesium	

There were no test article-related changes noted in clinical chemistry.

Urinalysis

specific gravity	glucose*	protein*
bilirubin*	ketones*	urobilinogen*
blood*	pH*	

*test strip determination

There were no test article-related changes in urinalysis.

Gross Pathology

Animals were fasted overnight (approximately 18 hours) prior to terminal necropsy. Fasted terminal body weights were collected. Complete necropsies were performed with a recording of macroscopic abnormalities for all protocol tissues.

Tissue list for collection, weighing (W), genomics (G) and/or processing (P)

W	P	adrenal	W	P	ovary (with oviduct)
	P	aorta		P	pancreas
	P	bone marrow (in bone)		P	parathyroid
	P	bone marrow smear	W	P	pituitary
W	P	brain	W	P	prostate
	P	cecum		P	rectum
	P	cervix		P	salivary gland
	P	colon		P	sciatic nerve
	P	duodenum		P	seminal vesicle
	P	epididymis		P	skeletal muscle
	P	esophagus		P	skin
	P	eye		P	spinal cord
	P	femur/tibia	W	P	spleen
	P	harderian gland		P	sternum
W	P	heart	G	P	stomach
	P	ileum	W	P	testis
	P	jejunum	W	P	thymus
W	P	kidney	W	P	thyroid
	P	lacrimal gland		P	tongue
	P	larynx-cross section		P	trachea
W	P	liver		P	ureter-cross section
	P	lung		P	urinary bladder
	P	lymph node - bronchial	W	P	uterus
	P	lymph node - mandibular		P	vagina
	P	lymph node - mesenteric		P	macroscopic lesions
	P	mammary gland area			animal identification
		nasal passage			

Organ Weights

Statistically significant decreased absolute and relative (to body and brain) heart weights were present after 13 weeks of dosing in both sexes dosed at 100 mg/kg/day. Females dosed at 30 mg/kg/day also had significantly decreased mean heart weights relative to brain weights.

No macroscopic or microscopic correlates were observed for these findings. The decrease in heart weights was considered reversible. Decreased absolute and relative (to body and/or brain) thyroid weights at the end of the dosing period in females at doses ≥ 30 mg/kg/day were of uncertain relationship to compound administration based on the lack of correlation between dose and magnitude of organ weight loss, the opposite (increase) but variable trend in males, and the absence of macroscopic or microscopic correlates. All other weight changes were interpreted to be consistent with random, spontaneous changes and unrelated to the test article.

Histopathology

Adequate Battery

Yes

Peer Review

All assessments were peer-reviewed per SOP

Histological Findings

No test article-related macroscopic lesions were present.

Test article-related microscopic findings were observed in the stomachs of both sexes dosed at 100 mg/kg/day. The stomach findings at 100 mg/kg/day consisted of an increased incidence and severity of minimal (both sexes) to slight (male only) mixed inflammatory cell infiltrates in the glandular stomachs. These infiltrates consisted primarily of admixtures of eosinophils, mononuclear inflammatory cells, and neutrophils in the submucosa of the glandular stomach. This lesion was considered reversible following a 4 week recovery period since similar incidences were observed in treated and control animals.

Toxicokinetics

Blood was obtained from all non-recovery study animals on study days 1-2 and in week 11. Two animals/sex/group were bled at 0.5, 1, 2, 6 and 24 hours post dose. Approximately 0.5 mL of whole blood was collected from the sublingual vein of isoflurane/O_2-anesthetized animals into tubes containing EDTA and approximately 10 to 20 mg of sodium fluoride (NaF) and placed on wet ice.

Toxicokinetic parameters of AHU377 in rat plasma

Dose	Study Day	Gender	$AUC_{(0\text{-}24h)}$	±SE	$AUC_{(0\text{-}24h)}$/Dose	±SE /Dose	C_{max}	C_{max}/Dose	t_{max}
10	1	Male	NA	NA	NA	NA	16.5	1.65	24.0
		Female	NA	NA	NA	NA	16.7	1.67	0.5
	76	Male	NA	NA	NA	NA	10.0	1.00	0.5
		Female	NA	NA	NA	NA	15.9	1.59	0.5
30	1	Male	NA	NA	NA	NA	41.7	1.39	0.5
		Female	NA	NA	NA	NA	38.2	1.27	0.5
	76	Male	NA	NA	NA	NA	19.0	0.633	0.5
		Female	NA	NA	NA	NA	11.9	0.397	0.5
100	1	Male	363	105	3.63	1.05	239	2.39	0.5
		Female	351	122	3.51	1.22	143	1.43	0.5
	76	Male	345	56.9	3.45	0.569	219	2.19	0.5
		Female	291	89.0	2.91	0.890	126	1.26	0.5

Units for Dose: mg/kg/day; $AUC_{(0\text{-}24h)}$±SE: (ng·h/mL); $AUC_{(0\text{-}24h)}$/Dose±SE/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h.

NA: not available. TK parameters not calculated due to lack of sufficient measurable AHU377 concentrations.

Toxicokinetic parameters of LBQ657 in rat plasma

Dose	Study Day	Gender	$AUC_{(0\text{-}24h)}$	±SE	$AUC_{(0\text{-}24h)}$/Dose	±SE /Dose	C_{max}	C_{max}/Dose	t_{max}
10	1	Male	2670	691	267	69.1	1130	113	1.0
		Female	1830	442	183	44.2	1330	133	0.5
	76	Male	2110	239	211	23.9	1650	165	0.5
		Female	1470	393	147	39.3	1480	148	0.5
30	1	Male	6290	2040	210	68.0	2840	94.7	1.0
		Female	4490	851	150	28.4	1430	47.7	0.5
	76	Male	5160	1380	172	46.0	1900	63.3	0.5
		Female	4800	739	160	24.6	1520	50.7	0.5
100	1	Male	22800	2720	228	27.2	9710	97.1	0.5
		Female	23600	5720	236	57.2	4670	46.7	0.5
	76	Male	36300	3630	363	36.3	12800	128	0.5
		Female	19300	3230	193	32.3	5740	57.4	0.5

Units for Dose: mg/kg/day; $AUC_{(0\text{-}24h)}$±SE: (ng·h/mL); $AUC_{(0\text{-}24h)}$/Dose±SE/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h.

Toxicokinetic parameters of valsartan in rat plasma

Dose	Study Day	Gender	$AUC_{(0\text{-}24h)}$	±SE	$AUC_{(0\text{-}24h)}$/Dose	±SE /Dose	C_{max}	C_{max}/Dose	t_{max}
10	1	Male	6210	1050	621	105	1690	169	1.0
		Female	3950	1060	395	106	1820	182	0.5
	76	Male	4840	726	484	72.6	1990	199	0.5
		Female	3170	431	317	43.1	1290	129	0.5
30	1	Male	17000	4400	565	147	3440	115	1.0
		Female	12600	2980	421	99.3	2030	67.7	0.5
	76	Male	11500	3090	382	103	3440	115	0.5
		Female	10300	978	344	32.6	1900	63.3	0.5
100	1	Male	47800	6430	478	64.3	12300	123	0.5
		Female	46600	4610	466	46.1	9080	90.8	1.0
	76	Male	53500	6620	535	66.2	8660	86.6	0.5
		Female	32600	9390	326	93.9	5640	56.4	0.5

Units for Dose: mg/kg/day; $AUC_{(0\text{-}24h)}$±SE: (ng·h/mL); $AUC_{(0\text{-}24h)}$/Dose±SE/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h.

Dosing Solution Analysis

Samples of the suspensions prepared for weeks 1, 3, and 13 were analyzed, and all results were within specifications. Two control samples were analyzed, and no LCZ696 was detected.

Study title: 13-week oral (gavage) toxicity study in monkeys with a 4-week recovery period

Study no.:	0670282
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover, New Jersey
Date of study initiation:	8/21/2006
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0650001, 95.2%

Key Study Findings

There were no test article-related macroscopic or organ weight changes. A microscopic test article-related change in the kidneys of males and females dosed at 300 mg/kg/day and consisted of minimal or slight hypertrophy of juxtaglomerular cells. This hypertrophy was considered to be consistent with the pharmacologic effect of angiotensin II receptor antagonists and since it was not observed after the 4-week recovery period was considered reversible.

Methods

Doses:	30, 100 and 300 mg/kg/day
Frequency of dosing:	daily
Route of administration:	Oral, gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% CMC
Species/Strain:	Cynomolgus monkey *(macaca fascicularis)*
Number/Sex/Group:	5/group – Control and High dose (Groups 1 & 4) #/group – Low and Mid dose (Groups 2 & 3)
Age:	2-3.5 yrs
Weight:	1.8 to 2.9 kg for males and 2 to 2.8 kg for females
Satellite groups:	no
Unique study design:	no
Deviation from study protocol:	Reported deviations did not impact the outcome of the study

Observations and Results

Mortality

Twice daily (AM and PM) on weekdays and once daily on weekends and holidays.

There was no mortality in the study.

Clinical Signs

At least twice daily (predose and approximately 2 hours postdose)

Test article-related minor occasional clinical signs of fecal changes and emesis were observed. Fecal changes of diarrhea were observed at doses ≥ 30 mg/kg/day in males and at doses ≥ 100 mg/kg/day in females. Soft feces were present in both males and females at doses ≥ 100 mg/kg/day. Soft feces and low grade diarrhea were also present in controls but present for longer periods or with increased severity with the test article in above mentioned groups.

Body Weights

Dosing period: Approximately once weekly
Recovery period: Approximately once weekly

Test article-related slight decrease in absolute body weight gain was observed at doses ≥ 100 mg/kg/day in males and at 300 mg/kg/day in females at the end of dosing period. The absolute body weight gain was in the range between 0 – 0.3 kg at both 100 and 300 mg/kg/day in males compared to the range of absolute body weight gain of 0.2 – 0.5 kg in controls.

Feed Consumption

Estimated daily, from the beginning of the pretest period to the end of the recovery period, except after any overnight fast.

There were no test article-related food consumption effects.

Ophthalmoscopy

Week 13: All surviving control and high-dose animals

There were no test article-related ophthalmology findings.

ECG

Week 14: All surviving animals between 2 to 2 hours and 45 minutes postdose. As per protocol, the ECG data should be collected approximately 2 hour post-dose. This deviation did not impact the outcome of the study.

There were no test article-related ECG findings.

Hematology

Hematology parameters: erythrocytes, Wintrobe indices, white blood cell count, Hematocrit, red cell distribution width (RDW), white blood cell differential, Hemoglobin, reticulocytes, platelets

Hemostasis/thrombosis parameters
prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen

There were no test article-related changes in hematology in male animals. At 300 mg/kg/day, one female (no. 4501) had a minimal decrease in the RBC count, hemoglobin concentration and hematocrit on day 89, when values were compared to those from the pretest period and concurrent controls.

Clinical Chemistry

Clinical pathology assessments were conducted pretest, during weeks 5 (urine samples were also collected during week 7), 13 and recovery week 4. Animals were fasted for approximately 18 hours prior to blood specimen collection.
Parameters:

alanine aminotransferase	globulins (G)	chloride
alkaline phosphatase	glucose	calcium
aspartate aminotransferase	blood urea nitrogen	inorganic phosphorus
total bilirubin	creatinine	triglycerides
total protein	sodium	cholesterol
albumin (A)	potassium	A/G ratio
creatine kinase		

One female animal (no. 4501) dosed at 300 mg/kg/day had a mild increase in serum urea (day 89) and creatinine (days 31 and 89) concentrations when values were compared to those from the pretest period and concurrent controls.

Urinalysis

specific gravity	glucose*	protein*
bilirubin*	ketones*	urobilinogen*
blood*	pH*	

*test strip determination

Urine specific gravity was in the isosthenuric range (osmolality similar to that of plasma) in animal no. 4501, which is significant given the presence of increased urea and creatinine concentrations in the serum and is considered a test article-related effect. There were no test article-related changes apparent in male animals.

Gross Pathology

Animals were fasted overnight (approximately 18 hours) prior to terminal necropsy. A prosector order was prepared per SOP. Complete necropsies were performed with a recording of macroscopic abnormalities for all protocol tissues.

Tissue list for collection, weighing (W), genomics (G) and/or processing (P)

W	P	adrenal	W	P	ovary (with oviduct)
	P	aorta		P	pancreas
	P	bone marrow (in bone)		P	parathyroid
	P	bone marrow smear	W	P	pituitary
W	P	brain	W	P	prostate
	P	cecum		P	rectum
	P	cervix		P	salivary gland
	P	colon		P	sciatic nerve
	P	duodenum		P	seminal vesicle
	P	epididymis		P	skeletal muscle
	P	esophagus		P	skin
	P	eye		P	spinal cord
	P	femur/tibia	W	P	spleen
	P	harderian gland		P	sternum
W	P	heart	G	P	stomach
	P	ileum	W	P	testis
	P	jejunum	W	P	thymus
W	P	kidney	W	P	thyroid
	P	lacrimal gland		P	tongue
	P	larynx-cross section		P	trachea
W	P	liver		P	ureter-cross section
	P	lung		P	urinary bladder
	P	lymph node - bronchial	W	P	uterus
	P	lymph node - mandibular		P	vagina
	P	lymph node - mesenteric		P	macroscopic lesions
	P	mammary gland area			animal identification
		nasal passage			

Organ Weights

There were no test article-related changes in organ weights.

Histopathology

Adequate Battery

yes

Peer Review

All assessments were peer-reviewed per SOP.

Histological Findings

Test article-related minimal or slight hypertrophy of the juxtaglomerular cells occurred in the kidney of all males and females dosed at 300 mg/kg/day. Individual cells typically had a plump, ovoid outline with a pale, eosinophilic, finely granular cytoplasm. They were arranged in tight circular to ovoid groups adjacent to glomeruli and within the wall

of the afferent arterioles. A spectrum of various other minimal or slight kidney changes occurred, including multifocal interstitial lymphoid cell infiltration, and tubular pigment deposition, mineralization and dilatation, however, their low severity, lack of any clear trend, and the presence of similar findings in concurrent control animals was not consistent with any of them being test article related.

Kidneys in the treated recovery animals were similar to concurrent controls.

Toxicokinetics

Blood was obtained from non-recovery animals on study days 1/2 and in week 11. Main study animals (non-recovery) were bled at 0.5, 1, 2, 6, and 24 hours postdose. Approximately 1 mL whole blood was collected and transferred into tubes containing EDTA and placed on wet ice. All blood samples were centrifuged at approximately 4°C within approximately 30 minutes after collection, plasma samples were obtained and samples were frozen at approximately -60°C or below before transfer to the Toxicokinetic investigator. All samples were analyzed. Concentrations of Valsartan, AHU377 and its active metabolite LBQ657 in plasma, AUC, AUC/dose, C_{max}, C_{max}/dose, t_{max} were determined.

Mean (n=3) toxicokinetic parameters of AHU377 in monkey plasma

Dose	Study Day	Gender	$AUC_{(0-24h)}$	±SD	$AUC_{(0-24h)}$/Dose	±SD /Dose	C_{max}	C_{max}/Dose	t_{max}
30	1	Male	6590	1650	219	55.2	3710	124	0.8
		Female	6450	2850	215	94.9	5800	193	0.5
	73	Male	5990	728	200	24.2	4920	164	0.5
		Female	5760	2700	192	90.0	4020	134	0.5
100	1	Male	24500	13900	245	139	8300	83.0	1.2
		Female	40100	19800	401	198	16000	160	0.8
	73	Male	24600	16800	246	168	11900	119	1.2
		Female	34500	22800	345	228	15500	155	0.5
300	1	Male	126000	67600	419	226	42400	142	1.2
		Female	134000	61600	445	204	49200	164	1.5
	73	Male	147000	21500	491	73.0	96000	320	0.5
		Female	154000	29400	515	98.5	71200	237	1.0

Units for Dose: mg/kg/day; $AUC_{(0-24h)}$±SD: (ng·h/mL); $AUC_{(0-24h)}$/Dose±SD/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h.

(cont.)

Mean (n=3) toxicokinetic parameters of LBQ657 in monkey plasma

Dose	Study Day	Gender	$AUC_{(0-24h)}$	±SD	$AUC_{(0-24h)}$/Dose	±SD/Dose	C_{max}	C_{max}/Dose	t_{max}
30	1	Male	47700	723	1590	26.5	18000	600	1.2
		Female	35800	8150	1190	272	17200	573	0.7
	73	Male	37800	529	1260	17.3	17600	587	0.8
		Female	34000	6550	1130	219	17700	591	0.5
100	1	Male	189000	27100	1890	271	53900	539	1.7
		Female	146000	32400	1460	324	40300	403	1.0
	73	Male	176000	35900	1760	359	47400	474	1.7
		Female	146000	34200	1460	342	48300	483	0.5
300	1	Male	687000	322000	2290	1080	129000	430	2.0
		Female	551000	206000	1840	688	126000	419	1.7
	73	Male	748000	137000	2490	458	183000	610	1.3
		Female	894000	482000	2990	1620	186000	620	1.7

Units for Dose: mg/kg/day; $AUC_{(0-24h)}$±SD: (ng·h/mL); $AUC_{(0-24h)}$/Dose±SD/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h.

Mean (n=3) toxicokinetic parameters of valsartan in monkey plasma

Dose	Study Day	Gender	$AUC_{(0-24h)}$	±SD	$AUC_{(0-24h)}$/Dose	±SD /Dose	C_{max}	C_{max}/Dose	t_{max}
30	1	Male	7900	1360	263	45.2	1310	43.8	1.0
		Female	8540	455	285	15.0	1700	56.8	1.5
	73	Male	5650	1500	188	50.2	1170	38.9	0.5
		Female	4920	1310	164	43.7	1110	36.9	0.5
100	1	Male	29200	8190	292	81.9	6250	62.5	2.3
		Female	15200	4120	152	41.2	3260	32.6	1.0
	73	Male	20500	3610	205	36.1	2990	29.9	1.5
		Female	12500	1810	125	18.1	3140	31.4	0.5
300	1	Male	97700	31700	326	107	30000	100	0.7
		Female	78300	32100	262	108	21700	72.4	1.7
	73	Male	74900	14100	250	46.6	33400	111	0.5
		Female	70300	36400	234	121	16200	54.1	0.7

Units for Dose: mg/kg/day; $AUC_{(0-24h)}$±SD: (ng·h/mL); $AUC_{(0-24h)}$/Dose±SD/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h.

Dosing Solution Analysis

Samples of the suspensions prepared for weeks 1, 9, and 13 were analyzed, and all results were within specifications. Two control samples were analyzed, and no LCZ696 was detected.

Study title: A 26-week oral (gavage) toxicity study in rats with a 4-week recovery period

Study no.:	0670620
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover, New Jersey
Date of study initiation:	12/12/2006
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0651004, 94.6%

Key Study Findings

LCZ696-related decreased heart weights were noted in rats of both sexes administered 100 mg/kg/day. Heart weights were still slightly lower in the high dose recovery males at the end of the 4-week recovery period. LCZ696-related microscopic findings consisted of increases in the incidence of minimal to slight focal erosion and minimal to moderate mixed inflammatory cell infiltrates in the glandular stomachs of both sexes at 100 mg/kg/day. Males generally appeared to be more often affected than females and these lesions were considered reversible.

Methods

Doses:	10, 30, 100mg/kg
Frequency of dosing:	daily
Route of administration:	Oral
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% CMC
Species/Strain:	IGS Wistar Hannover Rat; Crl: WI(Glx/BRL/Han)IGS BR
Number/Sex/Group:	20
Age:	8 weeks
Weight:	205.1 to 260.8 grams for males and 149.0 to 187.0 grams for females (at start of dosing)
Satellite groups:	10/group in Control and High dose groups for recovery
Unique study design:	no
Deviation from study protocol:	Reported deviations did not impact the study especially after the duration of the study is taken into account.

Observations and Results

Mortality

Twice daily (AM and PM) on weekdays and once daily on weekends and holidays.

There was no test article-related mortality noted during the study.

Clinical Signs

At least twice daily (predose and approximately 1 hour postdose).

Test article-related clinical signs observed during the study included hypersensitivity to touch at doses ≥ 10 mg/kg/day, increased locomotor activity at doses ≥ 30 mg/kg/day and piloerection at 100 mg/kg/day. The incidence and/or number of animals affected by these signs increased in a dose-proportional manner. These clinical signs were not present during the recovery period.

Body Weights

Approximately weekly during weeks 1-14 (days 1-92), every two weeks during weeks 16-26 (days 106-176) and week 27 (day 183).

During the recovery period: Approximately once weekly up to and including day 30.

Test article-related, statistically significant decreases in mean body weight were noted in males at 100 mg/kg/day beginning on day 15 and were 8.1% lower versus concurrent controls by day 183. Test article-related, statistically significant decreases in mean body weight gain of 8.3 and 16.1% by day 183 were noted in males at doses of 30 and 100 mg/kg/day, respectively.

The reduction in body weight parameters in males at 100 mg/kg/day was still evident after the 4-week recovery period.

Feed Consumption

Approximately weekly during weeks 1-14 (food consumed calculated weekly up to day 92), every two weeks during weeks 16-26 (food consumed calculated every other week during days 106-176), and week 27 (day 183).

Recovery period: Approximately once weekly up to and including day 30

Test article-related, statistically significant, reductions in food consumption were noted in males at 100 mg/kg/day in 10 of 20 measured intervals throughout the dosing period. There were no effects in females. Food consumption was comparable to controls during the recovery period.

Ophthalmoscopy

Pretest: All animals
Weeks 13 and 26: All surviving control and high-dose animals.

There were no test article-related effects on ophthalmology.

Hematology

Parameters:
Erythrocytes, Wintrobe indices, white blood cell count, hematocrit, red cell distribution width (RDW), white blood cell differential, hemoglobin, reticulocytes, platelets

There were no test article-related changes in hematology.

Clinical Chemistry

Clinical pathology assessments were conducted on the last 10 surviving animals/sex/group in weeks 14, 25 and recovery week 4. Blood specimens were collected from the sublingual vein from isoflurane/O_2-anesthetized animals into EDTA-containing tubes for hematology (approximately 0.5 mL) and serum collection tubes for clinical chemistry (approximately 1 mL).

alanine aminotransferase	globulins (G)	chloride
alkaline phosphatase	glucose	calcium
aspartate aminotransferase	blood urea nitrogen	inorganic phosphorus
total bilirubin	creatinine	triglycerides
total protein	sodium	cholesterol
albumin (A)	potassium	A/G ratio
creatine kinase	magnesium	

There were no test article-related changes in clinical chemistry.

Urinalysis

specific gravity	glucose*	protein*
bilirubin*	ketones*	urobilinogen*
blood*	pH*	

*test strip determination

There were no test article-related changes in urinalysis parameters.

Gross Pathology

Animals were fasted overnight (approximately 12-18 hours) prior to terminal necropsy. Fasted terminal body weights were collected by Pathology personnel prior to scheduled necropsies for the calculation of relative organ to body weight determinations. A prosector order was prepared per Pathology SOPs. Complete necropsies were performed with a recording of macroscopic abnormalities for all protocol tissues.

Tissue list for collection, weighing (W) and/or processing (P)

W	P	adrenal	W	P	ovary (with oviduct*)
	P	aorta		P	pancreas
	P	bone marrow (in bone)		P	parathyroid
	P	bone marrow smear	W	P	pituitary
W	P	brain	W	P	prostate
	P	cecum		P	rectum
	P	cervix		P	salivary gland
	P	colon		P	sciatic nerve
	P	duodenum		P	seminal vesicle
	P	epididymis		P	skeletal muscle
	P	esophagus		P	skin
	P	eye		P	spinal cord
	P	femur/tibia	W	P	spleen
	P	harderian gland		P	sternum
W	P	heart		P	stomach
	P	ileum	W	P	testis
	P	jejunum	W	P	thymus
W	P	kidney	W	P	thyroid
	P	lacrimal gland		P	tongue
	P	larynx-cross section		P	trachea
W	P	liver		P	ureter-cross section*
	P	lung		P	urinary bladder
	P	lymph node - bronchial	W	P	uterus
	P	lymph node - mandibular		P	vagina
	P	lymph node - mesenteric		P	macroscopic lesions
	P	mammary gland area			animal identification
		nasal passage			

* Bilateral collection, unilateral histopathology

Organ Weights

Statistically significant lower heart weights (absolute and relative to body and brain) in both sexes at 100 mg/kg/day. Slightly lower heart weights were still present in the high-dose recovery males at the end of the 4-week recovery period.

Reduced heart weight is commonly seen in studies of antihypertensive drugs and is a response to the decreased cardiac afterload.

Histopathology

Adequate Battery

Yes

Peer Review

All assessments were peer-reviewed per SOP.

Histological Findings

LCZ696-related microscopic findings of inflammation and minimal to slight focal erosions were observed in the stomachs of both sexes at 100 mg/kg/day. These changes were no longer present at the end of the 4-week recovery period. Inflammation occurred primarily along the muscularis mucosa and in the submucosa of the glandular stomach consisting of an increased incidence of generally minimal to moderate mixed inflammatory cell infiltrates composed of admixtures of mostly eosinophils and some neutrophils as well as some mononuclear inflammatory cells.

LCZ696-related findings in stomach

Group	1	2	3	4	1 Recovery	4 Recovery
Dose level (mg/kg/day)	0	10	30	100	0	100
Number of animals.	20	20	20	20	10	10
Males:						
Mixed Cell Inflammation	2	1	1	13	0	0
Erosion, Glandular Stomach	1	0	1	5	0	0
Females:						
Mixed Cell Inflammation	0	0	0	7	0	0
Erosion, Glandular Stomach	0	0	0	1	0	0

Toxicokinetics

Blood was obtained from the first 10 surviving animals/sex/group on study days 1-2 and in weeks 4 and 22. Two animals/sex/group were bled at 0.5, 1, 2, 6 and 24 hours post dose. Approximately 0.5 mL of whole blood were collected from the sublingual vein of isoflurane/O_2-anesthetized animals into tubes containing EDTA and approximately 10-20 mg of sodium fluoride (NaF) and placed on wet ice.

Toxicokinetic parameters for HUU377, valsartan and LBQ657 are summarized below.

Toxicokinetic parameters of AHU377 in rat plasma

Dose	Study Day	Gender	$AUC_{(0-24)}$	± SE	$AUC_{(0-24)}$/Dose	±SE/Dose	C_{max}	C_{max}/Dose	t_{max}
10	1	Male	18.8	11.6	1.88	1.16	15.4	1.54	1.0
		Female	12.4	9.14	1.24	0.914	10.8	1.08	1.0
	24	Male	NA	NA	NA	NA	24.6	2.46	0.5
		Female	NA	NA	NA	NA	16.2	1.62	0.5
	150	Male	NA	NA	NA	NA	7.70	0.770	0.5
		Female	NA	NA	NA	NA	7.25	0.725	0.5
30	1	Male	29.9	21.5	0.995	0.717	28.6	0.953	1.0
		Female	NA	NA	NA	NA	41.2	1.37	0.5
	24	Male	33.2	4.93	1.11	0.164	37.3	1.24	0.5
		Female	51.5	25.6	1.72	0.853	47.3	1.58	0.5
	150	Male	15.3	9.08	0.510	0.303	12.6	0.420	0.5
		Female	74.4	25.9	2.48	0.863	61.7	2.06	0.5
100	1	Male	374	174	3.74	1.74	202	2.02	0.5
		Female	184	77.0	1.84	0.770	128	1.28	0.5
	24	Male	163	NC	1.63	NC	155	1.55	0.5
		Female	483	273	4.83	2.73	312	3.12	0.5
	150	Male	253	134	2.53	1.34	87.2	0.872	0.5
		Female	239	59.4	2.39	0.594	206	2.06	0.5

Units for each parameter: Dose: mg/kg/day; $AUC_{(0-24h)}$±SE: (ng·h/mL); $AUC_{(0-24h)}$/Dose±SE/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h; NC = not calculable

NA: AUC were not calculated due to lack of sufficient measurable AHU377 concentrations.

Toxicokinetic parameters of valsartan in rat plasma

Dose	Study Day	Gender	$AUC_{(0-24)}$	± SE	$AUC_{(0-24)}$/Dose	±SE/Dose	C_{max}	C_{max}/Dose	t_{max}
10	1	Male	3210	743	321	74.3	1250	125	0.5
		Female	2710	451	271	45.1	1430	143	0.5
	24	Male	2410	619	241	61.9	1360	136	0.5
		Female	2740	474	274	47.4	1340	134	0.5
	150	Male	2710	584	271	58.4	1110	111	0.5
		Female	2240	345	224	34.5	1440	144	0.5
30	1	Male	12400	2730	413	91.0	2610	87.0	1.0
		Female	6220	664	207	22.1	4210	140	0.5
	24	Male	8660	1240	289	41.3	2530	84.3	0.5
		Female	5580	967	186	32.2	2470	82.3	0.5
	150	Male	8650	1980	288	66.0	1240	41.3	1.0
		Female	7560	2450	252	81.7	3080	103	0.5
100	1	Male	47200	7760	472	77.6	9600	96.0	0.5
		Female	31200	7410	312	74.1	6250	62.5	0.5
	24	Male	47400	NC	474	NC	11200	112	0.5
		Female	50800	27800	508	278	6970	69.7	0.5
	150	Male	28400	5680	284	56.8	4660	46.6	0.5
		Female	25900	4850	259	48.5	5060	50.6	0.5

Units for each parameter: Dose:mg/kg/day; $AUC_{(0-24h)}$±SE: (ng·h/mL); $AUC_{(0-24h)}$/Dose±SE/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h. NC: not calculable

Toxicokinetic parameters of LBQ657 in rat plasma

Dose	Study Day	Gender	$AUC_{(0-24)}$	± SE	$AUC_{(0-24)}$/Dose	±SE/Dose	C_{max}	C_{max}/Dose	t_{max}
10	1	Male	1630	346	163	34.6	1190	119	0.5
		Female	1170	305	117	30.5	1160	116	0.5
	24	Male	1470	678	147	67.8	1040	104	0.5
		Female	1580	556	158	55.6	1550	155	0.5
	150	Male	1620	496	162	49.6	1360	136	0.5
		Female	1730	452	173	45.2	1830	183	0.5
30	1	Male	5300	2160	177	72.0	1770	59.0	1.0
		Female	3410	557	114	18.6	3540	118	0.5
	24	Male	3830	867	128	28.9	2000	66.7	1.0
		Female	3310	486	110	16.2	2260	75.3	0.5
	150	Male	3620	670	121	22.3	1600	53.3	1.0
		Female	6720	1980	224	66.0	4140	138	0.5
100	1	Male	24700	6760	247	67.6	9400	94.0	0.5
		Female	11200	3900	112	39.0	4250	42.5	0.5
	24	Male	21100	NC	211	NC	15700	157	0.5
		Female	10900	2400	109	24.0	8090	80.9	0.5
	150	Male	14700	5280	147	52.8	4930	49.3	0.5
		Female	15700	5590	157	55.9	10600	106	0.5

Units for each parameter: Dose: mg/kg/day; $AUC_{(0-24h)}$±SE: (ng·h/mL); $AUC_{(0-24h)}$/Dose±SE/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h. NC: not calculable

Dosing Solution Analysis

Samples of the suspensions prepared for weeks 1, 13 and 26 were analyzed, and all results were within specifications. Three control samples were analyzed, and no LCZ696 was detected.

Study title: A 39-Week Toxicity Study of LCZ696 Administered by Nasal Gavage to Cynomolgus Monkeys, with a 4-Week Recovery Period

Study no.:	0670621
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2/20/2007
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0651002

Key Study Findings

LCZ696−related histologic changes on Day 274 consisted of juxtaglomerular cell hypertrophy/hyperplasia in the kidneys of cynomolgus monkeys at 100 or 300 mg/kg. The incidence, severity, and distribution within the kidney of these juxtaglomerular cell changes increased with increasing dose, consistent with a dose response. On Day 303, after a 4-week dose-free period, juxtaglomerular cell hypertrophy/hyperplasia had not resolved in 300 mg/kg females, but was lessened in distribution and severity in 300 mg/kg males.

Changes in hematology parameters included a mild decline in red cell mass (i.e., red blood cell count, hemoglobin concentration, and hematocrit) at 300 mg/kg (predominantly observed in female monkeys) on Days 91 and 267. Reticulocyte counts were diminished on Days 22, 91, and/or 267 when compared to the decline in circulating red cell mass. At the end of the recovery phase, all values for the 300 mg/kg monkeys had returned to baseline.

LCZ696-related changes in serum chemistry parameters included increased blood urea nitrogen (BUN) values in most monkeys at 300 mg/kg on Days 91 and 267. This finding correlated with the JG cell hypertrophy.

Methods

Doses:	30, 100, 300 mg/kg
Frequency of dosing:	daily
Route of administration:	nasal gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% CMC
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis)*
Number/Sex/Group:	See chart below
Age:	2.4 to 3.4 years of age for the males and 2.6 to 3.8 years ofage for the females (at start of dosing)
Weight:	2.0 to 3.1 kg for the males and 2.0 to 2.6 kgfor the females (Week -1)
Deviation from study protocol:	Deviations did not affect the overall integrity of the data obtained in this study or the overall conclusions of the study.

Group assignments and dose Levels

Group No.	Number of Males/Females	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Solution Conc. (mg/mL)
1	6/6	0 (control)	5	0
2	4/4	30	5	6
3	4/4	100	5	20
4	6/6	300	5	60

Observations and Results

Mortality and Clinical Signs

Cage side observation data were collected once daily (a.m., prior to dosing) and 2 hours post dose ± 30 minutes from Day -7 to Days 274 and 302

One 300 mg/kg male (4005) was found dead on Day 44 and one control female (1605) was humanely euthanized on Day 140. Early deaths were not LCZ696-related, although there were LCZ696-related histologic changes in the juxtaglomerular apparatus of the kidney for the 300 mg/kg male (4005) that were similar to, but more severe than those seen in other 300 mg/kg monkeys. This male (4005) was also listed as dying acutely from a gavage incident on the same day it was reported as found dead.

There were no LCZ696−related clinical signs, unscheduled observations or post dose observations, or changes in food consumption.

Body Weights

Week -1 and weekly thereafter.

There were no LCZ696−related changes in body weight.

Feed Consumption - not reported

Ophthalmoscopy

Prestudy and on Day 272. Direct ophthalmic exams were performed on all monkeys at approximately 3 hours post dose ± 30 minutes (following ECG measurements).

There were no LCZ696−related ophthalmic findings.

ECG

Prestudy and on Days 90, 181, and 272. Measurements were taken on all monkeys at 2 hours post dose ± 30 minutes (prior to any scheduled ophthalmic exams that may occur on the same day). Leads: I, II, III, aVR, aVL, and aVF.

All the electrocardiograms evaluated in this study were qualitatively considered normal for cynomolgus monkeys. No arrhythmias were found.

Hematology

Parameters: Red blood cell (RBC) count, Reticulocyte counts, Platelet counts, White blood cell (WBC) count,* Mean corpuscular hemoglobin (MCH), Blood cell morphology**, Hemoglobin concentration, Mean corpuscular volume (MCV), Hematocrit, and Mean corpuscular hemoglobin concentration (MCHC)

Hemostasis/thrombosis parameters: Prothrombin time (PT), Activated partial thromboplastin time (APTT), Fibrinogen

LCZ696-related changes in hematology parameters included a mild decline in red cell mass (i.e., red blood cell count, hemoglobin concentration, and hematocrit) at 300 mg/kg (predominantly observed in female monkeys) on Days 91 and 267 (statistically significant on Days 91 and/or 267). Reticulocyte counts were diminished on Days 22, 91, and/or 267 when compared to the decline in circulating red cell mass. At the end of the recovery phase, all values for the 300 mg/kg monkeys had returned to baseline.

There were no LCZ696-related changes in coagulation parameters.

Clinical Chemistry

Blood samples for evaluation of serum chemistry, hematology, and coagulation parameters were collected from all monkeys within two weeks prior to dose administration and on Days 22, 91, 267, and 302. Monkeys were fasted for at least 8 hours prior to blood collections for serum chemistry.

Serum chemistry parameters

Sodium	Alanine aminotransferase (ALT)	Albumin
Potassium	Gamma-glutamyltransferase (GGT)	Globulin
Chloride	Creatinine kinase (CK)	Albumin/Globulin ratio
Carbon dioxide	Calcium	Glucose
Total bilirubin	Phosphorus	Cholesterol
Alkaline phosphatase (ALP)	Urea nitrogen (BUN)	Triglycerides
Lactate dehydrogenase (LDH)	Creatinine	
Aspartate aminotransferase (AST)	Total protein	

LCZ696-related changes in serum chemistry parameters included increased blood urea nitrogen (BUN) values in most monkeys at 300 mg/kg on Days 91 and 267 (statistically significant on Day 267 for males).

The incidence of increased BUN in 300 mg/kg monkeys on Days 91 and 267 correlated with histologic observations of juxtaglomerular cell hypertrophy/hyperplasia noted histologically.

Urinalysis

Parameters: Color/character, Protein, Bilirubin, pH, Glucose, Occult blood, Specific gravity, Ketones, microscopics

There were no LCZ696-related changes in urinalysis parameters.

Gross Pathology

On Day 274, thirty monkeys (4/sex/Groups 2 and 3, 4 males and 3 females/Group 1 and 3 females and 4 males/Group 4) were euthanized. The remaining 8 monkeys (2/sex in Groups 1 and 4) continued on study, without further dosing for approximately 4 weeks, and were euthanized on Day 303.

Tissue list for collection, weighing (W) and/or processing (P)

W	P	Adrenal	W	P	Ovaries
	P	Aorta		P	Oviducts
		Monkey Number Tattoo		P	Pancreas
	P	Bone (femoral head)	W	P	Parathyroid
	P	Bone (sternum)	W	P	Pituitary
	P	Bone (7th rib)		P	Prostate
W	P	Brain		P	Rectum
	P	Cecum		P	Salivary gland (mandibular)
	P	Cervix		P	Sciatic nerve
	P	Colon		P	Seminal Vesicles
	P	Duodenum		P	Skeletal muscle (psoas and diaphragm)
	P	Epididymides		P	Skin
	P	Esophagus		P	Spinal cord (cervical, thoracic, lumbar)
	P	Eyes with optic nerve	W	P	Spleen
	P	Gall bladder		P	Stomach
	P	Gross Lesions	W	P	Testes
W	P	Heart	W	P	Thymus
	P	Ileum	W	P	Thyroid
	P	Jejunum		P	Tongue
W	P	Kidney		P	Trachea
	P	Larynx		P	Ureters
W	P	Liver		P	Urinary bladder
	P	Lung		P	Uterus
	P	Lymph node – bronchial		P	Vagina
	P	Lymph node – mandibular		P	Macroscopic lesions
	P	Lymph node – mesenteric			Monkey identification
	P	Mammary Gland			

W – weighed; P = processed

Organ Weights

Organs Weighed	
Adrenals	Heart
Kidneys	Liver
Pituitary	Ovaries
Testes	Spleen
Thyroid with parathyroids	Thymus
Brain	

There were no LCZ696-related alterations in organ weights or organ weight ratios in monkeys euthanized at Terminal Necropsy on Day 274 or after a 4 week dose-free period on Day 303.

Histopathology

Adequate Battery

Yes

Peer Review

The pathology results were formally peer reviewed by another [redacted] pathologist, [redacted].

Histological Findings

There were no LCZ696-related gross findings.

On Day 274, there was juxtaglomerular cell hypertrophy/hyperplasia in the kidneys of cynomolgus monkeys at 100 and 300 mg/kg. The incidence, severity, and distribution within the kidney of these juxtaglomerular changes increased with increasing dose, consistent with a dose response. Juxtaglomerular hypertrophy/hyperplasia has been described previously with angiotensin II antagonists, and is considered an exaggerated pharmacologic effect.

On Day 303, after a 4-week dose-free period, juxtaglomerular cell hypertrophy/hyperplasia had not resolved in 300 mg/kg females, but was lessened in distribution and severity in 300 mg/kg males. Due to the low number of monkeys (2/sex/group) at this time-point it was not possible to determine if this represented a gender difference.

Special Evaluation

Brain slices were prepared and immune-stained for β1-42-amyloid. Results are discussed under Special Toxicology Studies.

Toxicokinetics

Time Points: Predose and at 0.5, 1, 2, 6, and 24 hours post dose on Day 1 and predose and at 0.5, 1, 2, 6, and 24 hours post dose from all monkeys on Days 29 and 272.

The plasma profiles showed that monkeys in the LCZ696 treated dose groups were exposed to AHU377 (pro-drug for LBQ657), LBQ657 and valsartan (the active principals of LCZ696). The tmax values for AHU377, LBQ657, and valsartan ranged from 0.5 to 6.0 hour post dose. After oral administration of LCZ696 to monkeys, AHU377 was rapidly converted to its active metabolite LBQ657, as demonstrated by the short tmax values. Following single or multiple oral doses of LCZ696, no consistent difference in exposure to AHU377, LBQ657, and valsartan was observed between males and females. The increase in exposure (AUC and Cmax) to AHU377, LBQ657, and valsartan was generally proportional to the dose increase for both male and female monkeys after single and multiple doses within the dose range tested in this study. The mean exposure to AHU377, LBQ657, and valsartan on Days 29 and 272 was generally similar to the exposure on Day 1 in both male and female monkeys, suggesting no accumulation.

Mean (n=4) toxicokinetic parameters of AHU377 in monkey plasma

Dose	Study Day	Gender	$AUC_{(0\text{-}24h)}$	±SD	$AUC_{(0\text{-}24h)}$/Dose	±SD	C_{max}	±SD	C_{max}/Dose	±SD	t_{max}
30	1	Male	4650	2500	155	83.1	2310	1370	77.1	45.7	0.750
		Female	3130	1200	104	40.1	1340	516	44.6	17.2	0.500
	29	Male	5110	3270	170	109	4470	3670	149	122	0.500
		Female	4160	1530	138	50.8	1880	1060	62.5	35.3	0.625
	272	Male	2640	1010	87.9	33.9	1270	996	42.5	33.2	0.750
		Female	2000	594	66.5	19.8	1020	742	34.1	24.7	0.625
100	1	Male	19100	4550	191	45.5	11000	4970	110	49.7	0.750
		Female	23000	12100	230	121	14800	7900	148	79.0	0.750
	29	Male	24500	8490	245	84.9	12500	10900	125	109	0.750
		Female	28300	10400	283	104	16400	10800	164	108	0.625
	272	Male	20800	4630	208	46.3	13100	3680	131	36.8	0.625
		Female	20100	10800	201	108	11700	6990	117	69.9	0.625
300	1	Male	123000	45400	409	151	51800	24200	173	80.7	1.00
		Female	121000	9430	402	30.8	46400	29200	155	97.2	1.00
	29*	Male	119000	26100	397	87.6	41500	12400	139	41.3	1.42
	29*	Female	130000	23100	433	76.7	56800	20400	190	67.9	0.667
	272**	Male	93400	54200	311	180	36900	35100	123	117	1.60
	272*	Female	111000	41000	371	136	38300	20000	128	66.6	1.08

Units for Dose: mg/kg/day; $AUC_{(0\text{-}24h)}$±SD: (ng·h/mL); $AUC_{(0\text{-}24h)}$/Dose±SD/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h. *: n=6, **: n=5

Mean (n=4) toxicokinetic parameters of LBQ657 in monkey plasma

Dose	Study Day	Gender	$AUC_{(0\text{-}24h)}$	±SD	$AUC_{(0\text{-}24h)}$/Dose	±SD	C_{max}	±SD	C_{max}/Dose	±SD	t_{max}
30	1	Male	29300	4570	978	154	11400	1830	381	61.0	0.875
	1	Female	26500	6560	882	217	7940	2400	264	80.1	0.750
	29	Male	27900	4210	931	142	11200	2670	374	89.0	0.500
	29	Female	28800	8820	960	296	13400	6920	447	230	0.625
	272	Male	30300	7180	1010	239	8680	3310	289	110	1.00
	272	Female	24100	10700	802	356	6680	3720	223	124	0.875
100	1	Male	117000	37700	1170	377	38900	23400	389	234	1.13
	1	Female	140000	37200	1400	372	55300	33200	553	332	1.13
	29	Male	129000	31100	1290	311	37400	21500	374	215	1.13
	29	Female	135000	27400	1350	274	45800	15400	458	154	0.750
	272	Male	148000	50700	1480	507	44600	30400	446	304	0.750
	272	Female	156000	22100	1560	221	48500	15000	485	150	1.00
300	1	Male	673000	176000	2240	585	117000	32100	389	107	1.75
	1	Female	551000	119000	1840	394	102000	19000	340	63.4	1.75
	29*	Male	884000	676000	2950	2250	152000	18400	505	61.3	1.83
	29*	Female	564000	68700	1880	230	129000	26400	429	87.9	1.67
	272**	Male	659000	110000	2200	365	141000	51500	468	172	2.00
	272*	Female	569000	154000	1900	513	146000	60900	486	203	2.00

Units for Dose: mg/kg/day; $AUC_{(0\text{-}24h)}$±SD: (ng·h/mL); $AUC_{(0\text{-}24h)}$/Dose±SD/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h. *: n=6, **: n=5

Mean (n=4) toxicokinetic parameters of valsartan in monkey plasma

Dose	Study Day	Gender	$AUC_{(0\text{-}24h)}$	±SD	$AUC_{(0\text{-}24h)}$/Dose	±SD	C_{max}	±SD	C_{max}/Dose	±SD	t_{max}
30	1	Male	4970	2350	166	78.6	781	245	26.1	8.17	0.750
	1	Female	4820	3330	161	111	1450	1150	48.5	38.4	0.625
	29	Male	3740	965	125	32.2	1070	234	35.7	7.81	0.500
	29	Female	4330	1640	144	54.9	1280	761	42.5	25.4	0.625
	272	Male	9990	4650	333	155	813	252	27.1	8.39	4.63
	272	Female	7010	3050	234	102	825	442	27.5	14.7	2.00
100	1	Male	13900	2870	139	28.7	4980	2560	49.8	25.6	0.625
	1	Female	15600	10700	156	107	5580	4740	55.8	47.4	0.625
	29	Male	16900	3400	169	34.0	3410	2350	34.1	23.5	0.750
	29	Female	15500	4850	155	48.5	3710	1770	37.1	17.7	0.750
	272	Male	29100	16700	291	167	2900	1360	29.0	13.6	2.25
	272	Female	46200	27500	462	275	4270	1700	42.7	17.0	3.50
300	1	Male	84900	25200	283	83.2	34000	23800	113	79.3	1.00
	1	Female	77100	16500	257	54.8	28100	24200	93.7	80.9	0.625
	29*	Male	83800	59400	280	198	18700	12900	62.3	43.1	0.750
	29*	Female	64500	13900	215	46.5	16400	4120	54.7	13.7	1.00
	272**	Male	69800	27400	233	91.6	9330	6360	31.1	21.2	2.40
	272*	Female	65200	15700	217	52.2	10600	6340	35.2	21.1	1.58

Units for Dose: mg/kg/day; $AUC_{(0\text{-}24h)}$±SD: (ng·h/mL); $AUC_{(0\text{-}24h)}$/Dose±SD/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h. *: n=6, **: n=5

Dosing Solution Analysis

Samples of the dosing suspension prepared for Weeks 1, 4, 12, 24, and 36 were within specifications. Five control samples were analyzed, and no LCZ696 was detected.

6.2.2 Studies with AHU377

Study title: 13-week oral (gavage) toxicity study in rats

Study no.:	0570207
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover, New Jersey
Date of study initiation:	7/20/2005
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0451002, 97.3%

Key Study Findings

There was no test article-related mortality or moribundity during the study. There were no significant test article-related clinical signs, changes in body weight parameters, food consumption data or ophthalmoscopic examination in any treated groups. A minimal test article-related decrease in thyroid gland weights occurred in females at doses ≥ 100 mg/kg/day and males at 400 mg/kg/day, with no histological correlate. There were no test article-related microscopic or macroscopic observations.

Methods

Doses:	50, 100, 200, 400
Frequency of dosing:	daily
Route of administration:	oral
Dose volume:	20 mL/kg (Control and High dose) 5 mL/kg (Low, Mid and Mid-High dose)
Formulation/Vehicle:	0.5% CMC
Species/Strain:	IGS Wistar Hannover Rat; Crl: WI (Glx/BRL/Han) IGS BR
Number/Sex/Group:	10
Age:	8 weeks
Weight:	210.8 to 258.6 g for males 163.0 to 198.6 g for females
Satellite groups:	none
Unique study design:	no
Deviation from study protocol:	None reported

Observations and Results

Mortality

Twice daily (AM and PM) on weekdays and at least once daily on weekends and holidays

There was no test article-related mortality or moribundity during the study.

Clinical Signs

At least twice daily (predose and approximately 2 hours postdose)

There were no significant test article-related clinical signs noted in treated groups.

Body Weights

Once weekly

There were no significant test article-related changes noted in any treated groups during the study. Some minimal changes on body weight parameters noted in treated groups were not considered toxicologically significant.

Feed Consumption

Once weekly.

There were no test article-related changes noted in mean food consumption data from treated groups.

Ophthalmoscopy

Pretest: All animals
Week 13: All surviving control and high-dose animals

Ophthalmoscopic examinations performed during the study did not reveal any ocular changes attributable to treatment with AHU377.

Hematology

parameters: erythrocytes, Wintrobe indices, white blood cell count, hematocrit, red cell distribution width (RDW), white blood cell differential, hemoglobin, reticulocytes, platelets

Hematologic changes associated with the administration of AHU377 were minimal. Changes were seen only in males dosed at doses ≥ 200 mg/kg/day when compared to concurrent controls. A slight, dose-dependent increase in the absolute neutrophil count was present on day 30. All other values were within expected limits.

Clinical Chemistry

Clinical pathology assessments were conducted on all surviving study animals in weeks 5 and 13.

Parameters:

alanine aminotransferase	globulins (G)	Chloride
alkaline phosphatase	glucose	calcium

aspartate aminotransferase	Blood urea nitrogen	inorganic phosphorus
total bilirubin	creatinine	triglycerides
total protein	Sodium	cholesterol
albumin (A)	potassium	A/G ratio

Changes in clinical chemistry were also very mild and none are considered of significance. A minimal increase in serum alanine aminotransferase activity was apparent on both days 30 and 90 in males dosed at 400 mg/kg/day, but this increase was less than 70% over the concurrent control values and is therefore not considered significant.

Urinalysis

Parameters: specific gravity, glucose*, protein*,
bilirubin*, ketones*, urobilinogen*, blood*, pH*,

*test strip determinations

There were no test-article related changes in the urinalysis.

Gross Pathology

Animals were fasted overnight (approximately 18 hours) prior to terminal necropsy. Fasted terminal body weights were collected by Pathology personnel prior to scheduled necropsies for the calculation of relative organ to body weight determinations. A prosector order was prepared per Pathology SOPs. Complete necropsies were performed with a recording of macroscopic abnormalities for all protocol tissues.

Tissue list for collection, weighing (W), processing (P), and/or genomics (G)

W		P	adrenal	W		P	ovary
	G	P	aorta			P	pancreas
		P	bone marrow (in bone)			P	parathyroid
W		P	brain	W		P	pituitary
		P	cecum	W		P	prostate
		P	cervix			P	rectum
		P	colon			P	salivary gland
		P	duodenum			P	sciatic nerve
		P	epididymis			P	seminal vesicle
		P	esophagus			P	skeletal muscle
		P	eye			P	skin
		P	femur/tibia			P	spinal cord
		P	harderian gland	W	G	P	spleen
W	G	P	heart			P	sternum
		P	ileum		G	P	stomach
		P	jejunum	W		P	testis
W	G	P	kidney	W		P	thymus
		P	lacrimal gland	W		P	thyroid
W	G	P	liver			P	tongue
		P	lung			P	trachea
		P	lymph node - bronchial			P	urinary bladder
		P	lymph node - mandibular	W		P	uterus
	G	P	lymph node - mesenteric			P	vagina
		P	mammary gland			P	macroscopic lesions
			nasal passage				animal identification
					G		blood

Organ Weights

A minimal test article-related decrease in mean thyroid gland weights (absolute, and relative to body and brain) occurred in female animals dosed at doses ≥ 100 mg/kg/day and male animals dosed at 400 mg/kg/day.

Histopathology

Adequate Battery

yes

Peer Review

All assessments were peer-reviewed per SOP.

Histological Findings

There were no test article-related macroscopic observations.

There were no test article-related microscopic observations.

Toxicokinetics

Blood was obtained from animals on days 1/2 and in week 11 (day 76). Two animals/sex/group were bled at 0.5, 1, 2, 6, and 24 hours post dose. Approximately 0.5

mL of whole blood was collected from the sublingual vein of isoflurane/O_2-anesthetized animals into tubes containing EDTA and approximately 10-20 mg of sodium fluoride (NaF) and placed on wet ice.

Toxicokinetic parameters of AHU377 in rat plasma

Dose	Study Day	Gender	$AUC_{(0\text{-}24h)}$±SE	$AUC_{(0\text{-}24h)}$/Dose±SE/Dose	C_{max}	C_{max}/Dose	t_{max}
50	1	Male	308 ± 51.1	6.16 ± 1.02	104	2.08	0.5
		Female	164 ± 39.2	3.28 ± 0.784	109	2.18	0.5
	76	Male	212 ± 60.2	4.24 ± 1.20	107	2.14	0.5
		Female	111 ± 59.0	2.22 ± 1.18	123	2.46	1.0
100	1	Male	1250 ± 366	12.5 ± 3.66	172	1.72	0.5
		Female	1410 ± 592	14.1 ± 5.92	301	3.01	1.0
	76	Male	861 ± 383	8.61 ± 3.83	159	1.59	0.5
		Female	1140 ± 278	11.4 ± 2.78	256	2.56	1.0
200	1	Male	2400 ± 419	12.0 ± 2.10	329	1.65	1.0
		Female	2630 ± 312	13.2 ± 1.56	698	3.49	0.5
	76	Male	2030 ± 147	10.2 ± 0.735	300	1.50	0.5
		Female	3530 ± 765	17.7 ± 3.83	875	4.38	0.5
400	1	Male	5430 ± 2250	13.6 ± 5.63	1760	4.40	0.5
		Female	6360 ± 1230	15.9 ± 3.08	1980	4.95	0.5
	76	Male	5590 ± 1180	14.0 ± 2.95	2860	7.15	0.5
		Female	6060 ± 829	15.2 ± 2.07	1800	4.50	0.5

Units for dose: mg/kg/day; $AUC_{(0\text{-}24h)}$±SE: (ng·h/mL); $AUC_{(0\text{-}24h)}$/Dose±SE/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h

Except for t_{max}, all parameter values are reported to three significant figures.

Toxicokinetic parameters of LBQ657 in rat plasma

Dose	Study Day	Gender	$AUC_{(0\text{-}24h)}$±SE	$AUC_{(0\text{-}24h)}$/Dose±SE/Dose	C_{max}	C_{max}/Dose	t_{max}
50	1	Male	23500 ± 3390	470 ± 67.8	6790	136	0.5
		Female	16000 ± 1360	320 ± 27.2	5420	108	0.5
	76	Male	22300 ± 3950	446 ± 79.0	8300	166	0.5
		Female	16800 ± 3090	336 ± 61.8	5660	113	1.0
100	1	Male	67600 ± 16900	676 ± 169	10200	102	0.5
		Female	41100 ± 13900	411 ± 139	8250	82.5	1.0
	76	Male	73800 ± 20700	738 ± 207	16300	163	0.5
		Female	48400 ± 5340	484 ± 53.4	10200	102	1.0
200	1	Male	106000 ± 24900	530 ± 125	9510	47.6	1.0
		Female	98900 ± 6050	495 ± 30.3	16900	84.5	0.5
	76	Male	164000 ± 54300	820 ± 272	23200	116	0.5
		Female	171000 ± 57300	855 ± 287	29700	149	0.5
400	1	Male	173000 ± 54600	433 ± 137	41300	103	0.5
		Female	135000 ± 36000	338 ± 90.0	37200	93.0	0.5
	76	Male	255000 ± 16700	638 ± 41.8	86300	216	0.5
		Female	344000 ± 68100	860 ± 170	71300	178	0.5

Units for dose: mg/kg/day; $AUC_{(0\text{-}24h)}$±SE: (ng·h/mL); $AUC_{(0\text{-}24h)}$/Dose±SE/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h

Except for t_{max}, all parameter values are reported to three significant figures.

Dosing Solution Analysis

Samples of the formulations prepared for weeks 1, 5/6 and13 were analyzed. Sample concentrations were 98% to 107% of target concentrations. Three control samples were analyzed, and no AHU377 was detected.

Study title: A 26-week oral (gavage) toxicity study with a 4-week recovery period in the rat

Study no.:	1370484
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	10/1/2013
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0824011, 99.1%

Key Study Findings

Compound-related effects were limited to reversible clinical signs of salivation (both sexes) and body weight decreases in both sexes at 600 mg/kg/day. No compound-related clinical pathology or anatomic pathology changes were present. The no observed adverse effect level (NOAEL) in this study was considered to be 600 mg/kg/day.

Methods

Doses:	50, 150, 600 mg/kg
Frequency of dosing:	daily
Route of administration:	oral
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5 % (W/V) Hydroxypropylcellulose
Species/Strain:	Rat, Wistar Hannover Crl:WI (Han)
Number/Sex/Group:	20
Age:	8 weeks
Weight:	176 to 244 g (males) 149 to 189 g (females),
Satellite groups:	no
Unique study design:	No
Deviation from study protocol:	deviations had no impact on the outcome of the study or upon the interpretation of the results.

Observations and Results

Mortality

Twice daily (AM and PM) on weekdays and weekends. Once daily on the day of animal arrival and on the last day of necropsy.

There were three unscheduled deaths in the main study phase. None of these unscheduled deaths were attributed to test item administration.

Clinical Signs

Cageside observations: Daily, predose and 3 hours (± 15 minutes) postdose, relative to the last animal dosed in each group

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Detailed examinations: Weekly, starting during the last week of the predosing period.

Test item-related clinical signs were limited to observation of salivation in all males and females given 600 mg/kg/day. These observations were seen between weeks 4 and 22 with the females being slightly more affected base on the number of occurrences.

Body Weights

Individual body weights were measured weekly starting the last week of the prestudy period extending through the dosing and recovery periods.

Test item-related effects on body weight parameters were limited to males given 600 mg/kg/day. Minimally to slightly reduced body weight gains were seen at most intervals during the dosing period for males and females given 600 mg/kg/day which resulted in minimally decreased group mean body weights (-0.9 to -4.7%, control mean for males and -1.1 to -4.6%, control mean for females) and lower absolute group mean body weight gain (-8.5%, control mean for males and -12.4%, control mean for females). Reversibility from this effect was evidenced by the significantly higher absolute group mean body weight gains noted at the end of the recovery period for males previously given 600 mg/kg/day (249%, control mean).

Feed Consumption

The food consumption was performed weekly, starting during the last week of the prestudy period and extending throughout the dosing and recovery periods. Food consumption (cage measurement) was quantitatively measured on each occasion.

There were no AHU377-related effects on mean food consumption observed during the study.

Ophthalmoscopy

Ophthalmology examinations were performed once prior to the start of dosing (all animals) and again during weeks 13 and 26 of dosing (before dosing).

There were no test item-related ocular changes observed during the course of the study. The findings noted were age-related or incidental in origin and to be expected in this population of animals.

Hematology

Parameters: red blood cell count, mean corpuscular volume, reticulocyte count (absolute and percent), hemoglobin concentration, mean corpuscular hemoglobin concentration, platelet count, hematocrit, mean corpuscular hemoglobin, white blood cell count (total and absolute), microscopic blood cell morphology

Coagulation parameters: prothrombin time (PT), activated partial thromboplastin time (aPTT)

There were no differences in the hematology and coagulation parameters related to the administration of AHU377 for 26 weeks and following a 4-week recovery period.

Differences in the hematology and coagulation parameters, including those determined to be statistically significant, were judged to be due to biological variation.

Clinical Chemistry

Clinical pathology assessments were conducted on the last 10 surviving main study animals/sex/group and all recovery study animals. Blood was collected from the jugular vein of non-fasted animals during the dosing and recovery periods (weeks 13, 26 and recovery week 4), at approximately the same time each sampling day.

total CO2 (equivalent to bicarbonate)	alanine aminotransferase	aspartate aminotransferase
alkaline phosphatase	magnesium	cholesterol
total bilirubin	albumin	triglycerides
creatinine	globulin	sodium
calcium	albumin/globulin ratio	potassium
phosphorus	glucose	chloride
total protein	creatine kinase	

There were no differences in the clinical chemistry parameters related to the administration of AHU377 for 26 weeks and following a 4-week recovery period. There was a minimal increase (up to 1.5 fold control values) in alanine aminotransferase in males given 600 mg/kg/day on day 89 and day 177. This increase was judged to be not toxicologically significant due to the magnitude of the increase and lack of correlating microscopic findings.

Urinalysis

Parameters: specific gravity, glucose[a], protein[a], Bilirubin, ketones, volume, Blood, pH, appearance, Color,
a - Semi-quantitative measurement.

There were no differences in the urinalysis parameters related to the administration of AHU377.

Gross Pathology

Main study and recovery animals were subjected to a complete necropsy examination, which included evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues. Necropsy examinations were conducted under the supervision of a board-certified veterinary pathologist.

Tissue list for collection, weighing (W) and/or processing (P)

W	P	Adrenal[a]		P	Nasal cavities (1 level)[h]
	P	Artery, aorta (thoracic segment)	W	P	Ovary (weighed with oviduct)[a]
		Bone marrow smear (3)[b]		P	Oviduct[a]
	P	Bone marrow (femur)		P	Optic nerves[i]
	P	Bone marrow (sternum)		P	Pancreas
W	P	Brain (Forebrain, midbrain, cerebellum, and medulla oblongata. Hippocampus and cortex as per section 3.6.4)	W	P	Pituitary (weighed post fixation)
	P	Cecum	W	P	Prostate
	P	Cervix		P	Rectum
	P	Colon		P	Salivary gland (mandibular, parotid and sublingual)[a]
	P	Duodenum		P	Sciatic nerve[a]
W	P	Epididymis[a]		P	Seminal vesicle gland[a]
	P	Esophagus		P	Skeletal muscle (from thigh)
	P	Eye[a]		P	Skin (inguinal)
	P	Femur (distal w/ joint)		P	Spinal cord (cervical, thoracic and lumbar)
	P	Gut-associated lymphoid tissue (w/ small intestine)	W	P	Spleen
W	P	Heart		P	Sternum
	P	Harderian gland[a]		P	Stomach (glandular and non-glandular regions)
	P	Ileum	W	P	Testis[a]
	P	Jejunum	W	P	Thymus
W	P	Kidney[a]	W	P	Thyroid (and parathyroid; weighed post fixation)[ad]
	P	Larynx (1 level)		P	Tongue
	P	Lacrimal gland[a]		P	Trachea
W	P	Liver (2 lobes)		P	Ureters[a]
	P	Lung (all lobes)[f]		P	Urinary bladder
	P	Lymph node – axillary[a]	W	P	Uterus (horns and body)
	P	Lymph node – deep cervical[a]		P	Vagina
	P	Lymph node – mandibular[a]		P	Macroscopic lesions/masses
	P	Lymph node - mesenteric			Animal identification
	P	Mammary gland (inguinal)[c]			

a Paired weight (if required) and examination.
b Collected from femur for all euthanized animals for examination. Allowed to air dry and not fixed in formalin.
c Examined only when present in routine section of skin (males only).
d At least one parathyroid was examined.
e Bilateral for collection, unilateral for examination.
f Infused with 10% neutral buffered formalin. Samples of 2 lobes examined.
g Only one required for examination.
h Infused with 10% neutral buffered formalin.
i Examined only when present in routine section of the eye.

Organ Weights

No test item-related organ weight changes were noted. There were isolated organ weight values that were statistically different from their respective controls. There were, however, no patterns, trends, or correlating data to suggest these values were toxicologically relevant.

Histopathology

Adequate Battery

Yes

Peer Review

The pathology report and histopathological examinations were peer reviewed by a Novartis pathologist, including review of selected tissue samples.

Histological Findings

There were no test item-related macroscopic findings identified at the end of the main and recovery phases.

No test item-related microscopic findings were identified at the end of the main and recovery phase studies. The microscopic findings observed were considered incidental, did not present any microscopic correlate and/or were seen at similar incidence in treated and control animals and, therefore, were not considered AHU377-related.

Toxicokinetics

Blood samples were collected at the following 5 time points: 0.5, 1, 3, 7 and 24 hours post dose, and placed on wet ice following collection and before centrifugation. Parameters calculated are summarized in the following Tables for AHU377 and LBQ657.

Rat plasma AHU377 toxicokinetic parameters

Dose (mg/kg/day)	Study day	Gender	AUC0-24h±SE (ng*h/mL)	AUC0-24h±SE /Dose (ng*h/mL)/ (mg/kg/day)	Cmax (ng/mL)	Cmax/Dose (ng/mL)/ (mg/kg/day)	Tmax (h)
50	1	Male	309 ± 110	6.18 ± 2.20	148	2.96	0.5
		Female	215 ± 132	4.30 ± 2.64	87	1.74	1.0
	23	Male	121 ± 27.9	2.41 ± 0.558	120	2.40	0.5
		Female	177 ± 86.2	3.55 ± 1.72	246	4.92	0.5
	149	Male	294 ± 126	5.88 ± 2.52	151	3.02	0.5
		Female	301 ± 203	6.02 ± 4.06	189	3.78	0.5
150	1	Male	543 ± 123	3.62 ± 0.82	302	2.01	0.5
		Female	3610 ± 2150	24.0 ± 14.6	300	2.00	0.5
	23	Male	1360 ± 438	9.09 ± 2.92	514	3.43	0.5
		Female	2660 ± 1210	17.7 ± 8.07	372	2.48	0.5
	149	Male	2310 ± 1100	15.4 ± 7.33	803	5.35	0.5
		Female	3830 ± 2270	25.6 ± 15.1	627	4.18	1.0
600	1	Male	6510 ± 2110	10.9 ± 3.52	2330	3.88	1.0
		Female	5670 ± 2300	9.44 ± 3.83	1000	1.67	0.5
	23	Male	12100 ± 7760	20.2 ± 12.9	2470	4.12	0.5
		Female	5660 ± 567	9.43 ± 0.945	2860	4.77	0.5
	149	Male	11800 ± 3160	19.7 ± 5.27	7070	11.8	0.5
		Female	7480 ± 1870	12.5 ± 3.12	2940	4.90	1.0

Rat plasma LBQ657 toxicokinetic parameters

Dose (mg/kg/day)	Study day	Gender	AUC0-24h±SE (ng*h/mL)	AUC0-24h±SE /Dose (ng*h/mL)/ (mg/kg/day)	Cmax (ng/mL)	Cmax/Dose (ng/mL)/ (mg/kg/day)	Tmax (h)
50	1	Male	25900 ± 10900	517 ± 218	5000	100	0.5
		Female	17500 ± 6460	350 ± 129	4860	97.2	1.0
	23	Male	14900 ± 5190	298 ± 104	3530	70.6	0.5
		Female	15200 ± 5050	303 ± 101	8720	174	0.5
	149	Male	27100 ± 5000	541 ± 100	6320	126	0.5
		Female	35900 ± 15200	719 ± 304	12300	246	1.0
150	1	Male	36800 ± 7900	245 ± 52.7	12000	80.0	0.5
		Female	100000 ± 33700	669 ± 225	12000	80.0	1.0
	23	Male	46800 ± 3890	312 ± 25.9	18600	124	0.5
		Female	86900 ± 7770	579 ± 51.8	13600	90.7	0.5
	149	Male	108000 ± 31200	721 ± 208	42600	284	0.5
		Female	205000 ± 96600	1370 ± 644	35500	237	1.0
600	1	Male	167000 ± 46400	278 ± 77.3	51400	85.7	1.0
		Female	167000 ± 59700	278 ± 99.5	36200	60.3	0.5
	23	Male	298000 ± 155000	496 ± 258	38800	64.7	0.5
		Female	261000 ± 26100	435 ± 43.5	92500	154	0.5
	149	Male	341000 ± 103000	568 ± 172	48000	80.0	0.5
		Female	347000 ± 50400	578 ± 84.0	131000	218	0.5

Dosing Solution Analysis

All study samples analyzed had mean concentrations within the acceptance criteria of ±15% (individual values within ±20%) of their theoretical concentrations).

Study title: 52-week oral (gavage) toxicity study in marmosets with a 4-week recovery period.

Study no.:	0570307
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover, New Jersey
Date of study initiation:	11/29/2005
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	Batch nos. 0451002 and 0522003

Batch no.	Certificate of analysis no.	Retest date	Drug content %
0451002	(b) (4)	(b) (4)	97.3
0451002			96.8
0522003			99.1

Key Study Findings

No mortality occurred in any dose group and no moribundity occurred at the highest dose level evaluated (200 mg/kg/day). Clinical signs consisted of emesis with or without feed and apparent compound at the higher doses (100, 200 mg/kg/day). Persistent body weight losses were evident in individual females at 200 mg/kg/day and one male at 100 mg/kg/day. By the end of 52 weeks of treatment, treatment-related body weight losses of about 11-35% were noted. There were no ocular or electrocardiographic abnormalities associated with the administration of AHU377. There were no hematologic changes or changes in coagulation parameters associated with the administration of AHU377. There were no test article-related changes present in clinical chemistry, special chemistry or urine analyses.

Methods

Doses:	25, 100, 200 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% CMC
Species/Strain:	Marmoset *(Callithrix jacchus*)
Number/Sex/Group:	5
Age:	13 months to 6 years
Weight:	258.7 to 484.6 g for males and 249.8 to 468.9 g for females
Satellite groups:	Control – 2 M, 4F; High dose 3 M, 3 F; for recovery
Unique study design:	no
Deviation from study protocol:	None of the protocol deviations that occurred adversely impacted the study.

Observations and Results

Mortality

Twice daily (AM and PM) on weekdays and at least once daily on weekends, holidays and plant emergencies/closings.

No test article-related moribundity occurred during the study. Six animals were sacrificed prior to study termination for humane reasons and/or due to moribundity.

Clinical Signs

At least twice daily (prior to dosing and at approximately 2 hours postdose,

There were no clinical signs considered test article-related at 25 mg/kg/day in either sex. Emesis with or without feed and apparent compound was considered test article-related at the higher doses (100, 200 mg/kg/day) based on the increased number of animals affected and/or the increased frequency of occurrence, generally throughout the dosing period.

Body Weights

Days 1, 4, 8 and 15 and at least weekly thereafter. Recovery period: Once weekly

There were no test article-related effects on mean body weight and mean absolute body weight gains in AHU377-treated males during the course of the study. Mean body weights of the treated male dose groups displayed adequate body weight gain; whereas the controls struggled to maintain their baseline body weights until day 288.

In females, there were no test article-related effects on mean body weight and mean absolute body weight gains in AHU377-treated animals at doses ≤ 100 mg/kg/day. However at 200 mg/kg/day, body weight losses were evident in individual animals, notably animal nos. 4503 and 4504. By the end of 52 weeks of treatment, treatment-related body weight losses of about 35% and 11% were noted in animals 4503 and 4504, respectively. The body weight decreases in these and other animals during the course of the study may be secondary to the frequent episodes of postdose emesis with feed.

Feed Consumption

Estimated daily, from the beginning of the pretest period to the end of the recovery period.

There were no test article-related effects on estimated food consumption determinations as all groups, including controls, generally consumed 50-75% of the food presented.

Ophthalmoscopy

Pretest: All animals
End of dosing: All surviving animals

There were no ocular abnormalities associated with the administration of AHU377.

ECG

Pretest: All animals
Weeks 14, 26, 39 and 51: All surviving animals at approximately 1.5-2.5 hours postdose

There were no electrocardiographic abnormalities associated with the administration of AHU377.

Clinical Chemistry

Hematology, hemostasis/thrombosis and/or clinical chemistry assessments were conducted pretest and during weeks 13, 25, 52 and at the end of recovery.

Clinical chemistry parameters:

alanine aminotransferase	glucose	calcium
alkaline phosphatase	blood urea nitrogen	inorganic phosphorus
aspartate aminotransferase	creatinine	triglycerides
total bilirubin	creatine kinase (CK)*	cholesterol
total protein	sodium	A/G ratio
albumin (A)	potassium	
globulins (G)	chloride	

*Isoenzyme analysis was not performed as determined by the Clinical Pathologist.

There were no test article-related changes present in clinical chemistry.

Hematology

Parameters: erythrocytes, Wintrobe indices, white blood cell count, hematocrit, red cell distribution width (RDW), white blood cell differential, hemoglobin, reticulocytes, platelets

Hemostasis/thrombosis parameters: prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen

There were no hematologic changes or changes in coagulation parameters associated with the administration of AHU377.

Urinalysis

Parameters: specific gravity, glucose*, protein*, bilirubin*, ketones*, urobilinogen*, blood*, pH*
*test strip determinations

There were no test article-related changes in urinalysis parameters..

Gross Pathology

Animals were fasted overnight (approximately 18 hours) prior to terminal necropsy. A prosector order was prepared per SOP. Complete necropsies were performed on all animals with a recording of macroscopic abnormalities for all protocol tissues.

Tissue list for collection, weighing (W), processing (P)and/or genomics (G)

W		P	adrenal	W		P	ovary
		P	aorta			P	pancreas
		P	bone marrow (in bone)			P	parathyroid
		P	bone marrow smear	W		P	pituitary
W		P	brain	W		P	prostate
		P	cecum			P	rectum
		P	cervix			P	salivary gland
		P	colon			P	sciatic nerve
		P	duodenum			P	seminal vesicle
		P	epididymis			P	skeletal muscle
		P	esophagus			P	skin
		P	eye			P	spinal cord
		P	femur (distal w/joint)	W		P	spleen
		P	gall bladder			P	sternum
W	G	P	heart		G	P	stomach
		P	ileum	W		P	testis
		P	jejunum			P	thymus
W	G	P	kidney	W		P	thyroid
		P	lacrimal gland			P	tongue
W	G	P	liver			P	trachea
	G	P	lung			P	urinary bladder
		P	lymph node - bronchial			P	uterus
		P	lymph node – mandibular			P	vagina
		P	lymph node – mesenteric			P	macroscopic lesions
		P	mammary gland area				animal identification
					G		blood

Organ Weights

There was a decrease in mean absolute and relative (to brain) thyroid weights in the terminal sacrifice males at doses ≥ 25 mg/kg/day and in the high-dose recovery males. These organ weight decreases correlated with decreased amounts of intrafollicular colloid, considered to be a compound-related effect. Paradoxically, group mean thyroid weights were increased in the treated females but with no apparent histological correlate.

Mean pituitary weights (absolute and relative to brain weight) were also statistically significant in males at 200 mg/kg/day, however, these decreases were not considered to be biologically significant in the absence of correlative histopathology.

Histopathology

Adequate Battery

yes

Peer Review

All assessments were peer-reviewed per SOP.

Histological Findings

There were no apparent macroscopic findings at necropsy that were considered to be related to AHU377 administration.

The only microscopic finding worthy of mention occurred in the thyroid gland of one of five control males (animal no. 1002) and in all treated males except for animal no. 2001 and in all three high-dose recovery males. This change was characterized by a slight to moderate reduction in the amount of intra-follicular colloid. The epithelium of the follicles in the affected animals appeared morphologically intact, being low cuboidal to cuboidal in height as compared to the more flattened epithelial lining of the follicular walls caused by the presence of abundant colloid.

Toxicokinetics

Blood was obtained from study animals during weeks 1, 8, 39/40 and 50. Blood samples were collected at 1, 2, 6 and 24 hours postdose.

Toxicokinetic parameters for AHU377 and LBQ657 are summarized below.

Summary of mean toxicokinetic parameters of AHU377 in plasma

Dose	Study Day	Gender	AUC	SE	AUC/Dose	SE/Dose	C_{max}	C_{max}/Dose	t_{max}
25	1	Male	57600	NC	2300	NC	29100	1160	1.00
		Female	74100	NC	2960	NC	37800	1510	1.00
	50	Male	69100	NC	2760	NC	25100	1000	1.00
		Female	59500	NC	2380	NC	30300	1210	1.00
	273	Male	61200	NC	2450	NC	33100	1320	1.00
		Female	51100	NC	2040	NC	27400	1100	1.00
	346	Male	75200	NC	3010	NC	40700	1630	1.00
		Female	36000	NC	1440	NC	9990	400	1.00
100	1	Male	345000	NC	3450	NC	153000	1530	1.00
		Female	294000	NC	2940	NC	131000	1310	1.00
	50	Male	341000	NC	3410	NC	127000	1270	1.00
		Female	365000	NC	3650	NC	192000	1920	1.00
	273	Male	300000	NC	3000	NC	88100	881	2.00
		Female	342000	NC	3420	NC	185000	1850	1.00
	346	Male	266000	NC	2660	NC	53500	535	2.00
		Female	332000	NC	3320	NC	198000	1980	1.00
200	1	Male	920000	85300	4600	427	270000	1350	2.00
		Female	599000	200000	3000	1000	193000	965	1.00
	50	Male	1470000	198000	7330	990	327000	1640	2.00
		Female	1100000	471000	5500	2360	220000	1100	1.00
	273	Male	900000	53500	4500	268	341000	1710	1.00
		Female	891000	124000	4460	620	333000	1670	1.00
	346	Male	556000	102000	2780	510	236000	1180	1.00
		Female	694000	163000	3470	815	269000	1350	1.00

Units for each parameter: Dose: mg/kg/day; $AUC_{(0\text{-}24h)}$: (ng·h/mL); $AUC_{(0\text{-}24h)}$/Dose±SE/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h. NC: not calculable

Summary of mean toxicokinetic parameters of LBQ657 in plasma

Dose	Study Day	Gender	AUC	SE	AUC/Dose	SE/Dose	C_{max}	C_{max}/Dose	t_{max}
25	1	Male	32700	NC	1310	NC	19300	772	1.00
		Female	59600	NC	2380	NC	26000	1040	1.00
	50	Male	42700	NC	1710	NC	25200	1010	1.00
		Female	33000	NC	1320	NC	17200	688	1.00
	273	Male	35800	NC	1430	NC	19800	792	1.00
		Female	34400	NC	1370	NC	12500	500	1.00
	346	Male	34200	NC	1370	NC	17100	684	1.00
		Female	26600	NC	1060	NC	8440	338	2.00
100	1	Male	221000	NC	2210	NC	67400	674	2.00
		Female	208000	NC	2080	NC	62400	624	2.00
	50	Male	195000	NC	1950	NC	53000	530	1.00
		Female	186000	NC	1860	NC	58400	584	1.00
	273	Male	225000	NC	2250	NC	66300	663	2.00
		Female	189000	NC	1890	NC	57200	572	1.00
	346	Male	247000	NC	2470	NC	66400	664	2.00
		Female	193000	NC	1930	NC	59600	596	1.00
200	1	Male	685000	88200	3420	441	211000	1060	2.00
		Female	355000	121000	1780	605	109000	545	2.00
	50	Male	997000	183000	4980	915	244000	1220	2.00
		Female	741000	398000	3700	1990	114000	570	2.00
	273	Male	602000	37300	3010	187	175000	875	2.00
		Female	508000	56800	2540	284	138000	690	2.00
	346	Male	401000	45500	2000	228	122000	610	2.00
		Female	482000	111000	2410	555	137000	685	2.00

Units for each parameter: Dose: mg/kg/day; $AUC_{(0\text{-}24h)}$: (ng·h/mL); $AUC_{(0\text{-}24h)}$/Dose±SE/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h. NC: not calculable

Dosing Solution Analysis

The samples of the dose formulations for weeks 1, 4, 13, 26, 39 and 52 were analyzed. The concentrations were 95% to 107% of targets. Six control samples were analyzed, and no AHU377 was detected.

7 Genetic Toxicology

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

Study title: Mutagenicity test using *Salmonella typhimurium* **[LCZ696]**

Study no.:	0612017
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	Safety Profiling and Assessment, Exploratory Development (ED), Novartis Pharma AG, Basel, Switzerland
Date of study initiation:	7/27/2006
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0650001, 95.2 %

Key Study Findings: Treatment with LCZ696 did not notably increase the number of revertant colonies above the corresponding negative control values.

Methods

Strains:	*Salmonella typhimurium* strains TA1535,TA97a, TA98, TA100 and TA102
Concentrations in definitive study:	**First experiment (plate incorporation):** dose finding experiment using strain TA100 +/- S9 only): 5, 50, 500, 5000 µg/plate **Second experiment (plate incorporation):** 8, 40, 200, 1000, 5000 µg/plate **Third experiment (preincubation):** 312.5, 625, 1250, 2500, 5000 µg/plate **Fourth experiment (preincubation using strain TA102 +/- S9 only):** 312.5, 625, 1250, 2500, 5000 µg/plate
Basis of concentration selection:	toxicity
Negative control:	vehicle
Positive control:	All positive controls were dissolved in DMSO and used at one concentration level per strain only at 0.1 ml/plate. The same positive controls were used for all test items tested on the same day under either preincubation or plate incorporation conditions. ***2-Aminoanthracene***: This is an indirect-acting mutagen. At 3 µg/plate it is mutagenic for *Salmonella typhimurium* strains TA1535, TA98 and TA100. At 10 µg/plate it is mutagenic for *Salmonella typhimurium* strains TA97a and TA102. ***Benzo(a)pyrene*** : This is an indirect-acting

	frame-shift mutagen. At the concentration level used (3 µg/plate) it is mutagenic for strain TA98. ***Sodium azide***: This is a direct-acting agent which induces base-pair substitutions. At 3 µg/plate it is mutagenic for the *Salmonella typhimurium* strains TA1535 and TA100. ***9-Aminoacridine*** : This is a direct-acting frame-shift mutagen. At the concentration level used (100 µg/plate) it is mutagenic for strain TA97a. ***2-Nitrofluorene*** : This is a direct-acting frame-shift mutagen. At the concentration level used (2 µg/plate) it is mutagenic for the strain TA98. ***Mitomycin C*** : This is a direct acting agent which is mutagenic for strain TA102 at the concentration level used (0.5µg/plate).
Formulation/Vehicle:	DMSO
Incubation & sampling time:	In this assay, the bacteria, the test item solution, and either a phosphate buffer or a rat-liver S9-mix are plated together, directly or after a 20 minute preincubation in the liquid phase. All are plated in triplicate.

Study Validity

Study was valid with respect to strains, concentrations, and positive and negative controls.

Results

The figures represent mean colony numbers per concentration group.

Table 4-1 Experiment 1 (plate incorporation)

Strain:		TA100	
S9:		-	+
Concentration (µg/ plate):	0	152	156
	5	146	139
	50	150	148
	500	150	151
	5000	149	142

Table 4-2 Experiment 2 (plate incorporation)

Strain:		TA1535		TA97a		TA98		TA100		TA102	
S9:		-	+	-	+	-	+	-	+	-	+
Concentration (µg/ plate):	0	26	18	197	179	51*	60	151	132	334	386
	8	28	18	200	193	55	59	147	136	357	379
	40	28	15	201	180	58	58	143	118	369	408
	200	25	17	190	207	57	64	141	103	360	390
	1000	29	19	191	192	53	58	145	130	362	359
	5000	24	17	171	197	57	67	145	121	391	430

* value above historical negative control range

Experiment 3 (preincubation)

Strain:		TA1535		TA97a		TA98		TA100		TA102	
S9:		-	+	-	+	-	+	-	+	-	+
Concentration (µg/ plate):	0	21	22	198	179	52*	57	141	154	t	t
	312.5	29	16	190	187	38	61	141	154	t	t
	625	25	21	205	174	57	55	159	152	t	t
	1250	24	25	206	194	50	66	130	130	t	t
	2500	16t	23	212	159	26t	59	128t	148	t	t
	5000	22t	19t	204t	188	24t	49	135t	159	t	t

t: toxic, * value above historical negative control range

Experiment 4 (preincubation)

Strain:		TA102	
S9:		-	+
Concentration (µg/ plate):	0	304	350
	312.5	326	363
	625	331	366
	1250	312	371
	2500	333	361
	5000	159tp	333t

t: toxic, p: precipitation

Study title: Mutagenicity test using *Salmonella typhimurium* **[AHU377]**

Study no.:	0412008
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	Genetic Toxicology and Safety Pharmacology, NIBR, Basel, Switzerland
Date of study initiation:	7/8/2004
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0451002, 96.2%

Key Study Findings: AHU377 did not show evidence of a mutagenic potential under the experimental conditions used and applying standard mutagenicity criteria.

Methods

Strains:	*Salmonella typhimurium* strains TA1535, TA97a, TA98, TA100 and TA102
Concentrations in definitive study:	First experiment (plate incorporation): 8, 40, 200, 1000, 5000 µg/plate Second experiment (preincubation): 312.5, 625, 1250, 2500, 5000 µg/plate
Basis of concentration selection:	Precipitation at 5000 µg/plate
Negative control:	vehicle
Positive control:	All positive controls were dissolved in DMSO and used at one concentration level per strain only at 0.1 ml/plate. The same positive controls were used for all test items tested on the same day under either preincubation or plate incorporation conditions. ***2-Aminoanthracene***: This is an indirect-acting mutagen. At 3 µg/plate it is mutagenic for *Salmonella typhimurium* strains TA1535, TA98 and TA100. At 10 µg/plate it is mutagenic for *Salmonella typhimurium* strains TA97a and TA102. ***Benzo(a)pyrene*** : This is an indirect-acting frame-shift mutagen. At the concentration level used (3 µg/plate) it is mutagenic for strain TA98. ***Sodium azide***: This is a direct-acting agent which induces base-pair substitutions. At 3 µg/plate it is mutagenic for the *Salmonella typhimurium* strains TA1535 and TA100. ***9-Aminoacridine*** : This is a direct-acting frame-shift mutagen. At the concentration level used (100 µg/plate) it is mutagenic for strain TA97a. ***2-Nitrofluorene*** : This is a direct-acting

	frame-shift mutagen. At the concentration level used (2 µg/plate) it is mutagenic for the strain TA98. ***Mitomycin C*** : This is a direct acting agent which is mutagenic for strain TA102 at the concentration level used (0.5µg/plate).
Formulation/Vehicle:	DMSO
Incubation & sampling time:	In this assay, the bacteria, the test item solution, and either a phosphate buffer or a rat-liver S9-mix are plated together, directly or after a 20-minute preincubation in the liquid phase. All are plated in triplicate.

Study Validity

Study was valid with respect to strains, concentrations, and positive and negative controls.

Results

The figures represent mean colony numbers per concentration group.

Experiment 1 (plate incorporation)

Strain:		TA1535		TA97a		TA98		TA100		TA102	
S9:		-	+	-	+	-	+	-	+	-	+
Concentration (µg/ plate):	0	24	26	174	182	41	31	131	143	274	279
	8	22	13	165	157	32	29	127	129	234	253
	40	19	18	204	173	26	31	112	141	250	287
	200	25	14	141	189	24	29	128	144	240	291
	1000	24	16	165	160	29	32	130	145	241	261
	5000	19	18	162	158	29	30	129	124	222	261

Experiment 2 (preincubation)

Strain:		TA1535		TA97a		TA98		TA100		TA102	
S9:		-	+	-	+	-	+	-	+	-	+
Concentration (µg/ plate):	0	26	21	175	174	37	37	122	115	221	230
	312.5	25	21	165	172	40	32	130	113	225	231
	625	20	20	167	177	35	32	122	103	221	235
	1250	21	22	179	176	32	34	132	117	225	237
	2500	26	16	158	162	28	34	138	126	233	236
	5000	21p	17p	155p	189p	26p	29p	143p	122p	225p	222p

p: precipitation

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes **[LCZ696]**

Study no.:	0770050
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	1/16/2007
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0651004, 96.4%

Key Study Findings

LCZ696 did not induce chromosome aberrations in cultured human peripheral blood lymphocytes, when tested up to and in excess of the limits of cytotoxicity in both the absence and presence of a rat liver metabolic activation system (S-9).

Methods

Cell line:	Lymphocytes pooled from 3 healthy donors
Concentrations in definitive study:	See below
Basis of concentration selection:	A maximum treatment concentration of 5000 µg/mL was selected for the cytotoxicity range-finder experiment, in order that treatments were performed up to the maximum recommended concentration. Concentrations for the main experiment were selected based on the results of this cytotoxicity range-finding experiment.
Negative control:	water
Positive control:	Cells treated with 2.50 µg NQO/mL and 12.5 µg CPA/mL (Experiment 1) or 2.50 and 5.00 µg NQO/mL and 6.25 µg CPA/mL (Experiment 2) gave satisfactory responses in terms of quality and quantity of mitoses and extent of chromosomal damage. These were selected for analysis. (DMSO)
Formulation/Vehicle:	Sterile water
Incubation & sampling time:	See below

Experiment 1, LCZ696 Concentrations

Experiment 1 Concentration of treatment solution (mg/mL)	Final concentration (µg/mL)	Hours treatment + hours recovery 3+17 –S-9	3+17 +S-9
3.000	300.0	✓	
5.000	500.0	✓	
7.000	700.0	✓	
9.000	900.0	✓	
10.00	1000		✓
11.00	1100	✓	✓
12.00	1200	✓	✓
13.00	1300	✓	✓
14.00	1400	✓	✓
15.00	1500	✓	✓
16.00	1600	✓	✓
17.00	1700	✓	✓
18.00	1800		✓
19.00	1900	✓	
20.00	2000		✓
30.00	3000		✓
40.00	4000		✓

✓ Indicates concentration tested

Experiment 2, LCZ696 Concentrations

Experiment 2 Concentration of treatment solution (mg/mL)	Final concentration (µg/mL)	Hours treatment + hours recovery 20+0 -S-9	3+17 +S-9
1.000	100.0	✓	
2.000	200.0	✓	
2.500	250.0	✓	
3.000	300.0	✓	
3.500	350.0	✓	
4.000	400.0	✓	
4.500	450.0	✓	
5.000	500.0	✓	
6.000	600.0	✓	
10.00	1000		✓
12.00	1200		✓
14.00	1400		✓
15.00	1500		✓
20.00	2000		✓
25.00	2500		✓
30.00	3000		✓

✓ Indicates concentration tested

Study Validity

Study was valid with respect to strains, concentrations, and positive and negative controls.

Results

Range-Finding Experiment, Mitotic Index Determinations

Treatment (µg/mL)	Mitotic index (%) 3+17 hours, -S-9 A	B	MIH*	3+17 hours, +S-9 A	B	MIH*
Vehicle	11.7	9.8	-	8.3	9.6	-
18.14	10.2	NT	5	9.5	NT	0
30.23	12.2	NT	0	9.8	NT	0
50.39	11.0	NT	0	10.7	NT	0
83.98	8.9	NT	17	9.4	NT	0
140.0	9.1	NT	15	9.5	NT	0
233.3	10.3	NT	4	11.2	NT	0
388.8	8.8	NT	18	10.6	NT	0
648.0	7.4	NT	31	9.0	NT	0
1080	8.2	NT	24	10.1	NT	0
1800	1.4	NT	87	3.3	NT	63
3000	0.0	NT	100	3.3	NT	63
5000	0.0	NT	100	0.0	NT	100

NT = not tested
*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%
(where T = treatment and C = negative control)

A and B refers to the number of cultures treated (two [A and B] for vehicle controls and one [A] for test item)

Treatment (µg/mL)	Mitotic index (%) 20+0 hours, -S-9 A	B	MIH*
Vehicle	7.1	8.4	-
18.14	7.7	NT	1
30.23	9.8	NT	0
50.39	9.4	NT	0
83.98	9.7	NT	0
140.0	8.8	NT	0
233.3	5.5	NT	29
388.8	4.2	NT	43
648.0	1.5	NT	81
1080	0.5	NT	94
1800	0.0	NT	100
3000	0.0	NT	100
5000	0.0	NT	100

NT = not tested
*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%
(where T = treatment and C = negative control)

A and B refers to the number of cultures treated (two [A and B] for vehicle controls and one [A] for test item)

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

Treatment (μg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	MIH* (%)
Vehicle	A	100	2	2	
	B	100	0	0	
	Totals	200	2	2	-
300.0	A	100	0	0	
	B	100	1	1	
	Totals	200	1	1	4
700.0	A	100	2	2	
	B	100	1	1	
	Totals	200	3	3	25
1200	A	100	0	0	
	B	100	2	2	
	Totals	200	2	2	48
1300	A	100	2	1	
	B	100	2	2	
	Totals	200	4	3	52
NQO, 2.50	A	73	**20**	**19**	
	B	82	**20**	**20**	
	Totals	155	40	39[a]	

Binomial Dispersion Test $\chi^2 = 5.72$, not significant

a Statistical significance $p \leq 0.001$

Numbers highlighted exceed historical negative control range (Appendix 2: Historical control data)

*Mitotic inhibition (%) = [1 - (mean MIT/mean MIC)] x 100%

(where T = treatment and C = negative control)

3 hour treatment +S-9, 17 hour recovery (3+17), Experiment 1

Treatment (μg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	MIH* (%)
Vehicle	A	100	1	0	
	B	100	0	0	
	Totals	200	1	0	-
1100	A	100	1	0	
	B	100	1	1	
	Totals	200	2	1	3
1400	A	100	0	0	
	B	100	1	1	
	Totals	200	1	1	25
1800	A	100	2	2	
	B	100	1	1	
	Totals	200	3	3	41
CPA, 12.50	A	54	**20**	**20**	
	B	67	**20**	**20**	
	Totals	121	40	40[a]	

Binomial Dispersion Test $\chi^2 = 2.35$, not significant

[a] Statistical significance $p \leq 0.001$

Numbers highlighted exceed historical negative control range (Appendix 2: Historical control data)

*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%

(where T = treatment and C = negative control)

20 hour treatment -S-9, 0 hour recovery (20+0), Experiment 2

Treatment (µg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	MIH* (%)
Vehicle	A	100	0	0	
	B	100	2	1	
	Totals	200	2	1	-
100.0	A	100	0	0	
	B	100	0	0	
	Totals	200	0	0	0
200.0	A	100	1	1	
	B	100	1	1	
	Totals	200	2	2	29
300.0	A	100	2	2	
	B	100	2	1	
	Totals	200	4	3	52
NQO, 2.50[$]	A	100	**9**	**7**	
	B	100	**7**	**7**	
	Totals	200	16	14[a]	
NQO, 5.00[$]	A	96	**17**	**16**	
	B	88	**21**	**20**	
	Totals	184	38	36[a]	

Binomial Dispersion Test $\chi^2 = 1.34$, not significant

a Statistical significance $p \leq 0.001$

$ Due to uncertainty regarding suitability of positive control cultures both were scored.

Numbers highlighted exceed historical negative control range (Appendix 2: Historical control data)

*Mitotic inhibition (%) = [1 - (mean MIT/mean MIC)] x 100%

(where T = treatment and C = negative control)

3 hour treatment +S-9, 17 hour recovery (3+17), Experiment 2

Treatment (μg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	MIH* (%)
Vehicle	A	100	1	1	
	B	100	0	0	
	Totals	200	1	1	-
1000	A	100	2	2	
	B	100	0	0	
	Totals	200	2	2	14
1400	A	100	0	0	
	B	100	4	2	
	Totals	200	4	2	30
1500	A	100	1	1	
	B	100	1	1	
	Totals	200	2	2	37
2000	A	100	1	1	
	B	100	6	4	
	Totals	200	7	5	73
CPA, 6.25	A	48	21	20	
	B	48	21	20	
	Totals	96	42	40[a]	

Binomial Dispersion Test $\chi^2 = 6.89$, not significant

[a] Statistical significance $p \leq 0.001$

Numbers highlighted exceed historical negative control range (Appendix 2: Historical control data)

*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%

(where T = treatment and C = negative control)

Study title: Evaluation of the ability of AHU377 to induce chromosome aberrations in cultured peripheral human lymphocytes

Study no.:	0420038
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	(b) (4)
Date of study initiation:	6/28/2004
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0451002, 96.2%

Key Study Findings

It is concluded that this test is valid and that AHU377 is not clastogenic in human lymphocytes under the experimental conditions described in this report.

Methods

Cell line:	Cultured peripheral human lymphocytes
Concentrations in definitive study:	see below
Basis of concentration selection:	precipitation
Negative control:	DMSO
Positive control:	Without (-S9-mix): 4-nitroquinoline 1-oxide With (+S9-mix): Cyclophosphamide
Formulation/Vehicle:	DMSO
Incubation & sampling time:	see below

Study Validity

Study was valid with respect to strains, concentrations, and positive and negative controls.

Results

Mitotic index of donor cultures treated with AHU377 in the dose range finding test

AHU377 concentration (µg/ml)	Number of metaphases per 1000 cells	
	Absolute	Percentage of control
Without metabolic activation (-S9-mix)		
3 h exposure time, 24 h fixation time		
Control [a]	116	100
3	108	93
10	120	103
133	116	100
100	111	96
333 [b]	108	93
24 h exposure time, 24 h fixation time		
Control [a]	68	100
3	68	100
10	71	104
33	69	101
100	72	106
333 [b]	27	40
1000 [b]	0	0
With metabolic activation (+S9-mix)		
3 h exposure time, 24 h fixation time		
Control [a]	113	100
3	112	99
10	109	96
133	107	95
100	115	102
333 [b]	106	94

[a] Dimethyl sulfoxide
[b] AHU377 precipitated in the culture medium.

Mitotic index of donor cultures treated with AHU377 in the first cytogenetic assay

AHU377 concentration (µg/ml)	Number of metaphases per 1000 cells [a] Absolute	Percentage of control
Without metabolic activation (-S9-mix)		
3 h exposure time, 24 h fixation time		
Control [b]	64 - 73	100
33	69 - 70	101
100	71 - 74	106
333 [c]	64 - 66	95
MMC-C; 0.5 µg/ml	34 - 45	58
With metabolic activation (+S9-mix)		
3 h exposure time, 24 h fixation time		
Control [b]	52 - 54	100
33	54 - 60	108
100	53 - 60	107
333 [c]	59 - 60	112
CP; 15 µg/ml	22 - 28	47

[a] Duplicate cultures
[b] Dimethyl sulfoxide
[c] AHU377 precipitated in the culture medium.

Chromosome aberrations in donor cultures treated with AHU377 in the absence of S9 mix in the first cytogenetic assay (3 h exposure time, 24 h fixation time)

Conc µg/ml	DMSO (1.0% v/v)			33 µg/ml			100 µg/ml			333 µg/ml			MMC-C 0.5 µg/ml		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
Mitotic Index (%)		100			101			106			95			58	
No. of Cells scored	100	100	200	100	100	200	100	100	200	100	100	200	50	100	150
No. of Cells with aberrations (+ gaps) [a)]	0	0	0	0	0	0	0	0	0	1	0	1	27	46	73
No. of Cells with aberrations (- gaps)	0	0	0	0	0	0	0	0	0	1	0	1	27	44	***) 71
g'													1	3	
g"															
b'													21	40	
b"													3	5	
m'															
m"														3	
exch.													12	16	
dic															
d'															
misc.										p			3intra	2intra	
total aberr (+ gaps)	0	0		0	0		0	0		1	0		40	69	
total aberr (- gaps)	0	0		0	0		0	0		1	0		39	66	

a) Abbreviations used for various types of aberrations are listed in appendix I.
misc. = (miscellaneous) aberrations not belonging to the ones mentioned above.

*) Significantly different from control group (Chi-square test), * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$ (statistics was only performed on number of cells with aberrations without gaps).

Chromosome aberrations in donor cultures treated with AHU377 in the presence of S9-mix in the first cytogenetic assay (3 h exposure time, 24 h fixation time)

Conc µg/ml	DMSO (1.0% v/v)			33 µg/ml			100 µg/ml			333 µg/ml			CP 15 µg/ml		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
Mitotic Index (%)	100			108			107			112			47		
No. of Cells scored	100	100	200	100	100	200	100	100	200	100	100	200	100	100	200
No. of Cells with aberrations (+ gaps) [a)]	0	0	0	0	1	1	0	0	0	1	0	1	30	29	59
No. of Cells with aberrations (- gaps)	0	0	0	0	1	1	0	0	0	1	0	1	28	25	***) 53
g'													2	4	
g"															
b'													16	15	
b"					1					1			3	2	
m'													1	1	
m"													2	5	
exch.													8	7	
dic															
d'															
misc.													intra		
total aberr (+ gaps)	0	0		0	1		0	0		1	0		33	34	
total aberr (- gaps)	0	0		0	1		0	0		1	0		31	30	

a) Abbreviations used for various types of aberrations are listed in appendix I.
misc. = (miscellaneous) aberrations not belonging to the ones mentioned above.

*) Significantly different from control group (Chi-square test), * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$ (statistics was only performed on number of cells with aberrations without gaps).

Mitotic index of donor cultures treated with AHU377 in the second cytogenetic assay

AHU377 concentration (µg/ml)	Number of metaphases per 1000 cells [a] Absolute	Percentage of control
Without metabolic activation (-S9-mix)		
24 h exposure time, 24 h fixation time		
Control [b]	35 - 31	100
33	31 - 31	94
100	37 - 34	108
200 [c]	32 - 35	102
333 [c]	19 - 20	59
400 [c]	23 - 19	64
500 [c]	14 - 17	47
MMC-C; 0.2 µg/ml	17 - 22	59
With metabolic activation (+S9-mix)		
3 h exposure time, 24 h fixation time		
Control [b]	38 - 44	100
33	45 - 41	105
100	43 - 47	110
333 [c]	38 - 47	104
CP; 15 µg/ml	24 - 23	57

[a] Duplicate cultures
[b] Dimethyl sulfoxide
[c] AHU377 precipitated in the culture medium

Chromosome aberrations in donor cultures treated with AHU377 in the absence of S9-mix in the second cytogenetic assay (24 h exposure time, 24 h fixation time)

Conc µg/ml	DMSO (1.0% v/v)			100 µg/ml			400 µg/ml			500 µg/ml			MMC-C 0.2 µg/ml		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
Mitotic Index (%)		100			108			64			47			59	
No. of Cells scored	100	100	200	100	100	200	100	100	200	100	100	200	100	100	200
No. of Cells with aberrations (+ gaps) [a)]	0	1	1	3	1	4	6	3	9	4	3	7	31	29	60
No. of Cells with aberrations (- gaps)	0	1	1	2	1	3	4	2	6	3	3	6	30	29	59 ***
g'				1			2	1		1			1	1	
g"															
b'		1		2			4	2		2	3		22	24	
b"					1					1			8	11	
m'															
m"															
exch.													5	3	
dic															
d'															
misc.															
total aberr (+ gaps)	0	1		3	1		6	3		4	3		36	39	
total aberr (- gaps)	0	1		2	1		4	2		3	3		35	38	

a) Abbreviations used for various types of aberrations are listed in appendix I.
misc. = (miscellaneous) aberrations not belonging to the ones mentioned above.

*) Significantly different from control group (Chi-square test), * P < 0.05, ** P < 0.01 or *** P < 0.001 (statistics was only performed on number of cells with aberrations without gaps).

Chromosome aberrations in donor cultures treated with AHU377 in the presence of S9-mix in the second cytogenetic assay (3 h exposure time, 24 h fixation time)

Conc µg/ml	DMSO (1.0% v/v)			33 µg/ml			100 µg/ml			333 µg/ml			CP 15 µg/ml		
Culture	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
Mitotic Index (%)	100			105			110			104			57		
No. of Cells scored	100	100	200	100	100	200	100	100	200	100	100	200	100	100	200
No. of Cells with aberrations (+ gaps) [a)]	1	0	1	1	4	5	2	1	3	2	2	4	32	27	59
No. of Cells with aberrations (- gaps)	1	0	1	1	2	3	2	0	2	2	2	4	32	27	***) 59
g'					2			1							
g"															
b'				1	1		2			1	1		22	19	
b"	1				1					1	1		10	8	
m'															
m"															
exch.													6	3	
dic															
d"															
misc.													r		
total aberr (+ gaps)	1	0		1	4		2	1		2	2		39	30	
total aberr (- gaps)	1	0		1	2		2	0		2	2		39	30	

a) Abbreviations used for various types of aberrations are listed in appendix I.
misc. = (miscellaneous) aberrations not belonging to the ones mentioned above.

*) Significantly different from control group (Chi-square test), * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$ (statistics was only performed on number of cells with aberrations without gaps).

Study title: LBQ657: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes

Study no.:	1170562
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11/28/2011
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	LBQ657, alternative name (b) (4) (AHU377 related substance) batch number 11/3, 97.6%

Key Study Findings

It is concluded that this test is valid and that LBQ657 is not clastogenic in human lymphocytes under the experimental conditions described in this report.

Methods

Cell line:	Cultured peripheral human lymphocytes
Concentrations in definitive study:	see below
Basis of concentration selection:	precipitation
Negative control:	DMSO
Positive control:	Without (-S9-mix): Mitomycin C With (+S9-mix): Cyclophosphamide
Formulation/Vehicle:	DMSO
Incubation & sampling time:	see below

Test item concentrations

Experiment	Treatment	Concentration range (mg/mL)			Final concentration range (µg/mL)		
Range-Finder	3+17, -S-9	1.391	to	383.5	13.91	to	3835
	3+17, +S-9	1.391	to	383.5	13.91	to	3835
	20+0, -S-9	1.391	to	383.5	13.91	to	3835
Experiment 1	3+17, -S-9	30.00	to	383.5	300.0	to	3835
	3+17, +S-9	30.00	to	383.5	300.0	to	3835
Experiment 2	20+0, -S-9	30.00	to	383.5	300.0	to	3835
	3+17, +S-9	50.00	to	383.5	500.0	to	3835

Study Validity

Study was valid with respect to strains, concentrations, and positive and negative controls.

Results

The results of the MI determinations from the cytotoxicity Range-Finder were as follows:

Range-Finder Experiment, mitotic index determinations

Treatment (µg/mL)	Mitotic index (%)								
	3+17 hours, -S-9			3+17 hours, +S-9			20+0 hours, -S-9		
	A	B	MIH*	A	B	MIH*	A	B	MIH*
Vehicle	7.1	8.0	-	9.0	8.0	-	5.7	4.8	-
13.91	10.7	NT	0	10.7	NT	0	9.9	NT	0
23.19	9.2	NT	0	9.9	NT	0	6.2	NT	0
38.65	8.0	NT	0	7.8	NT	8	6.1	NT	0
64.41	10.3	NT	0	8.6	NT	0	5.5	NT	0
107.4	9.8	NT	0	9.3	NT	0	5.8	NT	0
178.9	8.6	NT	0	7.4	NT	13	3.9	NT	26
298.2	5.9	NT	22	8.4	NT	1	5.9	NT	0
497.0	6.8	NT	10 P	9.1	NT	0 P	5.2	NT	1 P
828.4	8.5	NT	0 P	7.3	NT	14 P	3.3	NT	37 P
1381	7.5	NT	1 P	6.8	NT	20 P	2.9	NT	45 P
2301	6.9	NT	9 P	6.5	NT	24 P	3.6	NT	31 P
3835	0.0	NT	100 PEH	0.5	NT	94 PEH	0.0	NT	100 PH

NT = Not tested

P Indicates precipitation observed at the beginning of the treatment incubation period

E Indicates precipitation observed at the end of treatment

H Indicates precipitation observed at harvest

*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%
(where T = treatment and C = negative control)

The results of the cytotoxicity Range-Finder Experiment were used to select suitable maximum concentrations for the Main Experiments.

Experiment 1, mitotic index determinations

Treatment (µg/mL)	Mitotic index (%) 3+17 hours, -S-9			3+17 hours, +S-9		
	A/C	B/D	MIH*	A/C	B/D	MIH*
Vehicle	10.0/9.7	9.1/9.1	-	7.8/8.5	11.2/9.3	-
300.0	NS	NS	-	NS	NS	-
600.0	NS	NS	-	NS	NS	-
1200	**9.1**	**9.9**	**0 P #**	**8.4**	**10.0**	**0 P #**
1800	**9.3**	**9.2**	**2 P #**	7.4	9.5	8 P
2400	**8.4**	**7.0**	**19 PEH #**	**7.6**	**8.4**	**13 P #**
2600	6.4	8.0	24 PEH	8.3	6.8	18 P
2800	7.2	7.4	23 PEH	**6.7**	**6.1**	**30 PE #**
3000	6.5	7.8	25 PEH	6.1	6.7	30 PEH
3200	6.3	6.1	35 PEH	5.2	6.5	36 PEH
3400	6.3	5.9	36 PEH	5.8	6.7	32 PEH
3600	6.2	5.1	40 PEH	6.1	5.6	36 PEH
3835	0.2	5.6	69 PEH	3.2	5.6	52 PEH

NS = Not scored

P Indicates precipitation observed at the beginning of the treatment incubation period

E Indicates precipitation observed at the end of treatment

H Indicates precipitation observed at harvest

*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%
(where T = treatment and C = negative control)

Highlighted concentrations were selected for analysis

Experiment 2, mitotic index determinations

Treatment (µg/mL)	Mitotic index (%) 20+0 hours, -S-9			3+17 hours, +S-9		
	A/C	B/D	MIH*	A/C	B/D	MIH*
Vehicle	3.7/8.2	5.6/7.2	-	9.5/11.1	7.6/8.7	-
300.0	**5.4**	**8.2**	**0 #**	NT	NT	-
500.0	NT	NT	-	9.0	8.3	6
600.0	**6.9**	**7.1**	**0 P #**	NT	NT	-
800.0	6.2	5.2	8 P	NT	NT	-
1000	**4.9**	**5.4**	**17 P #**	8.8	7.1	14 P
1200	4.7	3.7	32 P	NT	NT	-
1400	**3.7**	**2.5**	**50 P #**	NT	NT	-
1500	NT	NT	-	**7.9**	**7.8**	**15 P #**
1600	3.2	2.3	55 P	NT	NT	-
1800	3.6	4.4	35 P	NT	NT	-
2000	3.9	4.2	34 P	7.0	7.7	20 P
2300	3.6	3.4	43 P	**8.0**	**6.6**	**21 P #**
2600	4.1	2.7	45 P	7.2	6.4	26 P
2800	NT	NT	-	6.7	6.4	29 P
3000	3.6	3.1	46 P	5.4	7.0	33 P
3400	0.0	0.1	99 P	**5.2**	**8.3**	**27 PE #**
3835	0.0	0.1	99 PH	7.2	5.4	32 PE

NT = Not tested

P Indicates precipitation observed at the beginning of the treatment incubation period

E Indicates precipitation observed at the end of treatment

H Indicates precipitation observed at harvest

*Mitotic inhibition (%) = [1 - (mean MI_T/mean MI_C)] x 100%
(where T = treatment and C = negative control)

Highlighted concentrations were selected for analysis

Summary tables - cells with structural aberrations

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

Treatment (µg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	MIH (%)
Vehicle	A	100	3	3	
	B	100	1	0	
	Totals	200	4	3	-
1200	A	100	2	1	
	B	100	1	1	
	Totals	200	3	2	0
1800	A	100	0	0	
	B	100	0	0	
	Totals	200	0	0	2
2400	A	100	1	1	
	B	100	1	1	
	Totals	200	2	2	19
NQO, 5.00	A	85	**19 #**	**18 #**	
	B	96	**19 #**	**17 #**	
	Totals	181	38	35[a]	

Binomial Dispersion Test χ^2 = 3.05, Not significant

[a] Statistical significance $p \leq 0.001$

Numbers highlighted exceed historical negative control range (Appendix 2: Historical control data)

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

Treatment (µg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	MIH (%)
Vehicle	A	100	1	0	
	B	100	1	1	
	Totals	200	2	1	-
1200	A	100	0	0	
	B	100	1	1	
	Totals	200	1	1	0
2400	A	100	2	2	
	B	100	0	0	
	Totals	200	2	2	13
2800	A	100	0	0	
	B	100	2	2	
	Totals	200	2	2	30
CPA, 12.5	A	30	**20 #**	**20 #**	
	B	32	**20 #**	**20 #**	
	Totals	62	40	40[a]	

Binomial Dispersion Test χ^2 = 6.05, Not significant

[a] Statistical significance $p \leq 0.001$

Numbers highlighted exceed historical negative control range (Appendix 2: Historical control data)

20 hour treatment -S-9, 0 hour recovery (20+0), Experiment 2 donor sex: female

Treatment (µg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	MIH (%)
Vehicle	A	100	1	1	
	B	100	0	0	
	Totals	200	1	1	-
300.0	A	100	1	1	
	B	100	0	0	
	Totals	200	1	1	0
600.0	A	100	3	2	
	B	100	1	0	
	Totals	200	4	2	0
1000	A	100	2	0	
	B	100	1	1	
	Totals	200	3	1	17
1400	A	100	1	0	
	B	100	1	0	
	Totals	200	2	0	50
NQO, 5.00	A	94	**19 #**	**18 #**	
	B	97	**21 #**	**17 #**	
	Totals	191	40	35[a]	

Binomial Dispersion Test χ^2 = 5.04, Not significant

[a] Statistical significance $p \leq 0.001$

Numbers highlighted exceed historical negative control range (Appendix 2: Historical control data)

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

Treatment (µg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	MIH (%)
Vehicle	A	100	**5 #**	**5 #**	
	B	100	1	1	
	C	100	3	2	
	D	100	1	1	
	Totals	400	10	9	-
1500	A	100	1	0	
	B	100	0	0	
	Totals	200	1	0	15
2300	A	100	**5 #**	2	
	B	100	0	0	
	Totals	200	5	2	21
3400	A	100	4	**4 #**	
	B	100	2	1	
	Totals	200	6	5	27
CPA, 12.5	A	37	**20 #**	**20 #**	
	B	28	**21 #**	**21 #**	
	Totals	65	41	41[a]	

Binomial Dispersion Test χ^2 = 8.75, Not significant

[a] Statistical significance $p \leq 0.001$

Numbers highlighted exceed historical negative control range (Appendix 2: Historical control data)

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

Study title: LCZ696: Rat bone marrow micronucleus test after oral administration

Study no:	0770051
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	1/29/2007
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0651004 and 0651003, 94.6% and 94.5%, respectively. 0651004 was used for the range-finder experiment, 0651003 for the micronucleus test

Key Study Findings

LCZ696 did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of rats treated up to 2000 mg/kg/day (the maximum recommended)

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Methods

Doses in definitive study:	500, 1000, 2000 mg/kg
Frequency of dosing:	daily for 2 days
Route of administration:	oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% CMC
Species/Strain:	Wistar rat
Number/Sex/Group:	6M
Satellite groups:	na
Basis of dose selection:	Dose ranging study
Negative control:	0.5% CMC
Positive control:	Cyclophosphamide, 20 mg/kg

Micronucleus experiment treatment details

Treatment group	Dose administered (mg/kg/day) [a]	Dose volume (mL/kg)	Number of animals treated [b]
Vehicle	0	10	6M
LCZ696	500	10	6M
LCZ696	1000	10	6M
LCZ696	2000	10	6M
Positive control, CPA	20	10	6M

a Doses administered once daily for two consecutive days, approximately 24 hours apart (except positive control)
b Animals sampled 24 hours after final dose administration
CPA Cyclophosphamide, administered as a single dose
M Male

Study Validity

Study was valid with respect to strains, concentrations, and positive and negative controls.

Results

Test item- and vehicle-treated rats were sampled in groups, 24 hours after the second Administration. CPA-treated rats were sampled 24 hours after the single dose. Rats were killed by an overdose of sodium pentobarbitone, given via intraperitoneal injection and subsequently ensured by cervical dislocation, in the same sequence used for dosing.

One femur from each animal was exposed, removed, cleaned of adherent tissue and the ends removed from the shanks. Using a syringe and needle, bone marrow was flushed from the marrow cavity with 2 mL fetal bovine serum into appropriately labelled centrifuge tubes.

Slides from the CPA-treated rats were initially checked a (b) (4) to ensure the system was operating satisfactorily. The slides from all control and dose groups were arranged in numerical order by sampling time and analyzed by a person not connected with the dosing phase of the study.

Initially the relative proportions of polychromatic erythrocytes (PCE), seen as bright orange enucleate cells, and normochromatic erythrocytes (NCE), seen as smaller dark green enucleate cells, were determined until a total of at least 1000 cells (PCE plus NCE) had been analyzed.

Counting continued (but of PCE only) until at least 2000 PCE had been observed. All PCE containing micronuclei observed during these two phases of counting were recorded. The vernier coordinates of all cells containing micronuclei were recorded to a maximum of six per 2000 cells scored. Data are summarized below.

The range-finder experiment confirmed that 2000 mg/kg/day (the maximum dose currently recommended for *in vivo* genotoxicity studies) was non- toxic, consequently this dose was selected as the maximum dose for the micronucleus experiment. Two lower doses of 500 and 1000 mg/kg/day were also selected. As no substantial difference in toxicity was observed between males and females in the range-finder, males only were used in the micronucleus test.

No significant differences from control were observed in the percentage of micronucleated PCEs.

Summary of group mean data

Treatment group (mg/kg/day)	Kill Time (hours)	Sex	% PCE (±sd)	Group mean % micronucleated PCE per treatment group (±sd)
Vehicle Control	24	M	35.37 ± 19.07	0.22 ± 0.18
LCZ696 (500)	24	M	43.00 ± 5.12	0.08 ± 0.05
LCZ696 (1000)	24	M	41.13 ± 6.72	0.16 ± 0.18
LCZ696 (2000)	24	M	41.68 ± 7.01	0.08 ± 0.07
Positive control, CPA (20)+	24	M	31.38 ± 11.05	2.54 ± 1.59

\+ Administered as a single dose
sd Standard deviation

Study title: AHU377: Oral bone marrow micronucleus test in rats

Study no:	0412409
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	Safety Profiling & Assessment, Novartis Pharma AG, Basel, Switzerland
Date of study initiation:	12/1/2004
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0451002, 96.2%

Key Study Findings

LCZ696 did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of rats treated up to 1250 mg/kg/day (the maximum recommended)

Methods

Doses in definitive study:	125, 395 and 1250 mg/kg/day
Frequency of dosing:	Daily, 2 days
Route of administration:	oral
Dose volume:	20 mL/kg
Formulation/Vehicle:	1% CMC
Species/Strain:	CRL:WI (GLX/BRL/HAN)IGS BR rats
Number/Sex/Group:	7
Satellite groups:	na
Basis of dose selection:	MFD, based on solubility and dose volume
Negative control:	1% CMC
Positive control:	Cyclophosphamide (10 mg/kg)

The animals were killed 48 hours after the first application by CO2 asphyxiation. Immediately after death, one femur was excised and, after removal of muscle tissues, the proximal ends of the bones were cut off with scissors. A 2 ml plastic syringe was

connected via a latex tube to this end of the femur. The bone marrow canal at the distal end of the femur was opened, and was dipped in 2 ml FCS mix in a centrifuge tube.

Study Validity

Study was valid with respect to strains, concentrations, and positive and negative controls.

Results

The mean values of MPE were 0.18 ± 0.09 %, 0.14 ± 0.08 % and 0.18 ± 0.08 % at doses of 125, 395, and 1250 mg/kg, respectively. The corresponding negative control had a value of 0.18 ± 0.03 %. Biometric analysis did not show a statistically significant difference between the mean micronucleus frequencies in the treated groups and the vehicle control group. The mean percentage of MPE in the positive control (cyclophosphamide, 10 mg/kg) group was 2.94 ± 0.96 %, which was clearly higher than the negative control value results**.**

Summary of Data

Group	Dose (mg/kg)	Sex	No. of animals	Frequency (%)**	
				MPE	PCE
Vehicle	0	m	7	0.18 ± 0.03	49.3 ± 13.6
AHU377	125	m	7	0.18 ± 0.09	51.7 ± 9.7
AHU377	395	m	7	0.14 ± 0.08	51.6 ± 7.5
AHU377	1250	m	7	0.18 ± 0.08	46.5 ± 10.6
Cyclo-phosphamide	10	m	3	2.94 ± 0.96	35.1 ± 5.1

4000 cells/animal were analyzed.

Note:

Each value represents mean ± SD (standard deviation) of a treatment group.

**frequencies are calculated as follows:

Frequency MPE: number of MPE x 100 / number of PCE

Frequency PCE: number of PE x 100 / number of (PCE+NCE)

7.4 Other Genetic Toxicity Studies

none

8 Carcinogenicity

Carcinogenicity was evaluated for the New Molecular Entity AHU377, as the valsartan component has already been tested. Testing the component only allowed for higher doses of AHU377 to be tested, as the valsartan component was dose-limiting when the combination was tested.

Study title: A 104-week oral (gavage) carcinogenicity study in mice.

Study no.:	804258
Study report location:	NDA207620 SDN0001
Conducting laboratory and location:	(b) (4)
Date of study initiation:	3/18/10
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	AHU377 (also identified as AHU377 (b) (4) (b) (4) (b) (4), AHU377- (b) (4)), Batch # 0824011, 99.2% purity
CAC concurrence:	yes, under IND (b) (4)

Key Study Findings

No AHU377-related effects were seen in the incidence of any neoplastic lesion in male and female mice in this study. There were no statistically significant differences in incidence of any neoplasms.

Adequacy of Carcinogenicity Study

The study protocol design and dosing was approved by the Exec. CAC, and was conducted in an acceptable manner. The interpretation of the sponsor is consistent with the data as shown.

In the high (1200 mg/kg/day) groups, Cmax-based exposure ratios for AHU377 and LBQ657, relative to humans dosed at 200 mg LCZ696 bid (MRHD), were as follows:

	AHU377	LBQ657
Males	14x	16x
Females	46x	23x

(Human data was from Clinical study LCZ696A2117)

Methods

Doses:	150, 400 and 1200 mg/kg/day
Frequency of dosing:	daily
Dose volume:	10 mL/kg
Route of administration:	Oral by gavage
Formulation/Vehicle:	suspension in aqueous 0.5% (w/v) hydroxypropylcellulose
Basis of dose selection:	Maximum feasible dose based on solubility and dosing volume
Species/Strain:	mouse, CRl:CD1 (ICR)
Number/Sex/Group:	70
Age:	7 weeks (at start of dosing)
Animal housing:	Group housed (up to 3 animals of the same sex and same dosing group together) in polycarbonate bins containing appropriate bedding and equipped with an automatic watering valve.
Paradigm for dietary restriction:	none
Dual control employed:	no
Interim sacrifice:	no
Satellite groups:	10/sex/group for TK
Deviation from study protocol:	minor SOP deviations that did not impact the integrity of the study

Observations and Results

Mortality

Mice were observed twice daily (AM and PM) on weekdays and weekends, once daily on the day of animal arrival and on the last day of necropsy.

During the course of the study, between 26 and 34 male and 32 to 45 female mice per group died or were euthanized prior to the end of the study.

The mortality rate was comparable between all groups for both genders. For most pre-terminal decedent mouse, the most probable cause of death was determined. The cause of death could not be determined for a small number of animals per group.

No AHU377-related effect was seen in the distribution of neoplastic or non- neoplastic lesions contributory to pre-terminal death or euthanasia of animals in this study. The most frequent neoplastic causes of early death/euthanasia recorded in control animals and AHU377-treated animals were lymphoma and histiocytic sarcoma (recorded under hemolymphoreticular tissue) and pulmonary alveolar/bronchiolar carcinoma whereas the most frequent non-neoplastic cause of death/early euthanasia was obstructive uropathy in males and nephropathy in females.

Summary of survival ratios with % survival

Study week	Males Dose (mg/kg/day)				Females Dose (mg/kg/day)			
	0	150	400	1200	0	150	400	1200
16	70 (100%)	70 (100%)	70 (100%)	70 (100%)	70 (100%)	70 (100%)	70 (100%)	70 (100%)
24	70 (100%)	70 (100%)	69 (99%)	70 (100%)	69 (99%)	69 (99%)	69 (99%)	70 (100%)
52	70 (100%)	66 (94%)	65 (93%)	66 (94%)	67 (96%)	63 (90%)	66 (94%)	66 (94%)
80	63 (90%)	54 (77%)	53 (76%)	56 (80%)	58 (83%)	48 (69%)	56 (80%)	54 (77%)
Term	44 (63%)	36 (51%)	41 (59%)	37 (53%)	29 (41%)	25 (36%)	29 (41%)	38 (54%)

Note: n = 70 for each dose group.

Clinical Signs

Daily cage side observations were performed at least 2 hours post dose.

Clinical signs associated with generally poor or deteriorating condition were observed in animals from the control and AHU377-treated groups found dead, euthanized prior to the scheduled necropsies or at terminal necropsies. These clinical signs included, but were not limited to decreased activity, dehydration, prominent backbone, decreased muscle tone, lying on side/prostrate, hunched posture, thin body condition, weak condition, generalized skin pallor, partly closed eyes and breathing difficulties. There were no clinical signs clearly associated with AHU377.

Body Weights

Once weekly during weeks 1 to 14, every four weeks from weeks 18 to 78 and every 2 weeks thereafter up to and including week 104. Terminal body weights were collected from scheduled necropsy animals.

AHU377-related slight decreases in mean body weight were noted in male mice at doses ≥ 150 mg/kg/day, when compared to concurrent controls. These body weight effects had an onset at approximately week 22 (1200 mg/kg/day), week 34 (400 mg/kg/day) or week 66 (150 mg/kg/day). By the end of the study, body weights were slightly reduced by 4.7% (400 mg/kg/day) and 8.4% (1200 mg/kg/day), compared to concurrent controls, whereas mean body weights normalized in the 150 mg/kg/day group. These body weight changes correlated with decreases in mean absolute body weight gain in males at a dose of 1200 mg/kg/day compared to concurrent controls (-12.5%, -14.3%, -18.3% and -21.8% at the week 26, 54, 78 and 104 timepoints, respectively).

AHU377-related slight decreases in mean body weight were noted in female mice at a dose of 1200 mg/kg/day, when compared to concurrent controls. These body weight effects had an onset at approximately weeks 26-30 and, in general, persisted through the remainder of the dosing phase. By the end of the study, body weights were slightly reduced by 7.4%, compared to concurrent controls. These body weight changes in

females at 1200 mg/kg/day correlated with minor decreases in mean absolute body weight gain compared to concurrent controls (-14.1%, -17.2% and -16.8% at the week 54, 78 and 104 timepoints, respectively). Body weight data is appended.

Body weight data is appended.

Feed Consumption

Measured weekly during weeks 1 to 14, every fourth week from weeks 18 to 78 and every 2 weeks thereafter up to and including week 104.

There were no AHU377-related effects on food consumption observed during the study.

Gross Pathology

All animals found dead during the study had a necropsy performed and tissue samples were preserved. Prior to necropsy, the carcass was stored in a refrigerator set to maintain 4°C. The carcass of any animals euthanized in extremis was stored in a refrigerator set to maintain 4°C until necropsy and terminal procedures were performed as for animals found dead during the study.

Histopathology

All tissue sections processed were examined and assessed with all observations being recorded in the raw data. The optic nerves, parathyroid gland, mammary glands and thymic lymphoid tissue were examined histopathologically only if present in routine sections of eyes, thyroid lobes, skin or thymus, respectively. Ureters were submitted to a unilateral histopathological examination (although collected bilaterally).

Tissues were prepared for histopathological examination by embedding in paraffin wax, sectioning and staining with hematoxylin and eosin and examined from all animals. All tissue sections (except blood and bone marrow smears) were assessed with all observations being recorded in the raw data. Incidences of diagnosed lesions were tabulated.

All suspected tumors were diagnosed, and the incidences of benign and malignant tumors of different cell types in the various dosing groups were tabulated. Histopathological evaluation was performed by a board-certified veterinary pathologist.

Tissue list for collection and/or processing (P)

P Salivary gland (mandibular, parotid and sublingual)	P Spinal cord (cervical, thoracic and lumbar)	P Brain (forebrain, midbrain, cerebellum and medulla oblongata)
P Optic nerves	P Pituitary gland	P Sciatic nerve
P Artery, aorta (thoracic)	P Prostate gland	P Eyes
P Pancreas	P Adrenal, gland	P Bone and marrow, sternum
P Preputial gland	P Esophagus	P Femorotibial joint
P Clitoral gland	P Gross lesions/masses	P Skin (inguinal)
P Epididymis	P Gallbladder	P Small intestine, Ileum
P Skeletal muscle	P Harderian glands	P Kidneys
P Seminal vesicle	P Spleen	P Small intestine, jejunum
P Heart (including section of aorta)	P Uterus (cervix, horns and body)	P Thyroid gland (and parathyroid)
P Stomach	P Lacrimal gland (bilateral)	Small intestine, duodenum
P Large intestine, cecum	P Testis	P Large intestine, colon

P Large intestine, rectum	P Thymus/Thymic area	P Larynx (1 level)
P Liver (sample of two lobes)	P Tongue	P Lung (sample of two lobes)
P Trachea	P Lymph node - mandibular	P Urinary bladder
P Lymph node - mesenteric	P Mammary gland (inguinal)	P Vagina
P Ureter bilateral	P Nasal cavities (1 level)	P Ovaries
P Oviducts		

Peer Review

The pathology report and histopathological examinations were peer reviewed by a Sponsor-designated pathologist.

Neoplastic

The tumor incidences recorded were within the range of spontaneous occurrence reported for aged CD-1 mice, were generally randomly distributed in control and treated groups and/or lacked a dose-related pattern. In addition, no increase in the incidence of any hyperplastic lesions was noted. The various neoplastic lesions were generally typical of those commonly encountered in mice of this strain and age range.

Non Neoplastic

No AHU377-related effects were seen in the incidence of any non-neoplastic lesion in male and female mice in this study.

Toxicokinetics

During weeks 4 and 26, blood samples (2 mice/sex/group/timepoint) were collected at 0.5 and 1 hour post dose from the toxicokinetic animals from the abdominal aorta following isoflurane anesthesia. Approximately 0.5 mL of whole blood was collected into tubes containing K_2EDTA and approximately 10-20 mg of sodium fluoride (NaF). The samples were analyzed for AHU377 and metabolite LBQ657.

Dosage/Day/Gender	Time Post-Dose 0.5 h			Time Post-Dose 1 h		
	AHU377 (ng/mL)	LBQ657 (ng/mL)	AHU377/ LBQ657 %	AHU377 (ng/mL)	LBQ657 (ng/mL)	AHU377/ LBQ657 %
150 mg/kg/day / Day 28/Male	211	8610	2.5	107	4740	2.3
150 mg/kg/day / Day 28/Female	784	7720	10.2	108	1540	7.0
400 mg/kg/day / Day 28/Male	4830	55500	8.7	683	31200	2.2
400 mg/kg/day / Day 28/Female	13400	111000	12.1	1760	32200	5.5
1200 mg/kg/day / Day 28/Male	25800	154000	16.8	5930	110000	5.4
1200 mg/kg/day / Day 28/Female	66400	301000	22.1	13000	211000	6.2
150 mg/kg/day / Day 182/Male	1660	24600	6.7	70.1	1530	4.6
150 mg/kg/day / Day 182/Female	2170	35700	6.1	276	3600	7.7
400 mg/kg/day / Day 182/Male	3350	80600	4.2	1080	50700	2.1
400 mg/kg/day / Day 182/Female	38700	306000	12.6	4530[a]	100000[a]	4.5
1200 mg/kg/day / Day 182/Male	33700	264000	12.8	981[a]	81700[a]	1.2
1200 mg/kg/day / Day 182/Female	111000[a]	380000[a]	29.2	16700	199000	8.4

[a] n=1

Dosing Solution Analysis

Samples of AHU377 formulations from weeks 1, 9, 24, 39, 55, 69, 84 and 104 were analyzed for concentration. Concentrations were 88% to 105% of targets. Control samples were tested and no AHU377 was detected..

Summary of body weight

Males

Group 1 - Vehicle control
Group 2 - AHU377 150 mg/kg/day
Group 3 - AHU377 400 mg/kg/day
Group 4 - AHU377 1200 mg/kg/day

Group	Summary Information	Week -1	-1	1	2	3	4	5	6	7	8	9	10	11	12	13
1	Mean	31.83	33.33	34.09	35.46	37.15	37.26	38.44	39.24	39.80	40.84	41.54	42.40	42.93	43.70	43.43
	SD	1.86	2.11	2.13	2.30	2.50	2.37	2.52	2.76	2.95	2.99	3.10	3.39	3.62	3.67	3.79
	N	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
2	Mean	31.91	33.35	34.18	35.84	37.55	37.86	39.22	40.23	40.67	41.22	41.86	42.45	43.64	43.75	44.25
	SD	1.80	2.00	2.14	2.24	2.47	2.53	2.79	2.85	3.18	3.21	3.39	3.57	3.82	3.86	4.06
	N	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
3	Mean	31.87	33.19	33.99	35.80	37.45	37.51	38.60	39.64	40.11	40.86	41.56	42.19	43.02	43.45	44.12
	SD	1.81	2.10	2.27	2.57	2.95	3.08	3.30	3.67	3.92	4.34	4.73	4.80	5.07	5.12	5.52
	N	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
4	Mean	31.88	33.23	34.06	35.75	37.52	37.69	38.52	39.53	39.35	39.90	40.77	41.86	42.40	43.27	43.43
	SD	1.81	2.06	2.11	2.33	2.45	2.53	2.82	2.97	3.17	3.17	3.53	3.81	4.11	4.35	4.46
	N	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70

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

Males

Group 1 - Vehicle control
Group 2 - AHU377 150 mg/kg/day
Group 3 - AHU377 400 mg/kg/day
Group 4 - AHU377 1200 mg/kg/day

Group	Summary Information	Week 14	18	22	26	30	34	38	38	42	46	50	54	58	62	66
1	Mean	44.55	46.47	48.74	48.99	49.92	51.39		51.07	51.80	52.84	53.12	54.12	54.18	55.48	55.65
	SD	4.06	4.49	5.02	5.07	5.16	5.50		5.39	5.23	5.41	5.49	5.34	5.42	5.77	5.94
	N	70	70	70	70	70	70		70	70	70	70	70	70	70	67
2	Mean	44.97	46.65	48.74	48.72	49.95	50.76		50.31	50.82	52.29	52.60	53.75	53.23	53.77	52.91
	SD	4.35	4.60	4.78	4.86	5.37	5.55		5.77	5.84	6.06	5.79	5.97	5.72	6.15	6.10
	N	70	70	70	70	70	70		69	69	68	66	66	65	65	63
3	Mean	44.63	46.16	47.91	48.15	48.80	49.30 D	31.10	49.32 D	50.22	51.21	51.46	51.74 D	51.61	51.62 B	51.53 B
	SD	5.63	6.52	6.83	7.27	7.42	7.90	-	8.01	8.12	8.27	8.22	7.87	7.57	7.18	7.74
	N	70	70	69	69	69	69	1	67	66	66	65	63	62	61	59
4	Mean	43.96	45.06	46.40 D	46.93	47.71 D	48.33 E		48.19 D	48.78 E	49.49 E	49.56 E	50.99 D	51.08 A	51.17 C	50.59 C
	SD	4.55	5.13	5.50	5.82	5.99	6.50		6.42	6.43	6.55	7.09	6.75	7.03	6.99	7.22
	N	70	70	70	70	70	69		69	69	69	68	65	63	61	60

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

Males

Group 1 - Vehicle control
Group 2 - AHU377 150 mg/kg/day
Group 3 - AHU377 400 mg/kg/day
Group 4 - AHU377 1200 mg/kg/day

Group	Summary Information	Week 70	74	78	80	82	84	86	88	90	92	94	96	98	100	102	104
1	Mean	55.15	54.85	54.96	55.40	55.83	54.74	54.57	54.83	54.71	54.24	54.05	53.91	53.49	52.84	51.87	52.11
	SD	5.90	6.44	7.11	7.67	6.88	6.84	6.33	6.33	6.36	6.73	7.17	6.77	6.25	6.10	5.65	5.64
	N	67	66	65	65	59	59	58	57	56	55	54	51	50	46	45	44
2	Mean	52.85	52.53	52.99	52.62	52.38 A	52.03	51.94	52.36	53.16	52.39	51.61	51.45	51.50	51.10	51.95	52.05
	SD	6.47	6.24	6.32	6.32	6.52	6.52	6.52	6.46	6.62	6.76	6.68	6.84	6.41	6.64	6.27	6.62
	N	60	56	54	54	54	51	48	47	47	44	44	44	41	41	38	36
3	Mean	51.54 B	51.63 A	51.84	51.48 A	51.48 B	51.04 A	50.68 A	51.28 A	51.81	51.07	50.55 A	50.28 A	50.37	49.66	49.61	49.66
	SD	7.95	8.35	8.25	8.19	7.99	7.90	7.94	7.79	7.73	7.82	7.95	7.96	7.96	7.83	7.84	7.24
	N	57	56	54	53	52	52	51	50	49	49	48	47	45	43	43	41
4	Mean	50.64 C	51.18 A	50.72 B	50.94 B	50.86 B	50.10 B	49.63 C	49.48 C	50.79 A	49.66 B	49.25 B	49.37 B	49.10 B	48.25 B	48.44	47.73 B
	SD	6.69	7.42	7.70	7.61	7.97	7.57	7.64	7.95	7.76	7.39	7.39	7.28	7.23	7.17	7.13	6.27
	N	60	59	57	56	56	55	53	53	52	51	51	50	48	46	42	38

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

Females

Group 1 - Vehicle control
Group 2 - AHU377 150 mg/kg/day
Group 3 - AHU377 400 mg/kg/day
Group 4 - AHU377 1200 mg/kg/day

Group	Summary Information	Week -1	-1	1	2	3	4	5	6	7	8	9	10	11	12	13
1	Mean	23.50	24.16	24.36	25.61	26.92	27.21	28.32	28.23	28.66	29.13	29.84	30.75	31.03	31.32	31.45
	SD	1.32	1.37	1.67	1.76	2.04	2.02	2.25	2.37	2.55	2.87	2.86	3.28	3.13	3.17	3.64
	N	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
2	Mean	23.49	23.90	24.12	25.54	26.47	26.92	27.42	28.38	28.96	29.16	30.20	30.45	31.23	31.25	31.69
	SD	1.33	1.33	1.45	1.61	1.89	1.84	1.90	2.11	1.98	2.28	2.41	2.42	2.68	2.77	3.04
	N	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
3	Mean	23.51	23.89	24.58	26.15	27.43	27.58	28.02	28.96	29.52	30.10 A	30.51	31.03	31.71	32.11	32.02
	SD	1.35	1.26	1.36	1.50	1.75	1.77	1.90	1.97	2.37	2.17	2.65	2.71	2.94	3.07	3.22
	N	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
4	Mean	23.49	23.73	24.58	26.45 B	27.77 A	27.41	27.75	28.76	29.15	29.76	29.63	30.99	31.16	31.49	31.15
	SD	1.34	1.47	1.46	1.50	1.81	1.82	1.85	2.11	2.02	2.15	2.44	2.54	2.80	2.83	2.75
	N	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70

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

Females

Group 1 - Vehicle control
Group 2 - AHU377 150 mg/kg/day
Group 3 - AHU377 400 mg/kg/day
Group 4 - AHU377 1200 mg/kg/day

Group	Summary Information	Week 14	18	22	26	30	34	38	42	46	50	54	58	62	66	70
1	Mean	31.95	33.32	34.86	35.37	36.26	36.80	37.30	37.75	38.61	38.73	39.92	39.80	41.05	40.95	41.61
	SD	3.47	4.02	4.55	4.49	5.19	5.56	6.13	6.17	6.63	6.50	6.88	6.85	7.55	7.53	6.97
	N	70	70	69	69	69	69	69	68	67	67	67	67	67	65	64
2	Mean	31.68	33.90	35.57	36.22	36.88	37.80	38.37	39.07	39.58	39.63	39.73	40.10	40.95	41.23	41.03
	SD	3.13	3.80	4.47	4.75	5.34	5.48	5.74	5.99	6.14	6.46	7.18	7.12	7.55	7.52	7.79
	N	70	70	69	69	69	69	68	67	66	65	63	62	60	60	58
3	Mean	32.40	33.79	35.14	35.49	36.30	36.94	37.44	38.19	39.01	39.48	40.54	41.01	41.01	41.04	40.83
	SD	3.18	3.84	4.29	4.28	4.73	5.11	5.38	5.56	5.69	6.26	6.56	6.65	6.79	7.49	7.41
	N	70	70	69	69	68	68	68	67	67	67	65	62	62	62	62
4	Mean	31.51	32.85	33.39	33.80	34.24 A	34.78	35.04 A	35.96	36.07 A	36.09 A	37.21	36.82 A	37.36 B	36.83 B	37.78 B
	SD	3.15	3.35	4.03	3.97	4.04	4.52	4.56	5.32	5.66	5.64	6.42	6.26	6.33	6.17	5.80
	N	70	70	70	70	70	70	68	67	66	66	66	66	65	63	59

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

Females

Group 1 - Vehicle control
Group 2 - AHU377 150 mg/kg/day
Group 3 - AHU377 400 mg/kg/day
Group 4 - AHU377 1200 mg/kg/day

Group	Summary Information	Week 74	78	80	82	84	86	88	90	92	94	96	98	100	102	104
1	Mean	41.45	41.73	42.27	42.87	42.23	40.87	41.21	41.01	41.42	40.72	41.51	40.79	40.35	39.77	40.21
	SD	7.27	7.25	7.55	7.66	7.22	6.16	5.94	5.88	6.04	5.65	5.79	5.64	5.53	5.56	5.98
	N	60	60	59	55	53	49	48	45	44	43	40	38	35	33	29
2	Mean	41.14	41.74	42.15	42.25	41.51	41.30	41.72	42.84	42.08	42.38	42.15	42.03	41.24	41.10	41.12
	SD	8.35	8.50	8.63	8.96	8.87	8.78	8.88	8.87	8.99	8.80	8.83	8.29	8.41	8.49	8.14
	N	51	48	48	46	46	43	40	38	37	36	31	31	28	28	25
3	Mean	40.97	42.24	42.69	41.92	41.63	41.38	41.96	42.31	41.60	41.20	41.23	41.57	41.03	40.79	40.19
	SD	8.21	7.80	7.71	7.52	7.78	7.56	7.39	7.27	7.69	7.48	7.74	7.28	7.44	7.55	5.61
	N	59	56	56	55	53	52	51	49	47	46	43	39	35	31	29
4	Mean	38.09	38.26 A	38.35 A	37.83 B	38.08 A	37.55	38.32	37.98	37.87 A	37.85	37.92	38.06	37.77	36.75	37.25
	SD	6.15	6.28	6.57	6.33	5.80	5.55	5.82	5.79	5.63	5.80	5.61	5.89	5.55	4.97	4.89
	N	58	56	54	52	52	50	50	49	49	48	45	45	43	40	38

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

Table 2.6 Incidence of animals with neoplastic lesions by organ/group/sex
All animals

		MALE			
DOSE GROUP		**1**	**2**	**3**	**4**
NUMBER OF ANIMALS EXAMINED		**70**	**70**	**70**	**70**
ABDOMEN	NO.EXAM.:	1	2	1	1
- Hemangiosarcoma		-	-	-	1
ADRENAL	NO.EXAM.:	69	70	70	69
- Adenoma: subcapsular		-	1	1	2
AORTA	NO.EXAM.:	70	70	69	70
BONE MARROW	NO.EXAM.:	70	70	70	70
BONE MISCELLANEOUS	NO.EXAM.:	1	-	1	1
- Osteosarcoma		1	-	-	-
BONE-STERNUM	NO.EXAM.:	70	70	70	70
BRAIN	NO.EXAM.:	70	70	70	70
- Metastasis: astrocytoma		1	-	-	-
BRONCHUS	NO.EXAM.:	1	-	-	-
CAVITY PELVIC	NO.EXAM.:	-	1	1	2
CECUM	NO.EXAM.:	70	70	70	70
- Adenocarcinoma		-	-	1	1
COLON	NO.EXAM.:	70	70	70	70
DIAPHRAGM	NO.EXAM.:	-	1	1	-
DUODENUM	NO.EXAM.:	70	70	70	69
- Sarcoma (not otherwise specified)		-	-	1	-
- Adenocarcinoma		-	-	1	-
EAR	NO.EXAM.:	1	-	-	-
- Neural crest tumor		1	-	-	-
EPIDIDYMIS	NO.EXAM.:	70	70	70	70
ESOPHAGUS	NO.EXAM.:	70	70	70	70
- Papilloma: squamous cell		-	-	1	-
EYE	NO.EXAM.:	70	70	70	70
- Carcinoma (not otherwise specified)		-	1	-	-
FAT	NO.EXAM.:	8	5	4	4
- Carcinoma (not otherwise specified)		-	-	-	1
GALLBLADDER	NO.EXAM.:	70	66	67	64
HARDERIAN GLAND	NO.EXAM.:	70	70	70	70
- Adenoma		14	10	12	11
HEAD	NO.EXAM.:	-	1	-	-
- Carcinoma: metastasis		-	1	-	-
HEART	NO.EXAM.:	69	70	70	70
- Sarcoma: metastasis		-	1	-	1

MALE

DOSE GROUP NUMBER OF ANIMALS EXAMINED		1 70	2 70	3 70	4 70
HEMOLYM. TISSUE	NO.EXAM.:	70	70	70	70
- Mast cell tumor		1	-	-	1
- Histiocytic sarcoma		2	3	5	4
- Malignant lymphoma		11	4	10	6
ILEUM	NO.EXAM.:	70	69	70	70
- Adenocarcinoma		1	-	-	-
JEJUNUM	NO.EXAM.:	70	70	70	70
- Adenocarcinoma		-	-	-	2
JOINT	NO.EXAM.:	1	-	-	-
JOINT FEMOROTIB.	NO.EXAM.:	69	70	70	70
KIDNEY	NO.EXAM.:	70	70	70	70
- Adenoma: tubular cell		1	-	2	1
- Hemangiosarcoma		-	-	-	1
- Carcinoma: tubular cell		-	1	1	2
LACRIMAL GLAND	NO.EXAM.:	69	69	70	70
LARYNX	NO.EXAM.:	70	70	70	70
- Papilloma: squamous cell		-	-	1	-
LIVER	NO.EXAM.:	70	70	70	70
- Hemangioma		-	-	1	-
- Adenoma: hepatocellular		14	11	18	12
- Carcinoma: hepatocellular		3	5	4	-
- Hemangiosarcoma		4	4	1	3
- Sarcoma: metastasis		-	1	-	-
LUNG	NO.EXAM.:	70	70	70	70
- Carcinoma: metastasis		-	2	2	-
- Adenoma: alveolar/bronchiolar		18	9	14	8
- Metastasis: mesothelioma		-	-	-	1
- Sarcoma (not otherwise specified)		-	1	-	-
- Carcinoma: alveolar/bronchiolar		16	12	17	6
- Sarcoma: metastasis		-	-	-	1
LYMPH NODE	NO.EXAM.:	13	12	13	13
- Sarcoma: metastasis		-	1	-	-
L. NODE MANDIBULAR	NO.EXAM.:	67	69	68	66
L.NODE MESENTERIC	NO.EXAM.:	70	70	68	69
- Sarcoma: metastasis		-	1	-	-
MAMMARY GLAND	NO.EXAM.:	11	16	13	8
MUSCLE SKELETAL	NO.EXAM.:	69	69	68	69
MUSC.SKEL.MISC.	NO.EXAM.:	1	1	-	-
NERVE OPTIC	NO.EXAM.:	69	64	66	70
NERVE SCIATIC	NO.EXAM.:	70	70	70	70
PANCREAS	NO.EXAM.:	70	69	70	70

MALE

DOSE GROUP NUMBER OF ANIMALS EXAMINED		1 70	2 70	3 70	4 70
PARATHYROID GLAND	NO.EXAM.:	46	38	46	40
PENIS	NO.EXAM.:	-	-	1	-
PERICARDIUM	NO.EXAM.:	-	1	-	1
PITUITARY	NO.EXAM.:	69	68	68	67
- Adenoma: pars intermedia		1	1	1	-
PREPUTIAL GLAND	NO.EXAM.:	69	70	70	69
- Carcinoma		-	1	-	-
PROSTATE	NO.EXAM.:	70	70	70	70
- Adenoma		-	1	-	-
- Adenocarcinoma		1	-	-	-
RECTUM	NO.EXAM.:	70	68	69	70
SALIV.GL. MANDIBULAR	NO.EXAM.:	70	70	70	70
- Malignant myoepithelioma		1	-	-	-
- Carcinoma: metastasis		-	1	-	-
SAL. GL. PAROTID	NO.EXAM.:	70	69	70	70
- Sarcoma (not otherwise specified)		1	-	-	-
SAL.GL. SUBL.	NO.EXAM.:	70	70	70	70
SEMINAL VESICLE	NO.EXAM.:	70	70	70	70
- Sarcoma (not otherwise specified)		-	-	1	-
SKIN	NO.EXAM.:	70	70	70	70
SKIN MISCELLANEOUS	NO.EXAM.:	6	5	6	9
- Papilloma: squamous cell		-	-	1	-
- Carcinoma: squamous cell		1	-	-	1
SPINAL CORD CERVICAL	NO.EXAM.:	70	70	70	70
- Malignant astrocytoma		1	-	-	-
SPINAL CORD LUMBAR	NO.EXAM.:	70	69	70	70
SPINAL CORD THORACIC	NO.EXAM.:	70	69	70	70
SPLEEN	NO.EXAM.:	70	69	70	70
- Hemangiosarcoma		-	3	-	1
STOMACH	NO.EXAM.:	70	70	69	69
- Sarcoma (not otherwise specified)		1	-	-	-
- Adenocarcinoma		1	-	1	-
- Carcinoma: squamous cell		-	1	-	1
SUBCU. TISSUE	NO.EXAM.:	3	5	2	6
- Lipoma		-	-	-	1
- Fibrosarcoma		1	-	1	2
- Malignant histiocytic fibroma		-	-	1	-
TAIL	NO.EXAM.:	1	1	-	1

MALE

DOSE GROUP NUMBER OF ANIMALS EXAMINED		1 70	2 70	3 70	4 70
TESTIS	NO.EXAM.:	70	70	70	70
- Hemangioma		-	-	-	1
- Adenoma: interstitial cell		2	1	3	3
THORAX	NO.EXAM.:	-	-	1	2
- Mesothelioma(M)		-	-	-	1
THYMUS	NO.EXAM.:	67	68	65	67
- Metastasis: mesothelioma		-	-	-	1
THYROID	NO.EXAM.:	69	70	70	70
- Adenoma: follicular cell		-	-	-	2
- Carcinoma: follicular cell		1	-	1	-
TONGUE	NO.EXAM.:	70	70	70	70
- Papilloma: squamous cell		-	-	-	1
TRACHEA	NO.EXAM.:	70	70	70	70
URETER	NO.EXAM.:	65	70	68	68
URINARY BLADDER	NO.EXAM.:	70	70	70	70
- Leiomyosarcoma		-	-	-	1
- Hemangiosarcoma		1	-	-	-

Table 2.6 Incidence of animals with neoplastic lesions by organ/group/sex
All animals

		FEMALE			
DOSE GROUP		**1**	**2**	**3**	**4**
NUMBER OF ANIMALS EXAMINED		**70**	**70**	**70**	**70**
ABDOMEN	NO.EXAM.:	4	4	2	-
- Hemangiosarcoma		-	1	-	-
ADRENAL	NO.EXAM.:	70	70	70	69
- Benign pheochromocytoma		1	-	-	1
- Adenoma: subcapsular		-	1	-	-
- Adenoma: spindel cell		1	-	1	1
- Adenoma: cortical		1	-	-	-
- Sarcoma: metastasis		-	-	1	-
AORTA	NO.EXAM.:	70	70	70	69
- Sarcoma: metastasis		-	-	1	-
BILE DUCT	NO.EXAM.:	-	-	1	-
BONE MARROW	NO.EXAM.:	70	70	70	70
BONE MISCELLANEOUS	NO.EXAM.:	-	1	1	2
- Osteosarcoma		-	1	-	2
BONE-STERNUM	NO.EXAM.:	70	69	70	70
BRAIN	NO.EXAM.:	69	70	70	70
CAVITY CRANIAL	NO.EXAM.:	-	-	1	-
CECUM	NO.EXAM.:	70	70	70	69
CLITORAL GLAND	NO.EXAM.:	68	70	69	65
- Adenocarcinoma		-	1	-	-
COLON	NO.EXAM.:	70	70	70	70
DIAPHRAGM	NO.EXAM.:	-	-	1	-
- Sarcoma: metastasis		-	-	1	-
DUODENUM	NO.EXAM.:	70	70	70	70
- Sarcoma: metastasis		-	-	1	-
ESOPHAGUS	NO.EXAM.:	70	70	69	70
EYE	NO.EXAM.:	70	70	70	70
FAT	NO.EXAM.:	6	10	10	6
- Sarcoma: metastasis		-	-	1	-
GALLBLADDER	NO.EXAM.:	67	69	70	69
HARDERIAN GLAND	NO.EXAM.:	70	70	70	70
- Adenoma		2	4	4	3
- Adenocarcinoma		1	1	-	1
HEART	NO.EXAM.:	70	70	70	70
- Sarcoma (not otherwise specified)		-	-	1	-
- Carcinoma: metastasis		-	1	-	-
- Sarcoma: metastasis		-	-	-	1

FEMALE

DOSE GROUP NUMBER OF ANIMALS EXAMINED		1 70	2 70	3 70	4 70
HEMOLYM. TISSUE	NO.EXAM.:	70	70	70	70
- Histiocytic sarcoma		6	8	2	5
- Malignant lymphoma		16	19	21	20
ILEUM	NO.EXAM.:	69	70	70	70
JEJUNUM	NO.EXAM.:	70	70	70	70
JOINT	NO.EXAM.:	-	1	1	-
JOINT FEMOROTIB.	NO.EXAM.:	70	69	70	70
KIDNEY	NO.EXAM.:	70	70	70	70
LACRIMAL GLAND	NO.EXAM.:	70	68	70	68
LARYNX	NO.EXAM.:	70	70	70	69
LIVER	NO.EXAM.:	70	70	70	70
- Adenoma: hepatocellular		2	1	-	1
- Hemangiosarcoma		-	-	1	-
- Sarcoma: metastasis		-	1	-	-
LUNG	NO.EXAM.:	70	70	70	70
- Carcinoma: metastasis		2	1	-	-
- Adenoma: alveolar/bronchiolar		11	2	2	3
- Carcinoma: alveolar/bronchiolar		5	3	9	5
- Sarcoma: metastasis		1	1	1	-
LYMPH NODE	NO.EXAM.:	18	24	23	13
- Carcinoma: metastasis		-	-	1	-
L. NODE MANDIBULAR	NO.EXAM.:	68	70	68	67
L.NODE MESENTERIC	NO.EXAM.:	68	68	69	65
- Hemangioma		-	-	1	-
MAMMARY GLAND	NO.EXAM.:	68	70	68	67
- Adenocarcinoma		4	2	-	-
MUSCLE SKELETAL	NO.EXAM.:	70	70	68	70
MUSC.SKEL.MISC.	NO.EXAM.:	1	2	2	2
- Rhabdomyosarcoma		-	-	1	-
NERVE OPTIC	NO.EXAM.:	67	70	69	69
NERVE SCIATIC	NO.EXAM.:	70	69	70	70
OVARY	NO.EXAM.:	70	70	70	70
- Leiomyoma		-	1	-	-
- Cystadenoma		2	1	4	2
- Benign thecoma		-	-	1	-
- Benign luteoma		2	1	-	-
- Malignant luteoma		-	-	1	-
- Malignant granulosa-theca cell tumor		-	1	-	-
- Hemangiosarcoma		1	-	-	-

FEMALE

DOSE GROUP NUMBER OF ANIMALS EXAMINED		1 70	2 70	3 70	4 70
OVIDUCT	NO.EXAM.:	70	70	70	70
PANCREAS	NO.EXAM.:	70	70	70	69
- Sarcoma: metastasis		-	-	1	-
PARATHYROID GLAND	NO.EXAM.:	48	49	53	51
- Adenoma		1	-	-	-
PERICARDIUM	NO.EXAM.:	-	1	1	-
- Sarcoma: metastasis		-	-	1	-
PITUITARY	NO.EXAM.:	70	66	70	70
- Adenoma: pars distalis		4	3	-	1
- Adenoma: pars intermedia		1	1	-	2
PITUITARY	CONT'D.	70	66	70	70
- Carcinoma: pars intermedia		-	-	-	1
RECTUM	NO.EXAM.:	69	69	69	69
SALIV.GL. MANDIBULAR	NO.EXAM.:	70	70	70	69
SAL. GL. PAROTID	NO.EXAM.:	70	69	69	67
SAL.GL. SUBL.	NO.EXAM.:	70	70	70	69
SKIN	NO.EXAM.:	69	70	70	70
SKIN MISCELLANEOUS	NO.EXAM.:	3	7	5	1
- Carcinoma: basal cell		-	1	-	-
- Carcinoma: squamous cell		1	1	-	-
SPINAL CORD CERVICAL	NO.EXAM.:	70	70	70	70
SPINAL CORD LUMBAR	NO.EXAM.:	70	70	70	70
SPINAL CORD THORACIC	NO.EXAM.:	70	70	70	70
SPLEEN	NO.EXAM.:	70	70	70	70
- Sarcoma: metastasis		-	-	1	-
- Hemangiosarcoma		1	2	-	-
STOMACH	NO.EXAM.:	69	70	70	70
- Adenoma		1	-	-	-
- Carcinoma: squamous cell		-	-	1	-
SUBCU. TISSUE	NO.EXAM.:	13	15	8	8
- Fibrosarcoma		2	2	-	-
- Hemangiosarcoma		1	1	-	1
TAIL	NO.EXAM.:	-	-	-	1
THORAX	NO.EXAM.:	-	2	1	3
- Carcinoma (not otherwise specified)		-	-	-	1
THYMUS	NO.EXAM.:	70	70	68	67
- Sarcoma: metastasis		-	-	1	-
- Carcinoma: metastasis		-	1	-	-

FEMALE

DOSE GROUP NUMBER OF ANIMALS EXAMINED		1 70	2 70	3 70	4 70
THYROID	NO.EXAM.:	70	69	70	70
- Adenoma: follicular cell		-	1	1	-
TONGUE	NO.EXAM.:	70	69	70	68
TRACHEA	NO.EXAM.:	69	69	70	70
URETER	NO.EXAM.:	70	67	67	69
URINARY BLADDER	NO.EXAM.:	68	66	70	67
UTERUS	NO.EXAM.:	70	69	70	69
- Polyp: endometrial stromal		3	6	5	4
- Deciduoma		1	-	-	1
- Hemangioma		-	-	1	-
- Leiomyoma		3	2	1	4
- Leiomyosarcoma		1	-	2	2
- Sarcoma: endometrial stromal		-	2	-	2
- Hemangiosarcoma		2	-	1	-
- Adenocarcinoma: endometrial		-	-	1	-
VAGINA	NO.EXAM.:	69	69	69	69
- Polyp		1	-	1	-
- Sarcoma: metastasis		-	-	-	1

Study title: A 104 week oral (gavage) carcinogenicity study in the rat

Study no.:	804259
Study report location:	NDA207620 SDN0001
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	3/2/2010
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	AHU377 (also identified as AHU377 [redacted] (b) (4), AHU377-[redacted] (b) (4)) Lot no./Batch no.: 082401; 99.2% purity
CAC concurrence:	yes

Key Study Findings

No AHU377-related effects were seen in the incidence of any neoplastic lesion in male and female rats in this study. There were no statistically significant differences in incidence of any neoplasms.

Adequacy of Carcinogenicity Study

The study protocol design and dosing was approved by the Exec. CAC, and was conducted in an acceptable manner. The interpretation of the sponsor is consistent with the data as shown.

In the high (400 mg/kg/day) groups, Cmax-based exposure ratios for AHU377 and LBQ657, relative to humans dosed at 200 mg LCZ696 bid (MRHD), were as follows:

	AHU377	**LBQ657**
Males	0.63x	1.55x
Females	0.88x	3.55x

(Human data was from Clinical study LCZ696A2117)

Methods

Doses:	0, 50, 150 and 400 mg/kg/day
Frequency of dosing:	daily
Dose volume:	10 mL/kg
Route of administration:	Oral by gavage
Formulation/Vehicle:	Suspension in aqueous 0.5% (w/v) hydroxypropylcellulose
Basis of dose selection:	MTD, based on 13 week study
Species/Strain:	Rat, Wistar Hannover Crl:WI (Han)
Number/Sex/Group:	50
Age:	8 weeks (at start of dosing)
Animal housing:	Group housed (up to 3 animals of the same sex and same dosing group together) in polycarbonate bins containing appropriate bedding and equipped with an automatic watering valve
Paradigm for dietary restriction:	no
Dual control employed:	no
Interim sacrifice:	no
Satellite groups:	4/sex/group for TK
Deviation from study protocol:	reported deviations were considered to have no adverse impact on the study

Observations and Results

Mortality

Rats were examined twice daily (AM and PM) on weekdays and weekends, and once daily on the day of animal arrival and on the last day of necropsy.

There were no AHU377-related effects on survival. During the course of the study, between 12 and 20 rats per group/sex died or were euthanized prior to the end of the study. Mortality and survival data for all groups are summarized below:

Group no. identification	Dose level (mg/kg/day)	Survival %			
		Males	%	Females	%
1/ Control	0	13/50	74%	20/50	60%
2/ AHU377	50	12/50	76%	20/50	60%
3/ AHU377	150	15/50	70%	16/50	68%
4/ AHU377	400	19/50	62%	17/50	66%

The mortality rate was comparable between all groups for both genders. For each pre-terminal decedent rat, the most probable cause of death was determined. The cause of death could not be determined for a small number of animals per group.

Clinical Signs

Complete detailed examinations were performed weekly starting the week prior to the dosing period on main study animals. In addition, a detailed examination was conducted on all main study animals prior to necropsy.

Daily cage side observations were performed at least 2 hours post dose, during the dosing period. In addition, main study animals were examined for the presence of palpable masses during the detailed examination,

Body Weights

Determined once weekly during weeks 1 to 14, every four weeks from weeks 18 to 78 and every 2 weeks thereafter for the remainder of the dosing period.

Terminal body weights were collected from scheduled necropsy animals.

There were no AHU377-related effects on mean body weight or mean absolute body weight gain observed during the study.

Feed Consumption

Cage measurements, measured weekly during weeks 1 to 14, every fourth week from weeks 18 to 78 and every 2 weeks thereafter up to and including week 104. There were no AHU377-related effects on mean food consumption observed during the study.

Gross Pathology

Main study animals euthanized on completion of the dosing period, as well as those euthanized for humane reasons, underwent exsanguination from the abdominal aorta following isoflurane anesthesia and blood sample collection (from the abdominal aorta). A similar proportion of animals from each group and sex, as appropriate, were euthanized on any one day. In order to avoid autolytic change, a complete gross pathology examination of the carcass was conducted immediately on all animals which were euthanized. Where possible, the order of necropsy for each prosection group was vehicle control (group 1), high dose (group 4), mid dose (group 3) and low dose (group 2). All animals were not fasted overnight prior to scheduled necropsy. All necropsies were conducted under the supervision of a pathologist and necropsy consisted of an external examination, including identification of all clinically recorded lesions, as well as a detailed internal examination.

Histopathology

Tissue list for collection and/or processing (P)

P Salivary gland (mandibular, parotid and sublingual)	P Spinal cord (cervical, thoracic and lumbar)	P Brain (forebrain, midbrain, cerebellum and medulla oblongata)
P Optic nerves	P Pituitary gland	P Sciatic nerve
P Artery, aorta (thoracic)	P Prostate gland	P Eyes
P Pancreas	P Adrenal, gland	P Bone and marrow, sternum
P Preputial gland	P Esophagus	P Femorotibial joint
P Clitoral gland	P Gross lesions/masses	P Skin (inguinal)
P Epididymis	P Gallbladder	P Small intestine, Ileum
P Skeletal muscle	P Harderian glands	P Kidneys
P Seminal vesicle	P Spleen	P Small intestine, jejunum
P Heart (including section of	P Uterus (cervix, horns and	P Thyroid gland (and

aorta)	body)	parathyroid)
P Stomach	P Lacrimal gland (bilateral)	Small intestine, duodenum
P Large intestine, cecum	P Testis	P Large intestine, colon
P Large intestine, rectum	P Thymus/Thymic area	P Larynx (1 level)
P Liver (sample of two lobes)	P Tongue	P Lung (sample of two lobes)
P Trachea	P Lymph node - mandibular	P Urinary bladder
P Lymph node - mesenteric	P Mammary gland (inguinal)	P Vagina
P Ureter bilateral	P Nasal cavities (1 level)	P Ovaries
P Oviducts		

Tissues were prepared for histopathological examination by embedding in paraffin wax, sectioning and staining with hematoxylin and eosin and examined from all animals. The optic nerves, mammary glands (males) and thymic lymphoid tissue were examined histopathologically only if present in routine sections of eyes, skin or thymus, respectively. Oviducts, salivary glands and ureters were submitted to a unilateral histopathological examination (although collected bilaterally).

All tissue sections (except blood and bone marrow smears) were assessed with all observations being recorded in the raw data. Incidences of diagnosed lesions were tabulated.

All suspected tumors were diagnosed, and the incidences of benign and malignant tumors of different cell types in the various treatment groups were tabulated. Histopathological evaluation was performed by a board-certified veterinary pathologist.

No AHU377-related effects were seen in the incidence of any non-neoplastic lesion in male and female rats in this study.

The most frequent neoplastic causes of early death/euthanasia recorded in control animals and animals given AHU377 were pituitary adenoma/carcinoma, mammary gland tumor (fibroadenoma/adenocarcinoma) and sarcomas of various origin whereas the most frequent non-neoplastic cause of death/early euthanasia was obstructive uropathy.

Peer Review

The pathology report and histopathological examinations underwent peer review by a Sponsor-designated pathologist. The procedure was documented accordingly in the raw data.

Toxicokinetics

Mean concentrations of AHU377 in rat plasma (ng/mL)

	Group 2 (50 mg/kg/day)		Group 3 (150 mg/kg/day)		Group 4 (400 mg/kg/day)	
	Male	Female	Male	Female	Male	Female
	n = 2	n = 2	n = 2	n = 2	n = 2	n = 2
Time (h)	AHU377 concentration (ng/mL)					
	Week 4					
0.5	105	63.4	234	231	912	214
1	70.1	66.2	272	259	676	687
	Week 26					
0.5	124	88.9	411	598	1510	2110
1	87.4	91.5	132	457	531	1000

LLOQ: 10.0 ng/mL.

Mean concentrations of LBQ657 in rat plasma (ng/mL)

	Group 2 (50 mg/kg/day)		Group 3 (150 mg/kg/day)		Group 4 (400 mg/kg/day)	
	Male	Female	Male	Female	Male	Female
	n = 2	n = 2	n = 2	n = 2	n = 2	n = 2
Time (h)	LBQ657 concentration (ng/mL)					
	Week 4					
0.5	5230	3610	11500	9730	29100	12800
1	5380	5020	9540	14000	29800	23800
	Week 26					
0.5	11900	5840	15600	28700	25300	58100
1	5690	13200	8500	30500	27900	54200

LLOQ: 10.0 ng/mL.

All AHU377-treated animals displayed systemic exposure to the test item and to the active metabolite LBQ657 on study days 24 and 178 following multiple daily oral doses of AHU377. In general, mean plasma concentrations of AHU377 and LBQ657 increased approximately proportionally with increasing dose for the 0.5 h and 1 h samples within the dose range tested for both males and females.

The average plasma concentration of AHU377 was 2.1% of the LBQ657 plasma concentration (range 0.7% to 6.0%) at 0.5 h and 1 h post dose, suggesting rapid conversion of AHU377 to the metabolite LBQ657.

There was no consistent difference in exposure to AHU377 and LBQ657 between males and females. With few exceptions, the plasma concentrations for AHU377 and LBQ657 were generally higher on day 178 compared to day 24 for both males and females.

Dosing Solution Analysis

Samples of the formulations prepared for weeks 1, 9, 24, 39, 54, 69, 84 and 104 were analyzed. Concentrations were 85% to 103% of their nominal concentrations. Nine control samples were analyzed and no AHU377 was detected.

Summary of body weight

Males

Group 1 - Vehicle control
Group 2 - AHU377 50 mg/kg/day
Group 3 - AHU377 150 mg/kg/day
Group 4 - AHU377 400 mg/kg/day

Group	Summary Information	Week -2	-1	1	2	3	4	5	6	7	8	9	10
1	Mean	197.5	240.1	276.3	303.8	326.9	347.6	361.5	374.8	386.8	399.2	408.5	416.0
	SD	15.3	16.8	19.3	21.7	24.2	25.7	26.8	28.3	29.3	30.3	31.1	31.8
	N	50	50	50	50	50	50	50	50	50	50	50	50
2	Mean	196.9	238.3	272.8	298.6	321.0	339.6	354.0	365.2	377.3	387.7	396.3	403.6
	SD	14.7	16.1	17.2	19.3	21.6	23.1	24.1	25.7	26.0	28.0	28.8	29.1
	N	50	50	50	50	50	50	50	50	50	50	50	50
3	Mean	196.0	236.6	271.4	298.7	320.8	338.1	351.9	362.2	374.7	383.7	391.9	399.0
	SD	14.2	14.0	15.3	18.4	20.9	23.1	24.1	26.1	28.2	28.3	29.1	30.9
	N	50	50	50	50	50	50	50	50	50	50	50	50
4	Mean	197.6	238.3	273.4	301.3	323.3	339.9	354.9	366.7	376.9	386.6	396.6	405.5
	SD	15.0	16.3	18.0	19.6	22.8	26.7	29.3	31.5	33.2	33.9	34.7	35.4
	N	50	50	50	50	50	50	50	50	50	50	50	50

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

Males

Group 1 - Vehicle control
Group 2 - AHU377 50 mg/kg/day
Group 3 - AHU377 150 mg/kg/day
Group 4 - AHU377 400 mg/kg/day

Group	Summary Information	Week 11	12	13	14	18	22	26	30	34	38	42
1	Mean	423.5	431.3	437.8	442.4	463.2	484.7	502.6	516.8	530.6	545.9	560.5
	SD	32.7	33.0	33.4	33.9	36.4	38.5	41.3	44.4	45.6	47.5	49.4
	N	50	50	50	50	50	50	50	50	50	50	50
2	Mean	410.8	419.7	426.9	434.2	457.3	475.2	492.1	503.9	518.8	533.3	550.2
	SD	29.7	30.2	30.3	31.4	34.2	36.9	38.7	40.2	43.1	46.4	49.6
	N	50	50	50	50	50	50	50	50	50	50	50
3	Mean	406.9	416.0	422.9	428.5	450.3	468.5	487.8	500.1	516.9	534.4	552.6
	SD	31.7	33.3	34.1	35.2	37.9	41.6	46.7	51.1	54.4	58.2	62.1
	N	50	50	50	50	50	50	50	50	49	49	49
4	Mean	415.0	422.3	425.8	430.6	450.4	469.7	485.3	502.0	515.1	534.8	551.6
	SD	36.1	38.0	38.7	40.4	42.8	48.0	50.0	54.6	60.1	64.5	68.3
	N	50	50	50	50	50	50	50	50	50	50	49

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

Males

Group 1 - Vehicle control
Group 2 - AHU377 50 mg/kg/day
Group 3 - AHU377 150 mg/kg/day
Group 4 - AHU377 400 mg/kg/day

Group	Summary Information	Week 46	50	54	58	62	66	70	74	78	80	82
1	Mean	574.4	589.5	600.7	617.3	628.0	643.4	654.1	661.3	676.1	681.4	684.1
	SD	53.2	54.8	56.4	59.3	61.6	64.3	64.5	63.3	66.8	68.4	68.2
	N	50	50	50	50	49	49	48	48	48	48	47
2	Mean	565.9	580.7	590.5	609.7	619.8	634.6	637.1	641.3	652.3	659.1	666.6
	SD	51.8	53.7	58.5	61.3	62.7	65.6	67.8	72.0	75.2	76.7	79.5
	N	50	50	50	50	50	50	49	48	48	47	47
3	Mean	571.1	587.7	596.3	610.6	617.4	635.0	639.5	647.0	654.2	656.7	662.2
	SD	67.3	71.2	74.3	76.0	82.1	82.5	83.9	80.6	84.9	87.4	85.4
	N	49	49	49	49	49	48	48	46	45	45	44
4	Mean	566.0	577.3	585.0	603.3	617.3	631.7	638.6	647.4	662.9	665.3	661.2
	SD	75.0	78.3	81.8	83.4	86.6	88.8	94.7	98.3	104.7	101.1	102.3
	N	49	49	48	47	47	46	46	45	44	43	43

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

Males

Group 1 - Vehicle control
Group 2 - AHU377 50 mg/kg/day
Group 3 - AHU377 150 mg/kg/day
Group 4 - AHU377 400 mg/kg/day

Group	Summary Information	Week 84	86	88	90	92	94	96	98	100	102	104
1	Mean	689.2	690.6	693.0	690.0	694.9	699.3	700.0	698.4	700.5	699.3	703.0
	SD	68.7	69.6	70.6	72.7	75.1	70.7	74.8	77.9	82.1	91.9	79.9
	N	47	46	46	46	45	44	44	43	42	39	37
2	Mean	667.4	666.6	666.4	675.0	680.7	678.9	687.6	686.4	689.5	689.9	686.2
	SD	80.7	84.1	90.3	86.8	85.8	91.1	85.5	88.5	87.7	85.5	85.3
	N	47	46	44	41	40	40	38	38	38	38	38
3	Mean	669.1	669.5	664.0	662.7	664.2	671.8	672.7	675.4	679.0	678.4	671.1
	SD	84.3	89.8	94.9	99.5	100.8	91.8	92.9	94.6	96.1	99.8	98.2
	N	42	42	40	38	38	37	37	37	35	35	35
4	Mean	676.0	674.7	666.9	662.9	675.5	677.5	681.0	677.6	672.4	687.4	681.4
	SD	97.4	93.9	97.9	101.9	99.0	100.8	103.9	107.3	117.1	104.9	94.4
	N	39	38	38	38	35	34	34	34	34	32	31

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

Females

Group 1 - Vehicle control
Group 2 - AHU377 50 mg/kg/day
Group 3 - AHU377 150 mg/kg/day
Group 4 - AHU377 400 mg/kg/day

Group	Summary Information	Week -2	-1	1	2	3	4	5	6	7	8	9	10
1	Mean	151.9	174.2	191.3	203.4	214.4	222.6	228.6	234.1	239.4	242.7	244.9	247.8
	SD	10.5	11.0	12.4	12.3	12.8	12.7	14.0	13.7	14.1	14.3	14.2	15.0
	N	50	50	50	50	50	50	50	50	50	50	50	50
2	Mean	152.0	172.1	188.0	200.4	209.6	217.7	226.2	232.3	235.7	240.1	241.1	246.0
	SD	10.3	12.5	13.7	14.7	14.8	16.0	17.1	17.3	16.9	18.2	18.4	18.7
	N	50	50	50	50	50	50	50	50	50	50	50	50
3	Mean	152.5	174.3	191.5	202.7	212.6	220.3	229.0	234.8	238.4	243.5	245.4	249.5
	SD	10.3	12.2	12.5	13.5	15.0	16.1	15.3	17.0	16.4	17.4	16.7	17.8
	N	50	50	50	50	50	50	50	50	50	50	50	50
4	Mean	153.2	173.5	190.6	203.1	212.9	220.9	229.1	233.7	237.1	241.1	242.8	246.9
	SD	10.8	12.2	12.7	13.9	13.7	15.3	17.6	17.6	16.9	16.5	17.3	17.4
	N	50	50	50	50	50	50	50	50	50	50	50	50

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

Females

Group 1 - Vehicle control
Group 2 - AHU377 50 mg/kg/day
Group 3 - AHU377 150 mg/kg/day
Group 4 - AHU377 400 mg/kg/day

Group	Summary Information	Week 11	12	13	14	18	22	26	30	34	38	42
1	Mean	249.4	253.2	255.1	257.2	261.4	270.1	276.5	279.5	283.0	295.6	300.8
	SD	15.1	15.2	16.0	16.5	16.0	17.5	18.1	19.0	20.0	22.6	24.9
	N	50	50	50	50	50	50	50	50	50	50	50
2	Mean	247.2	250.5	252.6	255.8	261.5	266.1	272.2	278.8	287.2	295.8	298.9
	SD	18.0	18.7	19.4	19.7	19.3	20.7	23.3	23.9	29.0	29.3	30.9
	N	50	50	50	50	50	49	49	49	49	49	49
3	Mean	250.9	254.5	257.4	260.6	267.5	272.1	279.0	283.9	292.5	301.2	310.3
	SD	17.5	18.1	19.3	19.7	21.0	23.7	24.6	28.7	33.5	35.5	40.8
	N	50	50	50	50	50	50	49	49	49	49	49
4	Mean	249.8	252.9	255.3	260.0	266.0	270.7	276.3	284.0	290.3	298.5	308.6
	SD	18.2	18.1	17.4	18.9	21.3	20.9	22.4	23.9	26.1	29.6	34.3
	N	50	50	50	50	50	50	50	50	50	50	50

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

Females

Group 1 - Vehicle control
Group 2 - AHU377 50 mg/kg/day
Group 3 - AHU377 150 mg/kg/day
Group 4 - AHU377 400 mg/kg/day

	Summary	Week										
Group	Information	46	50	54	58	62	66	70	74	78	80	82
1	Mean	308.8	316.0	325.4	336.0	344.9	355.7	361.8	368.6	381.3	382.6	382.5
	SD	27.2	30.1	31.2	36.8	37.6	41.3	41.3	41.9	47.3	51.8	49.1
	N	50	50	50	50	50	50	50	48	47	46	44
2	Mean	308.1	317.7	329.2	342.1	352.5	362.7	368.3	375.0	386.1	391.5	394.5
	SD	34.9	38.7	43.0	43.7	48.9	49.7	50.6	52.3	54.7	56.4	57.6
	N	49	49	48	48	47	46	46	46	45	44	44
3	Mean	317.8	324.7	333.0	343.0	354.0	360.6	361.0	367.7	375.0	382.0	383.3
	SD	45.9	47.5	47.4	52.0	53.4	56.9	60.0	62.8	64.0	64.7	67.7
	N	49	49	49	48	48	47	46	45	44	44	44
4	Mean	315.1	319.8	328.1	338.4	346.5	354.8	365.6	371.4	377.7	381.2	380.8
	SD	35.5	38.8	40.6	43.7	45.2	52.4	53.9	50.9	52.2	52.9	53.6
	N	50	50	50	50	50	50	48	48	48	48	48

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

Females

Group 1 - Vehicle control
Group 2 - AHU377 50 mg/kg/day
Group 3 - AHU377 150 mg/kg/day
Group 4 - AHU377 400 mg/kg/day

	Summary	Week										
Group	Information	84	86	88	90	92	94	96	98	100	102	104
1	Mean	387.7	389.7	391.8	393.7	398.2	400.8	401.3	400.0	404.1	406.7	407.1
	SD	46.5	48.1	49.2	48.7	47.2	47.2	47.1	52.0	49.4	50.8	48.2
	N	41	40	40	40	38	38	36	35	32	31	30
2	Mean	397.3	398.5	400.1	403.5	404.6	411.2	412.7	415.7	422.9	424.7	424.3
	SD	57.3	60.0	60.1	60.3	61.7	60.6	62.0	67.7	59.3	62.1	58.3
	N	44	44	44	40	38	37	36	33	32	31	30
3	Mean	386.3	388.3	385.1	387.8	387.7	388.8	394.7	398.3	402.2	405.1	408.0
	SD	67.6	69.4	64.0	63.7	63.8	70.7	70.9	71.7	70.7	70.6	69.2
	N	44	44	41	41	41	40	37	35	34	34	34
4	Mean	386.0	387.7	392.7	393.6	396.4	396.2	396.2	395.9	395.3	390.7	388.5
	SD	54.1	54.3	54.5	51.1	53.4	53.7	55.4	59.6	67.5	52.8	50.3
	N	47	47	45	44	41	41	41	40	38	34	33

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

Table 2.6 Incidence of animals with neoplastic lesions by organ/group/sex
All Animals

		MALE			
DOSE GROUP		**1**	**2**	**3**	**4**
NUMBER OF ANIMALS EXAMINED		**50**	**50**	**50**	**50**
ABDOMEN	NO.EXAM.:	1	-	-	-
- Malignant schwannoma		1	-	-	-
ADRENAL	NO.EXAM.:	50	50	50	50
- Adenoma: cortical		4	3	2	-
- Carcinoma: cortical		-	-	2	-
- Benign pheochromocytoma		-	2	1	3
- Ganglioneuroma		-	-	-	1
AORTA	NO.EXAM.:	50	50	50	49
BILE DUCT	NO.EXAM.:	3	4	5	6
BONE MARROW	NO.EXAM.:	50	50	50	49
BONE MISCELLANEOUS	NO.EXAM.:	-	-	-	1
- Osteosarcoma		-	-	-	1
BONE-STERNUM	NO.EXAM.:	50	50	50	49
- Sarcoma: metastasis		-	1	-	-
BRAIN	NO.EXAM.:	50	50	50	49
- Malignant astrocytoma		-	1	-	-
- Malignant mixed glioma		-	-	-	1
- Benign granular cell tumor		2	-	-	-
- Malignant granular cell tumor		1	-	-	-
CAVITY PELVIC	NO.EXAM.:	-	-	-	1
CECUM	NO.EXAM.:	50	50	50	49
COLON	NO.EXAM.:	50	50	50	49
DUODENUM	NO.EXAM.:	50	50	50	49
EPIDIDYMIS	NO.EXAM.:	50	50	50	49
- Mesothelioma(B)		1	-	-	-
ESOPHAGUS	NO.EXAM.:	50	50	50	49
- Sarcoma: metastasis		-	1	-	-
EYE	NO.EXAM.:	49	50	50	49
FAT	NO.EXAM.:	6	7	4	5
- Lipoma		2	-	-	1
HARDERIAN GLAND	NO.EXAM.:	49	50	50	49
HEART	NO.EXAM.:	50	50	50	50
- Malignant schwannoma: endocardial		-	-	-	1
- Sarcoma: metastasis		-	1	-	1
HEMOLYM. TISSUE	NO.EXAM.:	50	50	50	50
- Malignant lymphoma		1	2	2	2
ILEUM	NO.EXAM.:	50	50	50	49

MALE

DOSE GROUP NUMBER OF ANIMALS EXAMINED		1 50	2 50	3 50	4 50
JEJUNUM	NO.EXAM.:	50	50	50	49
- Leiomyosarcoma		1	-	-	-
- Benign schwannoma		-	-	-	1
JOINT	NO.EXAM.:	-	1	1	-
JOINT FEMOROTIB.	NO.EXAM.:	50	50	50	49
KIDNEY	NO.EXAM.:	50	50	50	50
- Carcinoma: metastasis		1	-	-	-
- Papilloma: transitional cell		-	-	1	-
- Lipoma		-	-	1	-
LACRIMAL GLAND	NO.EXAM.:	50	50	50	50
LARYNX	NO.EXAM.:	50	50	50	50
LIVER	NO.EXAM.:	50	50	50	50
- Adenoma: hepatocellular		3	2	4	6
LUNG	NO.EXAM.:	50	50	50	50
- Carcinoma: alveolar/bronchiolar		1	-	-	-
- Sarcoma: metastasis		-	1	-	1
- Carcinoma: metastasis		-	-	-	1
- Adenoma: alveolar/bronchiolar		2	2	-	1
LYMPH NODE	NO.EXAM.:	4	6	7	12
- Hemangioma		-	-	1	1
L. NODE MANDIBULAR	NO.EXAM.:	50	50	50	49
L.NODE MESENTERIC	NO.EXAM.:	50	49	50	50
- Hemangioma		1	2	2	2
- Hemangiosarcoma		1	2	4	1
MAMMARY GLAND	NO.EXAM.:	43	44	43	42
MUSCLE SKELETAL	NO.EXAM.:	49	50	49	49
MUSC.SKEL.MISC.	NO.EXAM.:	-	-	1	-
- Hemangiosarcoma		-	-	1	-
NERVE OPTIC	NO.EXAM.:	50	50	50	48
NERVE SCIATIC	NO.EXAM.:	50	50	50	49
PANCREAS	NO.EXAM.:	50	50	50	49
- Adenoma: islet cell		-	1	2	1
- Carcinoma: islet cell		1	1	1	-
- Adenoma: acinar cell		-	-	-	2
PARATHYROID GLAND	NO.EXAM.:	47	49	50	49
- Adenoma		-	1	1	-

MALE

DOSE GROUP NUMBER OF ANIMALS EXAMINED		1 50	2 50	3 50	4 50
PITUITARY	NO.EXAM.:	49	49	50	49
- Adenoma: pars distalis		13	12	15	21
- Adenoma: pars intermedia		1	-	2	1
PREPUTIAL GLAND	NO.EXAM.:	49	50	50	48
PROSTATE	NO.EXAM.:	50	50	50	50
RECTUM	NO.EXAM.:	50	50	50	49
SALIV.GL. MANDIBULAR	NO.EXAM.:	50	50	50	49
- Adenoma		-	1	-	-
SAL. GL. PAROTID	NO.EXAM.:	50	50	50	49
SAL.GL. SUBL.	NO.EXAM.:	49	50	50	49
SEMINAL VESICLE	NO.EXAM.:	50	50	50	49
SKIN	NO.EXAM.:	50	50	50	50
SKIN MISCELLANEOUS	NO.EXAM.:	18	19	16	13
- Fibroma		-	1	-	1
- Adenoma: basal cell		1	2	-	-
- Carcinoma: basal cell		-	1	-	-
- Adenoma: sebaceous		-	2	-	-
- Papilloma: squamous cell		-	1	1	2
- Carcinoma: squamous cell		2	-	1	1
SKIN MISCELLANEOUS	CONT'D.	18	19	16	13
- Keratoacanthoma		10	2	8	5
- Hemangioma		-	-	1	-
SPINAL CORD CERVICAL	NO.EXAM.:	50	50	49	49
- Benign astrocytoma		-	-	-	1
SPINAL CORD LUMBAR	NO.EXAM.:	48	48	49	46
SPINAL CORD THORACIC	NO.EXAM.:	50	50	50	49
SPLEEN	NO.EXAM.:	50	50	50	49
STOMACH	NO.EXAM.:	50	50	50	50
- Papilloma: squamous cell		-	-	-	1
- Carcinoma: squamous cell		-	-	-	1
SUBCU. TISSUE	NO.EXAM.:	10	8	9	5
- Fibroma		4	5	6	3
- Fibrosarcoma		1	-	-	-
- Malignant schwannoma		2	1	-	-
- Lipoma		2	2	2	1
- Hemangiosarcoma		1	2	1	-

MALE

DOSE GROUP		1	2	3	4
NUMBER OF ANIMALS EXAMINED		50	50	50	50
TAIL	NO.EXAM.:	-	-	1	-
TESTIS	NO.EXAM.:	50	50	50	49
- Adenoma: interstitial cell		1	3	5	-
THORAX	NO.EXAM.:	1	2	-	1
- Mesothelioma(M)		-	1	-	-
- Carcinoma: metastasis		1	-	-	-
THYMUS	NO.EXAM.:	46	48	47	46
- Sarcoma: metastasis		-	1	-	-
- Carcinoma: metastasis		1	-	-	-
- Benign thymoma		2	3	1	1
- Malignant thymoma		-	1	-	-
THYROID	NO.EXAM.:	50	50	50	49
- Adenoma: C-cell		7	9	8	7
- Carcinoma: C-cell		1	1	1	1
- Adenoma: follicular cell		5	-	1	3
- Carcinoma: follicular cell		-	1	-	1
TONGUE	NO.EXAM.:	50	50	50	49
- Hemangiosarcoma		-	-	1	-
- Sarcoma: metastasis		-	1	-	-
TRACHEA	NO.EXAM.:	50	50	50	49
- Sarcoma: metastasis		-	1	-	-
URETER	NO.EXAM.:	49	45	45	44
URINARY BLADDER	NO.EXAM.:	50	50	50	49
- Carcinoma: transitional cell		-	-	-	1

Table 2.6 Incidence of animals with neoplastic lesions by organ/group/sex
All Animals

		FEMALE			
DOSE GROUP		**1**	**2**	**3**	**4**
NUMBER OF ANIMALS EXAMINED		**50**	**50**	**50**	**50**
ABDOMEN	NO.EXAM.:	-	-	1	1
- Carcinoma: metastasis		-	-	1	1
ADRENAL	NO.EXAM.:	50	50	50	50
- Adenoma: cortical		4	2	2	3
- Carcinoma: cortical		1	-	1	-
- Benign pheochromocytoma		-	-	1	-
- Ganglioneuroma		-	-	-	1
AORTA	NO.EXAM.:	50	50	50	50
BILE DUCT	NO.EXAM.:	5	4	4	1
BONE MARROW	NO.EXAM.:	50	50	50	50
BONE MISCELLANEOUS	NO.EXAM.:	-	2	-	-
- Osteosarcoma		-	1	-	-
BONE-STERNUM	NO.EXAM.:	50	50	50	50
BRAIN	NO.EXAM.:	50	50	50	50
- Malignant meningioma		-	1	-	-
- Carcinoma: metastasis		1	-	-	-
- Malignant oligodendroglioma		1	-	-	-
- Benign granular cell tumor		1	-	-	-
- Benign pineal gland tumor		-	-	1	-
CAVITY ORAL	NO.EXAM.:	-	-	1	-
CECUM	NO.EXAM.:	50	50	50	50
CLITORAL GLAND	NO.EXAM.:	50	50	49	50
COLON	NO.EXAM.:	50	50	50	50
DUODENUM	NO.EXAM.:	50	50	50	50
- Carcinoma: metastasis		-	1	-	-
ESOPHAGUS	NO.EXAM.:	50	50	50	50
EYE	NO.EXAM.:	49	50	50	50
- Sarcoma: metastasis		-	1	-	-
FAT	NO.EXAM.:	10	7	6	4
- Lipoma		1	-	1	-
- Liposarcoma		-	1	-	-
- Carcinoma: metastasis		-	1	-	-
- Sarcoma: metastasis		-	-	-	1
HARDERIAN GLAND	NO.EXAM.:	49	50	50	50
HEART	NO.EXAM.:	50	50	50	50
HEMOLYM. TISSUE	NO.EXAM.:	50	50	50	50
- Malignant lymphoma		1	-	1	-
- Histiocytic sarcoma		1	-	1	-

FEMALE

DOSE GROUP NUMBER OF ANIMALS EXAMINED		1 50	2 50	3 50	4 50
HEMOLYM. TISSUE	CONT'D.	50	50	50	50
- Leukemia: granulocytic		-	-	1	-
ILEUM	NO.EXAM.:	50	50	50	50
JEJUNUM	NO.EXAM.:	50	50	50	50
JOINT	NO.EXAM.:	1	-	2	1
JOINT FEMOROTIB.	NO.EXAM.:	50	50	50	50
KIDNEY	NO.EXAM.:	50	50	50	50
- Carcinoma: metastasis		-	1	-	-
- Carcinoma: tubular cell		-	1	-	1
- Lipoma		-	-	-	1
LACRIMAL GLAND	NO.EXAM.:	49	50	50	50
LARYNX	NO.EXAM.:	50	50	50	50
LIVER	NO.EXAM.:	50	50	50	50
- Adenoma: hepatocellular		3	5	3	5
- Carcinoma: hepatocellular		-	-	-	1
- Hemangiosarcoma		-	-	-	1
- Carcinoma: metastasis		-	1	1	1
LUNG	NO.EXAM.:	50	50	50	50
- Sarcoma: metastasis		-	1	-	1
- Carcinoma: metastasis		1	1	-	1
LYMPH NODE	NO.EXAM.:	8	11	9	5
- Sarcoma: metastasis		-	-	1	-
- Carcinoma: metastasis		1	-	-	1
L. NODE MANDIBULAR	NO.EXAM.:	49	50	50	50
L.NODE MESENTERIC	NO.EXAM.:	50	50	50	50
- Hemangioma		-	-	-	1
- Hemangiosarcoma		-	1	-	-
MAMMARY GLAND	NO.EXAM.:	50	50	50	50
- Adenoma		1	-	1	-
- Adenocarcinoma		6	2	6	2
MAMMARY GLAND	CONT'D.	50	50	50	50
- Fibroadenoma		13	9	15	19
MUSCLE SKELETAL	NO.EXAM.:	50	50	50	50
MUSC.SKEL.MISC.	NO.EXAM.:	-	1	1	-
NERVE OPTIC	NO.EXAM.:	50	50	50	50
NERVE SCIATIC	NO.EXAM.:	50	50	50	50
OVARY	NO.EXAM.:	50	50	50	50
- Adenoma: tubulostromal		1	2	2	1
- Adenoma: sertoliform tubular		1	-	-	-
- Carcinoma: yolk sac		1	-	-	-

		FEMALE			
DOSE GROUP		**1**	**2**	**3**	**4**
NUMBER OF ANIMALS EXAMINED		**50**	**50**	**50**	**50**
OVIDUCT	NO.EXAM.:	49	50	47	48
- Carcinoma: metastasis		-	1	-	-
PANCREAS	NO.EXAM.:	50	50	50	50
- Adenoma: islet cell		-	1	-	1
- Carcinoma: metastasis		-	1	1	1
PARATHYROID GLAND	NO.EXAM.:	49	50	49	49
- Adenoma		-	-	-	1
PERICARDIUM	NO.EXAM.:	-	-	-	1
PITUITARY	NO.EXAM.:	50	50	50	49
- Adenoma: pars distalis		33	32	32	30
- Carcinoma: pars distalis		1	-	-	-
- Adenoma: pars intermedia		-	-	1	1
- Carcinoma: pars intermedia		1	-	-	-
RECTUM	NO.EXAM.:	50	49	50	50
- Fibroma		-	1	-	-
SALIV.GL. MANDIBULAR	NO.EXAM.:	49	50	50	50
SAL. GL. PAROTID	NO.EXAM.:	49	50	50	49
SAL.GL. SUBL.	NO.EXAM.:	49	50	49	50
SKIN	NO.EXAM.:	50	50	50	50
- Adenoma: basal cell		-	-	1	-
SKIN MISCELLANEOUS	NO.EXAM.:	5	4	6	2
- Fibroma		-	1	1	-
- Benign melanoma		-	1	-	-
- Carcinoma: squamous cell		1	-	1	-
SPINAL CORD CERVICAL	NO.EXAM.:	50	50	48	50
SPINAL CORD LUMBAR	NO.EXAM.:	49	47	50	50
SPINAL CORD THORACIC	NO.EXAM.:	50	50	50	50
SPLEEN	NO.EXAM.:	50	50	50	50
- Carcinoma: metastasis		-	1	-	-
STOMACH	NO.EXAM.:	50	50	50	50
- Leiomyosarcoma		-	-	1	-
- Carcinoma: metastasis		-	1	-	1
SUBCU. TISSUE	NO.EXAM.:	3	-	3	2
- Fibroma		-	-	2	1
- Lipoma		-	-	-	1
- Osteosarcoma		1	-	-	-

FEMALE

DOSE GROUP NUMBER OF ANIMALS EXAMINED		1 50	2 50	3 50	4 50
THORAX	NO.EXAM.:	-	-	-	1
- Carcinoma: metastasis		-	-	-	1
THYMUS	NO.EXAM.:	47	49	49	49
- Sarcoma: metastasis		-	-	1	-
- Carcinoma: metastasis		-	-	-	1
- Benign thymoma		3	6	5	6
- Malignant thymoma		-	1	-	-
- Hemangiosarcoma		-	-	-	1
THYROID	NO.EXAM.:	50	50	50	50
- Adenoma: C-cell		6	5	4	7
- Carcinoma: C-cell		1	-	2	-
- Adenoma: follicular cell		1	-	1	4
TONGUE	NO.EXAM.:	50	50	50	50
TRACHEA	NO.EXAM.:	50	50	50	50
URETER	NO.EXAM.:	45	48	46	44
URINARY BLADDER	NO.EXAM.:	50	49	50	49
UTERUS	NO.EXAM.:	50	49	50	49
- Polyp: endometrial stromal		1	9	6	5
- Sarcoma: endometrial stromal		-	2	-	-
- Adenoma: endometrial		-	-	1	1
- Adenocarcinoma: endometrial		-	2	2	1
- Malignant schwannoma		1	-	2	-
- Hemangiosarcoma		-	-	1	-
VAGINA	NO.EXAM.:	50	49	50	49

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Rats were used to evaluate LCZ696 (Study 0970613) or AHU377 (Study 1370483) for effects on fertility and early embryonic development.

Study title: LCZ696: An oral (gavage) fertility and early embryonic development study in rats

Study no.:	0970613
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	The study was conducted at the Novartis East Hanover Facility. Statistical analysis of fertility and sperm analysis data was conducted by (b) (4)
Date of study initiation:	12/21/2009 – males 1/4/2010 - females
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0824074, 95.1%

Key Study Findings

Male reproductive parameters were unaffected by treatment at all doses. The only male parameters found to have a statistically significant effects were terminal body weights and relative epididymal weight (expressed as percentage of the terminal body weight), which were affected only at the high dose of 150 mg/kg/day.

Female reproductive parameters were unaffected at all doses.

Methods

Doses:	10, 50, 150 mg/kg/day
Frequency of dosing:	daily
Dose volume:	5 mL/kg
Route of administration:	oral
Formulation/Vehicle:	0.5% (w/v) Sodium carboxymethylcellulose (CMC)
Species/Strain:	Rat/Wistar Hannover
Number/Sex/Group:	25
Satellite groups:	na
Study design:	**Duration of dosing:** Males: Once daily for at least 28 days prior to mating, during the 2-week mating period and until terminal necropsy. Females: Once daily for the two week premating period during mating and through gestation day 6. **Terminal Sacrifice:** Males: Following the completion of mating and at the direction of the Study Director. Females: On gestation day 13 for sperm positive females.
Deviation from study protocol:	No significant deviations

Observations and Results

In the GLP embryo-fetal development study in rats at doses of 0, 30, 100 and 200 mg/kg from gestation day 6 – 17 [Study 0670390], there was no evidence of hydrocephaly. The maternal NOAEL was 200 mg/kg/day, with a fetal NOAEL of 30 mg/kg/day based on a slight increase in post-implantation loss at higher doses. Based upon these results, doses of 10, 50 and 150 mg/kg/day were selected for this study.

Mortality

Twice daily on weekdays (am and pm) and once daily on weekends, holidays and site closings due to weather. There were no mortalities associated with the test article.

Clinical Signs

Males: Twice daily, predose and within approximately 3 hours postdose.

Females: Twice daily during the dosing phase, predose and within approximately 3 hours postdose. Once daily during the postdosing phase.

Clinical observations were unremarkable and consisted of individual animals with hair loss across all doses, and material around the eyes or nose.

Body Weight

Males: Twice weekly until the initiation of terminal necropsies and on the day of necropsy

Females: Twice weekly on premating treatment days 1, 4, 8, 11, 15, etc., until mated or sacrificed Gestation days 0, 3, 6, 9 and 13

Male mean body weights were significantly decreased, as compared to controls, at a dose of 150 mg/kg/day beginning on day 11 (4.3% decrease), due to decreased body weight gains from days 1 to 15 at this dose. The body weight gains for these animals subsequently returned to normal, but the effect on group mean body weight persisted throughout the study (5.1% decrease at day 51). All other doses were similar to controls.
Female body weight parameters were unaffected by treatment prior to mating.

Feed Consumption

Males: Food consumption was calculated based on feeder weights collected weekly on treatment days 1, 8, 15, 22 and 29

Females: Food consumption was calculated based on feeder weights collected weekly on premating treatment days 1, 8, and 15. Gestation days 0, 3, 6, 9 and 13

Toxicokinetics

Not determined.

Dosing Solution Analysis

Samples of the formulations prepared for weeks 1 and 5 were analyzed, and concentrations were 99% to 103% of targets. Two control samples were analyzed, and no LCZ696 was detected in the controls.

Necropsy

Males
Right and left testis and epididymis were weighed. The right testes and epididymis were placed in Bouin's solution. After 1-3 days, the tissues were rinsed with cold tap water and transferred to 10% neutral buffered formalin for possible histopathological examination by Pathology at the discretion of the Study Director.

At the time of necropsy, the left testis and epididymis were excised, separated, trimmed, weighed, decapsulated and reweighed. The left testis was frozen for subsequent testicular sperm counts. The left epididymis was discarded. Homogenization resistant testicular sperm head counts were determined manually. Following sacrifice, a sperm sample was collected from the vas deferens and videotaped. The percent motile sperm

was determined manually from the videotaped images. The right testes and epididymis were discarded as no further analysis was necessary.

Females
On gestation day 13 the number of corpora lutea from the left and right ovaries were recorded for each pregnant female.

Uterine site description
At necropsy on gestation day 13, each uterine implant site was identified as either a live fetus or early resorption.

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

The estrous cycle, mating behavior, fecundity and fertility of the females were unaffected at all doses.

Male reproductive parameters were unaffected by treatment at all doses.

NOVARTIS STUDY NO. 0970613

SUMMARY STATISTICS FOR FERTILITY DATA / TEST: (FEMALES COHOUSED WITH MALES).

		Fertility				
Group	Summary	Precoital Interval (day)	Summary	Estrous Cycle	Mating	Fecundity	Fertility
1	Mean	2.5	Rate (%)	92.0	100.0	84.0	84.0
	S.D.	2.3	N Pos.	23	25	21	21
	N	24	N Neg.	2	0	4	4
2	Mean	3.0	Rate (%)	92.0	100.0	96.0	96.0
	S.D.	2.4	N Pos.	23	25	24	24
	N	25	N Neg.	2	0	1	1
3	Mean	3.4	Rate (%)	80.0	100.0	96.0	96.0
	S.D.	3.6	N Pos.	20	25	24	24
	N	25	N Neg.	5	0	1	1
4	Mean	2.3	Rate (%)	92.0	92.0	82.6	76.0
	S.D.	2.5	N Pos.	23	23	19	19
	N	23	N Neg.	2	2	4	6
Levene test (P): 0.4098							
Overall ANOVA F-test (P): 0.4744							
2x4 Fisher's Exact test (P):				0.5333	0.2424	0.2515	0.0944

* Indicate a significant test (P <= 0.05)

No overall ANOVA F-test was found significant (continuous data). Given that a protected post-hoc testing approach was planned for all analyses, no pairwise comparisons of interest (each treated group against Group 1) were conducted.

No overall 2x4 Fisher's exact test was found significant (binary data). Given that a protected post-hoc testing approach was planned for all analyses, no pairwise comparisons of interest (each treated group against Group 1) were conducted.

NOVARTIS STUDY NO. 0970613

SUMMARY STATISTICS FOR SPERM DATA (TREATED MALES).

Group	Summary	Terminal Body Weight (g)	Total Testis Weight (g)	Total Epididymal Weight (g)	Total Testis Weight (expressed as percentage of the terminal body weight)	Total Epididymal Weight (expressed as percentage of the terminal body weight)	Average Sperm Count	Sperm Count X 10E06 per gram of Testis	Sperm % Motility
1	Mean	481.2	4.017	1.640	0.837	0.342	90.8	123.9	79.0
	S.D.	38.5	0.411	0.107	0.089	0.023	23.4	32.4	14.6
	N	25	25	25	25	25	25	25	25
2	Mean	478.2	4.016	1.704	0.842	0.357	84.8	114.0	78.5
	S.D.	35.4	0.390	0.171	0.079	0.034	24.6	31.3	10.5
	N	24	24	24	24	24	24	24	24
3	Mean	460.4	3.901	1.651	0.850	0.359	81.4	114.2	79.2
	S.D.	37.2	0.322	0.171	0.072	0.032	14.5	20.6	17.1
	N	25	25	25	25	25	25	25	25
4	Mean	452.2 A	4.013	1.661	0.889	0.369 B	80.4	108.0	72.8
	S.D.	35.3	0.319	0.130	0.057	0.031	17.6	23.8	28.4
	N	25	25	25	25	25	25	25	25
Levene test (P):		0.9877	0.4285	0.0385 *	0.7125	0.0999	0.1335	0.1206	0.0488 *
Overall ANOVA F-test (P):		0.0163 *	0.6033	0.4907	0.0697	0.0217 *	0.2729	0.2346	0.7973

* Indicate a significant test (P <= 0.05)

Significantly different from Group 1 value: A - P <= 0.05 B - P <= 0.01 C - P <= 0.001 (Dunnett)

Study title: AHU377: An oral (gavage) fertility and early embryonic development study in the rat

Study no.:	1370483
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2/18/2014 – males 2/18/2014 - females
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0723009, 100.2% (from Study protocol) 0842011, 99.1% (from Cert. of Anal.)

Key Study Findings

Batch numbers from protocol and COA do not match, however both indicate AHU377 was used.

Administration of AHU377 by daily oral gavage at dosages of 75, 250 and 750 mg/kg/day to males and females resulted in adverse effects on body weights and food consumption in males, but not females, at 750 mg/kg/day. There was no evidence of effects on male or female reproductive function or embryo-lethality at any dose. The NOAEL for paternal toxicity was considered to be 250 mg/kg/day and for maternal toxicity, 750 mg/kg/day. The no-observed-adverse-effect level (NOAEL) for reproductive

function and early embryo-fetal development was considered to be 750 mg/kg/day, the highest dose level administered.

Methods

Doses:	75, 250, 750 mg/kg/day
Frequency of dosing:	daily
Dose volume:	5 mL/kg
Route of administration:	oral
Formulation/Vehicle:	0.5% (w/v) Sodium carboxymethylcellulose (CMC)
Species/Strain:	Rat/Wistar Hannover
Number/Sex/Group:	25
Satellite groups:	na
Study design:	**Duration of dosing:** Males: Once daily for at least 28 days prior to mating, during the 2-week mating period and until terminal necropsy. Females: Once daily for the two week premating period during mating and through gestation day 6. **Terminal Sacrifice:** Males: Following the completion of mating and at the direction of the Study Director. Females: On gestation day 13 for sperm positive females.
Deviation from study protocol:	Deviations had no impact on the outcome of the study or upon the interpretation of the results.

Observations and Results

Doses for this study were based on preliminary findings in the AHU377 26-week oral (gavage) toxicity study in rats conducted at doses of 50, 150 and 600 mg/kg/day [Novartis reference no. 1370484]; on the results of the AHU377 oral (gavage) embryo-fetal development study in rats conducted at doses of 75, 250 and 750 mg/kg/day [Novartis reference no. 0570301] and on the AHU377 oral (gavage) pre and postnatal study in rats conducted at doses of 50, 250 and 750 mg/kg/day [Novartis reference no. 1070349]. Based on results from those studies, doses of 75, 250 and 750 mg/kg/day were selected for the current study.

Mortality

Mortality was monitored twice daily on weekdays (am and pm) and weekends. There was no unscheduled mortality.

Clinical Signs

On non-dosing days, once daily starting on the day of randomization when no detailed examinations were scheduled; on days of dosing, predose (when no detailed examinations were scheduled) and within 1 to 3 hours post dose.

Detailed examinations: On the days of body weight assessment.

No observations considered to be adverse were noted.

Body Weight

Individual body weights were measured twice weekly commencing on the day of randomization and extending through the treatment period, including the day animals were placed for mating and, for males, the day of scheduled necropsy. Mated females were weighed on days 0, 3, 7, 10 and 13 postcoitum.

For males at 250 and 750 mg/kg/day, significantly lower body weight gains, compared to controls, were noted during the first week of the premating treatment period. At 750 mg/kg/day, this continued throughout the remaining 3 weeks of this period of the study. As a result, the overall weight gains (study days 1 to 28) were significantly decreased at 250 and 750 mg/kg/day, being 12 and 35% lower than controls. Body weight decreases in males at 750 mg/kg were of sufficient magnitude to be considered adverse.

Feed Consumption

Food consumption (cage measurements) was quantitatively measured twice weekly commencing the day of randomization and until initiation of the mating period. Food consumption for mated females was measured on days 0 to 3, 3 to 7, 7 to 10 and 10 to 13 post coitum. There were slightly lower food intakes for males at 250 and 750 mg/kg/day during the premating treatment period, particularly for the first week of treatment. Overall, all values (study days 1 to 28) were reduced by 5% and 8% at 250 and 750 mg/kg/day, respectively, compared to controls.

Toxicokinetics

Not determined.

Dosing Solution Analysis

All study samples analyzed had mean concentrations within the acceptance criteria of ±15% (individual values within ±20%) of their theoretical concentrations.

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

Estrous cycles
The estrous cycles of the AHU377-treated females were not affected.

Parental performance
There was no effect of AHU377 upon the day to mating, mating or fertility indices, or the conception rates.

Terminal evaluations

Macroscopic observations
No macroscopic observations attributed to the effect of treatment with AHU377 were observed; the miscellaneous macroscopic findings observed were considered incidental in origin and of no toxicological significance because findings were also observed in vehicle-treated control animals and/or because of a lack of a dose response relationship.

Organ weights
There were no toxicologically significant differences in mean absolute or relative organ weights between AHU377-treated animals and vehicle-treated control animals. Increased mean relative (to body) testis and epididymis weights were observed in AHU377-treated males at 750 mg/kg/day. These increases were interpreted to be related to the difference of terminal body weight which was decreased at that dose level compared to the control group and therefore, this difference of organ weight was considered of no toxicological significance.

Ovarian and uterine findings
For AHU377-treated females, there were no effects upon the numbers of corpora lutea, implantations, live embryos, dead embryos, resorptions or the pre or post-implantation losses. The increased pre or post-implantation loss values noted for the treated groups were not considered related to the test item since there was no dose response noted and because values were within the historical control values for these parameters.

Male reproductive assessments
There were no toxicologically significant differences in mean for sperm parameters, including sperm motility and concentration, between AHU377-treated animals and vehicle-treated control animals.

Summary of estrous cycle data

Group 1 - Vehicle Control
Group 2 - AHU377 75/78.45 mg/kg/day
Group 3 - AHU377 250/261.5 mg/kg/day
Group 4 - AHU377 750/784.5 mg/kg/day

Group	Summary Information	Number of Days in Estrus*	Number of Cycles Seen**	Average Cycle Length of Observed Cycles (Days)
1	Mean	4.4	3.5	4.16
	SD	1.1	0.5	0.53
	N	24	24	24
2	Mean	3.7	3.4	4.31
	SD	0.9	0.6	0.97
	N	24	24	24
3	Mean	3.9	3.3	4.35
	SD	1.1	0.6	0.99
	N	24	24	24
4	Mean	4.0	3.4	4.18
	SD	1.1	0.7	0.55
	N	24	24	23

* Includes only the days in estrous
** Includes actual cycles seen in estrous and the "unseen" cycles determined

Summary of parental performance data

Group 1 - Vehicle Control
Group 2 - AHU377 75/78.45 mg/kg/day
Group 3 - AHU377 250/261.5 mg/kg/day
Group 4 - AHU377 750/784.5 mg/kg/day

Group	Number Placed for Mating		Number Mating	Mean (SD) Day to Mating	Number Females Pregnant	Mating Index (%)	Fertility Index (%)	Conception Rate (%)
	Males	Females						
1	24	24	24	2.7 2.7 (N = 23)	23	100.0	95.8	95.8
2	24	24	24	2.3 0.9 (N = 22)	24	100.0	100.0	100.0
3	24	24	24	2.6 3.4 (N = 23)	24	100.0	100.0	100.0
4	24	24	24	2.8 2.3 (N = 22)	24	100.0	100.0	100.0

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn - day to mating only)
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

Summary of ovarian and uterine finding data

Group 1 - Vehicle Control
Group 2 - AHU377 75/78.45 mg/kg/day
Group 3 - AHU377 250/261.5 mg/kg/day
Group 4 - AHU377 750/784.5 mg/kg/day

Group	Summary Information	Total Number of Corpora Lutea	Total Number of Implantation Sites	Live Embryos	Dead Embryos
1	Mean	13.6	12.9	12.4	0.0
	SD	1.6	1.7	1.7	0.0
	N	22	22	22	22
2	Mean	13.7	12.2	11.2	0.0
	SD	1.7	2.4	2.6	0.0
	N	22	22	22	22
3	Mean	11.5	10.1	9.5	0.0
	SD	3.9	4.5	4.4	0.0
	N	23	23	23	23
4	Mean	12.5	11.2	10.7	0.0
	SD	3.0	4.0	4.2	0.2
	N	22	22	22	22

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

Group 1 - Vehicle Control
Group 2 - AHU377 75/78.45 mg/kg/day
Group 3 - AHU377 250/261.5 mg/kg/day
Group 4 - AHU377 750/784.5 mg/kg/day

Group	Summary Information	Early Resorptions	Sum of Early Resorptions and Dead Embryos	Preimplantation Loss (%)	Post Implantation Loss (%)
1	Mean	0.5	0.5	5.16	3.81
	SD	0.7	0.7	8.60	5.68
	N	22	22	22	22
2	Mean	1.0	1.0	11.73 D	9.50
	SD	0.8	0.8	10.00	12.16
	N	22	22	22	22
3	Mean	0.7	0.7	16.17	6.62
	SD	0.9	0.9	19.36	10.12
	N	23	23	23	23
4	Mean	0.5	0.5	13.93	7.73
	SD	0.8	0.8	21.58	13.34
	N	22	22	22	22

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

Summary of sperm evaluation data

Group 1 - Vehicle Control
Group 2 - AHU377 75/78.45 mg/kg/day
Group 3 - AHU377 250/261.5 mg/kg/day
Group 4 - AHU377 750/784.5 mg/kg/day

Group	Summary Information	Cauda Epididymis Weight (g)	Spermatozoa Count Per Gram (Millions)	Percent Motility
1	Mean	0.21908	813.112	84.6
	SD	0.02471	173.310	20.0
	N	24	24	24
2	Mean	0.22137	792.019	88.3
	SD	0.02190	178.625	6.8
	N	24	24	23
3	Mean	0.22682	714.784	90.0
	SD	0.03436	223.835	5.6
	N	24	24	24
4	Mean	0.22093	860.895	89.6
	SD	0.02432	225.349	9.1
	N	24	24	24

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

9.2 Embryonic Fetal Development

Studies with LCZ696

Study title: An oral (gavage) embryo-fetal development study in rats

Study no.:	0670390
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover, New Jersey 07936
Date of study initiation:	1/14/2007
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0651004, 94.6%

Key Study Findings

Treatment-related effects on reproductive parameters were limited to an increase in post-implantation loss at doses ≥ 100 mg/kg/day, however this effect was not strong enough to be statistically significant. All other parameters were unaffected by treatment. Fetal external, visceral and skeletal findings were unaffected by treatment at all dose levels.

The maternal no observed adverse effect level (NAOEL) was 200 mg/kg/day, with a fetal NOAEL of 30 mg/kg/day based on a slight increase in post-implantation loss at higher doses.

Methods

Doses:	30, 100 or 200 mg/kg/day30
Frequency of dosing:	daily (gestation days 6-17)
Dose volume:	5 mL/kg
Route of administration:	oral
Formulation/Vehicle:	0.5% CMC
Species/Strain:	Crl: WI(Glx/BRL/Han)IGS BR
Number/Sex/Group:	25
Satellite groups:	TK: 5 animals/group for the treated groups and 3 animals in the control group
Study design:	Duration of dosing: Gestation days 6-17 for the main study and toxicokinetic satellite animals Terminal C-section: Gestation day 21 for the main study animals. Gestation day 18 for the toxicokinetic satellite animals
Deviation from study protocol:	protocol deviations had no impact on the integrity or the interpretation of the data

Observations and Results

Mortality

Twice daily on weekdays (am and pm) and once daily on weekends.

There was no mortality associated with any dose. A single dam delivered early in the 30 mg/kg/day group and was sacrificed, which was unrelated to treatment.

Clinical Signs

Twice daily during the dosing phase: predose and within approximately 3 hours postdose.

Clinical signs associated with treatment were limited to decreased stool in all dose groups. This effect increased in frequency and duration with increasing dose.

Body Weight

Measured on Gestation days 0, 3, 6, 9, 12, 15, 18 and 21

Mean group body weight was significantly lower than controls at 200 mg/kg/day on GD18, supported by slightly decreased mean body weight gain from GD9 to GD18 at this dose level. All other groups were similar to controls. Gravid uterine weights and carcass weights were unaffected by treatment.

Feed Consumption

Food consumption was calculated based on feeder weights collected on gestation days 3, 6, 9, 12, 15, 18 and 21.

Mean food consumption was decreased from GD12 to GD18 at a dose of 200 mg/kg/day. All other groups were similar to controls.

Toxicokinetics

Samples were taken from each satellite animal on gestation day 17 at each of the following time points: 0.5, 2, 6 and 24 hours after the last dose.

Plasma concentrations of Valsartan, AHU377 and its active metabolite LBQ657 were measured using an LC-MS/MS method and AUC, AUC/dose, C_{max}, C_{max}/dose, and t_{max} were determined when possible.

Mean toxicokinetic parameters of AHU377 in rat plasma

Dose	n	$AUC_{(0\text{-}24h)}$	$AUC_{(0\text{-}24h)}$/Dose	±SD/Dose of AUC	C_{max}	C_{max}/Dose	±SD/Dose of C_{max}	t_{max}
100	3	509	5.09	3.19	157	1.57	0.485	0.500
200	3	821	4.11	3.39	214	1.07	0.511	2.33

Units for each parameter: Dose: mg/kg/day; $AUC_{(0\text{-}24h)}$: (ng-h/mL); $AUC_{(0\text{-}24h)}$ ±SD / Dose: (ng-h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max} ±SD / Dose: (ng/mL)/(mg/kg/day); t_{max}: h.

Mean toxicokinetic parameters of LBQ657 in rat plasma

Dose	n	$AUC_{(0-24h)}$	$AUC_{(0-24h)}$/Dose	±SD/Dose of AUC	C_{max}	C_{max}/Dose	±SD/Dose of C_{max}	t_{max}
30	4	5950	198	65.4	1970	65.8	10.8	0.500
100	5	19200	192	155	3290	32.9	23.9	1.60
200	3	29400	147	45.0	5200	26.0	13.5	2.33

Units for each parameter: Dose: mg/kg/day; $AUC_{(0-24h)}$: (ng--h/mL); $AUC_{(0-24h)}$ ±SD / Dose: (ng-h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max} ±SD / Dose: (ng/mL)/(mg/kg/day); t_{max}: h.

Mean toxicokinetic parameters of valsartan in rat plasma

Dose	n	$AUC_{(0-24h)}$	$AUC_{(0-24h)}$/Dose	±SD/Dose of AUC	C_{max}	C_{max}/Dose	±SD/Dose of C_{max}	t_{max}
30	4	13400	448	106	3020	101	15.6	0.500
100	5	60000	600	584	7770	77.7	68.9	1.60
200	3	119000	593	233	12100	60.5	43.1	1.00

Units for each parameter: Dose: mg/kg/day; $AUC_{(0-24h)}$: (ng--h/mL); $AUC_{(0-24h)}$ ± SD / Dose: (ng-h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max} ±SD / Dose: (ng/mL)/(mg/kg/day); t_{max}: h.

Dosing Solution Analysis

The uniformity of the suspensions was confirmed.

Necropsy

For all pregnant females sacrificed on gestation day 21, each uterine implant site was identified as either a live fetus, dead fetus, early or late resorption.

Live fetuses obtained on gestation day 21 were sexed, weighed individually, sacrificed and identified with a tag. They were examined for external findings. A fresh visceral examination was performed on approximately one-half of the fetuses from each litter. The remaining fetuses were placed in 70% ethanol for subsequent skeletal examinations.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Treatment related effects on reproductive parameters were limited to an increase in post-implantation loss at doses ≥ 100 mg/kg/day, however this effect was not strong enough to be statistically significant.

Offspring (Malformations, Variations, etc.)

The only fetal skeletal variation which demonstrated a dose response was an increase in incomplete ossification of the sternebrae at 200 mg/kg/day, however this is a fairly common finding and not considered adverse.

Study title: An oral (gavage) embryo-fetal development study in rabbits

Study no.:	0670280
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover, New Jersey 07936
Date of study initiation:	12/18/2006
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0651004, 94.6%

Key Study Findings

Mortalities were associated with doses ≥ 10 mg/kg/day.

Body weights were affected in a dose-dependent manner with mean body weight loss from GD7 to GD21 at 30 mg/kg/day, and a transient mean body weight loss from GD7 to GD14 at 10 mg/kg/day.

Late resorptions were significantly elevated at doses ≥ 10 mg/kg/day with a corresponding elevation of post-implantation loss and decrease in viable fetuses. Gravid uterine weights were decreased at a dose of 30 mg/kg/day.

Visceral malformations associated with treatment included three cases of hydrocephaly at 30 mg/kg/day and one case at 10 mg/kg/day.

Methods

Doses:	3, 10 and 30 mg/kg/day
Frequency of dosing:	daily
Dose volume:	5 mL/kg
Route of administration:	oral
Formulation/Vehicle:	0.5% (w/v) sodium carboxymethylcellulose (CMC)
Species/Strain:	Rabbits/New Zealand White, Hra:(NZW)SPF
Number/Sex/Group:	20
Satellite groups:	5/dose group for TK
Study design:	Duration of dosing: Gestation days 7-20 for the main study and toxicokinetic satellite animals. Terminal C-section: Gestation day 29 for the main study animals. Gestation day 21 for the toxicokinetic satellite animals.
Deviation from study protocol:	deviations had no impact on the study.

Observations and Results

Mortality

Twice daily on weekdays (am and pm) and once daily on weekends and holidays.

Mortalities were associated with doses ≥ 10 mg/kg/day. At a dose of 30 mg/kg/day, 4 does were found dead (ranging from GD20 to GD27), 1 doe was sacrificed after aborting on GD26 and 5 were sacrificed due to early deliveries. At a dose of 10 mg/kg/day, 1 doe was found dead on GD21 and 1 doe was sacrificed after aborting on GD26. All other animals survived to scheduled necropsy.

Clinical Signs

At least once daily during the pre- and postdose phases. Twice daily during the dosing phase: predose and within approximately 3 hours postdose.

Clinical signs associated with treatment included decreased locomotor activity in 2 does at 30 mg/kg/day and a dose dependent increase in soft and decreased stool as well as red stains in the cage at all dose levels.

Body Weight

Main study animals: Gestation days 0, 5, 7, 10, 14, 17, 21, 24 and 29.
Satellite females: Gestation days 7, 10, 14 and 17.

Body weights were affected in a dose-dependent manner. There was a pattern of mean body weight loss from GD7 to GD21 at 30 mg/kg/day, with statistical significance from GD14 to GD21, after which recovery was evident. A transient mean body weight loss was observed from GD7 to GD14 at 10 mg/kg/day after which recovery was evident. A dose of 3 mg/kg/day resulted in only a transient decrease in mean body weight gain from GD10 to GD14.

Feed Consumption

Food consumption was calculated based on feeder weights collected on gestation days 5 through 29.

Food consumption was significantly decreased at a dose of 30 mg/kg/day from GD11 to GD23. All other dose levels did not differ from controls.

Toxicokinetics

Maternal blood samples for toxicokinetics were collected from the marginal ear vein or artery into tubes containing lithium heparin. Samples of approximately 1 mL were taken from each satellite animal on gestation day 20 at each of the following time points: 1, 3, 7 and 24 hours after the last dose.

Daily dose (mg/kg)		0 (Control – Group 1)	3 (Group 2)	10 (Group 3)	30 (Group 4)
Dams/does	**Toxicokinetics: AHU377**				
	AUC0-24h (ng·h/mL)	NC	52.9	303	878
	Cmax (ng/mL)	NA	15.0	56.2	143
	Toxicokinetics: LBQ657				
	AUC0-24h (ng·h/mL)	NC	1930	8920	102000
	Cmax (ng/mL)	NA	491	1810	10500
	Toxicokinetics: Valsartan				
	AUC0-24h (ng·h/mL)	NC	41100	169000	400000
	Cmax (ng/mL)	NA	2850	10200	20100

Dosing Solution Analysis

Samples of the suspensions prepared for day 1 were analyzed, and all results were within specifications. One control sample was analyzed, and no LCZ696 was detected.

Necropsy

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

For all pregnant females sacrificed on gestation day 29, each uterine implant site was identified as either a live fetus, early or late resorption.

Offspring (Malformations, Variations, etc.)

Live fetuses obtained on gestation day 29 were weighed individually, sacrificed and identified with a tag. They were examined for external and internal findings. A fresh visceral examination was performed and the fetus were sexed. A mid-coronal slice was made in the head of each fetus to evaluate the contents of the skull. The eviscerated fetuses were stained with alizarin red for skeletal examination and retained in glycerine.

Fetal findings were classified as variations or malformations.

AN ORAL (GAVAGE) EMBRYO-FETAL DEVELOPMENT STUDY IN RABBITS
STUDY IDENTIFICATION NO.: 0670280

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	19	20	20	18
	%	95.0	100.0	100.0	90.0
Aborted	N	0	0	2	1
Premature Births	N	0	0	0	5
Female Mortality	N	0	0	1	4
Pregnant at C-section	N	19	20	18	8
Dams with Viable Fetuses	N	19	20	18	8
Dams with all Resorptions	N	0	0	0	0
Corpora Lutea	N	189	184	184	70
	MEAN	9.9	9.2	10.2	8.8
	S.D.	1.9	1.5	2.5	1.2
Implantation Sites	N	172	165	168	59
	MEAN	9.1	8.3	9.3	7.4
	S.D.	2.0	1.8	1.9	2.4
Preimplantation Loss	%	9.0	10.3	8.7	15.7
Postimplantation Loss	%	2.9	3.0	16.1	42.4
Dead Fetuses	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Resorptions, total	N	5	5	27**	25**
	%	2.9	3.0	16.1	42.4
	MEAN	0.3	0.3	1.5	3.1**
	S.D.	0.7	0.6	2.0	3.1
Early Resorptions	N	1	2	5	1
	%	0.6	1.2	3.0	1.7
	MEAN	0.1	0.1	0.3	0.1
	S.D.	0.2	0.3	0.8	0.4
Late Resorptions	N	4	3	22**	24**
	%	2.3	1.8	13.1	40.7
	MEAN	0.2	0.2	1.2	3.0**
	S.D.	0.7	0.5	2.0	3.2

Statistical key: ** = p<0.01

AN ORAL (GAVAGE) EMBRYO-FETAL DEVELOPMENT STUDY IN RABBITS
STUDY IDENTIFICATION NO.: 0670280

Summary of Cesarean Data [PCDSU]

	DOSAGE	0 MG/ KG/DAY	3 MG/ KG/DAY	10 MG/ KG/DAY	30 MG/ KG/DAY
Viable Fetuses	N	167	160	141**	34**
	%	97.1	97.0	83.9	57.6
	MEAN	8.8	8.0	7.8	4.3**
	S.D.	1.9	2.0	2.6	2.3
Viable Male Fetuses	N	92	81	74	19
	%	55.1	50.6	52.9	55.9
Live Fetal Body Weight (g)	MEAN	41.7	42.5	41.3	45.9*
	S.D.	3.4	4.5	3.7	3.6
Male Fetuses	MEAN	42.1	43.1	41.1	44.8
	S.D.	3.4	4.9	3.8	3.3
Female Fetuses	MEAN	41.1	41.4	40.3	45.8*
	S.D.	3.6	4.6	4.4	3.6

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

AN ORAL (GAVAGE) EMBRYO-FETAL DEVELOPMENT STUDY IN RABBITS
STUDY IDENTIFICATION NO.: 0670280

SUMMARY OF FETAL VISCERAL MALFORMATIONS

DOSAGE		0 MG/ KG/DAY	3 MG/ KG/DAY	10 MG/ KG/DAY	30 MG/ KG/DAY
Litters Evaluated	N	19	20	18	8
Fetuses Evaluated	N	167	160	140	34
Live	N	167	160	140	34
Dead	N	0	0	0	0
FLUID-FILLED ABDOMEN					
Fetal Incidence	N	0	3	0	2*
	%	0.0	1.9	0.0	5.9
Litter Incidence	N	0	3	0	1
	%	0.0	15.0	0.0	12.5
HYDROCEPHALY					
Fetal Incidence	N	0	0	1	3**
	%	0.0	0.0	0.7	8.8
Litter Incidence	N	0	0	1	2
	%	0.0	0.0	5.6	25.0
CEREBRAL VENTRICLE-DILATED					
Fetal Incidence	N	0	1	0	1
	%	0.0	0.6	0.0	2.9
Litter Incidence	N	0	1	0	1
	%	0.0	5.0	0.0	12.5
SMALL CEREBRUM					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	2.9
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	12.5
CARDIOMEGALY					
Fetal Incidence	N	0	2	0	0
	%	0.0	1.3	0.0	0.0
Litter Incidence	N	0	2	0	0
	%	0.0	10.0	0.0	0.0
ENLARGED ATRIAL CHAMBER					
Fetal Incidence	N	0	2	0	0
	%	0.0	1.3	0.0	0.0
Litter Incidence	N	0	2	0	0
	%	0.0	10.0	0.0	0.0

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

Studies with AHU377

Study title: An oral (gavage) embryo-fetal development study in rats

Study no.:	0570301
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover, New Jersey.
Date of study initiation:	11/20/2005
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0451002, 97.3%

Key Study Findings

Based upon clinical observations and changes in body weight gain and food consumption, the no observed adverse effect level (NOAEL) for the pregnant rat is 250 mg/kg/day. With no evidence of embryo-fetal toxicity or teratogenicity in the presence of maternal toxicity, the NOEL for embryo-fetal effects in the rat is considered to be at least 750 mg/kg/day.

Methods

Doses:	75, 250 and 750 mg/kg/day
Frequency of dosing:	daily (gestation days 6-17)
Dose volume:	15 mL/kg
Route of administration:	oral
Formulation/Vehicle:	50 mM Phosphate buffer; pH adjusted to approximately 8.8
Species/Strain:	Rat, Crl: WI (Glx/BRL/Han)IGS BR
Number/Sex/Group:	25
Satellite groups:	5/dose group for TK
Study design:	Duration of dosing: Gestation days 6-17 for the main study and toxicokinetic satellite animals Terminal C section: Gestation day 21 for the main study animals Gestation day 18 for the toxicokinetic satellite animals
Deviation from study protocol:	deviations had no impact on the study.

Observations and Results

Mortality

Twice daily (AM and PM) on weekdays and once daily on weekends and holidays.

There were no mortalities associated with treatment. One animal in each group delivered early and was sacrificed, which is considered unrelated to treatment. One animal at 250 mg/kg suffered an accidental death. All other animals survived to scheduled necropsy.

Clinical Signs

At least once daily during the pre- and postdose phases.
Twice daily during the dosing phase: predose and within approximately 3 hours postdose.

Dose-related clinical observations included salivation at a dose of 750 mg/kg/day with rales noted in 1, 3, and 8 animals at 75, 250 and 750 mg/kg/day, respectively.

Body Weight

Main study animals: Gestation days 0, 3, 6, 9, 12, 15, 18 and 21
Satellite animals: Gestation days 0, 6, 9, 12 and 15

Group mean body weights were generally unaffected by treatment, however, a trend towards lower group mean body weights at doses ≥ 250 mg/kg/day is evident and supported by significantly reduced body weight gain over the dosing period.

Feed Consumption

Food consumption was calculated based on feeder weights collected on gestation days 3, 6, 9, 12, 15, 18 and 21.

Food consumption was significantly reduced throughout dosing at 750 mg/kg/day. All other dose levels were similar to controls.

Toxicokinetics

Samples of approximately 0.5 mL were taken from each satellite animal on gestation day 17 at each of the following time points: 0.5, 1, 2 and 24 hours after the last dose

(Mean) toxicokinetic parameters of AHU377 in rat plasma

Dose (mg/kg/day)	$AUC_{(0\text{-}24h)}$ ± SD (ng*h/mL)	$AUC_{(0\text{-}24h)}$/Dose ± SD (ng*h/mL/mg/kg/day)	C_{max} (ng/mL)	C_{max}/Dose (ng/mL/mg/kg/day)	t_{max} (h)
75	2460 ± 785	32.8 ± 10.5	289	3.85	8.5
250	56800 ± 122000	227 ± 488	6600	26.4	0.8
750	38000 ± 52000	50.7 ± 69.3	24600	32.8	1.0

N = 3, 5 and 4 for 75, 250 and 750 mg/kg dose groups, respectively; except for t_{max} (reported one decimal place), all parameter values are reported to three significant figures.

(Mean) toxicokinetic parameters of LBQ657 in rat plasma

Dose (mg/kg/day)	$AUC_{(0\text{-}24h)}$ ± SD (ng*h/mL)	$AUC_{(0\text{-}24h)}$/Dose ± SD (ng*h/mL/mg/kg/day)	C_{max} (ng/mL)	C_{max}/Dose (ng/mL/mg/kg/day)	t_{max} (h)
75	60300 ± 34600	804 ± 461	10500	140	0.5
250	58700 ± 55000	235 ± 220	13400	53.6	0.5
750	659000 ± 896000	879 ± 1200	67000	89.3	0.9

N=3, 5 and 4 for 75, 250 and 750 mg/kg dose groups, respectively; except for t_{max} (reported to one decimal place), all parameter values are reported to three significant figures.

Dosing Solution Analysis

Samples of the formulations used for the first day of dosing were analyzed. Batch and container uniformity of the AHU377 formulations was confirmed. Concentrations were 101% to 104% of target concentrations. One control sample was analyzed and no AHU377 was detected.

Necropsy

Scheduled termination: Gestation day 21 for the main study animals; gestation day 18 for the toxicokinetic satellite animals.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

For all pregnant females sacrificed on gestation day 21, each uterine implant site was identified as a live fetus, dead fetus, early or late resorption. Uterine site descriptions were not recorded for animals that delivered early.

There were no dead fetuses in this study.

Live fetuses obtained on gestation day 21 were sexed, weighed individually, sacrificed and identified with a tag. They were examined for external findings. A fresh visceral examination was performed on approximately one-half of the fetuses from each litter. The remaining fetuses were placed in 70% ethanol for subsequent skeletal examinations.

Fetuses designated for skeletal examinations were processed, stained with alizarin red, examined, and retained in glycerin. Fetuses designated for visceral examinations were processed, stained with alizarin red and retained in glycerin.

Maternal necropsy findings were unremarkable. Gravid uterine weights and cesarean data were unaffected by treatment.

Offspring (Malformations, Variations, etc.)

Fetal external, visceral and skeletal findings were unaffected at all doses levels.

Study title: An oral (gavage) embryo-fetal development study in rabbits

Study no.:	0770644
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover, New Jersey 07936
Date of study initiation:	9/10/2007
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0522006, 99.0%

Key Study Findings

AHU377 was not found to be teratogenic in the rabbit in this study. Based upon the reduced body weight gain and food consumption at doses ≥ 200 mg/kg/day and mortalities at 500 mg/kg/day, the No Observed Adverse Effect Level (NOAEL) for maternal toxicity in this study is 50 mg/kg/day. Based upon abortion, the increase in resorptions, decreased fetal weights and fetal findings at 500 mg/kg/day, the embryo-fetal NOAEL in this study is 200 mg/kg/day.

Methods

Doses:	15, 50, 200 and 500 mg/kg/day
Frequency of dosing:	daily
Dose volume:	5 mL/kg
Route of administration:	oral
Formulation/Vehicle:	50mM Phosphate buffer,pH 8.8 +/- 0.1
Species/Strain:	rabbits (Hra:(NZW)SPF)
Number/Sex/Group:	20
Satellite groups:	5 TK

Study design:	Duration of dosing: Gestation days 7-20 for the main study and toxicokinetic satellite animals. Terminal C-section: Gestation day 29 for the main study animals. Gestation day 21 for the toxicokinetic satellite animals.
Deviation from study protocol:	deviations had no impact on the integrity of the study

Justification for selection of doses
In a previous study in pregnant rabbits with AHU377 [0570339], doses of 200, 500 and 1000 mg/kg/day were administered. 1000 mg/kg/day was not tolerated, with death in 5 of 6 animals prior to GD 12. Decreased body weight gains, food consumption and clinical signs were noted at all doses. Fetal weights were decreased at 500 mg/kg/day and no maternal NOAEL was established. Based upon these results, doses of 15, 50, 200 and 500 mg/kg/day were selected for this study.

Observations and Results

Mortality

Twice daily on weekdays (am and pm) and once daily on weekends and holidays.

Three animals at 500 mg/kg/day were moribund sacrificed with signs of decreased stool and red staining in the cage pan. An additional animal at 500 mg/kg/day (no.181) aborted on GD27 and was sacrificed. These three mortalities and the abortion are considered to be treatment-related.

Clinical Signs

At least once daily during the pre- and postdose phases.
Twice daily during the dosing phase: predose and within approximately 3 hours postdose.

Treatment-related clinical signs consisted of a dose-related increase in stool signs (decreased or no stool) and staining in the cage pan at doses. These signs were significantly higher than controls at 500 mg/kg/day, a dose which induced three moribund sacrifices and one abortion.

Body Weight

Main study animals: 0, 5, 7, 10, 14, 17, 21, 24 and 29.
Satellite females: 0, 7, 10, 17 and 21.

Group mean body weights were reduced, as compared to controls, in a dose dependent manner. Group means were significantly reduced by GD 14 at 500 mg/kg/day, by GD21 at 200 mg/kg/day and by GD29 at 50 mg/kg/day. These effects are consistent with the noted net body weight loss from GD7 to GD10 at doses ≥ 200 mg/kg/day and net body weight loss (ranging from no gain to 117 grams lost) at all doses from GD17 to GD21. Body weight gain was consistently lower over the entire dosing period at 500

mg/kg/day. Gravid uterine weights were significantly lower at 500 mg/kg/day, while carcass weights were reduced at doses ≥ 50 mg/kg/day.

Feed Consumption

Food consumption was calculated based on feeder weights collected on gestation days 5 through 29.

Food consumption was consistently decreased throughout dosing at doses ≥ 200 mg/kg/day and was decreased from GD13 through the completion of dosing at 50 mg/kg/day. Food consumption was also transiently decreased at 15 mg/kg/day, showing lower values than control from GD13 to GD14 and again from GD 17 to GD21. It took several days for food consumption values to return to control levels after the completion of dosing in all groups.

Toxicokinetics

Samples of approximately 1 mL were taken from each satellite animal on gestation day 20/21 at each of the following time points: 1, 3, 7 and 24 hours after the last dose.

Individual and mean ± SD (n=5) toxicokinetic parameters of AHU377 in rabbit plasma

Dose mg/kg/day	Animal no.	AUC	AUC/Dose	C_{max}	C_{max}/Dose	t_{max}
15	1711	686	45.7	170	11.3	1.00
	1713	968	64.5	96.5	6.43	3.00
	1715	807	53.8	133	8.87	1.00
	1717	842	56.2	69	4.60	3.00
	1719	912	60.8	101	6.73	3.00
	Mean ± SD	843 ± 108	56.2 ± 7.18	114 ± 38.7	7.59 ± 2.57	2.20
50	1725	501	10.0	143	2.86	1.00
	1727	1890	37.8	166	3.32	3.00
	1729	3410	68.3	628	12.6	1.00
	1731	3120	62.3	213	4.26	7.00
	1733	2280	45.6	389	7.78	1.00
	Mean ± SD	2240 ±1150	44.8 ± 23.0	308 ± 203	6.16 ± 4.08	2.60
200	1739	6650	33.3	1080	5.40	1.00
	1741	14100	70.3	1480	7.40	3.00
	1743	26500	133	5030	25.2	3.00
	1745	21000	105	1610	8.05	1.00
	1747	13600	67.9	938	4.69	3.00
	Mean ± SD	16400 ± 7610	81.9 ± 38.2	2030 ± 1700	10.1 ± 8.53	2.20
500	1753	38700	77.4	2460	4.92	7.00
	1755	89000	178	14900	29.8	3.00
	1757	107000	213	11000	22.0	1.00
	1759	51900	104	4710	9.42	3.00
	1761	69700	139	6230	12.5	3.00
	Mean ± SD	71300 ± 27500	142 ± 54.7	7860 ± 5030	15.7 ± 10.1	3.40

Units for Dose: mg/kg/day; $AUC_{(0\text{-}24h)}$: (ng·h/mL); $AUC_{(0\text{-}24h)}$/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h.

Individual and mean ± SD (n=5) toxicokinetic parameters of LBQ657 in rabbit plasma

Dose mg/kg/day	Animal no.	AUC	AUC/Dose	C_{max}	C_{max}/Dose	t_{max}
15	1711	28000	1860	5390	359	1.00
	1713	28400	1890	3260	217	3.00
	1715	29800	1990	4280	285	1.00
	1717	48000	3200	4210	281	3.00
	1719	41200	2750	5040	336	3.00
	Mean ± SD	35100 ± 9040	2340 ± 605	4440 ± 827	296 ± 55.1	2.20
50	1725	86800	1740	12300	246	1.00
	1727	143000	2850	11800	236	3.00
	1729	149000	2990	24100	482	1.00
	1731	173000	3460	13300	266	3.00
	1733	93800	1880	16200	324	1.00
	Mean ± SD	129000 ± 37300	2580 ± 743	15500 ± 5080	311 ± 102	1.80
200	1739	508000	2540	62500	313	1.00
	1741	743000	3720	66600	333	1.00
	1743	611000	3060	108000	540	3.00
	1745	877000	4390	62600	313	3.00
	1747	654000	3270	41300	207	7.00
	Mean ± SD	679000 ± 139000	3400 ± 699	68200 ± 24400	341 ± 122	3.00
500	1753	1620000	3240	106000	212	1.00
	1755	1270000	2550	192000	384	3.00
	1757	2180000	4360	185000	370	3.00
	1759	1700000	3400	172000	344	3.00
	1761	1890000	3770	198000	396	3.00
	Mean ± SD	1730000 ± 336000	3460 ± 668	171000 ± 37400	341 ± 74.8	2.60

Units for Dose: mg/kg/day; $AUC_{(0\text{-}24h)}$: (ng·h/mL); $AUC_{(0\text{-}24h)}$/Dose: (ng·h/mL)/(mg/kg/day); C_{max}: ng/mL; C_{max}/Dose: (ng/mL)/(mg/kg/day); t_{max}: h.

Dosing Solution Analysis

Samples of the formulations for the first day of dosing were analyzed. Concentrations were 100% to 102% of targets. One control sample was analyzed, and no AHU377 was detected.

Necropsy

Scheduled termination: Gestation day 29 for the main study animals; gestation day 21 for the toxicokinetic satellite animals.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

For all pregnant females sacrificed on gestation day 29, each uterine implant site was identified as either a live fetus, dead fetus, early or late resorption.

Live fetuses obtained on gestation day 29 were weighed individually, sacrificed and identified with a tag. They were examined for external and internal findings. A fresh visceral examination was performed and the fetus were sexed. A mid-coronal slice was

made in the head of each fetus to evaluate the contents of the skull. The eviscerated fetuses were stained with alizarin red for skeletal examination and retained in glycerine.

Post-implantation loss was increased at a dose of 500 mg/kg/day due to an increase in late resorptions. Mean fetal weights were also significantly reduced at this dose level. All other parameters were unaffected by treatment.

Offspring (Malformations, Variations, etc.)

A single pup at 500 mg/kg/day was found to have a short, curly tail. There were no other external observations.

Fetal visceral malformations were limited to 3 findings of absent gallbladders in individual pups from 3 litters at 500 mg/kg/day and a single enlarged, misshapen heart at 15 mg/kg/day that is considered sporadic an unrelated to treatment. There were no visceral variations associated with treatment.

Fetal malformations were limited to two individual findings of misshapen hyoid bone in separate litters and individual cases of a misshapen/fused cervical centrum and a hemicentric cervical centrum in different litters. Skeletal variations included a slight increase in bent hyoid at 500 mg/kg/day and a number of incomplete ossification findings that were inconsistent across dose groups. Due to the low incidence and absence of dose-response these findings are not considered to indicate developmental toxicity.

The increased incidence of delayed ossifications at 500 mg/kg/day is consistent with decreased fetal weights and is indicative of a broad developmental delay in ossification at this dose which produced maternal toxicity.

9.3 Prenatal and Postnatal Development

Study title: AHU377: An oral (gavage) pre and postnatal study in rats

Study no.:	1070349
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	(b) (4)
Date of study initiation:	7/2/2012
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0824011,

Key Study Findings

AHU377 was administered orally, by gavage, to F0 generation dams from day 7 of gestation to day 20 or 22 post partum at doses of 50, 250 and 750 mg/kg/day. In the F1 generation pups and weanlings at 750 mg/kg/day, body weights were slightly lower than controls, before recovering to be comparable. There were no adverse effects upon survival, behavioral or reproductive function of the F1 generation.

Based on these results, the no observed adverse effect level (NOAEL) for effects on the maternal (F0 generation) animals was considered to be 750 mg/kg/day and the no observed adverse effect level (NOAEL) for the development of their offspring (the F1 generation) was considered to be 250 mg/kg/day.

Methods

Doses:	50, 250 and 750 mg/kg/day
Frequency of dosing:	from day 7 of gestation to day 20 or 22 post partum
Dose volume:	10 mL/kg
Route of administration:	oral
Formulation/Vehicle:	0.5% (w/v) hydroxypropylcellulose
Species/Strain:	Wistar Han rats (Crl:WI[Han])
Number/Sex/Group:	24
Satellite groups:	na
Deviation from study protocol:	deviations had no impact on the outcome of the study

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Dosing solution analysis

The dose formulation concentrations were within specification. Homogeneity testing demonstrated that the formulation technique produced homogeneous preparations.

Observations and Results

The following parameters were evaluated: mortality, clinical signs, body weights, food evaluation, maternal performance, gross pathology (F0 generation); viability, clinical observations, body weights, food consumption, visual function, physical development,

reflexological development, behavioral and reproductive performance and gross pathology (F1 generation).

In-life examinations - F0 generation

Mortality

Clinical examination
A complete detailed examination was performed on the days of body weight assessment. Observed clinical signs were individually recorded. Cage-side observations were performed once daily on non-dosing days when no detailed examinations were scheduled. On dosing days, cage-side observations were performed twice daily, predose (on days when no detailed examination was conducted), and within 1-3 hours post dose, commencing on day 7 postcoitum.

There were no clinical observations attributed to treatment during the postcoitum and lactation periods.

Body weight
F0 generation females were were weighed individually on days 4, 7, 10, 13, 16, 19 and 21 postcoitum (if littering had not occurred), and on days 0, 4, 7, 10, 14, 17 and 21 of lactation.

The body weights and body weight gains during the postcoitum and lactation periods were not affected by treatment with AHU377.

Food consumption
Food consumption (cage measurement) was measured for the F0 generation on days 4 to 7, 7 to 10, 10 to 13, 13 to 16 and 16 to 19 of postcoitum and on days 0 to 7 and 7 to 14 of lactation.

The food consumption during the postcoitum and lactation periods was unaffected by AHU377 administration.

In-life examinations at parturition

Dams (F0 generation)
Females were observed at least 3 times each day beginning on day 21 postcoitum for signs of parturition. Where possible, parturition was observed, the time of onset and completion of parturition was recorded and signs of dystocia noted. The females' behavior immediately post partum was observed. The day of completion of littering was termed day 0 of lactation/post partum.

Pups (F1 generation)
On day 0 post partum, the pups were examined for malformations, sexed and the numbers of live and dead recorded. The live pups were weighed individually. No culling was performed.

Litter observations (F1 generation)

The pregnancy rate, gestation index, length of gestation, live birth index, number of live, dead and/or malformed pups per litter, sex ratio (% male), and number of implantation scars were unaffected by AHU377 administration.

Clinical examination
The general condition of the pups was evaluated each day during the lactation period. Any pups found dead or euthanized preterminally between days 0 and 7 post partum were stored in Bouin's fluid for subsequent examination.

The viability, survival and lactation indices showed no AHU377-related effects upon pup survival.

Body weight
In addition to the assessment of body weight at birth, the pups were weighed individually on days 4, 7, 10, 14 and 21 post partum.

Slightly lower pup weights were noted among males at 750 mg/kg/day between days 7 and 21 pp; on day 14 pp these differences (10% less than controls) achieved statistical significance. The values for female pups showed some slight differences most notably also on day 14 pp. As a result of these differences combined pup weight was also slightly lower on days 7 to 21 pp.

The pup body weights at 50 and 250 mg/kg/day were unaffected by treatment.

Physical development
The following parameters were assessed: pinna unfolding from day 1 post partum until all pups in the litter had a positive response; tooth eruption from day 7 post partum and eye opening from day 12 post partum until the pup tested showed signs of development.

The mean day of development of pinna unfolding and eye opening for males, females and total (genders combined) pups was unaffected by AHU377. Statistically significantly lower values for tooth eruption, indicating advanced development, were noted for the females at 250 mg/kg/day, but, in the absence of any dose dependency they were considered to be indicative of biological variation.

Reflexological development
The following parameters were assessed: righting reflex from day 2 post partum until all pups in the litter had a positive response or until the pup tested showed signs of development, after individual identification (5 post partum); negative geotaxis from day 8

post partum and the auricular startle response from day 12 post partum until the pup tested showed signs of development.

The mean day of development for negative geotaxis, righting reflex and auricular startle response were comparable to controls for all AHU377 treated groups.

F1 adult generation
On day 21 post partum, the F1 generation animals selected to form the F1 adult generation were separated from their dams. Shortly before weaning, 1 male and 1 female rat were randomly selected from each litter, where possible, using a computer-generated random series of numbers to provide the F1 adult generation. All other pups were euthanized on days 21 or 23 post partum and were given a gross pathological examination. No tissues were retained.

Clinical examination
All animals were examined twice daily for mortality and signs of ill health, except on the day of scheduled necropsy. All observations were recorded. A complete detailed examination was performed twice weekly, and on days 0, 6 and 13 pc for mated females. Death and observed clinical signs were individually recorded.

Body weight
Individual body weights were measured twice weekly following weaning for males and unmated females, until mating for mated females, and on days 0, 3, 6, 9 and 13 postcoitum for females once mated.

There were no significant differences for the AHU377 body weights. Slight differences post weaning at 750 mg/kg/day had diminished by day 35 pp for females and day 105 pp for males. Overall body weight gains were similar for the control and AHU377 groups. Gestation body weights were similar in the control and treated groups.

Food consumption
Food consumption (cage measurement) was measured once weekly for the males and females until day 77 post partum and for the mated females on days 0 to 3, 3 to 6, 6 to 9 and 9 to 13 postcoitum.

Overall food consumption values were for AHU377 groups were comparable to the controls. Gestation food intake was similar in the control and treated groups.

Visual function
On day 21 post partum, the pupillary closure and visual placing responses of the F1 adult generation were tested.

Tests of visual function, placing and papillary closure were normal for all treated animals.

Physical development
Vaginal opening was assessed from day 26 post partum until development for females. Preputial separation was assessed from day 35 post partum until development for males. The body weight was recorded on the day the animal was positive for physical development.

The days of vaginal opening and preputial separation were unaffected.

Behavioral performance
The following tests were performed on the animals of the adult F1 generation.

Motor activity
Locomotor activity was assessed using a San Diego Instruments Inc. system for 1 hour in figure 8 enclosures on days 35 (±1) and 60 (±2) post partum. Animals from all groups were balanced across chambers using a computer-generated random series of numbers. The sessions were of 1 hour's duration and were of six 10-minute intervals.

The motor activity of males and females was comparable to controls in the AHU377-treated groups.

Auditory startle habituation
At day 55 (±2) post partum, the startle habituation was measured using a San Diego Instruments Inc. SR-Lab Startle Response System. Animals from all groups were balanced across chambers using a computer-generated random series of numbers. The animals were given a 4-minute acclimation period and then the startle response measured in 50 identical trials at a sound level of 120dBA (±4) with an 8-second inter trial interval.

There were no AHU377-related effects on auditory startle habituation parameters.

'Cincinnati' water maze
Between days 63 and 80 post partum, the animal's ability to swim was assessed by measuring the time to swim a straight channel. The learning and memory tests were conducted using a Cincinnati water maze. The maze consisted of two paths. On the first day of testing, each animal was tested twice by measuring the time to complete the first path (Path A). This was repeated on two additional consecutive days (second trial on the first day and first trial on the second day were at least 25 hours apart) using the same path. The same paradigm (i.e., 3 days of testing) was repeated using a second path (Path B). Two days separated the testing of the two paths. Any abnormality in swimming was recorded.

Results were comparable to controls in all AHU377-treated groups.

Summary of maternal performance data

Incidence data
F0 Generation

Group 1 - Vehicle control
Group 2 - AHU377 50 mg/kg/day
Group 3 - AHU377 250 mg/kg/day
Group 4 - AHU377 750 mg/kg/day

Group	No. of Mated Females	No. of Pregnant Females	Pregnancy Rate (%)	Gestation Index (%)	Dead Pups		Malformed Pups	
					Litters Affected	Pups Affected	Litters Affected	Pups Affected
1	24	23	95.8	100.0	0	0	0	0
2	24	20	83.3	100.0	2	4	0	0
3	24	24	100.0	100.0	0	0	0	0
4	24	23	95.8	100.0	0	0	0	0

Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

(cont.)

Group	Summary Information	Length of Gestation (Days)	Sex Ratio (%)	Number of Pups at Birth/Litters			No. of Implant Scars	Live Birth Index (%)
				Live	Dead	Malformed		
1	Mean	21.3	52.68	8.4	0.0	0.0	8.8	95.25
	SD	0.5	17.74	2.6	0.0	0.0	2.6	9.25
	N	23	23	23	23	23	23	23
2	Mean	21.2	51.02	9.3	0.2	0.0	10.0	93.52
	SD	0.4	13.08	2.2	0.7	0.0	2.2	11.49
	N	20	20	20	20	20	20	20
3	Mean	21.4	47.65	9.0	0.0	0.0	9.6	93.18
	SD	0.5	19.60	2.1	0.0	0.0	2.1	8.21
	N	24	24	24	24	24	24	24
4	Mean	21.4	43.60	9.1	0.0	0.0	9.5	94.79
	SD	0.6	18.35	2.3	0.0	0.0	2.2	8.20
	N	23	23	23	23	23	23	23

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

Summary of viability data (%)

Group	Summary Information	Day 0			Day 4		
		Males	Females	Total	Males	Females	Total
1	Mean	4.4	4.0	8.4	4.3	3.9	8.2
	SD	1.8	2.0	2.6	1.8	2.0	2.5
	N	23	23	23	23	23	23
2	Mean	4.7	4.6	9.3	4.7	4.5	9.2
	SD	1.7	1.7	2.2	1.7	1.7	2.2
	N	20	20	20	20	20	20
3	Mean	4.2	4.8	9.0	4.1	4.8	8.9
	SD	2.0	2.1	2.1	2.0	2.1	2.1
	N	24	24	24	24	24	24
4	Mean	3.9	5.2	9.1	3.9	5.1	9.0
	SD	1.9	2.2	2.3	2.0	2.2	2.3
	N	23	23	23	23	23	23

Summary of ovarian and uterine findings data –F1 adults

F1 Generation adults

Group 1 - Vehicle control
Group 2 - AHU377 50 mg/kg/day
Group 3 - AHU377 250 mg/kg/day
Group 4 - AHU377 750 mg/kg/day

Group	Summary Information	Total Number of Corpora Lutea	Number of Implantation Sites	Live Embryos	Dead Embryos
1	Mean	11.8	10.6	10.2	0.0
	SD	1.9	3.1	3.0	0.0
	N	21	21	21	21
2	Mean	13.4	12.2	11.4	0.0
	SD	2.5	1.7	1.5	0.0
	N	18	18	18	18
3	Mean	12.7	12.0	11.6	0.0
	SD	1.7	1.5	1.7	0.0
	N	21	21	21	21
4	Mean	12.8	12.0	11.5	0.0
	SD	1.9	1.8	1.7	0.0
	N	20	20	20	20

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

Group	Summary Information	Early Resorptions	Sum of Early Resorptions and Dead Embryos	Preimplantation Loss %	Post Implantation Loss %
1	Mean	0.4	0.4	11.05	3.05
	SD	0.7	0.7	21.25	6.04
	N	21	21	21	21
2	Mean	0.8	0.8	7.86	5.97
	SD	1.1	1.1	7.57	8.38
	N	18	18	18	18
3	Mean	0.4	0.4	5.74	3.25
	SD	0.6	0.6	5.47	5.03
	N	21	21	21	21
4	Mean	0.4	0.4	6.12	3.69
	SD	0.7	0.7	6.10	5.42
	N	20	20	20	20

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

10 Special Toxicology Studies

Study title: LCZ696: A 16-day oral (gavage) investigational study of cerebrospinal fluid drug levels and amyloid beta in the female cynomolgus monkey

Study no.:	1270586
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	12/12/2006
GLP compliance:	no
QA statement:	no
Drug, lot #, and % purity:	C0001, 94.5%

Key Study Findings

The study was conducted in 2 parts, 1) surgery, conduct of the infusions, collections of plasma, CSF and brain tissue for PK analysis, and 2) analysis of plasma, CSF and brain for newly synthesized samples for β-amyloid (Study report #1270586A) in order to examine the effects of potential inhibition of CNS neprilysin activity, by LBQ657,on β-amyloid clearance.

This study is not fully reviewed as it was not GLP and the assays discussed are not universally standardized. It was conducted because neprilysin is thought to play a key role in normal clearance of β-amyloid, and that inhibition of neprilysin could result in increased amyloid deposition in the CNS.

Acute decreases in the CSF clearance of Aβ were observed, as demonstrated by an increase in the day 1 elimination half-life of Aβ peptides (38, 40, 42), and significant increases in CSF levels of newly generated (i.e., labeled) Aβ42.

Following two weeks of dosing, the elimination half-lives of all measured Aβ peptides in the LCZ696-treated animals were no different from day 15 vehicle-treated animals and numerically similar to day 1 vehicle-treated animals. However, day 15 levels of newly synthesized Aβ (labeled) as well as total Aβ (labeled and unlabeled) were increased for all measured peptides in the CSF. Plasma levels of Aβ40 were significantly increased at 2 hr (day 16) and 12 hr post dose (day 15), and Aβ42 also showed a trend to increase at 2 and 12 hr post dose (not statistically significant).

However, brain concentrations of Aβ40 and Aβ 42 were not increased. These results are mechanistically consistent with the CNS exposure to the NEPi LBQ657 at concentrations which exceed the *in vitro* IC_{50} for human NEP and a reduced proteolytic clearance of Aβ in the CNS. As Aβ was not increased in the brain, and the elimination half-life of CSF Aβ was unchanged on day 15, Aβ was cleared from brain tissue by

other clearance pathways, which is consistent with the increased appearance of Aβ in the CSF compartment.

The absence of an increase in brain β-amyloid, in response to LCZ696 treatment, was further strengthened by analysis of brain tissue from another monkey study. Monkeys were treated 39 weeks with LCZ696 as part of a general toxicology study. Brain tissue from this study was subjected to immunostaining for neprilysin substrate β-amyloid using two antibodies, [one specific for $A\beta_{(1-42)}$ and one for total Aβ (i.e. binds Aβ37, Aβ38, Aβ40 and Aβ42) indicated there were no compound-related increases in Aβ deposition or Aβ plaque formation.

A summary, largely copied from the study report, is provided below to clarify details of the (b) (4) assay and the interpretation of the results.

Fifty four (54) animals had surgical implantation of a femoral intravenous catheter for L-leucine infusion and a cisterna magna catheter for CSF collection.

Nine animals were assigned to the pilot phase (without LCZ696 treatment), of which 8 had patent ports. Thirty-six animals were assigned to the main study.

A pilot phase consisting of infusion of a stable isotope L-Leucine followed by serial blood and CSF sampling was conducted to confirm the adequacy of timepoints/ procedures to be used in the main phase.

At least 9 days after their surgery, 9 animals were selected for a pilot phase consisting in the infusion of L-leucine and serial CSF and blood draws. The L-leucine was administered via the femoral catheter as an initial bolus of 4 mg/kg (0.6 mL/kg) over a targeted period of 10 minutes, followed by a continuous infusion of 4 mg/kg/hr (0.6 mL/kg/hr) over period of 11 hours and 50 minutes. The infusions during the pilot phase of the study were performed at approximately the same time of the day as the infusions in the main phase.

CSF samples (target volume of 200 µL) were collected as described below at the following time points: 4, 12, 16, 20, 24, 28, 32, and 36 hours post initiation of infusion. Samples were frozen on dry ice then transferred in a freezer set to maintain -80°C until shipment.

Blood samples (target volume of 1000 µL, K_2 EDTA tubes) were collected as described below at the following timepoints: 0 min (pre-infusion), 6 minutes, 2 hours, 4 hours, 8 hours, 12 hours, 14 hours, and 24 hours post initiation of infusion. Plasma was separated and frozen on dry ice then transferred in a freezer set to maintain -80°C until shipment.

CSF and blood samples were shipped to (b) (4) for analysis of Aβ concentration for each of the following Aβ isoforms: Aβ 37, 38, 40, 42 and total. (b) (4)

measured concentrations of both labeled and unlabeled Aβ from the CSF. Following the last sample collection, animals were reassigned to their intended dose group for

Main phase

The $_{13}C_6$ L-leucine infusion (b) (4) was initiated 12 hours prior to the vehicle control and/or test item (LCZ696) gavage administration on days 1 and 15

Animals were treated at approximately the same time each day, 7 days a week for a minimum of 16 days. LCZ696 dose formulations were administered by oral gavage using a disposable catheter attached to a plastic syringe at a dose volume of 5 mL/kg. Following each daily dose, the gavage tube was rinsed with 6 mL of water into the animal's stomach. Each animal was dosed with a clean gavage tube on each dosing occasion. Animals were acclimated to the oral gavage procedure for at least 3 days prior to the commencement of dose formulation administration.

(b) (4) study overview

The amyloid beta stable isotope labeling kinetic (b) (4) assay is a pulse-chase type assay, wherein a labeled amino acid (stable isotope labeled $^{13}C_6$ leucine) is infused into the subject and the incorporation of the labeled amino acid into newly synthesized proteins is measured.

Leucine is an essential amino acid, which undergoes active transport into the brain. $^{13}C_6$ leucine is non-radioactive, harmless to humans and the environment, and is physically identical to unlabeled ($^{12}C_6$) leucine except for a 6 dalton difference in the mass of the amino acid. Since $_{13}C_6$ leucine is chemically identical to $_{12}C_6$ leucine, it is incorporated into newly synthesized proteins in the ratio that it is present in the system. All newly synthesized proteins will thus have a ratio of $^{13}C_6$ leucine to $^{12}C_6$ leucine that matches the ratio of these two isotopes of leucine in the system. As the labeled and unlabeled leucine is incorporated into newly synthesized proteins, the ratio of labeled to unlabeled protein in the biological sample will change. This ratio of labeled to unlabeled protein is called the tracer ($^{13}C_6$ leucine) to tracee ($^{12}C_6$ leucine) ratio or TTR. Since the maximal possible TTR for a protein depends on the ratio of labeled to unlabeled free leucine in that individual, we normalize the TTR of proteins based on the ratio of labeled to unlabeled leucine that was observed in the plasma during the $^{13}C_6$ leucine infusion period. The free leucine TTR is measured using GC/MS.

(b) (4) (b) (4)

The TTR and concentration of Aβ was measured using a combination of immunoprecipitation and mass spectrometry (IP/MS). The immunoprecipitation step is used to separate the Aβ from the complex mixture of proteins present in the CSF. The

TTR is measured using a mass spectrometer – the only instrument that can distinguish $^{13}C_6$ leucine containing peptides from $^{12}C_6$ leucine containing peptides. The mass spectrometer is set up to specifically monitor for the various Aβ peptides in the $^{12}C_6$ and $^{13}C_6$ leucine forms, as well as the quantitation peptide form, and the ion intensities of the peptides are used to calculate the TTR and the concentration. Table 1 shows a list of the Aβ peptides monitored by the mass spectrometer.

Table 1: Aβ peptides monitored by (b) (4)

Endopeptidase cleavage sites are shown with underscores (_). The monitored peptides are shown in bold. The leucines are highlighted in yellow. The Aβ total peptide is common to all Aβ species that contain the Aβ 16-27 domain.

(b) (4)

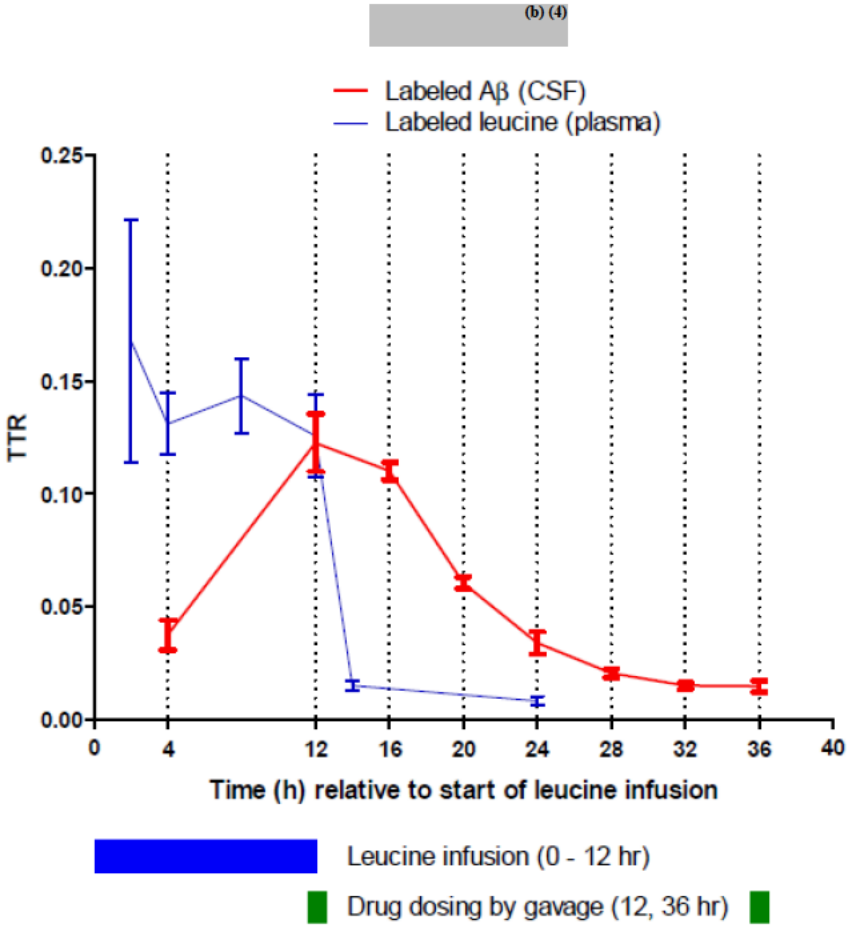
(b) (4)

Main phase study design

This study was designed to measure the effect of LCZ696 on the metabolism of Aβ as well as the concentrations of Aβ in the CSF of cynomolgus monkeys. For this purpose $^{13}C_6$ leucine was infused IV over 12 hours starting at 8PM (Figure 1). For Aβ analysis, CSF was collected at 4, 12, 16, 20, 24, 28, 32, and 36 hours after the start of the $^{13}C_6$ leucine IV infusion. For free leucine analysis, plasma samples were collected pre infusion (0 minutes) as well as 6 minutes and 2, 4, 8, 12, 14, and 24 hours after infusion. LCZ696 was given by oral gavage at 12 hours post $^{13}C_6$ leucine infusion (Figure 1).

Figure 1: Stable isotope labeling kinetics study design

All times shown are relative to start of leucine infusion. Leucine (blue line) is infused for 12 hours, illustrated by blue bar under the graph. Labeled leucine incorporation into Aβ is measured in CSF (red line). Drug or placebo was given by gavage at hour 12 and approximately every 24 hours, illustrated by the green bar under the graph. Graph shows average data from all the pilot study animals with error bars showing the standard deviation.



Stable isotope labeling kinetics and stable isotope spike absolute quantitation was used to measure the metabolic incorporation of $^{13}C_6$ leucine into Aβ in CSF. $^{13}C_6$ leucine infusion studies were run at the time of the first dose administration (acute phase, day 1) as well as after 15 days of daily dosing (we will refer to this phase as the chronic phase in this report although the 15 days of dosing may be better characterized as sub chronic).

A total of 52 monkeys were enrolled in the main phase study but by the time of initial dosing only 36 animals had CSF catheters that remained patent. Animals were assigned to one of two treatment arms: group no. 1 (n=18) was given placebo while group no. 2 (n=18) was given 50 mg/kg LCZ696. Dosing was given daily around 8AM by oral gavage. For the acute phase (day 1), 18 animals had patent catheters in group 1 and 16 animals had patent catheters in group 2. For the chronic phase, 17 animals had patent catheters in group 1 and 15 animals had patent catheters in group 2.

Acute decreases in the CSF clearance of Aβ were observed, as demonstrated by an increase in the day 1 elimination half-life of Aβ peptides (38, 40, 42), and significant increases in CSF levels of newly generated (i.e., labeled) Aβ42. Following two weeks of dosing, the elimination half-lives of all measured Aβ peptides in the LCZ696-treated animals were no different from day 15 vehicle-treated animals and numerically similar to day 1 vehicle-treated animals. However, day 15 levels of newly synthesized Aβ (labeled) as well as total Aβ (labeled and unlabeled) were increased for all measured peptides in the CSF. Plasma levels of Aβ40 were significantly increased at 2 hr (day 16) and 12 hr post dose (day 15), and Aβ42 also showed a trend to increase at 2 and 12 hr post dose (not statistically significant). However, brain concentrations of Aβ40 and Aβ 42 were not increased.

These results are mechanistically consistent with the CNS exposure to the NEPi LBQ657 at concentrations which exceed the *in vitro* IC_{50} for human NEP and a reduced proteolytic clearance of Aβ in the CNS. As Aβ was not increased in the brain, and the elimination half-life of CSF Aβ was unchanged on day 15, Aβ was cleared from brain tissue by other clearance pathways, which is consistent with the increased appearance of Aβ in the CSF compartment.

Study title: AHU377: An oral (gavage) toxicity study in the juvenile rats

Study no.:	0870734
Study report location:	eCTD, NDA 207620, SDN 0001
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	7/6/2009
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	0722008, 98.9%

Key Study Findings

Treatment of juvenile rats from days 7 through 70 *post partum at* doses of 100, 400 and/or 800 mg/kg/day, resulted in transient clinical signs, effects on body weight, food consumption and phosphorous levels. At the end of recovery, mild to marked increases in glucose and triglycerides for males and alanine aminotransferase and/or aspartate aminotransferase were observed in a few females at a dose of 800 mg/kg/day. Equivocal microscopic changes in the stomach of rats (hyperplasia and vacuolation of the squamous mucosa) in both sexes at doses ≥ 100 mg/kg/day were noted at the end of treatment, and at completion of the recovery period, hyperplasia of the squamous mucosa was observed in the stomach of rats with a minimally increased incidence noted at doses ≥ 400 mg/kg/day.

AHU377 administration resulted in generally dose dependent and slight decreases in bone length (femur and tibia) in animals at a dose of 800 mg/kg/day. Treatment related effects were noted on the bone mass (BMD and BMC) at the metaphysis (at ≥ 400 mg/kg/day for both males and females) and diaphysis (at ≥ 400 mg/kg/day for males and at 800 mg/kg/day for females).

At the end of the recovery period, the bone length, diameter and mass were generally comparable to vehicle controls. Based on the findings in this study, the No Observed Adverse Effect Level (NOAEL) was considered to be 100 mg/kg/day.

Methods

Doses:	100, 400, 800 mg/kg
Frequency of dosing:	daily
Route of administration:	gavage
Dose volume:	6.5 (groups 1 and 4) or 5 (groups 2 and 3) mL/kg/day
Formulation/Vehicle:	0.5% (w/v) hydroxypropylcellulose
Species/Strain:	Rat pups, Wistar Hannover Crl:WI (Han)
Number/Sex/Group:	See chart below
Age:	Pups were on day 7 *post partum* at the start of treatment.
Weight:	10.5 to 18.9 g at the start of treatment.
Deviation from study protocol:	Deviations did not affect the overall integrity of the data or the overall conclusions of the study.

Main study:

Group number identification	Dose level (mg/kg/day)	Dose conc. [a] (mg/mL) base/salt	Dose volume (mL/kg/day)	Minimum number of litters [d]	Number of pups			
					Main study[b]		Pathology subset[c]	
					Males	Females	Males	Females
1/ Vehicle control	0	0	6.5	13	20+20	20+20	12	12
2/ AHU377	100	20/20.9	5	13	20+20	20+20	12	12
3/ AHU377	400	80/83.7	5	13	20+20	20+20	12	12
4/ AHU377	800	123/128.7	6.5	13	20+20	20+20	12	12

a (Dose concentrations were not corrected for purity.) The salt:base ratio for AHU377 was 1.046.

b Main study included a reproduction and recovery subset: 20/sex/group were assigned to fertility assessments (reproduction subset) and 20/sex/group were assigned to behavioral and clinical pathology, gross and histopathological assessments (recovery subset).

c Animals assigned to the pathology subset underwent clinical pathology assessments during the last week of treatment and had gross and histopathological assessments.

d Dosed spares were included with all dose groups. Pups from the spare litters were assigned to groups and treated (2 litters/group). Any dosed spares that were not used by weaning were euthanized and discarded without examination.

Observations and Results

Mortality and Clinical Signs

A litter check was conducted daily until weaning. After weaning, animals were observed twice daily for mortality and signs of ill health or reaction to treatment

Pre-weaning: Several pups from the 100 and 800 mg/kg/day dose groups were found dead or euthanized between days 8 and 13 *post partum* due to gavage accident (nos. 251-2, 254-1, 255-3 (100 mg/kg/day) and 459-8 (800 mg/kg/day)). The causes of deterioration for pup nos. 256-8, 451-6 and 459-2 euthanized between days 8 and 11 *post partum* were undetermined. Body weight gains were decreased in some of these animals.

Post weaning: There was no mortality attributed to the administration of AHU377.

Clinical signs noted that were considered to be AHU377-related were a firm internal abdominal structure, wet fur and salivation. A firm internal abdominal structure was seen for both males and females primarily in animals at a dose of 800 mg/kg/day, commencing during the first week of treatment and generally abating by the end of the second week of dosing. Wet fur and salivation commenced post dose in animals given 400 and 800 mg/kg/day on day 21 *post partum* and continued through the treatment period.

Body Weights

Individual body weight was measured on day 4 *post partum* for randomization. In addition to the assessment for randomization, the pups were weighed individually, each day from days 7 to 21 *post partum* and then twice weekly thereafter until termination, and on the day of scheduled euthanasia.

Mated females assigned to the fertility phase were weighed on gestation days 0, 3, 6, 9 and 13. Toxicokinetic pups dosed once on day 7 *post partum* were weighed on days 4 and 7 *post partum.*

Pre-weaning period:
Daily body weight gains were significantly decreased in a dose dependent manner between days 7 and 11 *post partum* in both sexes in animals given 400 and 800 mg/kg/day and continuing until day 14 *post partum* at a dose of 800 mg/kg/day. These decreased body weight gains resulted in statistically significantly lower body weights for males and females from day 8 *post partum,* until day 19 *post partum* for animals given 400 mg/kg/day and lasting until weaning for animals given 800 mg/kg/day.

On day 21 *post partum*, the F_1 generation pups were separated from their dams and were housed 2 or 3 per sex/cage, according to dose level, until day 28 *post partum* and individually thereafter.

Post weaning period:
Overall, in all subsets, body weight gains were generally decreased in animals given 100, 400 and 800 mg/kg/day up to day 35 *post partum* for females and day 42 *post partum* for males, subsequently attaining levels comparable to controls.

Feed Consumption

Food consumption was measured on main study animals twice weekly from day 28 *post partum* onwards on days of body weight assessment until termination or, if selected for fertility assessment, until cohabitation. Mated females assigned to the reproductive subset had food consumption measured from days 0 to 3, 3 to 6, 6 to 9 and 9 to 13 of gestation.

Food intake in the treated groups, particularly in animals given 800 mg/kg/day, tended to be higher initially for the females and generally comparable to or higher than controls for the males commencing around day 52 during the post-weaning treatment period.

Overall values tended to be increased at a dose of 800 mg/kg/day in both sexes compared to control values for the post dosing period.

The food consumption in the reproductive subset females during gestation was unaffected.

Visual function
On day 21 *post partum*, the pupillary closure and visual placing responses were assessed on all main study animals.

There was no AHU377-related effect on visual placing or pupillary closure.

Physical development
On all main study animals, eye opening was assessed from days 14 to 17 *post partum.* Vaginal opening was assessed from day 26 *post partum* until development for females and preputial separation was assessed from day 35 *post partum* until development for males.

There was no AHU377-related effect on eye opening, vaginal opening or preputial separation.

Auditory startle
At day 28 (±1) *post partum*, the startle habituation (San Diego Instruments) was measured. The animals were given a 4-minute acclimation period and then the startle response was measured in 50 identical trials at a sound level of 120dBA with an 8-second inter-trial interval.

There was no AHU377-related effect on auditory startle habituation.

Motor activity
Locomotor activity was assessed for 1 hour in a figure 8 enclosure on day 91 (±5) *post partum*. Animals from the control and treated groups were balanced across chambers using a randomization procedure. The sessions were of 1 hour's duration and were of six 10-minute intervals. The sound level was kept constant at approximately 70dBA in the test room using exterior white noise generation. Room illumination was approximately 600 to 800 Lux.

There was no AHU377-related effect noted on motor activity.

'E' water maze
The 'E' water maze assessments were conducted commencing on day 98 (±5) *post partum*. The learning and memory tests were conducted using an 'E' water maze. The time to exit the maze and the number of errors (incorrect turns) were recorded for 5 tests, each of a maximum time of 1 minute on the first day of testing. Each test was performed at least 15 minutes apart. On the following day, two tests were performed (at

least 25 hours after the end of the first days' trials). Any abnormality in swimming was recorded.

There were no AHU377-related differences noted for performance in the 'E' water maze.

Peripheral quantitative computed tomography (pQCT)
In vivo
Peripheral QCT was performed on the right proximal tibia on day 28 and 105 (±1 day) *post partum* on the first ten surviving animals/sex/group assigned to the recovery subset.

Peripheral QCT was used to measure bone mineral content, bone mineral density, and geometric parameters of the right proximal tibia.

In vivo
The metaphysis scans were evaluated for area, bone mineral content and bone mineral density of the total slice and the trabecular and cortical/subcortical regions. The scans obtained at the diaphysis site were evaluated for cortical bone mineral content, cortical bone mineral density, total area, cortical area, cortical thickness, periosteal circumference and endosteal circumference.

Ex vivo
Peripheral QCT scans were performed *ex vivo* using an XCT Research SA or SA + bone scanner with software version 5.50D. Scans were obtained for the excised left tibia for all animals assigned to the pathology subset surviving to scheduled termination. A single scan was obtained of the proximal tibia metaphysis with an additional site in the diaphysis. The diaphysis site was analyzed using Cortmode 2 for cortical bone measurements. The exact position of the scan slices were documented in the raw data. All analyses was performed using the LOOP option of the analysis software. All other data generated as a result of the LOOP option was retained with the raw data but not reported. The most appropriate analysis mode for the metaphysis was used and documented in the raw data and the analysis details were included in the final report. All scan analysis data was retained.

Scanning parameters for excised left tibia were the same as the in vivo scanning parameters.

In vivo
Radiographs were performed on day 28 and day 105 (±1 day) *post partum* on the first ten surviving animals/sex/group assigned to the recovery subset, and measurements of the femur, tibia and axial skeleton were derived from the 2 radiographs taken from each animal. Radiographs were obtained while animals were under isoflurane anesthesia.

Ex vivo
Radiographs were performed *ex vivo* from the femur (right) and tibia (left) collected at scheduled necropsy from the pathology subset, and measurements of the femur and tibia were derived from 2 radiographs taken for each specimen.

Proximal tibia metaphysis
No test article related effect was noted in animals given 100 mg/kg/day.

On day 28 *post partum*, males and females at doses of 400 and 800 mg/kg/day had slightly lower (-7 to -20% and -6 to -19%, respectively, for total, trabecular and cortical/subcortical) bone mineral content (BMC) and bone mineral density (BMD) when compared to controls. The differences attained statistical significance for all parameters at 800 mg/kg/day and for total and cortical/subcortical BMD at a dose of 400 mg/kg/day. Slightly lower values were noted for the total slice area at a dose of 800 mg/kg/day.

In males at the end of the treatment period, no meaningful effect was noted at doses of 100 and 400 mg/kg/day, suggesting reversibility of effects at a dose of 400 mg/kg/day. However the mean BMC and BMD values remained lower (up to 13%) for AHU377-treated males compared to vehicle controls at a dose of 800 mg/kg/day, attaining statistical significance for cortical/subcortical and total BMC and BMD.

In females at the end of the treatment period, the mean BMC and BMD values for AHU377-treated animals were generally lower compared to vehicle controls at all dose levels, attaining statistical significance for total and trabecular BMC and BMD at a dose of 800 mg/kg/day, total and trabecular BMC at a dose of 400 mg/kg/day and total BMD and trabecular BMC and BMD at a dose of 100 mg/kg/day.

At the end of the recovery period, effects on pQCT parameters were generally normalized for bone densitometry values for all dose levels, however, trabecular BMC and BMD remained slightly lower compared to vehicle controls at doses of 400 and 800 mg/kg/day (6 - 16%-attaining statistical significance for BMC at 800 mg/kg/day). All other differences including slightly lower total and cortical/subcortical BMC at doses of 100 and 400 mg/kg/day were attributed to slightly lower total slice area.

Tibia diaphysis
On day 28 *post partum*, statistically lower periosteal circumference and total slice area (5 and 10% respectively) were noted for males at a dose of 800 mg/kg/day compared to vehicle controls. Endosteal circumference was also proportionally lower (6%) for treated males compared to vehicle control males, resulting in comparable cortical thickness. The mean cortical area and cortical BMC were also slightly lower, attaining statistical significance for cortical BMC, which was consistent with lower values for periosteal circumference and total slice area. A greater decrease in BMC, relative to cortical area, was associated with significantly lower (4%) cortical BMD. For males at a dose of 400 mg/kg/day, slightly (but statistically significant) lower values were noted for cortical BMD, consistent with the statistically lower cortical BMC value. Cortical thickness was slightly lower (5%) and associated with marginally higher endosteal circumference (2%)

and marginally lower (1%) periosteal circumference. No meaningful effect was noted at a dose of 100 mg/kg/day.

At the end of the treatment period in males, the effects on bone size and bone mass were generally reversed at a dose of 400 mg/kg/day. Evidence for normalization on bone mass and bone size (length and diameter) were also noted at a dose of 800 mg/kg/day. However the mean cortical BMC and BMD remained statistically significantly lower at a dose of 800 mg/kg/day when compared to vehicle controls. The mean cortical thickness and cortical area were also slightly lower and were attributed to the marginally lower periosteal circumference and higher endosteal circumference. No meaningful effect was noted at a dose of 100 mg/kg/day, except slightly lower cortical thickness attributed to slightly higher endosteal circumferences.

In females at a dose of 800 mg/kg/day at the end of the treatment period, the mean BMC and cortical area remained slightly lower (but still statistically significant) compared to vehicle controls. The mean cortical thickness was slightly lower (4%) and was attributed to marginally lower periosteal circumference and higher endosteal circumference. At the end of the treatment period, similar responses were also noted females at doses of 100 and 400 mg/kg/day. The mean cortical area, cortical BMC and cortical thickness were lower for treated females compared to vehicle controls, generally attaining statistical significance (except cortical area at a dose of 100 mg/kg/day). Similar to males, these effects seemed transient and evidence of recovery were noted for both bone mass and bone geometry parameters at the end of the treatment period.

No meaningful effect was noted at the end of the recovery period in males. At the end of the recovery period in females, the mean bone densitometry and bone geometry parameters were comparable to vehicle controls.

Radiographs
On day 28 *post partum*, mean bone length values for femur and tibia (left and right) were slightly lower (maximum of -4%) in treated males and females, attaining statistical significance at a dose of 800 mg/kg/day. Slightly lower values were also noted in the width of the long bones in animals (females up to -5%) at a dose of 800 mg/kg/day when compared to controls. Effects on the axial skeleton were limited to a slight reduction in length in males and females and in width only in females at a dose of 800 mg/kg/day.

At the end of the treatment period, a trend towards normalization was evident in both males and females when compared to controls although mean bone length was still minimally lower in males (-2%) and females at a dose of 800 mg/kg/day.

At the end of recovery, males at a dose of 800 mg/kg/day were completely recovered when compared to controls, although some differences persisted in the females.

The statistically significantly lower value for the right tibia width at the end of the recovery period in females was not consistent with the observations at doses of 100 or 800 mg/kg/day and, therefore, was likely incidental in nature.

Bone measurements
At the end of treatment, femur length was significantly lower for males (-2%) and slightly lower for females at a dose of 800 mg/kg/day attaining statistical significance only for the left femur of the males. Slightly lower femur width (-4%) was noted in males at a dose of 800 mg/kg/day.

At the end of recovery, there was no apparent effect noted in males, although in females the decreases in bone length persisted slightly (femur and tibia).

Reproductive phase (reproductive subset)

Mating procedures (F_1 adult generation)
At approximately 105 days of age, half of the main study animals were subjected to mating procedures whereby 1 female was placed with 1 male (sibling matings were avoided) in the same dosage group for up to 14 days. The females were examined for mating by examination of the vaginal lavage for spermatozoa and/or presence of vaginal plug. The day of positive identification of spermatozoa and/or presence of vaginal plug was termed day 0 of gestation. All unmated females were placed in solid-bottomed plastic cages at the end of the mating period.

No significant differences were noted between groups in terms of days to mating, the conception rate or mating and fertility indices.

Toxicokinetics
Toxicokinetic phase
On day 7 *post partum*, 4 pups/sex/treated group/time point and 2 control pups/sex/time point were bled at 0.5 1, 2, 6 and 24 hours after dose administration. Following anesthesia by an intraperitoneal injection of sodium pentobarbital, as much blood as possible was collected from the *vena cava* using a butterfly, needle and syringe apparatus. Blood samples were pooled for each sex/group/time point into tubes containing K_2-EDTA and placed on ice. Blood was collected once only from each animal because the blood collection exsanguinated the animal. The carcasses were discarded without further examination.

TK - Main study (recovery subset)
During the last week of dosing, blood was taken from 3 animals/sex/group/time point from the main study (recovery subset) at the following time points: 0.5, 1, 2, 6 and 24 hours post dose. At each time point, approximately 0.5 mL of whole blood was collected from the jugular vein into tubes containing K_2-EDTA and approximately 15 mg of sodium fluoride and placed on ice.

Hematology

The following parameters were assessed (using lithium heparin and citrate* as anticoagulant): activated partial thromboplastin time*, hematocrit, red blood cell count (erythrocyte count), blood cell morphology, hemoglobin, reticulocyte count (absolute and percent), erythrocyte indices (MCV, MCH and MCHC), platelet count, white blood cell count (total, absolute and percent differential), fibrinogen*, prothrombin time*.

Blood smears were prepared and those from high dose and controls were reviewed for any changes in morphology of red or white blood cells and platelets.

There were no test article-related changes in hematology parameters associated with administration of AHU377 at doses of 100, 400 or 800 mg/kg/day in males and females.

Clinical Chemistry

The following parameters were assessed:

A/G ratio (calculated)	chloride	magnesium
alanine aminotransferase	cholesterol	potassium
albumin	creatinine	sodium
alkaline phosphatase	creatine kinase	total bilirubin
aspartate aminotransferase	globulin (calculated)	total protein
blood urea nitrogen	glucose	triglycerides
calcium	inorganic phosphorus	

AHU377-related changes in clinical biochemistry were observed at doses ≥ 100 mg/kg/day compared to controls.

There were minimal increases in urea (UREA, 14% to 17%) in males and females at a dose of 800 mg/kg/day. There were mild non dose-related increases in phosphorus (PHOS, 13% to 20%) in males and females at all dose levels.

At the end of recovery there were no important changes in PHOS, indicating reversibility of the changes previously described for this parameter. There was a persistent mild increase in urea (16%) in males at a dose of 800 mg/kg/day. There were mild increases in glucose (40%) and triglycerides (55%) in males at a dose of 800 mg/kg/day. These changes were not observed during the treatment period, but were dose-related and considered AHU377-related. Moderate to marked increases in aspartate aminotransferase and mild increases in alanine

Urinalysis

The following parameters were assessed:

appearance	color	pH
bilirubin	glucose	proteins
blood	ketones	specific gravity

There were no test article-related changes in urinalysis parameters associated with administration of AHU377 at all doses tested in males and females.

Gross Pathology

Any pups found dead or euthanized during the pretreatment period (between days 0 to 6 *post partum*) were discarded without examination. At weaning, any spare treated pups unassigned to a study group were euthanized and the carcasses discarded without further examination. Treated pups from the main study and pathology subsets found dead or euthanized for humane reasons were subject to necropsy and tissue samples were preserved, where applicable. Before necropsy, the carcasses were stored at approximately 4°C.

All F_1 generation animals euthanized at scheduled termination (approximately on day 71 *post partum* for pathology subset, up to 3 weeks following the mating period for the reproductive subset, and after completion of behavioral assessments for the recovery subset), underwent exsanguination following CO_2 asphyxiation or anesthesia via isoflurane and blood collection, as applicable. In order to avoid autolytic change, a complete gross pathology examination of the carcass was conducted immediately on all euthanized animals.

Terminal body weight was recorded at scheduled necropsy for pathology and for the first 10 surviving animals/sex/group in the recovery subset. All necropsies were conducted under the supervision of a pathologist and necropsy consisted of an external examination, including identification of all clinically recorded lesions, as well as a detailed internal examination.

On day 13 of gestation, the female rats assigned to the fertility phase (reproductive subset) were evaluated in random order. The reproductive tract was dissected out, the ovaries removed, the corpora lutea counted. The uterine contents were examined and the number and position of live embryos, dead embryos and resorptions were recorded. Females not detected as having mated were euthanized at least 7 days after the end of the mating period and were examined in a similar manner as those found dead.

Histomorphometry

There were no microscopic abnormalities detected in the proximal tibia of the pathology and recovery subsets. The administration of AHU377 had no effects on the growth plate thickness in two subset groups (pathology and recovery). All variations noted were regarded as normal biological variations.

No macroscopic findings were considered to be related to treatment with AHU377 following the recovery period.

Tissue list for histopathology

abnormalities		sciatic nerve
animal identification [a]	kidneys	seminal vesicles
adrenals	lacrimal glands	skeletal muscle
aorta (thoracic)	larynx (1 level)	skin (inguinal)
bone (femurs and tibiae) [b,g]	liver (sample of 2 lobes)	spinal cord (cervical, thoracic and lumbar)
bone and marrow (sternum) [b]		
brain (forebrain, midbrain, cerebellum and medulla oblongata)	[d]lungs (sample of 2 lobes)	spleen
cecum	lymph nodes (mandibular, unilateral; mesenteric)	stomach
colon	mammary gland (thoracic and inguinal) [e,f]	testes [c]
duodenum	[a,d] nasal cavities (1 level)	thymus
epididymides [e]	optic nerves [e, c]	thyroid lobes (and parathyroids) [e]
esophagus	ovaries, oviducts [h]	tongue
eyes [c]	pancreas	trachea
harderian glands	pituitary	ureters [h]
heart (including section of aorta)	prostate	urinary bladder
ileum	rectum [a]	uterus (horns, body and cervix)
jejunum	salivary gland [h] (mandibular, parotid, sublingual)	vagina

a Retained but not processed.

b Bone decalcified prior to sectioning.

c Euthanized animals only: eyes and optic nerves fixed in Davidson's fluid and epididymides and testis fixed in modified Davidson's fluid.

d Infused with neutral buffered 10% formalin (all animals).

Organ Weights

For each animal from the pathology subset and from the first 10 surviving animals/sex/group in the recovery subset euthanized at completion of the observation period, the following organs were dissected free of fat and weighed:

adrenals	ovaries	testes
brain	pituitary*	thymus
heart	prostate	thyroid and parathyroid*
kidneys	spleen	uterus
liver		

* weighed post fixation

Paired organs were weighed together and organ weight ratios relative to body weights and to brain weight were calculated.

No organ weight changes were considered to be related to treatment with AHU377 in either examined subset.

Histopathology

Adequate Battery

Yes

Peer Review

The pathology report and histopathological examinations were peer reviewed by a Novartis pathologist, including review of selected tissue samples.

Histological Findings

There were no LCZ696-related gross findings.

Histopathological examination revealed a possible exacerbation of microscopic changes in the stomach of rats of both sexes at ≥ 100 mg/kg/day, as summarized in the table below.

In the stomach, there was a slight increase in incidence of hyperplasia of the squamous mucosa that was localized at the limiting ridge in all treated groups, with no dose-response compared to the control rats. In a few animals, one additional change at the same location was recorded as vacuolation and was usually seen in combination with submucosal inflammation. Because these changes (hyperplasia and vacuolation, squamous mucosa) were also observed in one control rat (no. 1951), the slightly increased, although comparable incidence observed in all treated groups was suggestive of an exacerbation by the test-article of a change seen associated with gavage administration.

Microscopic changes were still observed in the stomach of rats euthanized at completion of the recovery period. Minimal to slight hyperplasia of the squamous mucosa was observed in both controls and/or treated male and female rats with a low incidence.

Summary of bone densitometry data by peripheral quantitative computed tomography

F1 Generation - Recovery subset
Right proximal tibia - metaphysis site
Day 28 post partum
Males

		Total			Trabecular		Cortical/Subcortical		
Group	Summary Information	Area mm²	BMC mg/mm	BMD mg/cm³	BMC mg/mm	BMD mg/cm³	BMC mg/mm	BMD mg/cm³	
1	Mean	9.977	3.815	381.37	1.308	260.52	2.504	502.80	
	SD	0.874	0.478	20.04	0.200	21.86	0.298	27.80	
	N	10	10	10	10	10	10	10	
2	Mean	9.844	3.804	386.50	1.317	266.95	2.487	506.60	
	SD	0.879	0.391	18.27	0.178	24.27	0.222	15.09	
	N	10	10	10	10	10	10	10	
3	Mean	9.759	3.421	350.15 B	1.159	236.10	2.262	464.85	C
	SD	0.777	0.330	11.72	0.158	16.88	0.176	11.55	
	N	10	10	10	10	10	10	10	
4	Mean	9.141	3.177 B	346.64 C	1.042 A	225.34 B	2.137 B	468.47	B
	SD	0.971	0.477	22.37	0.230	27.96	0.249	22.55	
	N	10	10	10	10	10	10	10	

BMC: Bone mineral content BMD: Bone mineral density
Significantly different from control group (group 1) value: A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Summary of bone densitometry data by peripheral quantitative computed tomography

F1 Generation - Recovery subset
Right proximal tibia - diaphysis site
Day 28 post partum
Males

		Total	Cortical					
Group	Summary Information	Area mm²	Area mm²	BMC mg/mm	BMD mg/cm³	THICK mm	PERI_C mm	ENDO_C mm
1	Mean	2.802	1.606	1.413	881.13	0.3271	5.9288	3.8729
	SD	0.268	0.116	0.118	22.50	0.0130	0.2937	0.2801
	N	10	10	10	10	10	10	10
2	Mean	2.768	1.551	1.351	870.65	0.3173	5.8925	3.8989
	SD	0.228	0.098	0.096	25.55	0.0233	0.2436	0.3245
	N	10	10	10	10	10	10	10
3	Mean	2.760	1.522	1.278 A	839.63 C	0.3093	5.8870	3.9430
	SD	0.185	0.137	0.139	22.32	0.0253	0.2031	0.1893
	N	10	10	10	10	10	10	10
4	Mean	2.522 A	1.470	1.240 B	845.70 B	0.3175	5.6244 A	3.6292
	SD	0.235	0.092	0.096	22.60	0.0182	0.2612	0.3144
	N	10	10	10	10	10	10	10

BMC: Bone mineral content BMD: Bone mineral density ENDO: Endosteal circumference
PERI: Periosteal circumference THICK: Thickness
Significantly different from control group (group 1) value: A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Summary of bone densitometry data by peripheral quantitative computed tomography

F1 Generation - Recovery subset
Right proximal tibia - metaphysis site
Day 28 post partum
Females

		Total			Trabecular		Cortical/Subcortical	
Group	Summary Information	Area mm²	BMC mg/mm	BMD mg/cm³	BMC mg/mm	BMD mg/cm³	BMC mg/mm	BMD mg/cm³
1	Mean	8.208	3.036	369.64	0.943	228.39	2.094	511.71
	SD	0.542	0.286	18.79	0.144	26.94	0.161	17.13
	N	10	10	10	10	10	10	10
2	Mean	8.471	3.184	374.44	1.006	235.94	2.177	512.87
	SD	1.097	0.544	16.41	0.215	22.29	0.332	13.03
	N	10	10	10	10	10	10	10
3	Mean	8.299	2.837	341.92 A	0.864	207.85	1.973	476.24 B
	SD	0.688	0.304	22.83	0.112	18.88	0.200	30.39
	N	10	10	10	10	10	10	10
4	Mean	7.653	2.538 A	331.26 C	0.766 A	199.46 A	1.771 A	463.91 C
	SD	0.787	0.335	24.41	0.127	22.85	0.217	28.02
	N	10	10	10	10	10	10	10

BMC: Bone mineral content BMD: Bone mineral density
Significantly different from control group (group 1) value: A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Summary of bone densitometry data by peripheral quantitative computed tomography

F1 Generation - Recovery subset
Right proximal tibia - diaphysis site
Day 28 post partum
Females

		Total	Cortical					
Group	Summary Information	Area mm²	Area mm²	BMC mg/mm	BMD mg/cm³	THICK mm	PERI_C mm	ENDO_C mm
1	Mean	2.562	1.525	1.352	887.48	0.3296	5.6693	3.5991
	SD	0.236	0.082	0.096	23.19	0.0207	0.2633	0.3481
	N	10	10	10	10	10	10	10
2	Mean	2.620	1.588	1.394	878.93	0.3418	5.7290	3.5812
	SD	0.329	0.118	0.112	23.72	0.0244	0.3636	0.4490
	N	10	10	10	10	10	10	10
3	Mean	2.550	1.490	1.300	872.13	0.3204	5.6613	3.6490
	SD	0.091	0.075	0.083	22.97	0.0202	0.1019	0.1833
	N	10	10	10	10	10	10	10
4	Mean	2.274	1.391 A	1.176 B	844.64 C	0.3253	5.3316	3.2872
	SD	0.344	0.122	0.127	25.09	0.0371	0.4197	0.5825
	N	10	10	10	10	10	10	10

BMC: Bone mineral content BMD: Bone mineral density ENDO: Endosteal circumference
PERI: Periosteal circumference THICK: Thickness
Significantly different from control group (group 1) value: A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Toxicokinetics

Toxicokinetic Study (single dose)

Group number identification	Dose level (mg/kg/day)	Dose conc. [a] (mg/mL) base/salt	Dose volume. (mL/kg/day)	Minimum number of litters	Day 7 post partum	
					Males	Females
1/ Vehicle control	0	0	6.5	3	10	10
2/ AHU377	100	20/20.9	5	5	20	20
3/ AHU377	400	80/83.7	5	5	20	20
4/ AHU377	800	123/128.7	6.5	5	20	20

a (Dose concentrations were not corrected for purity.) Salt/base ratio for AHU377 was 1.046.

Following analysis of plasma samples from the control group, none of the control group samples showed quantifiable AHU377 or LBQ657 concentrations. The plasma profiles showed that all rats in the AHU377 treatment groups were exposed to AHU377 and/or LBQ657 with mean Tmax values ranging from 0.5 to 1 h post dose (AHU377) and 0.5 to 6 h post dose (LBQ657).

Following single or multiple oral doses of AHU377, no consistent difference in exposure to AHU377 or LBQ657 was observed between male and female rats.

Toxicokinetic parameters for AHU377 in rat plasma

Dose (mg/kg/day)	Study day	Gender	AUC (ng*h/mL)	±SE (ng*h/mL)	AUC/Dose (ng*h/mL)/ (mg/kg/day)	±SE/Dose (ng*h/mL)/ (mg/kg/day)	C_{max} (ng/mL)	C_{max}/Dose (ng/mL)/ (mg/kg/day)	T_{max} (h)
100	64	Male	874	198	8.74	1.98	252	2.52	0.500
		Female	402	125	4.02	1.25	226	2.26	1.00
400	64	Male	4590	1320	11.5	3.30	610	1.53	0.500
		Female	5360	1260	13.4	3.15	1010	2.53	0.500
800	64	Male	16300	6140	20.4	7.68	5440	6.80	0.500
		Female	12100	2890	15.1	3.61	1340	1.68	0.500

Toxicokinetic parameters for LBQ657 in rat plasma

Dose (mg/kg/day)	Study day	Gender	AUC (ng*h/mL)	±SE (ng*h/mL)	AUC/Dose (ng*h/mL)/ (mg/kg/day)	±SE/Dose (ng*h/mL)/ (mg/kg/day)	C_{max} (ng/mL)	C_{max}/Dose (ng/mL)/ (mg/kg/day)	T_{max} (h)
100	7	Male	435000	NC	4350	NC	51000	510	0.500
		Female	309000	NC	3090	NC	43900	439	0.500
	64	Male	46600	19700	466	197	10400	104	0.500
		Female	37300	15800	373	158	12200	122	1.00
400	7	Male	1780000	NC	4450	NC	145000	363	1.00
		Female	1990000	NC	4980	NC	202000	505	2.00
	64	Male	157000	44200	393	111	19300	48.3	0.500
		Female	207000	52500	518	131	27000	67.5	0.500
800	7	Male	3240000	NC	4060	NC	248000	310	2.00
		Female	3200000	NC	4000	NC	213000	266	6.00
	64	Male	465000	131000	581	164	75000	93.8	1.00
		Female	802000	273000	1000	341	77500	96.9	0.500

Dosing Solution Analysis

Deviations were considered to have had no impact on the study results

11 Integrated Summary and Safety Evaluation

Introduction

LCZ696 is a salt complex comprising sacubitril (AHU377, a new-molecular entity) and valsartan, sodium cations, and water molecules in the molar ratio of 1:1:3:2.5 (ratio of 6:6:18:15 in the asymmetric unit cell of the solid-state crystal). Each 200-mg dose of LCZ696 contains approximately 97 mg of AHU377 and 103 mg of valsartan.

Following oral administration, LCZ696 dissociates into valsartan and the pro-drug AHU377, which is further metabolized to the NEP inhibitor LBQ657. Exposures to both LBQ657 and valsartan are dose-proportional and predictable. LCZ696 doses of 50, 100, and 200 mg deliver valsartan exposures which are similar to those delivered from the Diovan® dose strengths of 40, 80, and 160 mg, respectively.

Upon solvation, LCZ696 dissociates into valsartan and AHU377; therefore analytical testing for formulation stability, homogeneity and concentration of LCZ696 is accomplished by measuring the individual components valsartan and AHU377. Exposure analyses were based on evaluation of blood levels of valsartan, sacubitril (AHU377, NEPi prodrug) and LBQ657 (neprilysin inhibitor).

The toxicity profile of LCZ696 has been characterized through a combined program of studies performed with LCZ696 itself, studies with AHU37) as well as studies supporting the original marketing application for valsartan. Studies performed with both LCZ696 and AHU377 included repeated dose toxicity studies, embryo-fetal development studies and genotoxicity studies. Where renal toxicity related to AT1 receptor blockade was dose-limiting, some assessments were performed with both LCZ696 and AHU377 in order to ensure adequate exposure to the neprilysin inhibitor LBQ657. Pre- and post-natal development studies, juvenile toxicity studies, and carcinogenicity studies were performed with AHU377 but not LCZ696.

The pharmacologic targets of LCZ696 (AT1 receptor and NEP) are evolutionarily conserved across mammalian species. Additionally, although there are some species differences in the rate of hydrolysis of AHU377 to LBQ657, all species are exposed to the same major compounds delivered by LCZ696. LCZ696 has been tested in a range of species including mice, rats, rabbits, and cynomolgus monkeys. The cynomolgus monkey was chosen as the principal nonrodent species for toxicity assessment of LCZ696 based on homology of NEP and NEP substrates to human, but AHU377 has also been evaluated in dogs and marmosets. Toxicology studies in the marmoset with AHU377 provided a basis for comparison to previous valsartan marmoset studies and helped define the specific toxicologic effects of these two LCZ696 components.

NEP exists as an ectoenzyme, preferentially hydrolyzing extracellular oligopeptides (<5 kDa)on the amino side of hydrophobic residues. NEP cleaves a variety of physiologically relevant substrates, including enkephalins, tachykinins, chemotactic peptide, adrenomedullin, and the NPs such as atrial natriuretic peptide (ANP), CNP and

to a lesser degree B-type natriuretic peptide (BNP). In mammals, NEP is widely expressed in kidney, lung, endothelial cells, vascular smooth muscle cells, cardiac myocytes, fibroblasts, neutrophils, adipocytes, testes, and brain, with the highest expression in the renal proximal tubule. NEP plays an important role in terminating peptide signaling events at the cell surface and NEP activity has been shown to contribute to ANP's short half-life (2-3 minutes). Inhibition of NEP increases circulating levels of NPs, with the potential to enhance their cardiovascular and renal actions, including reduction of blood pressure, vasodilation, natriuresis and diuresis, increased glomerular filtration and renal blood flow, inhibition of renin and aldosterone release, reduction of sympathetic activity, and anti-hypertrophic and anti-fibrotic effects.

AT1 receptors are primarily found in the brain, adrenal glands, heart, vasculature and kidney, and regulate blood pressure and electrolyte balance in response to angiotensin II binding. Competitive blockade of the AT1 receptor by valsartan inhibits the vasoconstrictive actions of angiotensin II, inhibits aldosterone release, reduces sodium and water retention, and inhibits cardiovascular hypertrophy and remodeling. Concomitant modulation of both systems would provide greater suppression of renin and aldosterone release, with the effect of further improving renal function through augmenting natriuresis and diuresis, reducing blood pressure, and inhibiting cardiovascular hypertrophy and vascular remodeling more than is achieved with single-agent therapy.

Primary pharmacodynamics

AHU377 (the pro-drug) is a poor inhibitor of human recombinant NEP enzyme activity *in vitro* (IC50 = 16,700 ± 2,300 nM), whereas its metabolite LBQ657 is a potent inhibitor (IC50 = 2.3 ± 0.4 nM). Both AHU377 and LBQ657 are poor inhibitors of human recombinant NEP-2 enzyme activity. Valsartan does not inhibit NEP or NEP-2 activity at concentrations up to 100,000 nM

Valsartan blocks angiotensin II binding to rat aortic smooth muscle cell membranes with a Ki of 2.4 nM and is more than 30,000-fold selective for the AT2 receptor subtype. Neither AHU377 nor LBQ657 inhibit binding to the human AT1 receptor at concentrations of 30 μM.

In rats, AHU377 (30 mg/kg p.o.) inhibits renal cortex NEP enzyme activity by 73%. A single dose of LCZ696 (60 mg/kg p.o.) increases circulating ANP in rats by an average of 89% over eight hours (peak increase of 132%). When valsartan is administered to rats (10 mg/kg p.o.), the pressor-response relationship to exogenous angiotensin II is shifted to the right by 100-fold after 4 hours, with a sustained 10-fold right shift after 24 hours

LCZ696 lowered blood pressure in rat models of hypertension with divergent etiologies. A single administration of LCZ696 (60 mg/kg) reduced MAP by 73 mmHg in double-transgenic rats overexpressing human renin and angiotensinogen (dTGR), a high-renin model of hypertension. LCZ696 (68 mg/kg/day) also reduced arterial pressure for the

duration of the two-week treatment period in a normal-renin, salt-insensitive model of hypertension (SHR). Likewise, LCZ696 (68 mg/kg/day) blunted or prevented the gradual rise in arterial pressure in two respective studies in a low-renin volume-dependent model of hypertension (DSS rat on highsalt diet).

In a rat post-myocardial infarction model, LCZ696 reduced the hypertrophy that occurs in response to injury. In stroke-prone spontaneously hypertensive rats (SHRSP), combined AHU377 and valsartan was more effective than valsartan alone in improving cardiac and vascular fibrosis and vascular remodeling. In DSS rats, LCZ696 significantly reduced the increase in left-ventricular weight caused by high-salt feeding and also prevented the salt-induced increase of N-terminal (NT)-proANP, consistent with reduced stretch-induced release of ANP. LCZ696 also prevented the salt-induced decrease in renal blood flow, improved glomerular filtration rate (GFR), and reduced renal injury markers]. In contrast, losartan and HCTZ were less effective on these cardiac and renal endpoints.

Secondary pharmacodynamics

AHU377, LBQ657, and valsartan show no meaningful inhibition (>2000-fold selective) of a set of ten proteases that are related to NEP or enzymes associated with NP/RAAS pathways. AHU377 and LBQ657 were assessed for their off-target activities on a panel of GPCRs, transporters, ion channels, nuclear receptors, and enzymes. No significant inhibitory effects were found for AHU377 at 30 μM or for LBQ657 at ≥10 μM for any of the targets tested

In addition to the NPs (ANP, BNP, and CNP), NEP is known to cleave other peptide substrates *in vitro*, including enkephalins, bradykinin (BK), substance P, neurotensin, Aβ, gastrin, and adrenomedullin. Other clearance mechanisms exist for these peptides *in vivo*, but inhibition of NEP could potentially increase some of these substrates. To evaluate the potential for adverse effects related to increases in BK, Aβ, and CNP, a series of investigative studies was conducted. Increased BK is associated with angioedema and may be an important causative factor in clinical angioedema induced by ACE inhibitors and which was observed with omapatrilat. In two rat models of angioedema (BK-induced rat paw edema or blood pressure lowering), AHU377 in the presence of valsartan did not potentiate BK action, whereas ACE inhibitors or omapatrilat did. Likewise, in another rat model of angioedema, omapatrilat or the ACE inhibitor captopril promoted tracheal plasma extravasation whereas the NEP inhibitor ecadotril or concomitant angiotensin AT_1 receptor blockade (valsartan) and NEP inhibition (candoxatril) had no effect.

Safety pharmacology

At the maximum feasible concentrations (3 mM for LCZ696; 1 mM for AHU377), no meaningful hERG inhibition was observed. Therefore, the risk for QT prolongation at anticipated exposures associated with the proposed clinical dose of 200 mg BID is perceived to be low.

AHU377 up to 250 mg/kg had no effects on blood pressure, heart rate or ECG parameters in normal, conscious dogs. Slight reductions in blood pressure (systolic, diastolic and mean arterial blood pressures) were observed in cynomolgus monkeys at a dose of 100 mg/kg LCZ696, consistent with the greater blood pressure lowering effect of the ARNI compared to single-acting therapy. There were no effects of LCZ696 on ECG parameters or heart rate.

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LCZ696 andAHU377 had no adverse effects on respiratory or CNS endpoints following single oral administration in rodents at doses which provided valsartan exposure multiples of ~1X, and LBQ657 exposure multiples in excess of 8X those associated with a 200 mg BID clinical dose.

In conclusion, no adverse effects on vital organ function are predicted at anticipated human exposures associated with the 200 mg BID clinical dose.

Absorption

LCZ696 or AHU377 was well absorbed in all animal species (65-100%) after p.o. dose. In human, absorption was estimated to be at least 61%. The absorption was relatively rapid in rate and onset in all species (mouse, rat, dog, rabbit, monkey, and human) with Tmax ranging from 0.25 - 2 hr.

Following an oral dose, the terminal half-life for AHU377 was short (1.3-3.2 h) in all species. The terminal half-life of LBQ657 or valsartan was relatively short in mice, rats and dogs (1.1-3.1 h), but was long in monkeys (~6 h) and humans (12-21 h).

Bioavailability of LBQ657 was moderate to high (41-100%) in animals and was estimated to be greater than 50% in human. Dose-normalized plasma or blood exposure values (AUC) for LCZ696-associated components (AHU377 or LBQ657) in monkey were higher than other species (rank order: monkey >dog >rat ≈mouse). The lower dose-normalized exposure seen in mouse, rat, and dog was associated with its high clearance (as high as hepatic blood flow).

Plasma exposure (AHU377, LBQ657 and valsartan) is generally proportional to the dose in all tested animal species (mice, rats, rabbits, and monkeys). In dogs, the exposure of valsartan following LCZ696 dosing was ~3-fold higher than the exposure following administration of the molar equivalent of valsartan. In humans [CLCZ696A2101], [CLCZ696A2102], [CLCZ696A1101], Cmax and AUC values of

AHU377 and LBQ657 are also approximately dose proportional up to 600 mg although valsartan is slightly less than dose-proportional at high dose.

Distribution

Uptake of AHU377, LBQ657, and valsartan into blood cells was not significant and therefore drug concentrations in plasma were higher than in blood. Both AHU377 and LBQ657 were moderately to highly bound to plasma proteins with some species differences (90%, 80%, 93% and 97% in rat, dog, monkey, and human, respectively for LBQ657 and 91% and 97% in monkey and human, respectively, for AHU377). Similarly, valsartan was bound to serum proteins with some species differences (82-97%). In human, serum albumin was found to be the primary binding protein for AHU377, LBQ657 and valsartan.

***In vivo* distribution in non-pregnant animals**

In rats, drug-related radioactivity was widely and rapidly distributed to most tissues following a single i.v. or p.o. dose of radiolabelled AHU377. The highest tissue radioactivity (2-4 fold higher than blood) was found in kidney and liver 5 min after i.v. dosing. The lowest radioactivity levels were observed in brain, eye, seminal vesicles and spinal cord. Based on AHU377-derived radioactivity, brain and testis penetration is minimal (tissue: blood ratios were 0.02 and 0.05, respectively) and affinity to melanin-rich tissues (pigmented skin and uveal tract) is low. Tissue distribution patterns of drug-derived radioactivity following AHU377 p.o. dosing were similar to those following i.v. dosing. The peak tissue radioactivity concentration was reached at 1 h for most tissues, with the highest concentrations observed in the kidney and liver. After 24 h, tissue radioactivity was below the limit of quantification, except for the GI and kidneys.

After i.v. dosing of valsartan, relatively high concentrations of radioactivity were found in blood, plasma and well perfused t issues including liver and kidney. Valsartan-derived radioactivity showed minimal brain and testis penetration. After p.o. dosing, the pattern of distribution was similar to that following i.v. dosing. By either route of administration, residual radioactivity after 7 days was at or below the limits of detection in all tissues investigated except liver.

***In vivo* maternal-fetal and milk transfer**

In pregnant rats dosed with [^{14}C]LCZ696, the extent of the transfer of AHU377-derived radioactivity from maternal blood into the embryo-fetal compartment was moderate (fetus-tomaternal blood ratio at fetus Tmax: 0.509 on day 12 and 0.246 on day 17).

In pregnant rabbits dosed with LCZ696, the fetal exposure relative to maternal plasma was low (~0.06 - 0.21 for LBQ657 and ~0.01 for valsartan), indicating that LCZ696 related materials was poorly transferred into the fetus

Following an oral dose (30 mg/kg) of [^{14}C]LCZ696 to lactating rats, transfer of LBQ657 into milk was observed. The overall milk:plasma concentration ratio of total radioactivity was 0.91 based on $AUC_{0-\infty}$ values. Projecting the rat data to humans, it is estimated that

a breast-fed infant could be exposed to ~0.889% of an adult total daily dose of 400 mg (or 200 mg BID) by ingesting 1 L of milk daily. LBQ657 was the major drug related compound in rat milk.

After a single oral administration of 3 mg/kg [^{14}C] valsartan to lactating rats, transfer of valsartan into milk was observed. As both LBQ657 and valsartan are transferred into milk, administration of LCZ696 is not recommended during breast feeding, and a decision should be made to discontinue nursing or discontinue LCZ696.

Metabolism

Following oral administration of LCZ696 to rabbit, monkey and human, valsartan, AHU377 and LBQ657 were detected in plasma.

Similarly, AHU377 and LBQ657 were detected in plasma after oral dosing of AHU377 to mouse, rat and dog. AHU377 primarily underwent ethyl ester hydrolysis to form LBQ657 in all species. The rate of conversion from AHU377 to LBQ657 was high in mouse, rat and human, and the major compound detected in plasma/blood was LBQ657 (mouse: ~73% of AUC, rat: ~80% AUC, human: 93-98% of AUC comparing to AHU377). In contrast, the rate of conversion was moderate in dog and monkey where both AHU377 and LBQ657 were present in plasma/blood (AHU377:LBQ657 = ~34%:~46% of AUC in dogs, AHU377:LBQ657 = ~37%:~62% of AUC in monkeys).

AHU377 and LBQ657 undergo further hydroxylation, glucuronidation, sulfation, and glycine/taurine conjugation to generate several minor metabolites in plasma/blood of various species. LBQ657 was the major component recovered in excreta from all species studied, accounting for ~70-100% of the oral dose. Unchanged AHU377 recovered in excreta was minimal in all species studied, accounting for <1-14% of the oral dose. The metabolic profiling studies did not reveal any major unique human metabolite.

Excretion

In mice, rats and dogs, AHU377-derived radioactivity was predominantly excreted in the feces (~74-98% of the dose. In monkeys and humans, however, AHU377-derived radioactivity had higher excretion into urine (~42-65% of the dose).

LCZ696 was eliminated primarily as LBQ657 and valsartan with minimal AHU377 (~3% of dose, except for ~13% in dog) and some minor metabolites in both animals and humans. There was no indication of enterohepatic recycling based on the plasma pharmacokinetics. Valsartan was excreted mainly via the biliary-fecal route in mice, rats, dogs, marmosets, and humans. In human, 9% and 86% of the valsartan dose was excreted in urine and feces, respectively.

Drug interactions

Enzyme inhibition

AHU377 showed little or no inhibition (IC_{50} >100 μM) in *in vitro* assays using human CYP enzymes 1A2, 2C9, 2D6, 2E1, 3A4/5, and only a weak inhibition against CYP2C8 (IC_{50} ~15 μM) and 2C19 (IC_{50} ~20 μM). Based on the Cmax of AHU377 (5.9 μM) observed in patients following dosing of LCZ696 200 mg BID [Summary of Clinical Pharmacology-Section 3.1.9.1], no drug-drug interactions (DDI) between AHU377 and co-medications (as CYP substrates) are expected. LBQ657 demonstrated weak inhibition of CYP2C9 (IC_{50} ~40 μM) in *in vitro* assays. The potential inhibition of CYP2C9 was investigated in the clinical trial [CLCZ696B2112] in which no interaction occurred between LCZ696 and warfarin, a drug metabolized by CYP2C9. Since LBQ657 is mainly eliminated unchanged, its elimination is unlikely to be influenced by CYP enzyme inhibitors. Therefore, the potential for a DDI between LBQ657 and concomitantly administered drugs is considered to be low. Valsartan did not inhibit CYP enzymes 1A2, 2A6, 2C19, 2D6, 2E1, or 3A4/5 to any significant extent. It marginally inhibited CYP2C9 with a relatively high Ki value (135 μM). No interaction was observed in a clinical DDI study with warfarin [CLCZ696B2112]. CYP2C9 is the enzyme responsible for the formation of 4-hydroxyvaleryl metabolite of valsartan in human microsomes. However, valsartan was predominately excreted unchanged in urine and feces. CYP-mediated DDIs between valsartan and co-administered drugs are negligible.

Enzyme induction

AHU377, LBQ657 and valsartan at concentrations up to 100 μM did not induce the expression and/or catalytic activities of CYP1A2, CYP2B6, CYP2C9, or CYP3A in primary human hepatocytes.

Transporters

The *in vitro* inhibition studies suggested likely involvement of P-glycoprotein (P-gp) in AHU377 transport; however, this is not anticipated to have a significant effect on its oral absorption because the affinity of AHU377 for P-gp was low (>100 μM) and a high absorption (65-100%) was observed in animals. These data also imply that potential inhibition of AHU377 absorption by other P-gp inhibitors is expected to be low. AHU377, up to a concentration of 50 μM, did not inhibit multi-drug resistance protein (MRP2) or P-gp transport activity and only very weakly inhibited breast cancer resistance protein (BCRP)-mediated transport activity. These data suggest that AHU377 is unlikely to significantly inhibit BCRP, P-gp or MRP2. Similarly, LBQ657 was found not to be an inhibitor of P-gp and BCRP activities, suggesting a low likelihood of pharmacokinetic interactions when co-administered with P-gp and BCRP substrates. The *in vitro* results suggest that AHU377 or LBQ657 are weak inhibitors of multidrug and toxin extrusion transporter 1 (MATE1) and 2-K (MATE2-K) but these effects are unlikely to be clinically relevant.

The *in vitro* results suggest that active transport by hepatic organic anion-transporting polypeptide 1B1 (OATP1B1) and 1B3 (OATP1B3) contributes to the systemic clearance of LBQ657 which may be altered when LBQ657 is co-administered with drugs that inhibit these transporters such as cyclosporine and a number of protease inhibitors. LBQ657 was shown to be an *in vitro* inhibitor of OATP1B1 (IC_{50} = 126 ± 26 µM) but not of OATP1B3. Based on the Cmax of LBQ657 (16531 ng/mL = 43 µM) observed therapeutically (Summary of Clinical Pharmacology-Section 3.1.9.1), it is unlikely that LBQ657 will increase the systemic exposure of OATP1B1 substrates. AHU377, LBQ657, and valsartan did not inhibit the hepatic organic cation transporters 1 (OCT1) or renal OCT2 in the *in vitro* assays. AHU377 has an *in vitro* inhibitory effect on OATP1B1, OATP1B3, and organic anion transporter 3 (OAT3) with IC_{50} values of 1.91 ± 0.56 µM, 3.81 ± 2.2 µM, and 0.795 ± 0.058 µM, respectively. As a result, AHU377 may increase the systemic exposure of OATP1B substrates (not for OAT3 *in vivo,* see below) in view of the Cmax of AHU377 (5.9 µM; unbound Cmax = ~0.18 µM) observed therapeutically and when considering the site of interaction at the liver inlet which is exposed to higher concentrations following oral absorption. The clinical study (LCZ696B2115) demonstrated an up to 2-fold increase in Cmax and up to 34% increase in AUC of atorvastatin (OATP1B1 and 1B3 substrate) and its active metabolites when co-administered with LCZ696 200 mg BID.

Valsartan was found to be a substrate of OAT1 and OAT3 *in vitro* but it was transported poorly by OAT1. LBQ657 was found to be an *in vitro* inhibitor (IC_{50} 15.2 ± 5.60 µM) and substrate of OAT3 but not OAT1. These *in vitro* findings did not appear to be clinically relevant as indicated by the clinical observations (i.e., similar urinary excretion of LBQ657 or valsartan after LCZ696 administration vs. AHU377 or valsartan alone) [(b) (4)] [CLCZ696A1101]. In the clinical study [CLCZ696B2116], Cmax and AUC of furosemide (40 mg dose) were somewhat decreased by 50% and 26%, respectively, when co-administered with LCZ696 (200 mg BID steady state) indicating that changes were not associated with the inhibition of OAT3. If OAT3 was inhibited, then the exposure of furosemide should increase. Since the 24 hrs diuresis is unchanged, the observed PK changes are not clinically relevant.

Valsartan was found to be an *in vitro* substrate of OATP1B1, OATP1B3 (hepatic uptake transporter) and MRP2 (efflux transporter) in the test system of human hepatocytes and doubly transfected (OATP1B1/MRP2) Madin-Darby Canine Kidney II (MDCKII) cells. Based on this information, although clinical relevance has not yet been demonstrated, co-administration of the inhibitors of these uptake or efflux transporters with LCZ696 may increase the systemic exposure to valsartan. The *in vitro* results suggest that valsartan poorly inhibited the transport activity of MATE1 and MATE2-K and the inhibition is unlikely to be clinically relevant.

Toxicokinetics

In the toxicokinetic studies, with respect to AHU377, LBQ657 and valsartan concentrations, the AUC values in all tested species (mouse, rat, rabbit, and monkey including pregnant rat and rabbit) were generally proportional to dose. No clear

evidence of accumulation in rats and monkeys was observed following multiple daily dosing for 26 or 39 weeks. There was no evidence of gender differences.

Toxicology

Single dose toxicity studies, no longer required as per (ICH M3 (R2)), were not performed for LCZ696. However, single dose studies were performed for AHU377 and valsartan prior to the issue of this guidance, and these studies are included for transparency. Single oral doses LCZ696 up to 600 mg/kg were tested and tolerated in respiratory and CNS safety pharmacology studies conducted in rats. Single dose toxicity studies performed with AHU377 indicate that AHU377 generally well tolerated following oral administration to rats (doses up to 2000 mg/kg) and marmosets (doses up to 1000 mg/kg) with clinical signs limited to gastrointestinal changes in marmosets (emesis at 1000 mg/kg).

The pivotal oral administration repeated dose toxicity studies for LCZ696 were conducted for up to 26-weeks in rats and up to 39-weeks in cynomolgus monkeys. Pivotal oral administration repeated dose toxicity studies for AHU377 were conducted for up to 26-weeks in rats and 52-weeks in marmoset monkeys; higher exposure to LBQ657 was achieved in these studies. Both the cynomolgus monkey and the marmoset are considered relevant non-rodent species. Marmoset studies with AHU377 allow for meaningful comparisons of target organ toxicity profiles to marmoset studies conducted with valsartan. Valsartan repeat dose toxicology studies are not described in detail in this submission; reference is made to the Diovan NDA (NDA 20665).

In repeated dose oral toxicity studies with LCZ696 up to 26-weeks in duration in rats, and 39-weeks in duration in cynomolgus monkeys, LCZ696 was tolerated at doses up to 100 mg/kg (rat) and 300 mg/kg (monkey). The NOAEL was 30 mg/kg in both species. Although exposure to valsartan, AHU377, and LBQ657 at the NOAEL were below those observed following a 200 mg BID clinical dose, LCZ696 has been well tolerated at clinical doses up to 200 mg BID in heart failure patients.

In repeated dose oral toxicity studies with AHU377 up to 26-weeks in duration in rats and 52-weeks in duration in marmosets, relatively high doses of AHU377 were generally well tolerated and no new target organs were identified. Clinically relevant exposures (~1X) were attained in the rat at the NOAEL (600 mg/kg/day in the 26-week rat study); in the marmoset exposure to LBQ657 at the NOAEL was ≤1X based on based on $AUC_{(0\text{-}24hr)}$ and Cmax, relative to LBQ657 exposure at a 200 mg BID clinical dose. Targets for LCZ696 and/or AHU377 were kidney, red blood cells, heart, and the gastrointestinal tract.

Kidney

Renal juxtaglomerular hypertrophy/hyperplasia is a well-recognized adaptive response in preclinical studies with agents that interact with RAAS. Valsartan-mediated angiotensin receptor blockade results in dilation of the renal efferent arteriole and a compensatory stimulation of the RAAS. This results in stimulation of renin production by

the renin producing cells in the juxtaglomerular apparatus and over time this will manifest as juxtaglomerular hypertrophy/hyperplasia. This finding was observed at oral LCZ696 doses ≥100 mg/kg in the cynomolgus monkey and ≥ 200 mg/kg in the rat. The NOEL for adaptive renal juxtaglomerular changes is 30 mg/kg in the cynomolgus monkey and 100 mg/kg/day in the rat (corresponding exposure multiples for valsartan of <1X based on AUC and Cmax).

In the cynomolgus monkey (but not the rodent), higher LCZ696 doses (600 mg/kg) were associated with renal tubular changes comprised of tubular basophilia, cytoplasmic vacuolation and single cell necrosis (valsartan $AUC_{(0\text{-}24hr)}$ ≥274000 ng*hr/mL or 3.3X; AHU377 $AUC_{(0\text{-}24hr)}$ ≥ 269000 ng*hr/mL or 41X; LBQ657 $AUC_{(0\text{-}24hr)}$ ≥2990000 ng*hr/mL or 10X the human exposure at 200 mg BID).

No renal changes were observed in preclinical studies with AHU377 alone in marmosets (high dose of 200 mg/kg for 52-weeks), rats (high dose of 600 mg/kg for 26-weeks; high dose of 400 mg/kg in 2-year carcinogenicity study), or mice (high dose of 1200 mg/kg in 2-year carcinogenicity study). There is no evidence from preclinical studies with LCZ696 to suggest that angiotensin receptor blockade-mediated renal findings are potentiated by NEP inhibition.

Gastrointestinal tract
Oral ingestion of solutions/suspensions prepared with LCZ696 was associated with reversible microscopic changes of focal glandular stomach mucosal erosion and mixed cell inflammation in rats (doses ≥50 mg/kg/day) and with emesis and diarrhea without histologic correlates in the cynomolgus monkey (doses ≥30 mg/kg/day).

While the sponsor's opinion is that AHU377 is responsible for the gastric effects, the possibility that irritant effects of LCZ696 are attributable to valsartan cannot be excluded. Gastritis was observed after high non-tolerated doses of valsartan in rats (doses ≥600 mg/kg) and marmosets (doses ≥200 kg) and vomiting has been observed in marmosets at doses ≥40 mg/kg (Diovan, NDA 20665). Whereas LCZ696-related microscopic gastrointestinal changes (focal erosion, mixed inflammatory cell infiltrate of glandular stomach) were still observed in the 26-week rat study at 100 mg/kg (0.5% CMC vehicle) [Study 0670620], AHU377 doses of 50-800 mg/kg (0.5% CMC or 0.5% hydroxypropylcellulose vehicle) were not associated with gastritis in 13-week repeated dose studies in rats [Study 0570207] [Study 0770711], and AHU377 high doses of 400-600 mg/kg/day (0.5% hydroxypropylcellulose vehicle) were not associated with gastritis in 26-week repeated dose [Study 1370484] and 104-week oral (gavage) carcinogenicity studies in rats [Study 0870373].

Red Blood Cells
Hematology changes indicative of decreased red cell mass (decreases in red blood cell count, hemoglobin concentration, hematocrit, and reticulocytes) were present in the cynomolgus monkey at LCZ696 doses ≥ 300 mg/kg [Study 0670276] [Study 0670282] and in the rat at LCZ696 doses ≥200 mg/kg (decreases in red cell parameters and reticulocyte counts) [Study 0670220]. Reticulocyte counts were increased

(regeneration) at 800 mg/kg in a 2-week study in the No changes in red blood cell parameters were observed in marmosets and rodents administered AHU377. Reductions in red blood cell parameters after LCZ696 treatment, which were reversible, are a recognized effect of valsartan but generally without clinical consequences and attributed to the effect of the renin angiotensin system on production of erythropoietin by the kidney.

Heart
Heart weights were decreased in mice (doses ≥400 mg/kg/day) and rats (doses ≥100 mg/kg/day) treated with LCZ696 and in rats treated with AHU377 (doses ≥400 mg/kg/day) or valsartan (doses≥200 mg/kg/day) and may be related to the expected effects of AHU377 and valsartan in decreasing blood pressure and afterload thereby resulting in decreased cardiac work load. There were no histopathological findings accompanying the decreased heart weight.

Genetic toxicology

These studies, which were compliant with (ICH S2 (R1)), included *in vivo* testing and *in vitro* assays in bacterial and mammalian systems, with and without S9 metabolic activation. Reverse mutation (Ames), chromosomal aberrations in human lymphocytes, and the rat micronucleus assay were performed on both LCZ696 and AHU377. All assays were adequately performed and uniformly negative.

The S9 fraction enables conversion of AHU377 to LBQ657 thereby ensuring *in vitro* characterization. An *in vitro* chromosome aberration study was performed with LBQ657 to ensure characterization of the clastogenic potential of LBQ657 under the 20 h incubation conditions in the absence of S9.

Valsartan genetic toxicology studies did not reveal any evidence for genotoxic potential [NDA 20665].

Carcinogenicity

Assessment of the carcinogenicity potential of LCZ696 was based on carcinogenicity studies performed in rats and mice with AHU377 and valsartan [NDA 20665}. Dose selection for carcinogenicity studies performed with AHU377 was based on the results of 13-week oral (gavage) dose range-finding studies. The highest dose selected was either the maximum feasible dose (mouse high dose of 1200 mg/kg/day) or the MTD (rat high dose of 400 mg/kg/day).

There was no evidence of carcinogenicity when AHU377 was administered by oral gavage for a minimum of 104 weeks to mice at doses up to 1200 mg/kg/day (LBQ657 exposure multiples 14x (males) and 46X (females) based on Cmax relative to 200 mg BID clinical dose), and to rats at doses up to 400 mg/kg/day (LBQ657 exposure multiples 1.6x (males) and 3.6 (females) based on Cmax). Exposure multiples in the rat would increase by a factor of three if one takes into account species differences in

protein binding between rats (90% bound; 10% free) and humans (97% bound; 3% free).

There was no evidence of carcinogenicity when valsartan was administered in the diet to mice and rats for up to 2 years at doses up to 160 and 200 mg/kg/day, respectively (Diovan® US prescribing information).

Reproductive toxicology

Fertility and early embryonic development
No effects on fertility and early embryonic development were present in rats treated with LCZ696 (doses up to 150 mg/kg/day), AHU377 (doses up to 750 mg/kg/day) or valsartan (doses up to 200 mg/kg/day)

Embryo-fetal development
In embryo-fetal development studies, LCZ696 treatment was associated with increased embryo lethality in both rats and rabbits.

LCZ696 was teratogenic in rabbits at doses ≥10 mg/kg based on a low but dose-dependent increase in hydrocephaly. This finding is attributed to valsartan which produced similar fetal findings (increased incidence of dilated lateral brain ventricles) at equivalent maternally toxic doses (doses ≥5 mg/kg/day) [NDA 20665.]

AHU377 treatment was also associated with fetal toxicity or fetal death in the context of maternal toxicity, but was not teratogenic. The NOAEL for embryo-fetal toxicity in studies with LCZ696 was 30 mg/kg/day in rats and 3 mg/kg in rabbits (corresponding exposure multiples of ≤1 based on Cmax and AUC relative to 200 mg BID clinical dose); in studies with AHU377 the NOAEL was 750 mg/kg/day in rats and 200 mg/kg/day in rabbits (corresponding LBQ657 exposure multiples of 4.5 and 4.6X based on AUC(0-24 hr), respectively.

Pre- and postnatal development
Results of pre- and postnatal development studies with AHU377 and valsartan suggest that LCZ696 administration during these periods may impair fetal development and survival.

In a pre- and postnatal development study with valsartan in rats, there were slight reductions in pup development and survival in the presence of reduced maternal body weight gain at 600 mg/kg/day.

Administration of AHU377 to rats during pre- and postnatal development resulted in slight body weight effects at 750 mg/kg/day although there were no adverse effects on survival. The NOAEL was 250 mg/kg/day for AHU377 and 200 mg/kg/day for valsartan (exposure multiples of ≤2X based on Cmax and AUC relative to a 200 mg BID clinical dose). Neither compound had effects on behavioral or reproductive function of the F1 generation.

Juvenile toxicity studies
Results of neonatal and juvenile studies with AHU377 and valsartan suggest that LCZ696 treatment may impact bone growth and development and impair kidney development. Kidney changes (renal tubular and pelvic changes) were observed at all valsartan dose levels (1, 20, 150 mg/kg/day) in juvenile rats [NDA 21283-Valsartan Nonclinical Overview (pediatrics 2007), Section 5.5.4]. This contrasts with general toxicity studies performed in adult rats in which the NOAEL was determined to be 200 mg/kg/day. Kidney changes in valsartan treated juvenile rats represent an expected exaggerated pharmacological effect of ACE inhibitors and ARBs that are observed if rats are treated during the first 13 days of life. Since nephrogenesis is generally completed by birth in humans, this safety concern is most relevant to children under one year of age.

The slight decreases in body weight gain, bone length (femur and tibia) and bone mass were observed at doses ≥400 mg/kg/day in AHU377-treated juvenile rats but not in juvenile rabbits administered AHU377 at lower doses. These changes were generally transient in nature and reversible provided there was sufficient time for catch up growth prior to growth plate closure. Bone changes in juvenile rats treated with AHU37 coincided with periods of highest AHU377/LBQ657 exposure and greatest body weight decreases, suggesting these changes are potentially secondary to decreases in body weight gain; however the mechanism is not well understood.

Studies investigating the effects of LCZ696 on Aβ clearance in CSF and brain
The potential for LCZ696 to affect CNS Aβ levels in cynomolgus monkeys was evaluated in a 2-week investigative toxicology study using serial sampling of CSF through cisterna magna ports and measuring CSF Aβ using the (b) (4) assay. Measuring the pool of Aβ in the CSF is assumed to reflect Aβ metabolism within the brain parenchyma in a qualitative and correlative manner.

In this study, a clinically relevant LCZ696 dose of 50 mg/kg (plasma and CSF exposure to LBQ657 similar to 400 mg QD dose) resulted in CSF levels of the NEPi LBQ657 that exceeded the *in vitro* IC_{50} for human recombinant NEP inhibition (2.3 nM) from 0.5 through 24 hr post-dose. Brain concentrations of LBQ657 were 5- to 7-fold higher than CSF concentrations. Acute decreases in the CSF clearance of Aβ were observed, as demonstrated by an increase in the day 1 elimination half-life of Aβ peptides (38, 40, 42), and significant increases in CSF levels of newly generated (i.e., labeled) Aβ42. Following two weeks of dosing, the elimination half-lives of all measured Aβ peptides in the LCZ696-treated animals were no different from day 15 vehicle-treated animals and numerically similar to day 1 vehicle-treated animals. However, day 15 levels of newly synthesized Aβ (labeled) as well as total Aβ (labeled and unlabeled) were increased for all measured peptides in the CSF.

Plasma levels of Aβ40 were significantly increased at 2 hr (day 16) and 12 hr post dose (day 15), and Aβ42 also showed a trend to increase at 2 and 12 hr post dose (not statistically significant). However, brain concentrations of Aβ40 and Aβ 42 were not

increased. These results are mechanistically consistent with the CNS exposure to the NEPi LBQ657 at concentrations which exceed the *in vitro* IC_{50} for human NEP and a reduced proteolytic clearance of Aβ in the CNS. As Aβ was not increased in the brain, and the elimination half-life of CSF Aβ was unchanged on day 15, Aβ was cleared from brain tissue by other clearance pathways, which is consistent with the increased appearance of Aβ in the CSF compartment.

The absence of elevations in brain Aβ in a 2-week study with LCZ696 in cynomolgus monkeys is consistent with results of brain immunostaining for Aβ42 in the 39-week toxicology study with LCZ696 in cynomolgus monkeys. In this chronic study in young (2-4 year old) cynomolgus monkeys treated for 39 weeks with LCZ696 at 300 mg/kg/day there were no compound-related microscopic brain changes, increases in brain or cerebral vascular Aβ content, or plaque formation as assessed by anti-Aβ immunostaining. The 300 mg/kg cynomolgus monkey dose results in AUC exposure ~2X, and Cmax exposure ~9X the exposures associated with a 200 mg BID clinical dose.

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/s/

WILLIAM T LINK
05/15/2015

ALBERT F DEFELICE
05/15/2015

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 207620 **Applicant:** **Stamp Date:** 12/17/2014

Drug Name: LCZ696 (sacubitril/valsartan) **NDA/BLA Type:** Priority NDA

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

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

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		
11	Has the applicant addressed any abuse potential issues in the submission?		x	Low abuse potential
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			na

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

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

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

William T. Link 1-26-15

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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

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