CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

205598Orig1s000

NON-CLINICAL REVIEW(S)

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:	205598
Supporting document/s:	SN0031
Applicant's letter date:	June 23rd 2017
CDER stamp date:	June 30th 2017
Product:	Macimorelin Acetate (AEZS-130)
Indication:	Diagnostic for Adult Growth Hormone Deficiency
Applicant:	AEterna Zentaris
Review Division:	DMEP
Reviewer:	Jeffrey Quinn
Supervisor/Team Leader:	Todd Bourcier
Division Director:	Jean-Marc Guettier
Project Managers:	Abolade Adeolu/Meghna Jairath

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Except as specifically identified, all data and information discussed below and necessary for approval of NDA 205598 are owned by AEterna Zentaris or are data for which AEterna Zentaris has obtained a written right of reference.

Any information or data necessary for approval of NDA 205598 that AEterna Zentaris does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 205598.

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1 Executive Summary

1.1 Introduction

AEterna Zentaris resubmitted NDA 205590 on June 30th 2017 after receiving a Complete Response letter from the FDA on November 5th 2014 following the first-round submission in which the Sponsor was denied approval of the application based on the deficiencies identified in the clinical data and the lack of a thorough QT study.

Macimorelin acetate (AEZS-130) is a synthetic, peptidomimetic which stimulates the release of GH by binding to the GHS receptor. AEterna Zentaris is seeking approval for the proposed indication: "Diagnosis of adult growth hormone deficiency (AGHD) (b) (4) ' under this NDA.

1.2 Brief Discussion of Nonclinical Findings

The Sponsor submitted a new pharmacology study with this resubmission that did not alter Pharm/Tox's decision to support approval of NDA 205598 (Macimorelin Acetate). A full review of the Sponsor's nonclinical data package was completed on June 5th 2014 following the first-round submission of NDA 205598.

1.3 Recommendations

1.3.1 Approvability

Pharmacology/Toxicology supports approval of NDA 205598 (Macimorelin Acetate).

1.3.2 Additional Nonclinical Recommendations

No additional nonclinical studies are required.

1.3.3 Labeling

Nonclinical labeling recommendation have not changed following resubmission of NDA 205598

2 Drug Information

2.1 Drug

Generic Name

Macimorelin acetate (AEZS-130)

Code Name(s)

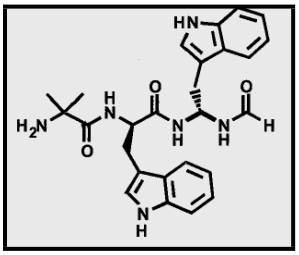
EP-01572, JMV1843, GHS, ARD-07, D-87575, D-106760, ET-1

Chemical Name

Amino isobutiryl-D-tryptophanyl-gem-diamino-D-tryptophanylformaldehyde acetate salt

Molecular Formula/Molecular Weight: Aib-D-Trp-D-gTrp-CHO/474.56 amu

Structure or Biochemical Description



Pharmacologic Class

Synthetic tripeptide GH secretagogue - Growth hormone secretagogue receptor agonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

Macimorelin was developed under IND 73196. (b) (4)

2.3 Drug Formulation

The proposed commercial formulation is a (b) (4) containing (b) (4) mg macimorelin which will be dissolved in 120 mL water, resulting in a solution with a nominal concentration of 0.5 mg/mL administered orally to the patient at the same dose (0.5 mg macimorelin/kg body weight) used in the Phase III study.

2.6 Proposed Clinical Population and Dosing Regimen

Diagnosis of growth hormone deficiency in adult patients suspected of having AGHD. Patients will be administered a single 0.5 mg/mL oral solution of macimorelin in water (0.5 mg macimorelin/kg body weight). Systemic exposure at the 0.5 mg macimorelin/kg body weight dose is: $AUC_{0-\alpha}$ = 1200 ng.min/mL / 60 = 20 ng.hr/mL and C_{max} = 7.59ng/mL (16nM).

2.7 Regulatory Background

The IND for macimorelin was opened in 2007 under a prior sponsor. At that time, the Division agreed that 28 day repeat dose oral toxicity studies in the rat and dog were sufficient to support clinical trials under the IND and eventual submission of the NDA. Reproductive toxicity studies and carcinogenicity studies were not required for this program based on the intended single-dose use of the product and on the known pharmacology/toxicology of the drug target.

The IND was subsequently inactivated in 2008, and was reactivated under the current sponsor AEterna Zentaris in 2009. The Division confirmed in November of 2011 that the previously agreed-to nonclinical program was adequate to support NDA submission. In addition to the nonclinical studies previously discussed, AEterna conducted two *in vitro* hERG assays and an *in vitro* histamine release assay to augment their safety pharmacology program. A complete listing of nonclinical studies submitted in support of this NDA can be found in the full NDA review completed on June 5th 2015.

3 Studies Submitted

3.1 Studies Reviewed Under Resubmission

Study	Route	Species	Primary Review
Activity of AEZS-130 synthesis byproducts, impurities, stereoisomers and degradation product for the human Ghrelin Receptor	*in vitro*	Human	NDA Resubmission

3.2 Studies Not Reviewed

All nonclinical studies submitted by AEterna Zentaris have been reviewed.

3.3 Previous Reviews Referenced

Pharmacology/Toxicology reviews (1 thru 4) under IND 73196.
Pharmacology/Toxicology NDA review (first-round)

4 Pharmacology

4.1 Primary Pharmacology

Activity of AEZS-130 Synthesis Byproducts, Impurities, Stereoisomers and Degradation Product for the Human Ghrelin Receptor Expressed in Mouse LTK cells (non-GLP)

Key study findings:

- Two separate functional assays [induction of CRE-dependent reporter gene expression (EC_{50} = 3.8 nM) and calcium release (EC_{50} = 0.88 nM)] demonstrated that AEZS-130 (D, D-diastereomer) elicited an agonistic interaction with the human ghrelin receptor.

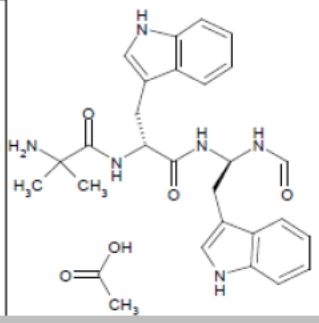
- The EC_{50s} associated with the AEZS-130 synthesis byproducts/impurities, stereoisomers and the degradation product tended to be higher (less agonistic activity) than those associated with AEZ-130.

- The antagonist assay (competition with 10 nM Ghrelin) yielded no examples of notable receptor activity inhibition.

Study no.: 8100-2014-997
Sequence # and Date: SN0031 (6/30/2017)
Conducting laboratory and location: Aeterna Zentaris GmbH, Germany
Date of study: December 2nd 2014
GLP compliance: No
QA report: No
Drug, Lot/Batch #, and % purity: See Sponsor Tables Below

(Objective)

The aim of this study was to explore the potential agonistic ghrelin activity of AEZS-130 related synthesis byproducts/ impurities, stereoisomers and degradation product versus the functional agonistic interaction of AEZS-130 to human GHS-R1A. To exclude potential antagonistic activities of the AEZS-130 related compounds a CRE-dependent luciferase reporter gene expression antagonist assay was also conducted (competition with 10 nM Ghrelin).

compound	structure	D-no.	batch
AEZS-130, macimorelin, D,D-diastereomer		D-87575	S155058

(b) (4)

compound	structure	D-no.	batch
	(b) (4)		

(Methods)

Mouse LTK- cells were stably transfected with a plasmid containing a CMV promoter linked to three cAMP response elements (CRE) followed by a luciferase (Luc) reporter gene and clones stably overexpressing the human ghrelin receptor (GHS-R1A) were established. For the CRE/Luc reporter gene assay ATP bioluminescence was measured and for the determination of calcium release Fluo-4 was employed.

(Results)

The ghrelin receptor activities obtained for the AEZS-130 related compounds are summarized in the table below as mean EC_{50} values. The inter-assay results (induction of CRE-dependent reporter gene expression vs calcium release) were to some extent comparable with respect to the relative differences in fold-activity. The EC_{50s} of [redacted] (b) (4) and the [redacted] (b) (4) comparable to AEZ-130 (roughly 2 to 4-fold less active) while the remaining compounds were considerably less active.

Agonistic Activity for the Human Ghrelin Receptor of AEZS-130 Related Compounds

D-no.	compound	human ghrelin receptor EC50 [µM]*		fold activity§	
		CRE/Luc induction	calcium release	CRE/Luc induction	calcium release
D-87575	AEZS-130 D,D-diastereomer	0.00376 ±0.00128	0.00088 ±0.00015	1	1
[redacted] (b) (4)					

(*rounded values, §fold activity calculated with non-rounded values)

No significant inhibition of receptor activity was elicited by the AEZS-130 related compounds in the presence of 10 nM Ghrelin (data not shown).

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/s/

JEFFREY A QUINN
10/23/2017

TODD M BOURCIER
10/24/2017
I concur

Tertiary Pharmacology/Toxicology Review

From: Timothy J. McGovern, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 205598
Agency receipt date: November 5, 2013
Drug: Macimorelin acetate (AEZS-130)
Sponsor: AEterna Zentaris

Indication: Diagnosis of adult growth hormone deficiency (AGHD) (b) (4)

Reviewing Division: Division of Metabolism and Endocrinology Products

Introductory Comments: Macimorelin acetate (AEZS-130) is a synthetic, peptidomimetic which stimulates the release of GH by binding to the GHSR. The pharmacology/toxicology reviewer and supervisor concluded that the nonclinical data support approval of macimorelin for the indication listed above.

The recommended pharmacologic class for macimorelin is a growth hormone secretagogue receptor (GHSR) agonist.

A complete nonclinical program was conducted to support the proposed clinical dosing regimen of a single oral dose. The program included pharmacology and safety pharmacology studies, general toxicology studies up to 28 days duration in rats and dogs, and in vitro genetic toxicology studies. Safety pharmacology studies using the IV route of administration identified CNS, respiratory, cardiovascular and immunomodulatory effects at exposures significantly greater than anticipated clinical exposures. No significant findings were observed in repeat dose oral toxicity studies that achieved systemic exposures that were at least 8-fold greater than the anticipated clinical exposure.

In vitro genetic toxicology studies with macimorelin were negative. Carcinogenicity studies were not warranted given the proposed clinical use. It is noted that activation of the GHSR is known to induce GH secretion and elicit numerous biological functions including cell proliferation.

The standard battery of reproductive and developmental toxicity studies was not required; the proposed clinical use and the resulting transient activation of GHSRs present minimal developmental or fertility risk.

Conclusion:
I agree with the division pharmacology/toxicology conclusion that dulaglutide can be approved from the pharmacology/toxicology perspective. I have reviewed the proposed labeling and agree with the recommendations made by the division regarding the relevant nonclinical sections.

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/s/

TIMOTHY J MCGOVERN
10/31/2014

Tertiary Pharmacology/Toxicology Review - Addendum

From: Timothy J. McGovern, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 205598
Agency receipt date: November 5, 2013
Drug: Macimorelin acetate (AEZS-130)
Sponsor: AEterna Zentaris

The purpose of this addendum is to correct an error in the conclusion of the original tertiary review. The conclusion incorrectly indicates that the drug substance is dulaglutide rather than macimorelin. The corrected conclusion is provided below.

Conclusion:
I agree with the division pharmacology/toxicology conclusion that macimorelin can be approved from the pharmacology/toxicology perspective. I have reviewed the proposed labeling and agree with the recommendations made by the division regarding the relevant nonclinical sections.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

TIMOTHY J MCGOVERN
10/31/2014

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:	205598
Supporting document/s:	
Applicant's letter date:	November 4th 2013
CDER stamp date:	November 5th 2013
Product:	Macimorelin Acetate (AEZS-130)
Indication:	Diagnostic for Adult Growth Hormone Deficiency
Applicant:	AEterna Zentaris
Review Division:	DMEP
Reviewer:	Jeffrey Quinn
Supervisor/Team Leader:	Todd Bourcier
Division Director:	Jean-Marc Guettier
Project Manager:	Abolade Adeolu

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TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

Macimorelin acetate (AEZS-130) is a synthetic, peptidomimetic which stimulates the release of GH by binding to the GHS receptor. Aeterna Zentaris is seeking approval for the proposed indication: "Diagnosis of adult growth hormone deficiency (AGHD) (b) (4) ' under this NDA.

1.2 Brief Discussion of Nonclinical Findings

Pharmacology

The GHS receptor binding characteristics of macimorelin are comparable to the natural ligand ghrelin, although its relative oral bioavailability and elimination half-life are proposed to enhance its efficacy during oral administration. Signal transduction consistent with GHS receptor activation is achieved by macimorelin *in vitro*.

Macimorelin is rapidly degraded in rat plasma and consequently the PD activity that is clearly identifiable *in vitro* is short lived *in vivo*. Pharmacokinetic measurements in the rat do not accurately reflect intact macimorelin exposures and combined with the loss of PD activity the usefulness of this model to assess pharmacodynamically driven toxicity is limited. Macimorelin is stable in dog and human plasma and despite its poor oral bioavailability the release of GH is maintained in dogs dosed orally or intravenously. The dog is currently the most appropriate model to evaluate short-term pharmacodynamically driven toxicity.

Safety pharmacology assessments of the neurological, pulmonary, cardiovascular and immunomodulatory effects of macimorelin revealed extensive toxicological findings related to the intravenous (IV) administration of the compound to rats and dogs. While the IV route of administration differs from the intended oral route to be used in human subjects the extremely low oral bioavailability of macimorelin in rats and dogs dictated the alternate route.

Neurological (tremors/stupor, etc.) and respiratory toxicity (increased inspiratory/expiratory times, etc.) were observed in Wistar rats following single IV doses (LOAEL = 70x MRHD) of macimorelin during safety pharmacology assessments. Pharmacokinetic data generated in rats with drug product comparable to the clinical lot indicates that clinical exposure is approximately 3-fold lower than the NOAEL identified in rats for intravenous neurological and respiratory toxicity.

The respiratory safety pharmacology assessment of macimorelin administered intravenously to unconscious dogs yielded results that were consistent with those observed in the conscious rat. In the concurrent cardiovascular safety pharmacology assessment unconscious dogs presented with rapid, but transient episodes of hypotension characterized by increased HR, decreased BP, and increased carotid blood flow (LOAEL = 300x MRHD). A sustained decrease in the maximum ventricular dP/dT was noted in dogs and indicates a paradoxical negative inotropic effect of macimorelin. These effects were amplified in the two high dose dogs (600x MRHD), and led to severe adverse events (convulsive spasms and cardiac/respiratory arrest) and death in one dog and convulsions, tremors and hypersalivation with vomiting, in the other. Clinical exposure is approximately 15-fold lower than the NOAEL identified in dogs for IV pulmonary and cardiovascular toxicity.

The immunomodulatory safety pharmacology assessment of macimorelin revealed that hypersensitivity reactions (lying down, reddening of the mucous membranes and excessive salivation) were present in dogs intravenously dosed with the original drug product formulation (300x MRHD). These findings were replicated in a subsequent SD/repeat dose IV dog study of macimorelin (≥ 600x MRHD) in which clinical signs progressed in severity to include unsteady gait or poor motor coordination and tremors at the highest dose tested (2100x MRHD). Histamine release was not evaluated during either of these IV dog studies. Hypersensitivity reactions were not observed in a parallel dog study utilizing the oral route of administration and despite significantly lower drug exposures (≤ 18x MRHD) a 2-fold increase in serum GH levels was achieved at the highest dose tested.

The safety pharmacology assessments indicate that hypersensitivity reactions were likely present in the rat; however, macimorelin did not induce histamine release from rat mast cells (*in vitro*) at concentrations ≤ 178 μM (11000x MRHD). Though not demonstrated, it is feasible that breakdown products formed *in vivo* provoked hypersensitivity reactions in the rat. Based on the significant safety margins to the IV NOAELs and the lack of hypersensitivity reactions in orally dosed animals these effects are not likely to occur in human subjects at the MRHD.

Absorption, Distribution, Metabolism, and Excretion

Macimorelin is poorly absorbed by rats and dogs via the oral route of administration (bioavailability < 1%). Metabolism is CYP450 and NADPH-dependent in dogs, rats, humans and mice and occurs independently of Phase II enzymes (*in vitro*). The noted instability of macimorelin in rat plasma is likely due to a species specific plasma protease activity.

Macimorelin is predominantly metabolized by CYP3A4 and concomitant use of drugs that inhibit this enzyme may decrease its metabolism.

General Toxicology (MRHD, Maximum Recommended Human Dose, or 0.5 mg/kg)

- Oral toxicology studies were pivotal, as they incorporated a clinically comparable formulation of macimorelin and utilized the intended clinical route.

- Oral administration achieved sufficient blood drug levels to adequately assess toxicity of clinically relevant exposures.

- Lack of dose-limiting toxicity from oral studies that dosed up to 8x (males) and 36x (females) the MRHD in rats and up to 55x (males) and 35x (females) the MRHD in dogs provides the pivotal toxicity data that supports our assessment of safety, and is the basis for our recommendation of approval.

- Gender-related differences in drug exposures were noted during the pivotal toxicity studies and may represent a complication when using this compound as a diagnostic agent in human subjects.

- Intravenous studies demonstrate the capacity of the drug to cause extensive toxicity when significantly higher blood drug levels are achieved, but are otherwise essentially irrelevant to the intended clinical indication and labeled use of the drug.

Reproductive Toxicology

The endogenous ligand (ghrelin) for the growth hormone secretagogue receptor (GHSR) is primarily secreted from the stomach into the circulation but it is also synthesized by reproductive tissues suggesting local activity (autocrine and/or paracrine) at these locations. The GHS receptor is expressed at different levels along the hypothalamic-pituitary-gonadal axis and activation of this receptor appears to play a role in the regulation of different aspects of the female and male reproductive functions from germ cell production to embryo development.

Ovarian weights were dose-dependently (LOAEL = 8x MRHD) and significantly decreased (36x MRHD) in the absence of microscopic changes during the pivotal repeat dose oral toxicity study in rats. Prolonged exposure to macimorelin appears to incite hormone disruption in rats and dogs. Hormone levels were not assessed during single dose oral toxicity studies in these species, but no finding consistent with a disruption of hormone homeostasis was noted.

Definitive reproductive or developmental toxicity studies were not conducted; however, the weight of evidence indicates that the single dose use of macimorelin and the resulting transient activation of GHSRs present minimal developmental or fertility risk to WOCBP.

Genetic Toxicology & Carcinogenicity

Macimorelin is a synthetic, modified tri-peptide that was not mutagenic or clastogenic in three *in vitro* assays (Ames, mouse lymphoma mutation assay and micronucleus assay (CHO-K1 Cells). No *in vivo* genotoxicity assays were conducted.

Macimorelin and the related structures/impurities assessed by DEREK NEXUS did not trigger any alerts for mutagenicity or genotoxicity *in silico*. Positive structural alerts were triggered in MCASE for the (b) (4) and (b) (4) These compounds yielded similar alerts (b) (4) in multiple databases and were classified as potential genotoxic impurities based on MCASE prediction for bacterial mutagenicity. Levels of (b) (4) ((b) (4) µg) following a single dose of macimorelin will not exceed the acceptable daily intake of a genotoxic impurity set forth in the relevant guidances for single exposures (120 µg/day) nor exceed the threshold of toxicological concern (TTC) of 1.5ug/day. (b) (4) is ultimately removed from the API (b) (4) and therefore does not present a risk to human subjects.

Activation of the GHS receptor is known to induce GH secretion and elicit numerous biological functions including cell proliferation. No definitive carcinogenicity studies were conducted and while the weight of evidence indicates that the use of macimorelin does not pose a carcinogenic risk to human subjects, prolonged exposure may promote the growth of existing tumors.

1.3 Recommendations

1.3.1 Approvability

Pharmacology/Toxicology supports approval of NDA 205598 (Macrilen®)

1.3.2 Additional Non Clinical Recommendations

No additional nonclinical studies are required.

1.3.3 Labeling

Established Pharmaceutical Class

MACRILEN will be described as a "growth hormone secretagogue receptor (GHSR) agonist".

1 INDICATIONS AND USAGE

MACRILEN is a growth hormone secretagogue receptor (GHSR) agonist.

Note: Sponsor's description of (b) (4) *replaced with proposed EPC.*

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

(b) (4)

Animal reproduction studies have not been conducted with macimorelin. (b) (4)

Note: The Sponsor's proposed language for section 8.1, 8.2, 8.3, and 8.4 is consistent with 21 CFR 201 and is acceptable.

10 OVERDOSAGE

(b) (4)

In the event of an overdose, symptomatic and supportive measures should be employed.

Note: Sponsor's sentence (b) (4) *has been deleted, as it is* (b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

Long-term carcinogenesis studies in rodents have not been conducted.

Mutagenesis

Macimorelin did not cause mutations in bacteria under assay conditions with or without metabolic activation. There were also no mutations or clastogenic effects in mouse lymphoma cells with or without metabolic activation.

Impairment of Fertility

No studies have been conducted to assess the effect of macimorelin on fertility.

(b) (4)

2 Drug Information

2.1 Drug

Generic Name

Macimorelin acetate (AEZS-130)

Code Name(s)

EP-01572, JMV1843, GHS, ARD-07, D-87575, D-106760, ET-1

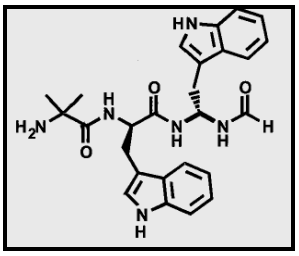
Chemical Name

Amino isobutiryl-D-tryptophanyl-gem-diamino-D-tryptophanylformaldehyde acetate salt

Molecular Formula/Molecular Weight

Aib-D-Trp-D-gTrp-CHO – (474.56 amu)

Structure or Biochemical Description



Pharmacologic Class

Synthetic tripeptide GH secretagogue - Growth hormone secretagogue receptor agonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

Macimorelin was developed under IND 73196. (b) (4)

2.3 Drug Formulation

The proposed commercial formulation is a (b) (4) containing (b) (4) mg macimorelin which will be dissolved in 120 mL water, resulting in a solution with a nominal concentration of 0.5 mg/mL administered orally to the patient at the same dose (0.5 mg macimorelin/kg body weight) used in the Phase III study.

2.4 Comments on Novel Excipients

The reported excipients are commonly used and do not exceed the daily recommended exposure levels.

Composition	Function	Standard	Mass / Sachet
Macimorelin (as acetate)	API	Internal	(b) (4)
Lactose monohydrate, (b) (4)	(b) (4)	USP-NF	
Crospovidone, (b) (4)		USP-NF	
Colloidal silicon dioxide		USP-NF	
Sodium stearyl fumarate		USP-NF	
Saccharin sodium, (b) (4)		USP-NF	
Total			1817.2 mg

(b) (4)

2.5 Comments on Impurities/Degradants of Concern

(b) (4)

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2.6 Proposed Clinical Population and Dosing Regimen

Diagnosis of growth hormone deficiency in adult patients suspected of having AGHD. Patients will be administered a single 0.5 mg/mL oral solution of macimorelin in water (0.5 mg macimorelin/kg body weight). Systemic exposure at the 0.5 mg macimorelin/kg body weight dose is: $AUC_{0-\alpha}$ = 1200 ng.min/mL / 60 = 20 ng.hr/mL and C_{max} = 7.59ng/mL (16nM).

2.7 Regulatory Background

The IND for macimorelin was opened in 2007 under a prior sponsor. At that time, the Division agreed that 28 day repeat dose oral toxicity studies in the rat and dog were sufficient to support clinical trials under the IND and eventual submission of the NDA. Reproductive toxicity studies and carcinogenicity studies were not required for this program based on the intended single-dose use of the product and on the known pharmacology/toxicology of the drug target.

The IND was subsequently inactivated in 2008, and was reactivated under the current sponsor AEterna Zentaris in 2009. The Division confirmed in November of 2011 that the previously agreed-to nonclinical program was adequate to support NDA submission. In addition to the nonclinical studies previously discussed, AEterna conducted two *in vitro* hERG assays and an *in vitro* histamine release assay to augment their safety pharmacology program. A complete listing of nonclinical studies submitted in support of this NDA is found in the next section.

3 Studies Submitted

3.1 Studies Reviewed

Study	Route	Species	Primary Review
Pharmacology			
Primary Pharmacodynamics			
Activity of Ghrelin Receptors	*in vitro*	Hu, Ms, Rt	IND 73196 P/T Review 4
Safety Pharmacology			
Irwin Test	IV	Rat	IND 73196 P/T Review 2
Respiratory Function	IV	Rat	IND 73196 P/T Review 2
Hemo and Pulmo Evaluation	IV	Dog	IND 73196 P/T Review 2
Adverse Event Profiling (*in vivo)*	Oral/*ex vivo*	Ms, Rt, Gn Pig	**NDA Review**
Histamine Release	*in vitro*	Rat Mast Cells	IND 73196 P/T Review 4
hERG Channel Binding	*in vitro*	Membrane Fracs	IND 73196 P/T Review 4
Patch Clamp hERG	*in vitro*	HEK293	IND 73196 P/T Review 4
Pharmacokinetics			
Absorption			
Permeability Assay	*in vitro*	CaCo2 cells	**NDA Review**
SD Rat PK Study	Oral	Wistar Rat	**NDA Review**
SD Dog PK/PD Study	Oral/IV	Beagle Dog	IND 73196 P/T Review 2
SD Rat PK/PD Study	Oral/IV	Wistar Rat	IND 73196 P/T Review 2
SD Rat PK Study	IV	Wistar Rat	IND 73196 P/T Review 4
Metabolism			
Liver Microsomes + CYP Inhibition	*in vitro*	Hu, Ms, Rt, Dg	**NDA Review**
PK Drug Interactions			
Hepatocyte CYP450 Induction	*in vitro*	Human	**NDA Review**
Pgp (MDR-1) Inhibition	*in vitro*	Human	IND 73196 P/T Review 4
Toxicology			
Single Dose Rat Toxicity	IV	Sprague Dawley	**NDA Review**
PK Dose Range Dog	Oral	Beagle Dog	**NDA Review**
MTD IV Dog	IV	Beagle Dog	**NDA Review**
MTD Oral Dog	Oral	Beagle Dog	**NDA Review**
5 Day Range Finder Rat	Oral	Wistar Rat	P/T Review 2
5 Day Range Finder Dog	Oral	Beagle Dog	IND 73196 P/T Review 2+3
14 Day Range Finder Rat	IV	Sprague Dawley	**NDA Review**
14 Day Range Finder Rat	Oral	Wistar Rat	**NDA Review**
28 Day Rat Pivotal Toxicity	Oral	Sprague Dawley	IND 73196 P/T Review 4
28 Day Dog Pivotal Toxicity	Oral	Beagle Dog	IND 73196 P/T Review 4

Genotoxicity			
Ames Test	*in vitro*	S. typhimurium	IND 73196 P/T Review 2
Mouse Lymphoma Assay	*in vitro*	Mouse	IND 73196 P/T Review 2
Micronucleus Assay	*in vitro*	CHO cells	IND 73196 P/T Review 4
DEREK NEXUS Analysis	*in silico*	Mammals/Bact	**NDA Review**
QSAR MCASE Analysis	*in silico*	SARs	**NDA Review**

3.2 Studies Not Reviewed

All preclinical studies submitted by AEterna Zentaris have been reviewed.

3.3 Previous Reviews Referenced

Pharmacology/Toxicology reviews (1 thru 4) under IND 73196.

APPEARS THIS WAY ON ORIGINAL

4 Pharmacology

4.1 Primary Pharmacology

Macimorelin is a synthetic, peptidomimetic which stimulates the release of Growth Hormone (GH) by binding to the Growth Hormone Secretagogue (GHS) receptor.

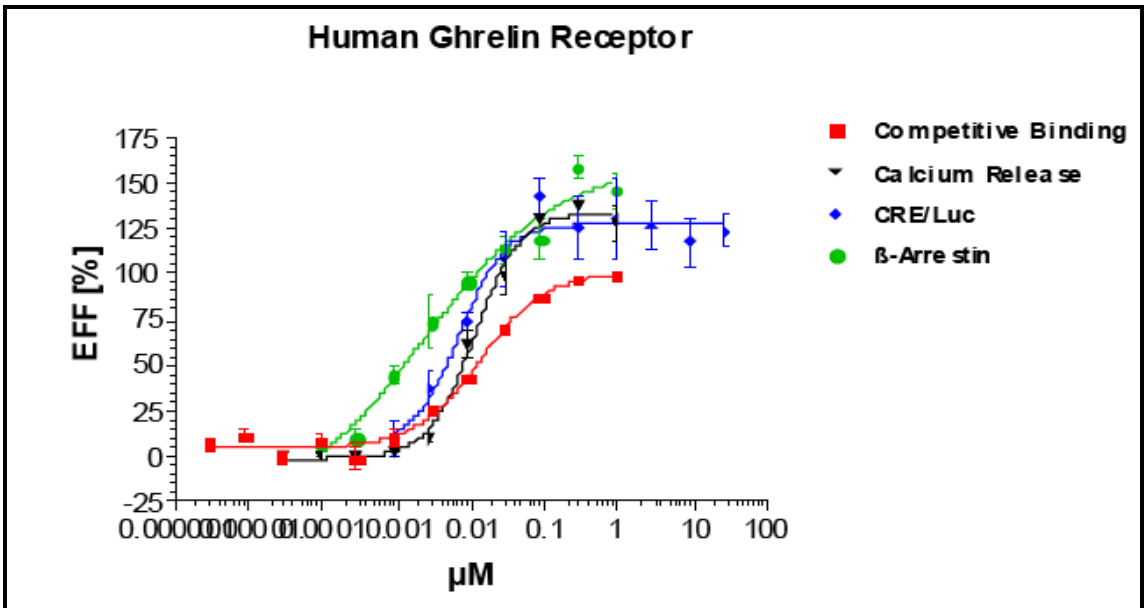
Macimorelin GHS receptor binding characteristics are similar to ghrelin, although its oral bioavailability and elimination half-life is proposed to be better than hexarelin (GHS hexapeptide). Macimorelin exhibits receptor binding characteristics at the cloned hGHS receptor similar to ghrelin.

For competitive binding studies, iodinated ghrelin was used as a tracer (80% saturable binding) and increasing concentrations of macimorelin were analyzed for tracer displacement. The IC_{50} for *in vitro* binding of macimorelin to the human ghrelin receptor is 13.3 nM (C_{max} at MRHD – 16 nM). Macimorelin induced signal transduction was confirmed through *in vitro* functional assays although receptor specificity was not addressed by receptor inhibition, competitive ligand inhibition or loss of signal in recombinant cell lines expressing empty vector.

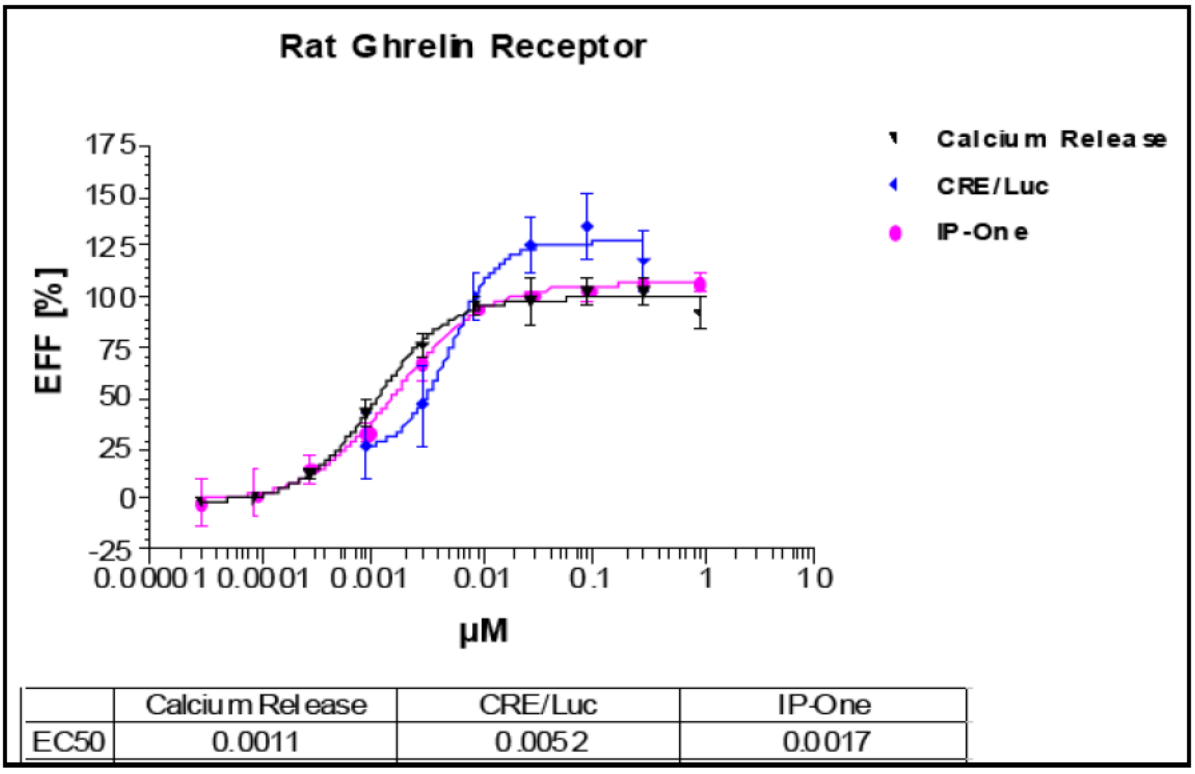
Macimorelin ghrelin agonistic activity for the human, rat and mouse ghrelin receptor

Ghrelin Receptor	Human				Rat			Mouse
Assay	Competitive Binding	Calcium Release	CRE/Luc	ß-Arrestin	Calcium Release	CRE/Luc	IP-One	Calcium Release
EC50 [µM]	0.0133	0.0009 ± 0.0003	0.0084 ± 0.0016	0.0022 ± 0.0001	0.0012 ± 0.0001	0.0052	0.0017	0.0016 ± 0.0001

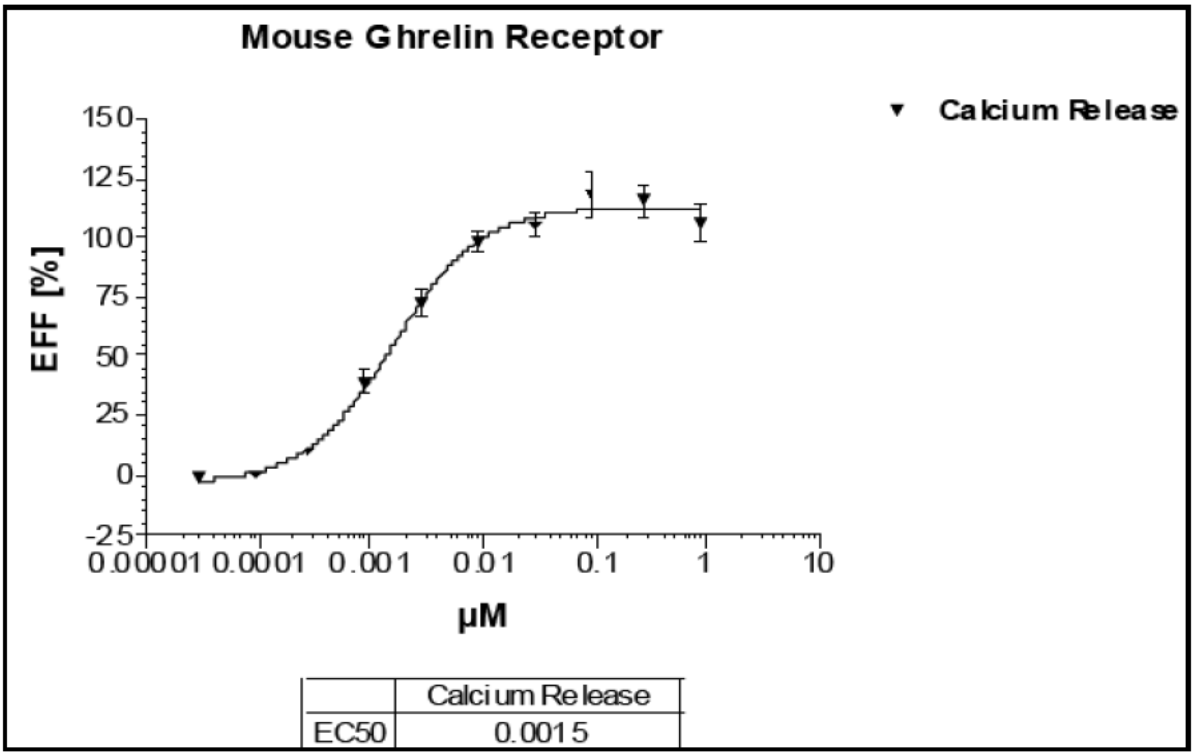
The figures below depict representative EC_{50} curves in % efficacy related to saturating concentrations of ghrelin as obtained for the human, rat and mouse ghrelin receptors.



	Competitive Binding	Calcium Release	CRE/Luc	ß-Arrestin
EC50	0.0133	0.0011	0.0071	0.0023



	Calcium Release	CRE/Luc	IP-One
EC50	0.0011	0.0052	0.0017



	Calcium Release
EC50	0.0015

4.2 Secondary Pharmacology

Secondary pharmacology studies have not been submitted in support of NDA 205598.

4.3 Safety Pharmacology

The intravenous (IV) route was selected for the majority of the *in vivo* safety pharmacology program and differs from the intended oral route to be used in human subjects. The oral bioavailability of macimorelin is extremely low in rats and dogs and likely explains this methodological choice.

Neurological effects

The effects of macimorelin on rats were evaluated using a series of behavioral and neurological tests (Irwin profile) in a GLP compliant neurological safety pharmacology study. Effects were monitored at 0.5, 1, 2, 5 and 24 hours after a single IV dose of drug-substance in male Wistar rats (6 per group). The doses tested were 1, 10 and 30 mg/kg (No PK data). The NOAEL was set at 1 mg/kg due to the decreases in body temperature, flattened posture, lateral decubitus, impaired gait, soiled fur, passivity, decreased rearing, intermittent tremors, decreased grip strength and the slow to moderate stupor induced at higher doses. It is interesting to note that neurological toxicity occurred over a period of 24 hrs (IV dosing) and based on the rat plasma stability data, the compound was likely undergoing rapid degradation.

Pulmonary effects

The respiratory effects of macimorelin on rats were evaluated by plethysmography in a GLP compliant respiratory function study. Effects were monitored in 15 minute intervals over a period of 4 hours after a single IV dose of drug-substance in freely moving male Wistar rats (8/group). The doses tested were 1, 10 and 30 mg/kg (No PK data). The NOAEL was 1 mg/kg due to the dramatic effects (increased respiratory rate, tidal volume and minute volume and decreased inspiratory/expiratory times and peak inspiratory flow) macimorelin had on respiration parameters in rats dosed at ≥ 10 mg/kg. Findings occurred within 4 hrs post IV dosing and based on the rat plasma stability data, about 50% of the compound would have been degraded.

The respiratory effects of macimorelin on dogs were evaluated in a GLP compliant respiratory/cardiovascular function study. Measurements were collected continuously and averaged in 5 min intervals over a period of 0.5 to 1 hr after a single escalating IV dose of in male beagle dogs (5 dogs). Escalating doses (0, 0.3, 1, 10 and 30 mg/kg) were administered with a minimum wash-out period of 30 min in between (minimal PK data). Macimorelin at ≥ 10 mg/kg caused a marked increase in respiratory rate and minute volume, and a decrease in tidal volume, and shorter inspiratory and expiratory times, in anesthetized dogs.

Cardiovascular effects

Macimorelin displaced (↓25%) hERG receptor-associated ligand from membrane fractions at 57 μM (3500X MRHD). The IC_{50} for macimorelin induced inhibition of recombinant hERG channels expressed in human embryonic kidney cells is > 300 μM (> 18750X MRHD).

The *in vitro* portion of a preliminary adverse events profile of macimorelin (30μM – 1900X MRHD) revealed no significant activity (≥ 50% change) in the following non-GLP tissue assays related to cardiac function: guinea pig atria cardiac inotropy, guinea pig atria cardiac chronotropy, aorta rat contractile agonism or antagonism of KCl-induced contractions, ileum guinea pig contractile agonism or antagonism of KCl-induced contractions, trachea guinea pig contractile agonism or antagonism of KCl-induced contractions and portal vein rat contractile agonism or antagonism of KCl-induced contractions.

The cardiovascular effects of macimorelin on rats were evaluated in a non-GLP compliant preliminary adverse events profile study. The blood pressure (BP) and heart rate (HR) were measured at 0, 0.5, 1 and 2 hours following a single oral dose of 30 mg/kg in 3 normotensive male Wistar rats (No Pk Data). The systolic BP of rats decreased significantly at 30 min (↓10%) and increased at 2 hrs (↑17%) while the HR was decreased (↓21%) only at 30 min post dose. Additional *in vivo* studies performed during the preliminary adverse events profile in ICR mice, Wistar and LE rats and Guinea pigs did not yield any significant findings.

The cardiovascular effects of macimorelin on dogs were evaluated in a GLP compliant respiratory/cardiovascular function study. Measurements were collected continuously and averaged in 5 minute intervals over a period of 0.5 to 1 hour after a single escalating intravenous dose of drug-substance in male beagle dogs (5 dogs). Escalating doses (0, 0.3, 1, 10 and 30 mg/kg) were administered with a minimum wash-out period of 30 minutes in between each dose (minimal PK data). Rapid, but transient episodes of hypotension were observed in dogs administered 10 mg/kg macimorelin IV, characterized by increased heart rate, decreased blood pressure, and increased carotid blood flow. A sustained decrease in the maximum ventricular dP/dT was noted in dogs and indicates a paradoxical negative inotropic effect of macimorelin. These effects were amplified in the two animals dosed at 30 mg/kg intravenously, and led to severe adverse events (convulsive spasms and cardiac/respiratory arrest) and death in one dog and convulsions, tremors and hypersalivation with vomiting, in the other.

Immunomodulatory Effects

Macimorelin did not induce histamine release from rat mast cells (*in vitro*) at concentrations ≤ 178 μM (11000X).

In a GLP-complaint single ascending dose (0.5, 10 and 40 mg/kg) comparative (IV vs Oral) PK/PD study, the IV administration of macimorelin to beagle dogs (1/sex/dose) had a NOAEL of 10 mg/kg due to the observed hypersensitivity reaction characterized by lying down and reddening of the mucous membranes around the eyes and excessive salivation. Histamine release was not evaluated during this study.

The NOAEL for administration of macimorelin to beagle dogs by oral gavage was 40 mg/kg due to the lack of toxicological findings (including hypersensitivity reactions) at any oral dose in this arm of the study. While this result is not surprising due to the low oral bioavailability in dogs, a 2-fold increase in serum growth hormone levels was achieved at the highest oral dose.

Preliminary Adverse Event Profiling of Macimorelin (non-GLP)

Key study findings:

- Systolic BP in rats was altered by a single oral dose of macimorelin (30 mg/kg) at 30 min (↓10%) and 2 hrs (↑17%) while HR was decreased (↓21%) only at 30 min post dose

Study no.:	1015758
Sequence # and Date:	SD1 (11/5/2013)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 21 2001
GLP compliance:	No
QA report:	No
Drug, Lot/Batch #, and % purity:	EP 01572 (Coded ET-1), Unknown

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

A GLP-compliant comparative pharmacokinetic study was performed in Wistar rats to evaluate the efficiency of oral administration of macimorelin compared to intravenous administration.

Macimorelin (AEZS-130) was poorly absorbed by Wistar rats via oral administration. A comparison of $AUC_{0\text{-}6h}/AUC_{0\text{-}t}$ values at 50 mg/kg demonstrated that bioavailability of macimorelin after oral administration was low compared to that observed after IV delivery (approximately 0.04%) in rats. A comparison of C_{max} values demonstrated that the maximum serum concentration of macimorelin obtainable by oral administration (50 mg/kg) was low (approximately 5400-fold lower) compared to those concentrations obtainable by intravenous dosing in rats. Macimorelin concentrations were below the detectible limit at the 0.5 mg/kg oral dose. AUC values were roughly similar between male and female Wistar rats, but C_{max} values tended to be higher in females than in males after 50 mg/kg oral or IV administration. It should be noted that while IV dosing appears to achieve higher exposure levels (AUC and C_{max}) and bioavailability, macimorelin is rapidly degraded in rat plasma and consequently PK values are likely derived from a significant amount of inactive compound.

In a subsequent non-GLP compliant pharmacokinetic study in Wistar rats utilizing reformulated drug product a single IV dose (max dose - 30 mg/kg) yielded a C_{max} that was 2-fold lower than expected based on the previous Wistar rat PK data (IV - plasma degradation).

In an attempt to establish the lowest oral dose at which systemic exposure is maximized in Wistar rats a GLP compliant study of macimorelin at five escalating oral doses was conducted. A saturating dose was not established (max dose - 1000 mg/kg) and systemic exposures tended to be elevated in HD females relative to males.

A GLP-compliant comparative PK study was performed in Beagle dogs to evaluate the efficiency of oral administration of macimorelin compared to IV. The oral bioavailability of macimorelin was low (< 1%) in Beagle dogs. A comparison of C_{max} values demonstrated that the maximum serum concentration of macimorelin obtainable by oral delivery of 50 mg/kg in dogs was low (470-fold less) compared to those values measured after IV administration.

Metabolism

Metabolism of macimorelin is CYP450 and NADPH-dependent in dogs, rats, humans and mice and occurs independently of Phase II enzymes (*in vitro*).

Macimorelin is stable in dog and human plasma but is significantly degraded in rat plasma (likely due to rat plasma protease activity)

Pharmacodynamic Note: Dogs have demonstrated an increase in GH levels in the blood after single IV doses of 0.5, 10, and 40 mg/kg and oral doses of 10, and 40 mg/kg macimorelin. Rats lack a pharmacological response at any dose level up to 50 mg/kg (IV). Macimorelin has been demonstrated to bind to rat ghrelin receptors *in vitro* and promote signaling indicative of GPCR activation. Proteolytic degradation of macimorelin in rats may explain the apparent lack of concordance between the *in vitro* and *in vivo* pharmacodynamic responses to macimorelin dosing and should be considered when evaluating nonclinical studies in the rat model.

Pharmacokinetic Drug Interactions

Macimorelin is predominantly metabolized by CYP3A4 and concomitant use of drugs that inhibit this enzyme may affect the metabolism of this compound.

Macimorelin inhibited CYP2C19, CYP1A2 and CYP3A4 and enhanced the enzymatic activity of CYP2C9 at IC_{50s} above 30 μM. The risk for drug-drug interactions is negligible considering this concentration represents a dose that greatly exceeds (1600X) the C_{max} value obtained in human subjects.

Macimorelin is a weak inducer of CYP1A2, CYP2B6 and CYP3A4 mRNA expression in cultured human hepatocytes (concentration exceeding 1000X MRHD - C_{max}) and represents a minimal risk for drug-drug interactions occurring in human subjects.

Exposure of insect cell membranes expressing Pgp to macimorelin caused minimal and dose-dependent inhibition of Pgp ATPase activity.

Distribution

No preclinical distribution studies were conducted with macimorelin.

Excretion

No preclinical excretion studies were conducted with macimorelin.

Pharmacokinetics

Single Ascending Dose Oral (Gavage) Administration PK Study in Wistar Rats (GLP)

Study Number, Species, Doses, Vehicle, Age, Route, Gender and Animal Data	**NOAEL – 500 mg/kg (4X MRHD - AUC) (9X MRHD - C_{MAX})**
Study 2732/003 January 24th 2007 (b) (4) HsdRccHan:Wistar Rat EPO1572 (Single Oral Dose) 50,100,250,500,1000 mg/kg MZ77032 (062080), 86.5% 0.5% methyl cellulose in sterile water - 10 mL/kg N=6 Sex/group Age – 7 to 8 Weeks Weight – Males -165 to 206g Females -125 to 174 g	**Macimorelin PK in Rats Following a Single Oral Dose**

Dose (mg/kg)	Sex	$AUC_{0\text{-}6\ h}$ (ng.h/mL)	$AUC_{0\text{-}6\ h}$ (norm)	C_{max} (ng/mL)	C_{max} (norm)
50	M	13.4	0.268	5.15	0.103
	F	11.9	0.238	5.17	0.103
100	M	16.2	0.162	6.48	0.0648
	F	15.8	0.158	6.87	0.0687
250	M	40.1	0.161	13.6	0.0545
	F	34.3	0.137	13.1	0.0523
500	M	71.6	0.143	74.2	0.148
	F	93.0	0.186	55.7	0.111
1000	M	118	0.118	110	0.110
	F	213	0.213	369	0.369

C_{max} (norm) = C_{max} [ng/mL] / dose [mg/kg]
$AUC_{0\text{-}6\ h}$ (norm) = AUC [ng.h/mL] / dose [mg/kg]

Objective: To establish the lowest oral dose at which systemic exposure becomes maximal by determining the pharmacokinetic profile of macimorelin (EPO1572) at five escalating dose levels following oral (gavage) administration to the Wistar rat.

Reviewer's Comments: A saturating oral dose was not established and macimorelin was generally well tolerated (≤ 1000 mg/kg) in Wistar rats. GH levels were not assessed.

Mortality: There were no unscheduled deaths during this study

Clinical Assessments: Salivation was observed in rats immediately post-dose at 1000 mg/kg

Body Weight: As expected significant body weight changes were not observed after a SD

Pharmacokinetics: $AUC_{0\text{-}6h}$ was less than dose proportional in males and roughly dose proportional in females. C_{max} was roughly dose proportional in males and greater than dose proportional in females. Female systemic exposures tended to be higher than males $AUC_{0\text{-}6h}$ (2X) and C_{max} (3X) at the high dose. The t_{max} ranged of 0.25 to 1 hr.

Single Ascending Dose Oral (Gavage) Administration PK Study in Beagle Dogs (GLP)

Study Number, Species, Doses, Vehicle, Age, Route, Gender and Animal Data	**NOAEL – 250 mg/kg [290X (AUC) + 650X (C_{MAX}) MRHD]**
Study 2732/007 January 15th 2007 (b) (4) Beagle Dog EPO1572 (Single Oral Dose) 50,100,250,500,1000 mg/kg MZ77031/032 (062030/80), 86.6%/86.5%, 0.5% methyl cellulose in sterile water - 5 mL/kg N=2 Sex/group Age – 25 to 28 Weeks Weight – 9 to 11.2 kg	**Macimorelin PK in Dogs Following a Single Oral Dose** EPO1572 Dose Level mg/kg / Sex / $AUC_{0\text{-}8\ h}$ (ng.h/mL) / $AUC_{0\text{-}8\ h}$ (norm) / $AUC_{0\text{-}24\ h}$ (ng.h/mL) / $AUC_{0\text{-}24\ h}$ (norm) / C_{max} (ng/mL) / C_{max} (norm) / t_{max} (h) / $t_{1/2}$ (h) 50 N 2 2 2 2 2 2 2 1 50 Mean Male 353 7.07 581 11.6 419 8.38 0.250 2.48 50 N 2 2 2 2 2 2 2 2 50 Mean Female 425 8.50 624 12.5 348 6.96 0.250 3.88 100 N 2 2 2 2 2 2 2 2 100 Mean Male 2190 21.9 2440 24.4 3330 33.3 0.250 5.07 100 N 2 2 2 2 2 2 2 2 100 Mean Female 991 9.91 1470 14.7 1270 12.7 0.250 7.05 250 N 2 2 2 2 2 2 2 2 250 Mean Male 4420 17.7 5820 23.3 4970 19.9 0.250 5.32 250 N 2 2 2 2 2 2 2 2 250 Mean Female 4630 18.5 5920 23.7 4960 19.8 0.375 7.95 500 N 2 2 2 2 2 2 2 1 500 Mean Male 32600 65.1 34900 69.8 18500 36.9 0.250 3.33 500 N 2 2 2 2 2 2 2 2 500 Mean Female 11200 22.4 13000 26.0 15100 30.2 0.250 6.55 1000 N 2 2 2 2 2 2 2 2 1000 Mean Male 76900 76.9 78300 78.3 54200 54.2 0.250 1.43 1000 N 2 2 2 2 2 2 2 2 1000 Mean Female 42700 42.7 43600 43.6 32800 32.8 0.250 2.46 C_{max} (norm) = C_{max} [ng/mL] / dose [mg/kg] AUC (norm) = AUC [ng.h/mL] / dose [mg/kg]
Objective: To establish the lowest oral dose at which systemic exposure becomes maximal by determining the pharmacokinetic profile of macimorelin (EPO1572) at five escalating dose levels following oral (gavage) administration to beagle dogs.	
Reviewer's Comments: A saturating oral dose was not established and potential dose-limiting toxicity (emesis) was apparent at single oral doses ≥ 500 mg/kg. GH levels were not assessed.	
Mortality: There were no unscheduled deaths during this study	
Clinical Assessments: Vomiting was observed in 2 dogs immediately or 1-2 hours post dosing at the 500 mg/kg dose and immediately in all dogs at the 1000 mg/kg dose. Pale stools were observed in 3 dogs at 2 hours post dose following the administration of 1000 mg/kg.	
Body Weight/Food Consumption: No significant changes in body weight or food consumption	
Pharmacokinetics: Systemic exposure ($AUC_{0\text{-}8\ h}$ and C_{max}) increased in a supra-proportional manner across the 50 to 1000 mg/kg dose range and was more pronounced in males. Pre-dose samples for each of the dose levels were < 2% of the respective C_{max} values indicating that the increased exposures were not due to drug accumulation. Respective mean $AUC_{0\text{-}8\ h}$ and C_{max} values were found to increase by 218- and 129-fold in males and 100- and 94-fold in females for the 20-fold increase in dose.	

In vitro CaCo-2 Permeability Assay (non-GLP)

Key study findings:

- Macimorelin is partially cell permeant and undergoes moderate efflux in the human colon carcinoma cell line (CaCo-2)

Study no.: 8100-2013-950
Sequence # and Date: SD1 (11/5/2013)
Conducting laboratory and location: Aeterna Zentaris
Date of study initiation: July 1st 2013 (Issue Date)
GLP compliance: No
QA report: No
Drug, Lot/Batch #, and % purity: AEZS-130, Unknown

Methods:

The goal of this study was to utilize that CaCo-2 permeability assay to evaluate the transport characteristics for macimorelin over a broad concentration range (2.5 to 1000 μM).

"Using LC-MS/MS for the analysis of samples derived from CaCo-2 cell studies allows the rapid and accurate determination of drug transport across the CaCo-2 cell monolayer. Assessing transport in both directions (apical to basolateral (a→b) and basolateral to apical (b→a) across the cell monolayer enables influx as well as efflux ratios to be determined, which provides an indicator as to whether a compound undergoes active efflux."

Results:

Permeability values for macimorelin were similar over the dose range analyzed and the mean ratio of Papp values (efflux/influx) indicates moderate efflux. The final concentration of macimorelin in the acceptor compartment demonstrated a linear relationship to the compound start concentration in the donor compartment up to the maximum concentration tested.

CaCo-2 Permeability Assay Results for Macimorelin (AEZS-130)

AEZS-130 Concentration [μM]	2.5	5	10	20	30	40	50	75	100	1000	Mean ± SD
Papp (a->b) [cm/s]x10^{-6}	1.38	1.10	1.15	1.17	1.33	1.38	1.53	1.41	1.10	1.73	1.33 ± 0.19
Papp (b->a) [cm/s]x10^{-6}	2.08	1.78	2.00	2.47	2.75	2.82	2.66	2.79	2.28	2.11	2.37 ± 0.36
Papp ratio (b->a/a->b)	1.5	1.6	1.7	2.1	2.1	2.0	1.7	2.0	2.1	1.2	1.8 ± 0.3
Concentration in Acceptor Compartment a->b (2h)	0.020 μM	0.018 μM	0.061 μM	0.104 μM	0.173 μM	0.233 μM	0.291 μM	0.377 μM	0.441 μM	6.565 μM	-

Measurement of the transport characteristics of fluorescent dyes confirmed that the assay conditions were valid.

Metabolism

In vitro Metabolism and Cytochrome P450 Inhibition of Macimorelin (non-GLP)

Key study findings:

- Macimorelin was metabolized by hepatic microsomal CYPs in a NADPH-dependent manner with the rank order of stability being: dog > rat > human > mouse
- Phase II enzymes did not contribute to the overall metabolism of macimorelin
- Macimorelin is predominantly metabolized by CYP3A4. Concomitant use of drugs that inhibit CYP3A4 may affect the metabolism of macimorelin
- Macimorelin is stable in dog and human plasma but is significantly degraded in rat plasma (likely due to rat plasma protease activity)
- Macimorelin inhibited CYP2C19 (IC_{50} 79 μM), CYP1A2 (IC_{50} >27 μM) and CYP3A4 (IC_{50} >30 μM). The 30 μM value represents a concentration of 16 μg/mL, which greatly exceeds (1600X) the C_{max} values of 5-10 ng/mL obtained in human clinical studies.
- Macimorelin (> 30 μM) enhanced the enzymatic activity of CYP2C9. Concomitant use of drugs that are metabolized by CYP2C9 will likely be unaffected at the levels of macimorelin expected to be achieved in the clinic.

Study no.:	ADME-2012-01
Sequence # and Date:	SD1 (11/5/2013)
Conducting laboratory and location:	Aeterna Zentaris
Report Date:	July 16th 2012
GLP compliance:	No
QA report:	No
Drug, Lot/Batch #, and % purity:	AEZS-130, S131754/S143980, 98%

Methods:

The goal of this study was to examine the *in vitro* Phase I metabolism of macimorelin in mixed-gender liver microsomes of mouse, rat, dog and human and to identify the cytochrome P450 (CYP) enzyme or enzymes responsible for metabolizing the drug by reaction phenotyping in the human context (human microsomes and recombinant CYPs) in the event that CYP enzymes were found to be involved in the metabolism.

Secondary metabolism (conjugation) was further assessed thru the impact of Phase II metabolic reactions (direct and Phase-I-dependent Phase II metabolism) on the overall *in vitro* metabolism of macimorelin in mixed-gender human S9 fractions.

Metabolic stability in common matrices in species used for pharmacokinetic and toxicokinetic evaluations and stability of macimorelin in plasma of rat, dog and man were also evaluated.

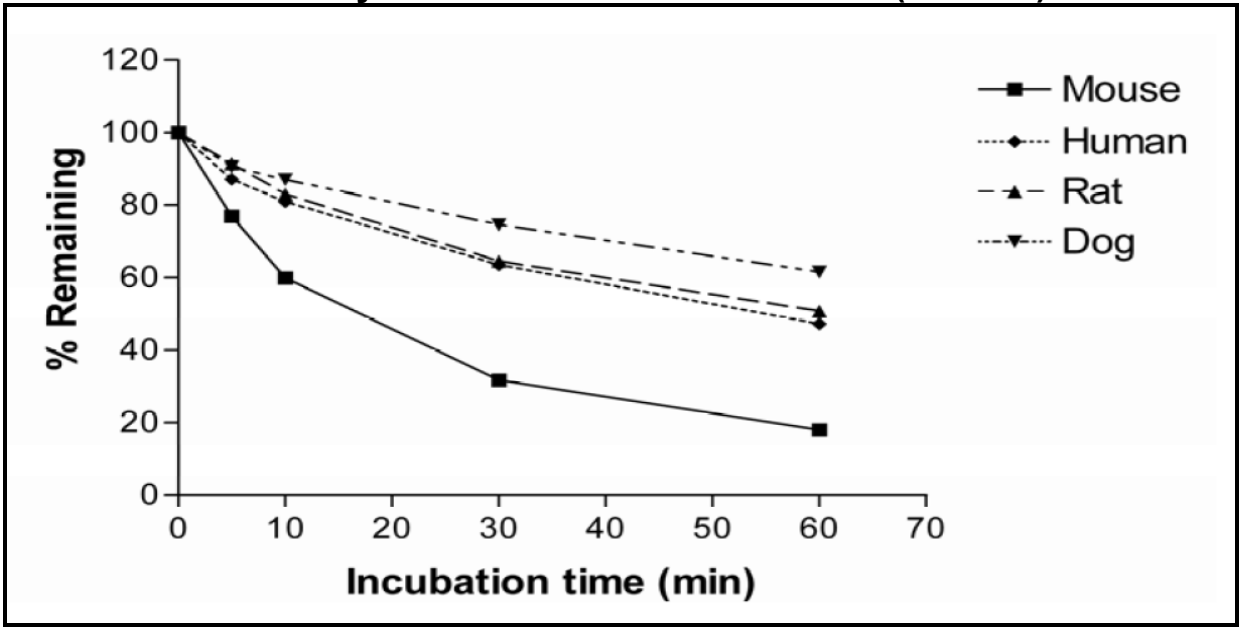
The potential of macimorelin to inhibit cytochrome P450 activity was investigated using common marker substrates in a direct and (only for CYP3A4/5) time-dependent manner to determine the likeliness of drug-drug interaction (DDI).

Results:

Metabolic Stability (Phase I)

The stability of macimorelin was assessed in mixed-gender liver microsomes of mouse, rat, dog and human. Macimorelin was metabolized by hepatic microsomal CYPs in a NADPH-dependent manner with a rank order of stability being: dog > rat > human > mouse after 1 hour of incubation.

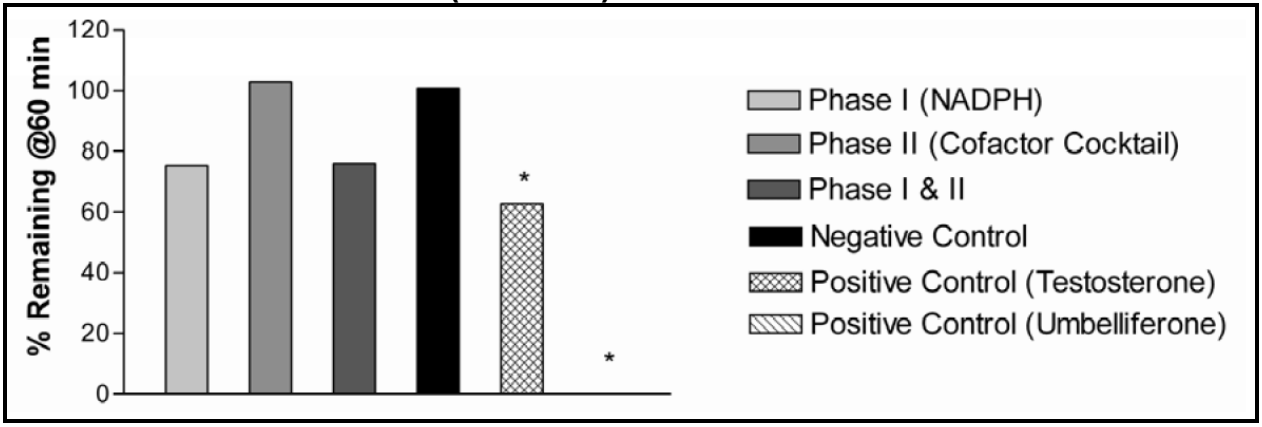
Macimorelin Stability in Pooled Liver Microsomes (Phase I)



Metabolism (Phase II)

Macimorelin was tested for its stability in human liver S9 fraction. Following Phase I metabolism (+NADPH) 75% of macimorelin remained after 1 hour. The addition of Phase II metabolism cofactors (GSH, UDPGA, PAPS) did not enhance the metabolism of macimorelin. Controls indicated the experimental conditions were valid.

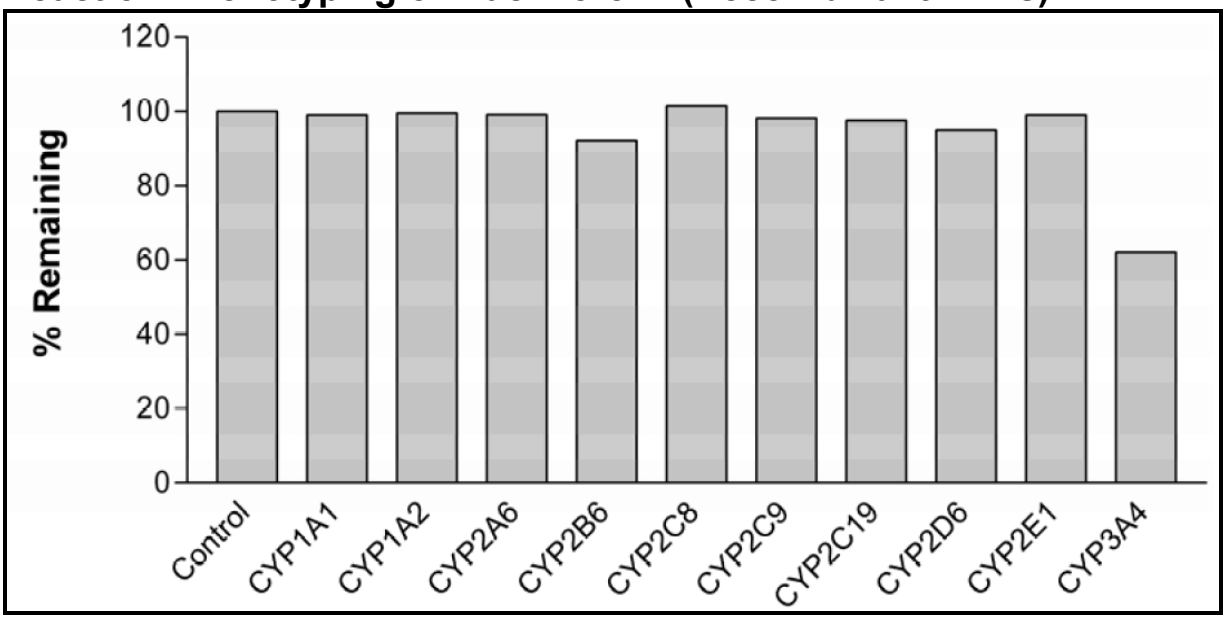
Macimorelin Metabolism (Phase II)



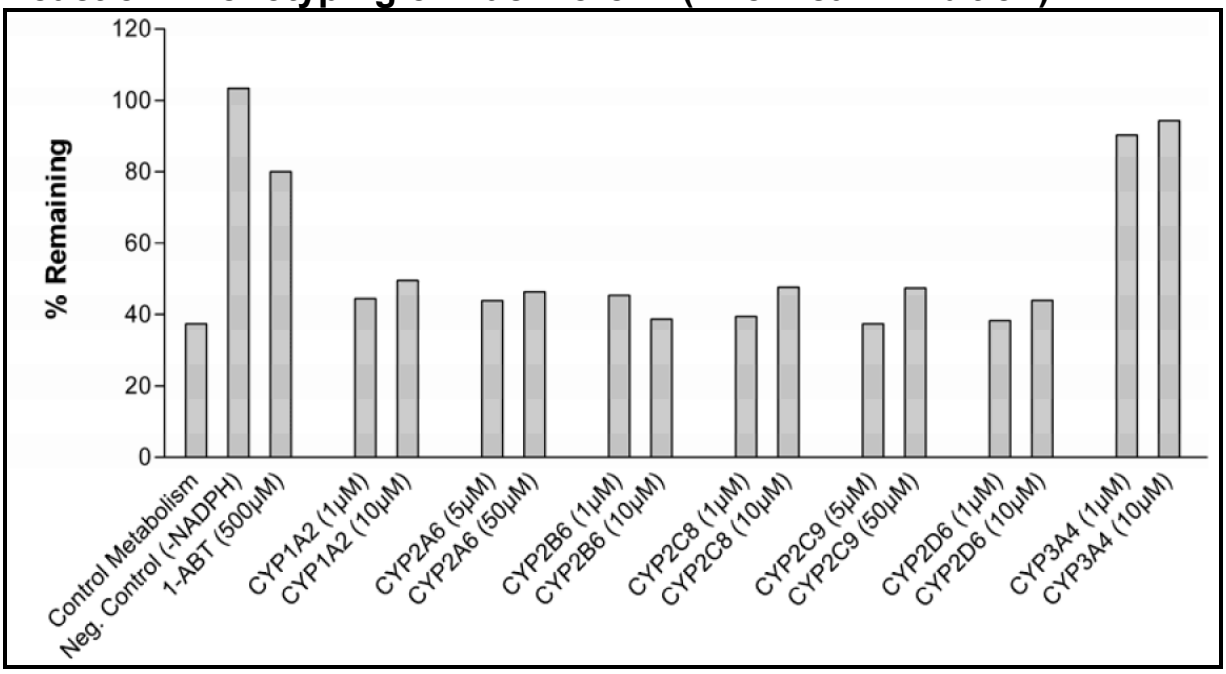
Reaction Phenotyping

The CYP enzymes involved in the Phase I metabolism of macimorelin were determined using recombinant human CYPs and chemical inhibition in human liver microsomes. Under these experimental conditions, macimorelin appeared to be predominantly metabolized by CYP3A4.

Reaction Phenotyping of Macimorelin (Recombinant CYPs)



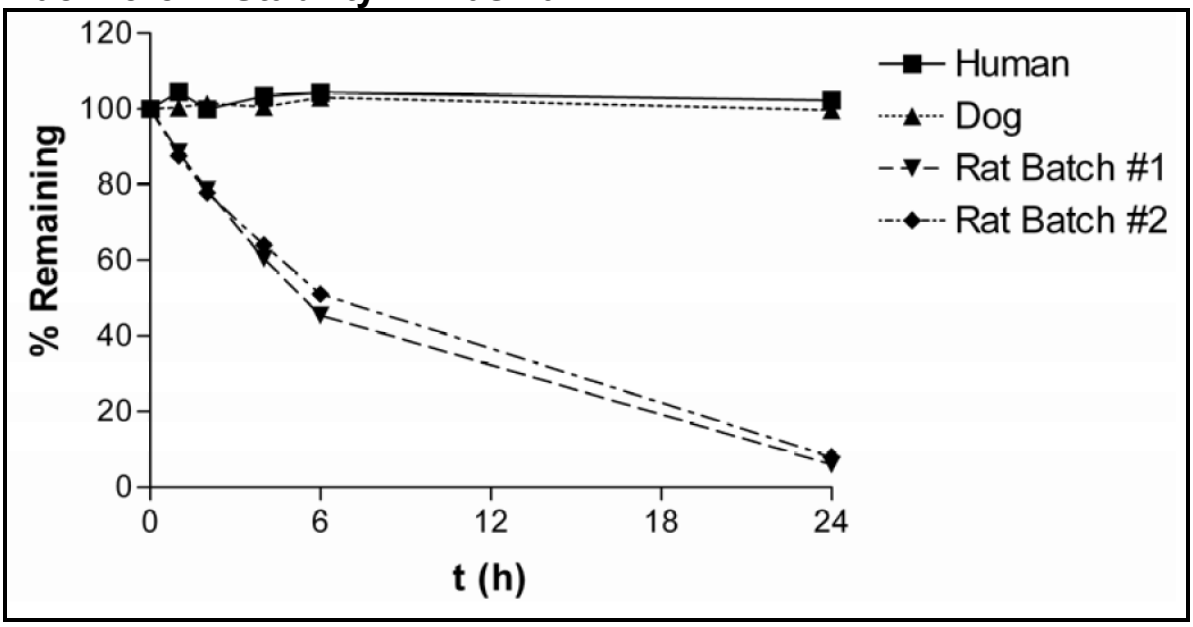
Reaction Phenotyping of Macimorelin (Chemical Inhibition)



Macimorelin Stability in Plasma

Macimorelin was stable in pooled mixed gender dog and human plasma but degraded in rat plasma after 24 hours of incubation. Increased degradation in rats was confirmed in a second plasma batch, indicating the instability of macimorelin was species specific. Mouse plasma was not examined.

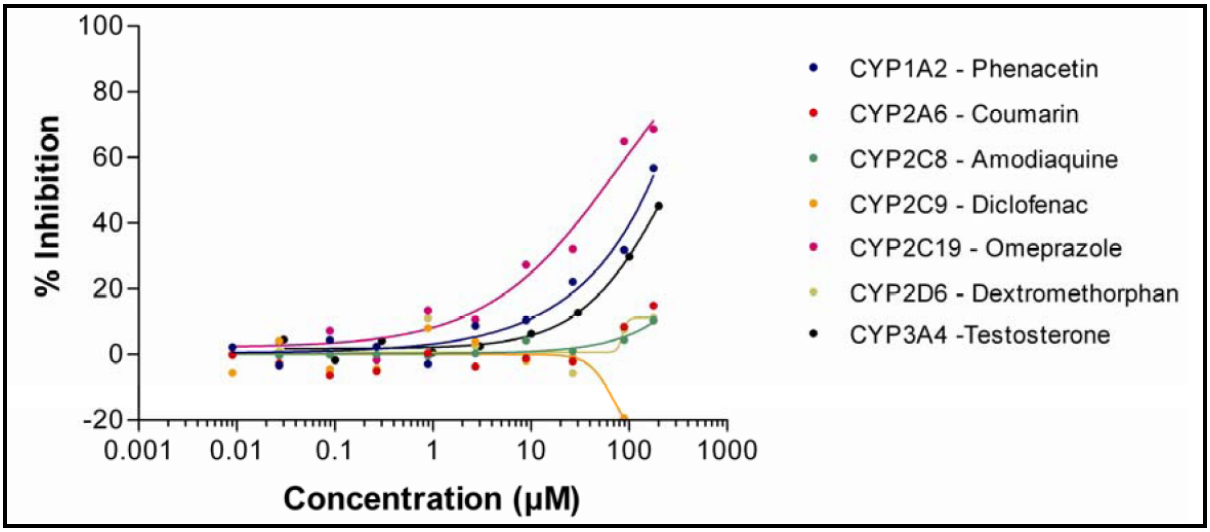
Macimorelin Stability in Plasma



CYP Inhibition

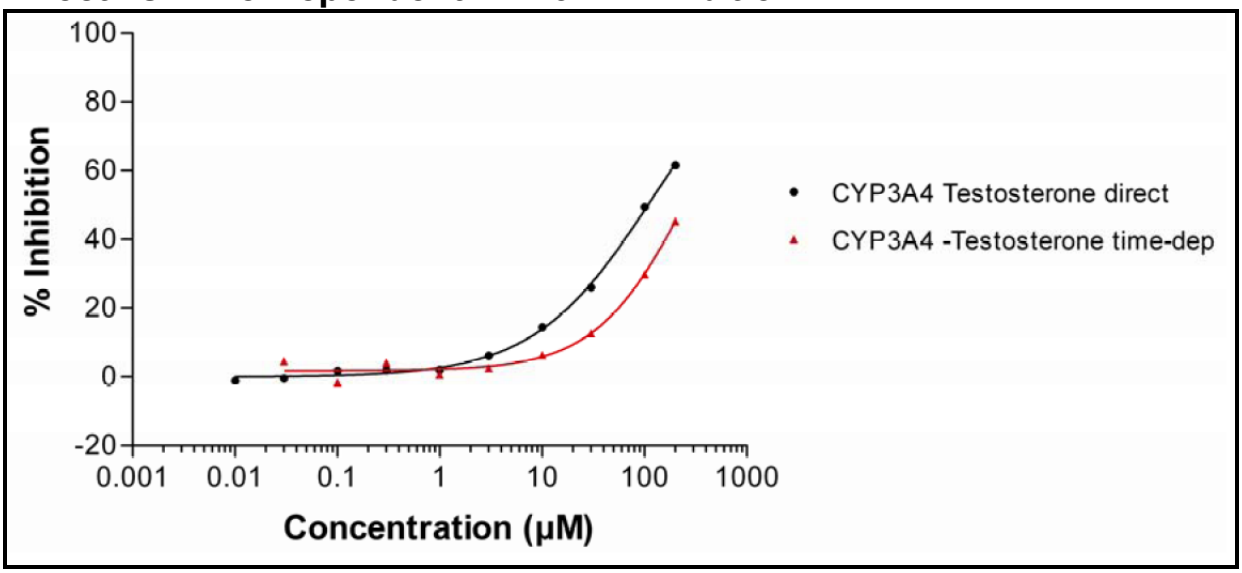
Macimorelin inhibited CYP2C19, CYP1A2 and CYP3A4 over a concentration range of 0.01 to 200 μM. A valid IC_{50} value was established for CYP2C19 only (78 μM). At doses above 30 μM macimorelin enhanced the enzymatic activity of CYP2C9.

Direct CYP Inhibition



Direct and time-dependent metabolism of testosterone by CYP3A4 was comparable.

Direct vs Time-Dependent CYP3A4 Inhibition



Although the FDA recommends that CYP3A4 inhibition studies to be carried out by two, structurally unrelated marker substrates, the methods in place did not support the use of midazolam as a secondary substrate.

Direct and Time-dependent CYP450 Inhibition by Macimorelin

	IC_{50}	
CYP Enzyme	**direct**	**time-dep.**
CYP1A2	>27 µM	n.d.
CYP2A6	>89 -178 µM	n.d.
CYP2C8	>89 -178 µM	n.d.
CYP2C9	>89 µM	n.d.
CYP2C19	78 µM	n.d.
CYP2D6	>178 µM	n.d.
CYP3A4	>30 µM	>30 µM

In vitro Evaluation of Macimorelin as an Inducer of Cytochrome P450 Expression in Cultured Human Hepatocytes (non-GLP)

Key study findings:

- Macimorelin (31.6 μM) was a weak inducer of CYP1A2, CYP2B6 and CYP3A4 mRNA expression in cultured human hepatocytes (> 1000X MRHD - C_{max})
- Macimorelin will likely not induce these specific CYPs in human subjects

Study no.:	ADME-2013-01
Sequence # and Date:	SD1 (11/5/2013)
Conducting laboratory and location:	Aeterna Zentaris
Report Date:	February 14th 2013
GLP compliance:	No
QA report:	No
Drug, Lot/Batch #, and % purity:	AEZS-130, S151314, Purity Unknown

Methods:

The goal of this study was to investigate the ability of macimorelin (AEZS-130) to induce increases in the mRNA of the P450 isozymes CYP1A2, CYP2B6 and CYP3A4/5 in primary cultures of cryopreserved human hepatocytes.

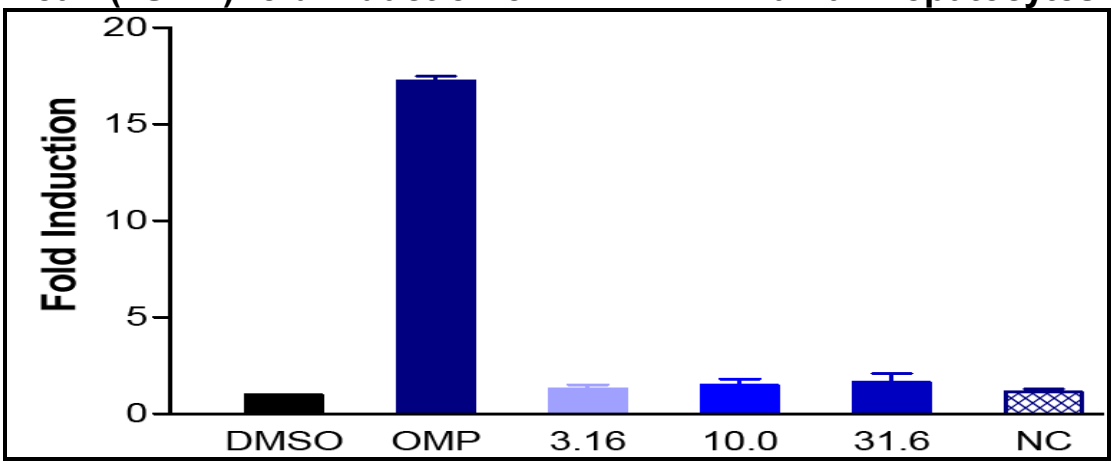
Cultures from a single donor were exposed daily (two consecutive days) to DMSO, macimorelin (3.16, 10 and 31.6 μM), CYP inducers (omeprazole, phenobarbital and rifampicin) or an internal negative control in duplicate. Following exposure, RNA was isolated from cells and analyzed by qRT-PCR to assess the effect of macimorelin on CYP1A2, CYP2B6 and CYP3A4 mRNA levels.

Results:

Human CYP1A2 mRNA

Macimorelin was a weak (< 2-Fold over Control) inducer of human CYP1A2 mRNA levels.

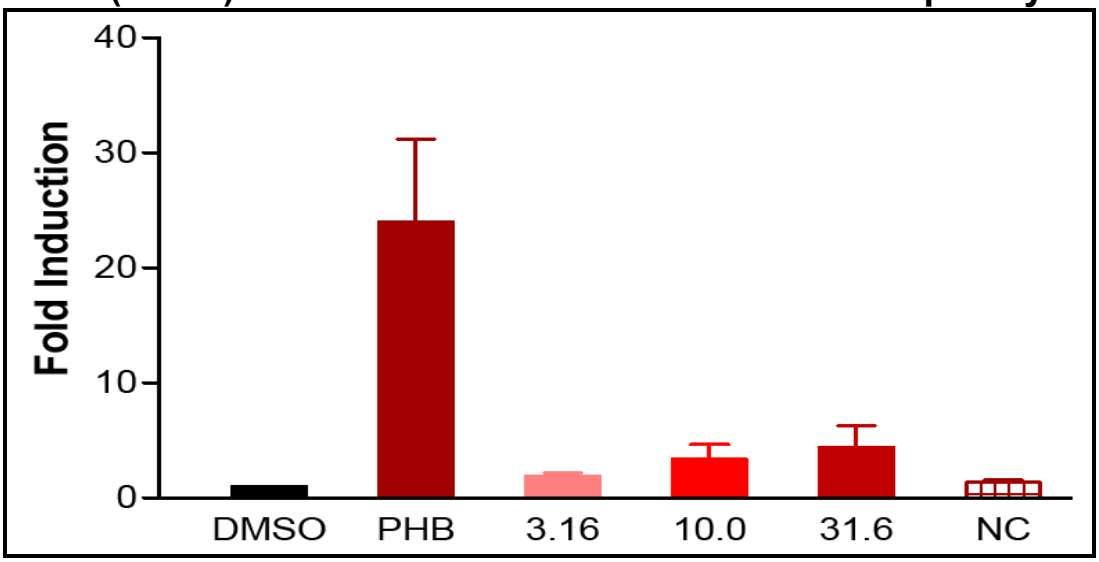
Mean (±SEM) fold induction of CYP1A2 in human hepatocytes



Human CYP2B6 mRNA

Macimorelin was a weak (4-Fold over Control) inducer of human CYP2B6 mRNA levels.

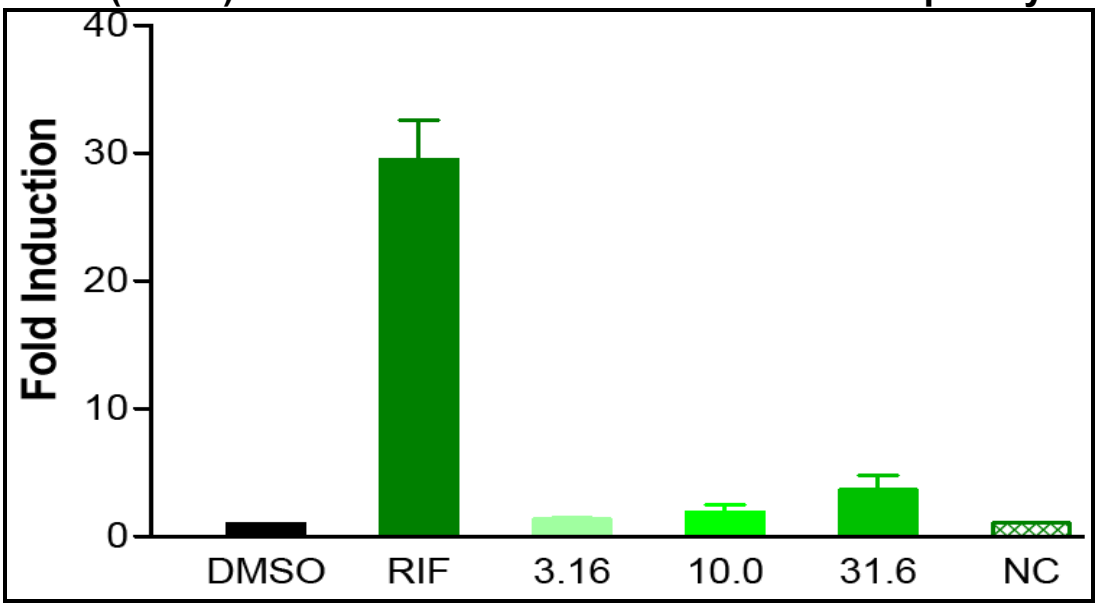
Mean (±SEM) fold induction of CYP2B6 in human hepatocytes



Human CYP3A4 mRNA

Macimorelin was a weak (< 4-Fold over Control) inducer of human CYP3A4 mRNA levels.

Mean (±SEM) fold induction of CYP3A4 in human hepatocytes



An assessment of cell viability (post thaw), development and morphology indicated that the hepatocyte conditions were acceptable and the treatments and procedures utilized during this study did not significantly alter these parameters. Thus the study appears valid.

5.2 Toxicokinetics

After a single 50 mg/kg oral or intravenous dose AUC values were roughly similar between male and female Wistar rats, but C_{max} values tended to be higher in females than in males.

Early Formulation Macimorelin PK after Single IV or PO Administration to Wistar Rats

i.v.	AUC_{0-6h} (ng*hr/mL)			C_{max} (ng/mL)			$T_{1/2}$ (h)		
Sex	Male	Female	Mean	Male	Female	Mean	Male	Female	Mean
50 mg/kg	24000	26000	25000	24000	39000	32000	1.08	NA	NA
p.o.	AUC_{0-t}			C_{max}			T_{max}		
Sex	Male	Female	Mean	Male	Female	Mean	Male	Female	Mean
5 mg/kg	0.8	0.8	0.8	0.6	0.5	0.5	0.5	1.0	0.8
50 mg/kg	9.1	12.4	10.8	3.3	8.6	5.9	0.5	0.3	0.4

Reformulation of macimorelin led to lower than anticipated exposures in Wistar rats following IV administration. Despite concentrations of macimorelin as high as 39000ng/mL in the blood, there were no changes in growth hormone levels among rats in either of these IV studies.

Reformulated Macimorelin PK after a Single IV Administration to Wistar Rats

Dose [mg/kg]	Sex	C_{max} [ng/ml]	t_{max} [h]	AUC_{0-last} [ng·h/ml]	AUC [ng·h/ml]	AUC_{expo} [%]	$t_{1/2}$ [h]
1	**males**	279.27	0.08	64.99	66.58	2.38	1.86
	females	214.73	0.08	57.22	58.19	1.66	1.35
3	**males**	747.13	0.08	189.89	192.54	1.38	1.28
	females	701.13	0.08	224.04	229.84	2.52	1.76
10	**males**	3669.80	0.08	1485.70	1492.98	0.49	1.02
	females	2780.13	0.08	1330.96	1338.54	0.57	0.94
30	**males**	14546.67	0.08	7104.34	7129.90	0.36	0.90
	females	12913.33	0.08	6079.44	6117.28	0.62	1.00

AUC and C_{max} values were comparable between male and female dogs after intravenous administration, but exposures tended to be higher in males after 40 mg/kg oral administration.

Macimorelin Pharmacokinetics after Single IV or PO Administration to Beagle Dogs

i.v.	AUC_{0-8h} (ng*hr/mL)			C_{max} (ng/mL)			$T_{1/2}$ (h)		
Sex	Male	Female	Mean	Male	Female	Mean	Male	Female	Mean
0.5 mg/kg	130	180	160	130	180	160	1.7	1.7	1.7
10 mg/kg	5400	6900	6200	5900	7500	6700	NA	1.4	NA
40 mg/kg	53000	52000	53000	47000	47000	47000	1.5	1.3	1.4
p.o.	AUC_{0-t} (ng*hr/mL)			C_{max} (ng/mL)			T_{max} (h)		
Sex	Male	Female	Mean	Male	Female	Mean	Male	Female	Mean
0.5 mg/kg	1.3	1.3	1.3	0.6	0.9	0.8	1.0	1.0	1.0
10 mg/kg	130	110	120	70	20	50	0.5	1.0	0.8
40 mg/kg	410	300	360	130	70	100	2.0	1.0	1.5

Pharmacodynamic Note: GH levels increased in dogs following intravenous doses of macimorelin at 0.5, 10, and 40 mg/kg (↑5.6-, 23.3-, and 17.3-fold, respectively, compared to no-dose controls). GH increased upon oral administration of macimorelin to dogs at 10 and 40 mg/kg (↑3.2- and 1.8-fold) compared to no-dose controls. No significant stimulation of growth hormone was observed in dogs administered macimorelin orally at 0.5 mg/kg.

6 General Toxicology

6.1 Single-Dose Toxicity

Single dose toxicity studies were conducted in Sprague Dawley rats, Wistar rats and Beagle dogs with two different formulations of macimorelin: [redacted] (b) (4) (early formulation) and [redacted] (b) (4) formulation (new formulation) [redacted] (b) (4) (latter identical to clinical formulation). Macimorelin was administered by IV and/or oral gavage in these studies. The IV route differs from the intended oral route to be used in human subjects and was likely utilized to address the low oral bioavailability of macimorelin.

(Oral Administration)

A lethal oral dose was not attained following single dose oral administrations of macimorelin up to 1000 mg/kg in rats and dogs.

Single oral administrations of macimorelin were generally well tolerated in Wistar rats at doses ≤ 1000 mg/kg, corresponding to average exposures of ≤ 166 ng.hr/mL $AUC_{0\text{-}6\ hr}$ (8x MRHD).

Dose-limiting toxicity (emesis) was present in beagle dogs following single oral doses of ≥ 500 mg/kg, corresponding to average exposures ≥ 24000 ng.hr/mL $AUC_{0\text{-}24\ hr}$ (1200x MRHD). The single oral dose NOAEL (250 mg/kg) in dogs exceeds the MRHD (20 ng.hr/mL) by 290x (AUC).

(Intravenous Administration)

The early formulation of macimorelin caused mortalities (22%) in Wistar rats administered a single IV dose of 50 mg/kg, corresponding to an average exposure of 25000 ng.hr/mL $AUC_{0\text{-}6hr.}$ Clinical signs included: noisy resp., subdued behavior, paleness, cyanosis, and piloerection.

Reformulation of macimorelin led to lower than anticipated exposures in Wistar rats following IV administration (based on the previous IV Wistar rat data). Newly formulated macimorelin caused mortalities at doses ≥ 1 mg/kg (≥ 61 ng.hr/mL $AUC_{0\text{-}last}$ = 3x MRHD).

A sedative effect was observed in Sprague Dawley rats administered macimorelin at IV doses ranging from 30 to 60 mg/kg (No PK data). Additional clinical signs noted at the 60 mg/kg IV dose included: shallow breathing, hunched posture and blood in the urine.

In a respiratory/cardiovascular safety pharmacology study, after a single IV dose (0.3, 1, 10 and 30 mg/kg) of macimorelin to male beagle dogs, rapid, but transient episodes of hypotension were observed at 10 mg/kg. These changes were characterized by increased HR, decreased BP, and increased carotid blood flow. These effects were amplified in the two dogs dosed at 30 mg/kg, and led to severe adverse events (convulsive spasms and cardiac/respiratory arrest) and death in one dog and convulsions, tremors and hypersalivation with vomiting, in the other.

The early formulation of macimorelin caused hypersensitivity reactions in dogs at an IV dose of 40 mg/kg (53000 ng.hr/mL $AUC_{0\text{-}8\ hr}$) that were characterized by lying down, reddening of the mucous membranes around the eyes and excessive salvation. These results were confirmed at IV doses ≥ 30 mg/kg (≥ 12000 ng.hr/mL $AUC_{0\text{-}6\ hr}$) in a non-GLP SD/repeat dose study in dogs administered macimorelin. Clinical signs progressed in severity at 60 mg/kg (43000ng.hr/mL, $AUC_{0\text{-}6}$) and included unsteady gait or poor motor coordination and tremors.

Single Dose Toxicity Study Intravenous Administration in Sprague Dawley Rat (GLP)

Study Number, Species, Doses, Vehicle, Age, Route, Gender and Animal Data	**NOAEL – 45 mg/kg**
Study R14810 April 13th 2001 (b) (4) Crl:CD(SD)BR Rat EPO1572 (Single IV Dose) 30, 45 and 60 mg/kg Batch (402), Unknown 20% DMSO/0.9% NaCl N=5 Sex/group Age – Unknown Weight – Males -165 to 215g Females -160 to 196 g	Pharmacokinetics was not evaluated during this study Based on the instability of macimorelin in rat plasma (Wistar) and the lack of GH activity in previous IV rat studies it is conceivable that the majority of the exposure was to degraded drug product. GH release was not measured during this study
Objective: To evaluate the acute intravenous toxicity of macimorelin (EP01572)	
Reviewer's Comments: The MTD in rats by intravenous route exceeds 60 mg/kg.	
Mortality: There were no unscheduled deaths during this study	
Clinical Assessments: A sedative effect was observed in all rats exposed to macimorelin. Shallow breathing, hunched posture and blood in the urine were observed only at 60 mg/kg. Hypoactivity and piloerection were observed in all groups including control animals.	
Body Weight: As expected significant body weight changes were not observed after a SD	
Gross Observations: No macroscopic changes were noted in rats at doses ≤ 60 mg/kg (IV)	

Note: Single dose toxicity following oral administration of macimorelin to Wistar rats was also evaluated in the GLP study 2732/003 described in the pharmacokinetics section above.

Oral Toxicity Study of Macimorelin in a Single Beagle Dog (non-GLP)

<table>
<tr><th>Study Number, Species, Doses, Vehicle, Age, Route, Gender and Animal Data</th><th>NOAEL – 200 mg/kg [23X (AUC) + 91X (C_{MAX}) MRHD]</th></tr>
<tr><td>Study AA32386
January 30th 2006
(b) (4)

Beagle Dog

EPO1572 (Oral)
Dosed 2 consecutive days
5, 20, 50, 100, 200 mg/kg/day

10 Days Total Dosing

MZ09431(SP001572B)98.6%
0.5% methyl cellulose in sterile water - 5 mL/kg/day

N=1 Male
Age – 7 months
Weight – 9.7 kg</td><td>Toxicokinetics were determined only at the 200 mg/kg/day dose.

Blood samples for the toxicokinetic evaluation were taken on day 10, before dosing and then, 0.25, 0.5, 1, 2, 4, 6 and 8 hours PD.

<table><tr><th>Dose (mg/kg/day)</th><th>Sex</th><th>Animal numbers</th><th>C_{max} (ng/mL)</th><th>T_{max} (h)</th><th>t (h)</th><th>AUC_{0-8h} (ng.h/mL)</th></tr><tr><td>200</td><td>Male</td><td>471</td><td>691</td><td>0.25</td><td>8</td><td>468</td></tr></table></td></tr>
<tr><td colspan="2">Objective: Evaluate the MTD of macimorelin (EP01572) by oral route in the Beagle dog.</td></tr>
<tr><td colspan="2">Reviewer’s Comments: The MTD in Beagle dogs by oral route exceeds 200 mg/kg/day.</td></tr>
<tr><td colspan="2">Mortality: There were no unscheduled deaths during this study</td></tr>
<tr><td colspan="2">Clinical Assessments: No adverse clinical signs were noted at oral doses ≤ 200 mg/kg/day</td></tr>
<tr><td colspan="2">Body Weight/Food Consumption: No significant changes in body weight or food consumption.</td></tr>
<tr><td colspan="2">Gross Observations: No macroscopic changes were noted in dogs at doses ≤ 200 mg/kg (oral)</td></tr>
</table>

Note: Single dose toxicity following oral administration of macimorelin to Beagle dogs was evaluated further in the GLP study 2732/007 described in the pharmacokinetics section above.

Intravenous Toxicity Study of Macimorelin in Beagle Dogs (non-GLP)

<table>
<tr><th>Study Number, Species, Doses, Vehicle, Age, Route, Gender and Animal Data</th><th>NOAEL (< 30 mg/kg) Intravenous Dosing</th></tr>
<tr><td>Study AA33826
April 18th 2006
(b) (4)

Beagle Dog

EPO1572 (IV)

Phase I - Single Dose
(1 - 2 Day Wash Out Period)
(Between Dose Escalations)
30, 40, 60 mg/kg/day

Phase II - 7 Day Dosing
40 mg/kg/day

MZ09431- 97.89%
Buffered 1N NaCl

N=1 Sex/Phase
Age – 8 to 10 Months
Weight – Males -10 kg
Females -7 to 9 kg</td><td>Blood samples for the toxicokinetic evaluation for Phase I were taken on day 0 then after each change of dose and Phase II after the first and last doses at the following time-points: Before dosing, 5, 15, 30 minutes then 1, 2, 4 and 6 hours after dosing.
<table>
<tr><th>Occasion</th><th>Dose</th><th>Sex</th><th>Animal numbers</th><th>C_{max} (ng/mL)</th><th>$AUC_{0.083\text{-}6h}$ (ng.h/mL)</th></tr>
<tr><td rowspan="2">Phase I - day 0</td><td rowspan="2">40 mg/kg</td><td>Male</td><td>341</td><td>57905</td><td>23292</td></tr>
<tr><td>Female</td><td>342</td><td>52790</td><td>19420</td></tr>
<tr><td rowspan="2">Phase I - day 2</td><td rowspan="2">60 mg/kg</td><td>Male</td><td>341</td><td>120955</td><td>46206</td></tr>
<tr><td>Female</td><td>342</td><td>113370</td><td>39568</td></tr>
<tr><td rowspan="2">Phase I - day 5</td><td rowspan="2">30 mg/kg</td><td>Male</td><td>341</td><td>44655</td><td>13880</td></tr>
<tr><td>Female</td><td>342</td><td>35870</td><td>10795</td></tr>
<tr><td rowspan="2">Phase II - day 0</td><td rowspan="4">40 mg/kg/day</td><td>Male</td><td>343</td><td>65180</td><td>28821</td></tr>
<tr><td>Female</td><td>344</td><td>93625</td><td>37231</td></tr>
<tr><td rowspan="2">Phase II - day 6</td><td>Male</td><td>343</td><td>70985</td><td>26890</td></tr>
<tr><td>Female</td><td>344</td><td>97620</td><td>30380</td></tr>
</table></td></tr>
<tr><td colspan="2">Objective: Evaluate the MTD of macimorelin by intravenous route in Beagle dogs and to define dose levels for a subsequent intravenous toxicity study.</td></tr>
<tr><td colspan="2">Reviewer's Comments: The hypersensitivity reactions observed during this study at doses ≥ 30 mg/kg (IV) were similar to those noted in beagle dogs administered 40 mg/kg (IV) during the SAD PK/PD study (GLP-complaint) discussed above (NOAEL, 10 mg/kg). Histamine release was not evaluated during either of these studies. GH levels were not determined during this study.</td></tr>
<tr><td colspan="2">Mortality: There were no unscheduled deaths during this study</td></tr>
<tr><td colspan="2">Clinical Assessments (Phase I): Clinical signs were observed at all single dose levels and included: Hypersalivation, redness of mucosa, vomiting and head shaking during and/or after injection. These signs were associated with unsteady gait or poor motor coordination and tremors at the 60 mg/kg dose. Head shaking, stiffness of hind limbs, abdominal breathing, subdued behavior and vomiting were observed at doses ≥ 40 mg/kg (within 15 minutes after injection). Tremors and head shaking persisted for up to 1 hour after administration (60 mg/kg), and hypersalivation was still observed on days 3, 4 and 6 (days without treatment) in the male. Hyperventilation, half-closed eyes and visible third eyelid were observed sporadically.</td></tr>
</table>

Clinical Assessments (Phase II): The administration of 40 mg/kg/day macimorelin for 7 days induced hypersalivation, redness of mucosa and head shaking on each day of dosing. These signs appeared during the injection and generally lasted for < 1 hour, but could persist for up to 2 hours PD. Lateral decubitus was observed in the female within 5 minutes after the first dose followed by tremors on days 1 and 2.
Body Weight/Food Consumption: All animals gained weight during the study and food consumption was not affected by dosing.
Hematology: RBC counts, hemoglobin levels and pack cell volumes declined minimally during Phase II. These changes were accompanied by a reticulocyte regenerative response.
Clinical Chemistry: Minimal increases in cholesterol and ALP activity were noted during Phase II and were accompanied by a slight decrease in BUN.
Organ Weight: There were no significant changes in organ weights reported.
Gross Observations: Necropsy findings were limited to local reactions at the injection sites and no histopathological examination was performed.

6.2 Repeat-Dose Toxicity

The general toxicity of macimorelin was assessed in Sprague Dawley rats and Beagle dogs in 28 day repeat dose oral toxicity studies (GLP). Systemic exposure (AUC) to macimorelin ranged from 1X to 36X (rat) and 7X to 55X (dog) to the 0.5 mg/kg MRHD. Additional repeat-dose range finding studies were conducted in Wistar rats (Oral dosing – 5 and 14 days), Beagle dogs (Oral dosing – 5 days) and Sprague Dawley rats (IV dosing – 14 days).

Note that the pivotal 28 day repeat dose toxicity studies incorporated a formulation of macimorelin that was similar to the clinical formulation and the oral route of administration utilized in these toxicity studies is the intended route to be used in the clinic.

Target organs of toxicity were not identified during repeat dose toxicity studies in rats and dogs.

Gender-related differences in exposure in the pivotal rat study indicate that the pharmacokinetic characteristics of macimorelin may represent a complication when using this compound as a diagnostic agent in human subjects. The NOAEL of 1000 mg/kg/day (HD) is associated with an exposure of 163ng.hr/mL (8X) and 719ng.hr/mL (36X) for male and female rats, respectively.

The pharmacodynamic activity of macimorelin in the pivotal rat study was limited to a minimal increase in food consumption at the high dose (1000 mg/kg/day) and occurred in the absence of any changes in body weight or body weight gain. Paradoxically, macimorelin drove growth hormone levels down in rats which triggered downstream increases in T4 (males) and corticosterone (both genders). ACTH levels tended to decline in females indicating a failure to release this hormone following repeat dosing of macimorelin to rats.

Note that macimorelin is poorly absorbed by rats via the oral route of administration and upon achieving absorption the compound is subjected to rapid degradation in the plasma. These two forces drive the pharmacodynamic activity of macimorelin downward and limit the usefulness of the rat model as an indicator PD driven toxicity.

The pharmacodynamic activity of macimorelin in the dog manifested itself as increased body weight/body weight gains and increased food consumption for the first 2 weeks of the pivotal toxicology study. These effects occurred independently of dose and were observed only in males where exposures were notably elevated at the highest dose (100 mg/kg/day). Food consumption tended to normalize after 2 weeks of dosing indicating an adaptive response in dogs and/or the loss of the pharmacodynamic activity of macimorelin.

The results of the hormone analysis in the dog were consistent with the results noted in rats. Growth hormone levels tended to decline (dose-independently) in dogs and the levels of downstream target hormones (TSH, free T4 levels and ACTH) were not significantly altered by repeat macimorelin dosing. A dose-dependent decrease in cortisol was observed in males (Day 28) and females (Day 15) administered macimorelin. Changes in cortisol indicate hormone signaling may be disrupted by repeat administration of macimorelin.

Note that macimorelin is poorly absorbed by dogs via the oral route of administration. However, macimorelin is relatively stable in dog and human plasma (unlike the rat). The mechanism underlying the gradual loss of pharmacodynamic activity in the dog is unclear. The dog is currently the most appropriate model to evaluate the PD driven toxicity of macimorelin, however pharmacodynamic activity will likely begin to subside beyond 2 weeks of dosing.

2 Week Oral Dose Range-Finding Study of Macimorelin in Wistar Rats (non-GLP)

<table>
<tr><th>Study Number, Species, Doses, Vehicle, Age, Route, Gender and Animal Data</th><th>NOAEL (150 mg/kg) Oral Dosing</th></tr>
<tr><td>Study AA32387
February 6th 2006
(b) (4)

Wistar Rat

EPO1572 (Oral) - 2 Weeks
10, 50, 150 mg/kg/day

MZ09431- 98.6%
0.5% methyl cellulose in sterile water - 5 mL/kg/day

N=5 or 6 Sex/grp (Main/PK)
Age – 6 Weeks
Weight – Males -167 to 197g
Females -131 to 153 g</td><td>Blood samples for the TK evaluation were taken on day 0 and day 11 at the following time-points: Before dosing, 0.5, 1, 2, 6 and 8 hours after dosing.

<table>
<tr><th>Occasion</th><th>Dose (mg/kg/day)</th><th>Sex</th><th>C_{max} (ng/mL)</th><th>T_{max} (h)</th><th>AUC_{0-8h} (ng.h/mL)</th></tr>
<tr><td rowspan="6">day 0</td><td rowspan="2">10</td><td>Male</td><td>1.00</td><td>1.0</td><td>1.46*</td></tr>
<tr><td>Female</td><td>1.56</td><td>0.5</td><td>1.86*</td></tr>
<tr><td rowspan="2">50</td><td>Male</td><td>3.57</td><td>0.5</td><td>10.47</td></tr>
<tr><td>Female</td><td>4.26</td><td>0.5</td><td>10.20</td></tr>
<tr><td rowspan="2">150</td><td>Male</td><td>10.09</td><td>0.5</td><td>29.80</td></tr>
<tr><td>Female</td><td>17.31</td><td>0.5</td><td>30.59</td></tr>
<tr><td rowspan="6">day 11</td><td rowspan="2">10</td><td>Male</td><td>0.65</td><td>1.0</td><td>1.02*</td></tr>
<tr><td>Female</td><td>0.96</td><td>0.5</td><td>1.18*</td></tr>
<tr><td rowspan="2">50</td><td>Male</td><td>3.56</td><td>0.5</td><td>11.21</td></tr>
<tr><td>Female</td><td>4.09</td><td>0.5</td><td>9.44</td></tr>
<tr><td rowspan="2">150</td><td>Male</td><td>5.83</td><td>0.5</td><td>22.15</td></tr>
<tr><td>Female</td><td>11.93</td><td>0.5</td><td>22.45</td></tr>
<tr><td colspan="6">* AUC_{0-2h} were reported instead of AUC_{0-8h}</td></tr>
</table>
</td></tr>
<tr><td colspan="2">Objective: Evaluate the toxicity of macimorelin following daily oral (gavage) administration to the Wistar rat for 2 weeks and to define dose levels for a subsequent oral toxicity studies in rats.</td></tr>
<tr><td colspan="2">Reviewer's Comments: The pituitary gland was not assessed for changes in weight or morphology and growth hormone release was not measured during this study.</td></tr>
<tr><td colspan="2">Mortality: There were no unscheduled deaths during this study</td></tr>
<tr><td colspan="2">Clinical Assessments: No adverse clinical signs were noted at oral doses ≤ 150 mg/kg/day</td></tr>
<tr><td colspan="2">Body Weight/Food Consumption: No dose-related changes in body weight gain or food consumption were noted at oral doses ≤ 150 mg/kg/day. Male mean terminal body weights tended to be lower than controls at all doses. This trend was less apparent in females.</td></tr>
<tr><td colspan="2">Hematology: A dose-dependent decline in MCV (↓4% - HD) was observed in males on day 14. WBCs tended to be lower in males dosed at ≥ 50 mg/kg/day (↓13% - HD) and in females at doses ≥ 10 mg/kg/day (dose-independent, ↓32%). Reticulocytes counts tended to decline with dose in males and females on day 14 (↓15% - HD).</td></tr>
<tr><td colspan="2">Clinical Chemistry: Total bilirubin tended to increase with dose in males (↑18% - HD) and cholesterol tended to decrease with dose in females (↓24% - HD) on day 14.</td></tr>
<tr><td colspan="2">Organ Weight: There were no dose-dependent organ weight changes noted in the adrenal glands, brain, heart, kidneys, liver, thymus and uterus.</td></tr>
<tr><td colspan="2">Gross Observations: No significant changes were noted during gross examinations.</td></tr>
<tr><td colspan="2">Histology: No histopathological examination was performed.</td></tr>
</table>

2 Week Intravenous Dose Range-Finding Study in Sprague Dawley Rats (GLP)

Study Number, Species, Doses, Vehicle, Age, Route, Gender and Animal Data	**NOAEL (3 mg/kg) Intravenous Dosing**
Study R14820 June 11th 2001 (b) (4) Crl:CD(SD)BR Rat EPO1572 (IV) - 2 Weeks 3, 10, 30 mg/kg/day Batch (402), 97.3% 20% DMSO/0.9% NaCl 5 ml/kg/day N=4 Sex/group Age – 5wks Weight – Males -120 to 130g Females -110 to 120 g	Pharmacokinetics was not evaluated during this study Based on instability of macimorelin in rat plasma (Wistar) and the lack of growth hormone activity in previous rat studies utilizing the IV route of administration it is conceivable that the Sprague Dawley rats in this study were not exposed to active compound for the duration of the study. Growth hormone release was not measured during this study
Objective: Evaluate the toxicity of macimorelin by intravenous route to Sprague Dawley rats.	
Reviewer's Comments: Increased pituitary gland weights are likely related to the activation of GHS receptors however *in vivo* rat studies of macimorelin have never demonstrated GH release.	
Mortality: There were no unscheduled deaths during this study	
Clinical Assessments: Clinical signs were observed at intravenous doses ≥ 10 mg/kg/day and included: sedation/hypoactivity and transient ear redness in rats immediately after dosing during the first week of the study. These changes were accompanied by tachypnea (rapid breathing) and hematuria (blood in the urine) at the high dose (30 mg/kg/day). A single high dose female presented with congestion of the injection site that was trending towards recovery by the end of the study. No significant clinical changes were noted in rats dosed at ≤ 3 mg/kg/day.	
Body Weight/Food Consumption: All animals displayed similar weight gains and food consumption was not affected by 2 weeks of intravenous dosing.	
Hematology: Platelet counts were lowered minimally at the high dose (↓15%) and a dose-related decrease in leukocyte counts was observed in males (↓26% - HD).	
Clinical Chemistry: Glucose (↑15% - HD) and beta globulin (↑17% - HD) levels tended to increase dose-dependently in males. Triglyceride levels were significantly increased in high dose males (↑64%) and dose-dependently increased in females (↑38% - HD). ALT levels increased (↑170% - HD) and serum calcium levels decreased (↓9% - HD) dose-dependently in females only.	
Urinalysis: There were no significant changes in urinalysis parameters reported.	
Organ Weight: Absolute pituitary gland weights increased at doses ≥ 3 mg/kg/day in males (↑37% - HD) and at doses ≥ 10 mg/kg/day in females (↑15% - HD). Sporadic decreases (dose-independent) in adrenal weight were noted in females only (↓20% - max).	
Gross Observations: No significant or dose-related changes were noted	
Histology: The histological examination was limited to the pituitary gland to assess the morphological changes associated with the observed increased organ weight. No microscopic changes were detected in the pituitary gland of intravenously dosed rats.	

28 Day Pivotal Oral Toxicity Study in Sprague Dawley Rats (GLP - No Recovery)

Study no.:	1502-002
Study report location:	SD51/SN43 (3/12/2012)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 27th 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AEZS-130 (ARD-07), MZ77050, 99.1%

Key Study Findings

- After 27 days male Sprague Dawley rats administered 250, 500, and 1000 mg/kg/day had exposures of 14, 45, and 163ng.hr/mL ($AUC_{0\text{-}tlast}$) and 13, 31 and 256ng/mL (C_{max}). Exposures in females were considerably higher at equivalent doses of macimorelin: 152, 260, and 719ng.hr/mL ($AUC_{0\text{-}tlast}$) and 939, 1317 and 2075ng/mL (C_{max}).
- Macimorelin tended to accumulate in females but not in males following 27 days of dosing and it is not clear whether the difference in systemic exposures are uniquely do to accumulation of macimorelin in females or an increase in metabolism/elimination of the parent compound in males.
- Food consumption was significantly increased in males administered 1000 mg/kg/day macimorelin and this trend was absent in females. Increased food consumption did not alter male mean body weights and is likely related to the pharmacology of macimorelin.
- GH levels did not increase following the administration of macimorelin for 28 days to rats. GH levels tended to decline in rats and were accompanied by increases in T4 (males), and corticosterone (both genders). ACTH levels tended to decline in females indicating a failure to release this hormone following repeat dosing of macimorelin to rats. Lack of a pharmacodynamic effect here is not surprising based on the low oral bioavailability of macimorelin and its rapid degradation in rat plasma.
- Ovarian weights were dose-dependently (≥ 250 mkd) and significantly decreased at 1000 mg/kg/day. Macimorelin exposures were significantly higher in females and may have contributed to inhibition of reproductive tissue growth. No histopathological correlates were noted in female reproductive tissues.

Reviewer Comments: The gender-related differences in exposure and accumulation are of paramount concern and may represent a complication when administering macimorelin to human subjects. The NOAEL of 1000 mg/kg/day is associated with an exposure of 163ng.hr/mL (8X) and 719ng.hr/mL (36X) for males and females, respectively.

Rat (28 Day) (250, 500 and 1000 mg/kg)	NOAEL	Multiple of MRHD 35 mg (20 ng.hr/mL)
Increased Food Consumption (Males)	500 mg/kg (45ng.hr/mL)	2X
Ovarian Weight Increased (Females)	500 mg/kg (260μg.hr/mL)	13X

28 Day Pivotal Oral Toxicity Study in Beagle Dogs (GLP - No Recovery)

Study no.:	1502-001
Study report location:	SD51/SN43 (3/12/2012)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 2nd 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AEZS-130 (ARD-07), MZ77050, 99.1%

Key Study Findings

- After 28 days male dogs administered 25, 50, and 100 mg/kg/day had exposures of 145, 327, and 1107ng.h/mL (AUC_{last}) and 90, 364 and 775ng/mL (C_{max}). In females exposures were 196, 450, and 704ng.h/mL (AUC_{last}) and 203, 291 and 476ng/mL (C_{max}). Macimorelin did not accumulate and gender-related differences were minimal.

- Body weight and body weight gain increased (dose-independently) in males and were accompanied by an increase in food consumption for the first two weeks of dosing. Food consumption tended to normalize after 2 weeks indicating an adaptation and/or a functional loss of pharmacodynamic activity.

- Heart rate increased in a single female (#132) dosed at 100 mg/kg/day and progressed to sinus tachycardia 24 hrs following the final dose. The C_{max} for the 100 mg/kg/day dose occurred 12 min post dose on Day 28. This finding was likely unrelated to macimorelin dosing but is noted here due to the QT prolongation observed clinically.

- GH levels declined (dose-independently) in dogs administered macimorelin. TSH, free T4 and ACTH did not change significantly in dogs. A dose-dependent decrease in cortisol was observed in males at termination and in females following day 15 of dosing. Reduced cortisol levels may indicate that hormone signaling is disrupted by repeat administration of macimorelin to dogs.

- Moderate dilatation of the brain was observed in a single female (#132) dosed at 100 mg/kg/day and correlated with hydrocephalus microscopically. Hydrocephalus has been associated with growth hormone deficiency and may be related to the disruption of GH release following multiple administrations of macimorelin.

- Cysts were observed in multiple tissues (parathyroid, thyroid, pituitary) and may be related to the disruption of growth hormone related signaling pathways.

Reviewer Comments: The NOAEL of 100 mg/kg/day is associated with an average macimorelin exposure of 1107ng.hr/mL (55X) and 704ng.hr/mL (35X) for males and females, respectively.

Dog (28 Day) (25, 50 and 100 mg/kg/day)	NOAEL	Multiple of MRHD 35 mg (20 ng.hr/mL)
Increased Food Consumption (Males)	< 25 mg/kg (< 145ng.hr/mL)	< 7X

7 Genetic Toxicology

Genetic toxicity of macimorelin was evaluated by three *in vitro* assays (Ames, mouse lymphoma assay and micronucleus Assay). No *in vivo* genotoxicity assays were conducted. The genetic toxicity of macimorelin was also evaluated in two *in silico* assays (DEREK Nexus analysis and QSAR MCASE analysis).

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells

Ames Assay

The reversion frequencies were not significantly different from negative controls for any macimorelin dose tested (5 to 5000μg/plate) in the Ames assay. Macimorelin is considered to be Ames negative in the presence and absence of S9 activation at concentrations up to 1500 μg/plate or approximately 158 μM. Concentrations of macimorelin of 1500 and 5000 μg/plate led to precipitation upon addition of top-agar. Under the conditions of this study, there were no effects on the viability of any strain utilized in these assays, either with or without S9 activation and macimorelin is not considered mutagenic.

7.2 *In Vitro* Assays in Mammalian Cells

Mouse Lymphoma Mutation Assay

There were no instances where macimorelin was mutagenic in L5178Y T/K+/- mouse lymphoma cells at concentrations which produced 10% reduction in viability or less. In Phase 1 (4 hour incubation with macimorelin), the highest dose yielding < 10% reduced viability was 1000 μg/mL both with and without S9. In Phase 2 (4 hour incubation with macimorelin), the highest acceptable dose was 1700 μg/mL (+/- S9). In Phase 3 (24 hour incubation with and without S9, 750 μg/mL (-S9) and 1800 μg/mL (+S9) were the highest non-lethal, non-mutagenic concentrations, respectively. All negative controls were in the range of historical background, and all positive controls performed as expected. Macimorelin is thus considered negative for mutagenicity in an *in vitro* L5178V TK +/- mouse lymphoma mutation assay.

In Vitro Micronucleus Assay (CHO-K1 Cells)

In the preliminary assay macimorelin had no effect on cell survival at doses ≤ 18 μM (1125X - MRHD) and a minimal effect on cell survival (↓15%) at the 36 μM dose. There was no indication that the formation of micronuclei was increasing with ascending doses of macimorelin, although no positive control was utilized in this initial experiment. In the definitive experiment, macimorelin had no effect on cell survival at doses ≤ 18 μM and a significant affect (↓52%) at 53 μM (3300X - MRHD) although there was no increase in the number of apoptotic and/or necrotic cells at any dose. There was no indication that the formation of micronuclei was increasing with increasing doses of macimorelin, although a single positive control failed to illicit a response. A second positive control, vinblastine, induced cell death, increased the number of apoptotic and/or necrotic cells and increased the formation of micronuclei validating the assay. Macimorelin is thus considered negative for *in vitro* clastogenic and/or disruption of the mitotic apparatus in dividing CHO-K1 cells.

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

No *in vivo* assays were conducted to evaluate the genetic toxicity of macimorelin.

Other Genetic Toxicity Studies

DEREK Evaluation of the Mutagenicity and Genotoxicity of Macimorelin and its related compounds/impurities

The potential mutagenicity and genotoxicity of macimorelin (AEZS-130 base), (b) (4) were assessed using the toxicity prediction program DEREK NEXUS.

The endpoints searched included: Chromosome damage, Genotoxicity, Mutagenicity and *in vitro* chromosome damage relevant to mammals and bacteria.

DEREK NEXUS is a knowledge-based expert system designed to apply structure-activity relationships to compounds for which little or no data exists and hence to aid in the assessment of their potential toxicity. The DEREK NEXUS knowledge base contains a large number of rules that associate a chemical structure with one or more toxicity endpoints. When a structural alert is identified a reasoning program assigns a probability to the expression of toxicity by the test compound.

Macimorelin and the related structures/impurities assessed by DEREK NEXUS did not trigger any alerts for mutagenicity or genotoxicity *in silico*.

MCASE Evaluation of the Mutagenicity and Genotoxicity of Macimorelin and its related compounds/impurities

The goal of this study was to provide an initial safety assessment for bacterial mutagenicity potential based on QSAR prediction for macimorelin (AEZS-130 base), (b) (4) that were identified during the manufacturing process of active pharmaceutical ingredient macimorelin.

MCASE is based on structure activity relationships with structures in various databases, either known for presence or absence of alerting effects for specific endpoints. It breaks down all input chemical structures into non-cyclic fragments of 2–10 atoms. Fragments with activating activity ('biophores') and deactivating activity ('biophobes') are reported, as well as fractions which are unknown to activity.

Positive structural alerts were triggered in MCASE for the (b) (4) These compounds yielded similar alerts (biophores) in multiple databases and were classified as potential genotoxic impurities based on MCASE prediction for bacterial mutagenicity. Levels of (b) (4) following a single dose of macimorelin ((b) (4) µg) will not exceed the acceptable daily intake of a genotoxic impurity set forth in ICH M7 (120µg/day). The other potential genotoxic compound, (b) (4) is ultimately removed from the API through purification and should not present a risk to human subjects.

The MCASE report stated that the molecular structure of the biophores was not related to the API, therefore these molecules can be classified as Class 3 impurities (ICH M7) until additional data is provided that indicates that they are no longer potential genotoxic impurities.

8 Carcinogenicity

Activation of the GHS receptor is known to induce GH secretion and elicit numerous biological functions including cell proliferation. No definitive carcinogenicity studies were conducted and while the weight of evidence indicates that the use of macimorelin does not pose a carcinogenic risk to human subjects, prolonged exposure may promote the growth of existing tumors.

9 Reproductive and Developmental Toxicology

The endogenous ligand (ghrelin) for the growth hormone secretagogue (GHS) receptor is primarily secreted from the stomach into the circulation but it is also synthesized by reproductive tissues suggesting local activity (autocrine and/or paracrine) at these locations. The GHS receptor is expressed at different levels along the hypothalamic-pituitary-gonadal axis and activation of this receptor appears to play a role in the regulation of different aspects of the female and male reproductive functions from germ cell production to embryo development.

Ovarian weights were dose-dependently (LOAEL = 8x MRHD) and significantly decreased (36x MRHD) in the absence of microscopic changes during the pivotal repeat dose oral toxicity study in rats. Prolonged exposure to macimorelin appears to incite hormone disruption in rats and dogs and while hormone levels were not assessed during single dose oral toxicity studies in these species no finding consistent with a disruption of hormone homeostasis was noted.

Definitive reproductive or developmental toxicity studies were not conducted, however the weight of evidence indicates that the single dose use of macimorelin and the resulting transient activation of GHSRs present minimal developmental or fertility risk to WOCBP.

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

Macimorelin acetate is a synthetic, peptidomimetic which stimulates the release of GH by binding to the GHS receptor (ghrelin receptor). Aeterna Zentaris is seeking approval for the proposed indication: "Diagnosis of adult growth hormone deficiency (AGHD) (b) (4) ' under this NDA. Macimorelin was safe and well tolerated in 40 healthy volunteers and 40 patients with growth hormone deficiency up to a single oral dose of 0.5 mg/kg ($AUC_{(0-\alpha)}$ = 20 ng.hr/mL).

Marked toxicological findings were observed during the single dose intravenous (IV) safety pharmacology evaluations of macimorelin. The IV route of administration was selected for the bulk of the safety pharmacology program and differs from the intended oral route to be used in human subjects. The use of the IV route of administration in these studies was likely driven by the extremely low oral bioavailability (< 1%) of macimorelin in rats and dogs. While IV dosing appears to achieve higher exposures (AUC and C_{max}) and bioavailability in the rat, macimorelin is rapidly degraded in rat plasma and consequently the PD activity that is readily obtained *in vitro* quickly diminishes *in vivo*. Based on these conditions the rat model may be suitable to assess only the non-PD driven effects of macimorelin (i.e., off-target toxicology).

In contrast to the rat, macimorelin is relatively stable in dog and human plasma and despite the poor oral bioavailability in dogs the pharmacodynamic activity of this compound is maintained in short term studies where GH levels increase in the blood after single IV doses (0.5, 10, and 40 mg/kg) and single oral doses (10, and 40 mg/kg). Based on these conditions the dog is currently the most appropriate model to evaluate short term PD driven toxicity.

Neurological toxicity was present in Wistar rats following single IV doses (≥ 10 mg/kg) and manifested as decreased body temperatures, flattened postures, lateral decubitus, impaired gait, soiled fur, passivity, decreased rearing, intermittent tremors, decreased grip strength and a slow to moderate stupor. Toxicity occurred over a period of 24 hours and based on the rat plasma stability data, the compound was likely undergoing rapid degradation.

Respiratory toxicity was observed in Wistar rats at similar IV doses of macimorelin (≥ 10 mg/kg) and presented as increases in respiratory rates, tidal volumes and minute volumes and decreased inspiratory - expiratory times and peak inspiratory flow. Toxicity occurred within 4 hours post IV dosing and based on the rat plasma stability data, roughly 50% of the plasma associated macimorelin would have been degraded by this time.

Based on the intravenous PK data generated in Wistar rats with the reformulated drug product (comparable to the clinical lot) and the poor oral absorption of macimorelin, clinical exposures following a single 0.5 mg/kg oral dose of macimorelin should not approach the rat NOAEL (IV) for neurological and respiratory toxicity (1 mg/kg, 61 ng.hr/mL $AUC_{0\text{-last}}$ = 3x MRHD).

Respiratory toxicity presented similarly in anesthetized dogs where IV dosing with macimorelin (≥ 10 mg/kg) incited increases in respiratory rate and minute volume, decreases the tidal volume and shortened inspiratory and expiratory times. Cardiovascular effects presented as rapid, but transient episodes of hypotension (10 mg/kg) characterized by increased heart rate, decreased blood pressure, and increased carotid blood flow. A sustained decrease in the maximum ventricular dP/dT was noted in dogs and indicates a paradoxical negative inotropic effect of macimorelin. These effects were amplified in the two animals dosed at 30 mg/kg, and led to severe adverse events (convulsive spasms and cardiac/respiratory arrest) and death in one dog and convulsions, tremors and hypersalivation with vomiting, in the other.

Based on an extrapolation of the intravenous PK data generated in dogs with macimorelin (early formulation) and the poor oral absorption of the drug substance, clinical exposures following a single 0.5 mg/kg oral dose of macimorelin should not approach the dog NOAEL (IV) for pulmonary and cardiovascular toxicity (1 mg/kg, 300 ng.hr/mL $AUC_{0\text{-last}}$ = 15x MRHD).

Hypersensitivity reactions were triggered by intravenous dosing (10 mg/kg) in dogs with the original formulation of macimorelin and were characterized by lying down and reddening of the mucous membranes around the eyes and excessive salivation. These results were confirmed at intravenous doses ≥ 30 mg/kg (12000 ng.hr/mL $AUC_{0\text{-}6\ hr}$ = 600x MRHD) in a non-GLP SD/repeat dose study in dogs administered macimorelin. Clinical signs progressed in severity during this study to include unsteady gait or poor motor coordination and tremors at the 60 mg/kg dose (43000ng.hr/mL, $AUC_{0\text{-}6\ hr}$ – 2100x MRHD). Histamine release was not evaluated during either of these intravenous dog studies.

Hypersensitivity reactions were not observed at oral doses ≤ 40 mg/kg (18x MRHD) in a parallel dog study. While this result is not surprising due to the low oral bioavailability in dogs, a 2-fold increase in serum growth hormone levels was achieved at the 40 mg/kg oral dose.

The extensive toxicological findings that manifested in the rat indicate that hypersensitivity reactions were likely present. Macimorelin did not induce histamine release from rat mast cells (*in vitro*) at concentrations ≤ 178 μM (11000X) indicating that the metabolism of macimorelin may increase the sensitivity to this compound or its breakdown products *in vivo.* Hypersensitivity reactions are not likely to occur in human subjects at the MRHD.

Target organs of toxicity were not identified during the pivotal 28 day repeat dose oral toxicity studies in rats and dogs.

Gender-related differences in exposure during the pivotal 28 day oral toxicity study in Sprague Dawley rats indicate that the pharmacokinetic characteristics of macimorelin may represent a complication when using this compound as a diagnostic agent in human subjects. The NOAEL of 1000 mg/kg/day (HD) is associated with an exposure of 163ng.hr/mL (8X) and 719ng.hr/mL (36X) for male and female rats, respectively.

Nominal signs of pharmacodynamic activity were observed in the rat and repeat oral administration of macimorelin tended to drive growth hormone levels down which triggered downstream increases in T4 (males) and corticosterone (both genders). ACTH levels tended to decline in females indicating that repeat oral dosing with macimorelin had instigated hormone dysregulation in the rat.

Pharmacodynamic activity began to dwindle after 2 weeks of oral dosing in dogs indicating an adaptive response and/or a loss of the pharmacodynamic activity of macimorelin. Growth hormone levels tended to decline (dose-independently) in dogs following repeat oral administration of macimorelin. Unlike the rat, the downstream target hormones: TSH, free T4 levels and ACTH were not significantly altered by repeat oral administration of macimorelin. A dose-dependent decrease in cortisol was observed in males (Day 28) and females (Day 15) administered macimorelin. Changes in cortisol indicate that hormone signaling may be disrupted by repeat oral administration of macimorelin. The mechanism underlying the loss of the pharmacodynamic activity of macimorelin in the dog is unclear. The NOAEL for repeat oral administration of macimorelin to dogs is 100 mg/kg/day with AUCs of 1107ng.hr/mL (55X) and 704ng.hr/mL (35X) for males and females, respectively. Systemic exposures to macimorelin in the dog were elevated relative to the rat, despite orally dosing with 10-fold less drug product.

SPECIES TOXICOLOGY STUDIES			
SPECIES/ STUDY	**NOAEL**	**MULTIPLE MRHD (AUC)***	**BASIS**
Rat 28 Day (GLP) No Recovery 250, 500, 1000 mg/kg Oral Dosing (M): 14, 45, 163 ng.h/mL (F): 152, 260, 719 ng.h/mL	**1000 mg/kg** M: 163 ng.hr/mL F: 719 ng.hr/mL	M:8X F:36X	**≥ 250 mg/kg:** Increased Exposure (F) Increased Accumulation (F) Hormone Disruption (Both Genders) **1000 mg/kg:** Increased Food Consumption (Males Only) Decreased Ovarian Weights (No Histology Correlate) Hyperplasia Bladder (Single HD Female -1/10)
Dog 28 Day (GLP) No Recovery 25, 50, 100 mg/kg Oral Dosing (M): 145, 327, 1107 ng.h/mL (F): 196, 450, 704ng.h/mL	**100 mg/kg** M: 1107 ng.hr/mL F: 704 ng.hr/mL	M:55X F:35X	**≥ 25 mg/kg:** Hormone Disruption (Both Genders) **100 mg/kg:** Increased Food Consumption (Males Only) Increased BW + BWG (Males Only)

*AUC in human: S.A.D. – 20ng.hr/mL at 35 mg (0.5 mg/kg) - MRHD

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/s/

JEFFREY A QUINN
06/04/2014

TODD M BOURCIER
06/05/2014
Pharm/tox supports approval

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 205598 **Applicant: Aeterna Zentaris** **Stamp Date: November 4th 2013**

Drug Name: Macimorelin acetate/AEZ-130 **NDA Type: 505(b)1**

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		Consistent with a single dose diagnostic agent: No carcinogenicity studies or reproductive and developmental toxicity studies have been conducted Pivotal studies are 28 days in duration *In vivo* metabolism studies have not been conducted in nonclinical test species or humans
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		
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		Oral dose administration (gavage for animal studies and granules in an oral solution in the clinic)
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		Pivotal Safety Pharm and Toxicology studies were performed under GLP.

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
8	Has the applicant submitted all special Studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Impurities above the qualification threshold (0.15%) were qualified in nonclinical studies as per ICH Q3A(R2). Degradant (b) (4) was qualified *in silico,* although levels of (b) (4) were not determined in preclinical drug lots.
11	Has the applicant addressed any abuse potential issues in the submission?		X	Macimorelin acetate abuse potential studies were not carried out.
12	If this NDA/BLA is to support an Rx to OTC switch, have all relevant studies been submitted?			Not applicable. Macimorelin acetate is a new molecular entity that will not be marketed OTC.

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

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.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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

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/s/

JEFFREY A QUINN
12/06/2013

TODD M BOURCIER
12/06/2013
pharm/tox supports filing