CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208325Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

Date: August 22, 2016
From: Timothy J. McGovern, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 208325
Agency receipt date: August 24, 2015
Drug: Parsabiv **(**Etelcalcetide) injection
Sponsor: Amgen

Indication: Secondary hyperparathyroidism (HPT) in adult patients with chronic kidney disease (CKD) on hemodialysis

Reviewing Division: Division of Metabolism and Endocrinology Products

Introductory Comments: The nonclinical review team concludes that the nonclinical program supports approval of etelcalcetide for the indication listed above. Etelcalcetide is a synthetic peptide that is intravenously bolus injected at doses up to 15 mg three times per week. The recommended established pharmacologic class (EPC) for etelcalcetide is a calcium-sensing receptor (CaSR) agonist. Sensipar (cinacalcet) is another drug in this class that was previously approved in the US.

A complete general toxicology program was conducted in rats and dogs at durations up to 6 months. All of the observed findings were related to the pharmacologic activity of the drug, namely hypocalcemia resulting from suppression of PTH secretion. No off-target toxicity was identified. Resulting animal to human exposure margins were < 1-fold but healthy animals are more sensitive to the hypocalcemic effects of etelcalcetide.

Based on findings of fatal stomach bleeding in the phase 3 clinical program, the nonclinical studies were evaluated for associated findings. No gastrointestinal findings were observed in dogs and only reversible, minimal to mild stomach erosions were observed in rats. The nonclinical review team evaluated the plausibility of a relationship between CaSR agonism and gastrointestinal bleeding and determined that a plausible link does exist although no significant concern regarding gastrointestinal bleeding was observed in the nonclinical program. Given the identified clinical concern and lack of definitive effects in animals, I agree with the reviewers that inclusion of the nonclinical findings in the product label is not warranted.

Etelcalcetide was positive in the Ames assay but negative in in vitro and in vivo chromosome aberration and mutation assays. Carcinogenicity studies in rats and mice did not result in any drug-related tumors.

A complete battery of reproductive and developmental toxicity studies was conducted in rats and rabbits. Exposure to etelcalcetide did not result in any fertility or developmental effects at doses not associated with maternal toxicity. At doses associated with maternal toxicity, effects included reduced fetal growth, increased perinatal pup mortality, and a delay in parturition.

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Conclusion:
I agree with the division pharmacology/toxicology conclusion that etelcalcetide can be approved from the pharmacology/toxicology perspective. The designated EPC is appropriate. I have reviewed and discussed the proposed labeling recommendations made by the division regarding the relevant nonclinical sections.

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/s/

TIMOTHY J MCGOVERN
08/22/2016

DEPARTMENT OF HEALTH & HUMAN SERVICES Food and Drug Administration

Memorandum

PHARMACOLOGY/TOXICOLOGY
MEMO TO FILE

Date: July 13, 2016
From: Miyun Tsai-Turton, PhD, Pharmacology/Toxicology Reviewer
Through: C. Lee Elmore, PhD; Supervisory Toxicologist

Subject: Nonclinical assessment of a clinical signal for fatal gastrointestinal bleeding observed in phase 3 trials with etelcalcetide

To: NDA 208325 for Parsabiv® (Etelcalcetide), Amgen

Refer to: Pharmacology/Toxicology Review & Evaluation of NDA 208325 (Finalized in DARRTS on April 27, 2016)

Background

Etelcalcetide is a calcium sensing receptor (CaSR) agonist intended for the treatment of secondary hyperparathyroidism (HPT) in patients with chronic kidney disease (CKD) on hemodialysis. The pharmacology of etelcalcetide is similar to that of cinacalcet tablets (Sensipar®), the only previously approved calcimimetic agent for the treatment of secondary HPT in patients with CKD undergoing dialysis. Both drugs are highly specific for the CaSR, with limited off-target toxicity identified.

On June 15, 2016, Pharmacology/Toxicology was notified by the clinical team of concern for an imbalance in fatal gastrointestinal (stomach) bleeding events in phase 3 clinical trials with etelcalcetide. Across the phase 3 program, three patients died from stomach bleeding who were randomized to etelcalcetide versus none on placebo.

In nonclinical studies with etelcalcetide, no gastrointestinal toxicity was observed in dogs administered etelcalcetide for any duration (except for sporadic emesis), and no severe stomach erosions, ulcerations or bleeding were observed in rats at any exposure level. However, a dose-related signal was observed in the chronic rat toxicity study for stomach erosions of minimal to mild severity in a few animals, which were recoverable with cessation of dosing. It is notable that a similar signal was observed in rats chronically administered cinacalcet. While the nonclinical signal for stomach erosions with etelcalcetide was of limited incidence and severity and was observed previously with the prototype drug in the CaSR class (i.e., cinacalcet), it is undeniable that these findings correlate with the clinical signal of concern identified in the phase 3 program for etelcalcetide. This memo seeks to address the biological plausibility of a relationship between CaSR agonism and GI bleeding observed in clinical trial patients administered etelcalcetide, based on the established mechanism of action and observations in nonclinical studies.

Pharmacology

Both cinacalcet and etelcalcetide are highly specific for the CaSR, where they act to sensitize the receptor to the presence of calcium. Both drugs are broadly lacking in off-target toxicity, based on extensive toxicology programs conducted to support marketing,

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including chronic toxicology, lifetime carcinogenicity and reproductive toxicology studies.

Etelcalcetide is a synthetic peptide that functions as an allosteric activator of the CaSR in the parathyroid gland and elsewhere. Stimulation of the CaSR in the stomach and small intestine has been studied for its role in stimulating stomach acid secretion (*Alfadda TI, et al. (2014)*). Indeed, extracellular calcium stimulates secretion of the hormone gastrin in animals and humans through activation of the CaSR (*Ward BK, et al. (2012)*). Greater gastrin production leads to increased stomach acid secretion into the lumen of the stomach. Thus, etelcalcetide-related, CaSR-dependent increases in gastrin secretion could represent a plausible biological link between etelcalcetide exposure and worsening stomach erosion, ulceration, and increased risk of stomach bleeding in sensitive patients.

GI bleedings with Sensipar

Given the common mechanism of action of both etelcalcetide and cinacalcet, one might expect a clinical signal observed with etelcalcetide to be similarly observed in the clinical program for Sensipar. According to approved product labeling, in one single-arm trial with 46 patients administered Sensipar (29 patients with parathyroid carcinoma and 17 with intractable primary hyperparathyroidism), the most frequent adverse reactions and most frequent cause of withdrawal were nausea and vomiting. Eight patients died during treatment with Sensipar in this study. Causes of death were cardiovascular (5/8), multi-organ failure (1/8), GI hemorrhage (1/8) and metastatic carcinoma (1/8). No cases of gastrointestinal hemorrhage were reported in the label for three double-blind, placebo-controlled trials with 1126 patients (646 on Sensipar) with CKD on dialysis with dosing up to 6 months.

Nonclinical stomach erosions observed in rats

Stomach erosions (partial thickness of epithelial loss superficial to the muscularis mucosa) were documented in rats in both the etelcalcetide and cinacalcet nonclinical programs (see Table 1). Rats showed dose-related increased incidences of predominantly minimal stomach erosions with both drugs, while non-rodents (dogs and monkeys) showed dose-related increased incidence of emesis without the presence of erosions. No ulcerations (entire epithelial thickness lost down to or through the basement membrane and muscularis mucosa) or bleeding were observed in any species. No concern for increased risk of gastrointestinal bleeding was included for cinacalcet in approved product labeling. Common alternative etiologies for stomach erosions in rats include bacterial infections, severe stress, food deprivation, high-fat diets and others. However, given the dose-related increase in erosions, as well as normal diet, ad libitum feeding, etc. these alternative etiologies are considered unlikely.

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Table 1: Incidence and severity of stomach erosions in rats (sexes combined) administered CaSR agonists

	Severity	Clinical exposure multiples for stomach erosions				Recoverable?
		0	0.2-fold	0.7-fold	2.7-fold	
Etelcalcetide	N	30	29	29	29	
	Minimal	0	0	1	4	Yes
	Mild	0	0	0	1	

	Severity	Clinical exposure multiple for stomach erosions				Recoverable?
		0	0.1-fold	1.4-fold	7.5-fold	
Cinacalcet	N	30	30	30	30	
	Minimal	0	1	0	3	Yes
	Mild	0	0	0	0	

Conclusions

While no significant safety concern regarding GI bleeding was identified in the nonclinical program conducted for etelcalcetide, Pharmacology/Toxicology concludes that a biologically plausible link between etelcalcetide and increased clinical bleeding risk exists, which is based on the established effect of CaSR agonism on stimulation of gastrin secretion and observations of dose-related increases in incidence of minimal to mild stomach erosions in healthy rats administered this drug.

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Appendix

Nonclinical data

The following section contains primary data extracted from the nonclinical NDA reviews for Sensipar (NDA 021688) and Parsabiv (NDA 208325). The incidences and severities of gastrointestinal findings are shown for rodents and non-rodents.

Parsabiv (NDA 208325 PDUFA date: August 2016)

- The maximum recommended clinical dose of Parsabiv is 15 mg, administered three times a week via the intravenous route (AUC_{human} = 33867 ng·hr/mL)
- Chronic (6 month) intravenous rat toxicity study
  - Doses: 0.3, 1, 3 mg/kg (corresponding to 0.2-, 0.7- and 2.7-fold human exposure, respectively)
  - **Incidence of minimal to mild stomach erosions**: 0/14, 1/15, 3/14 (0%, 7%, 21%) in males and 0/15, 0/14, and 2/15 (0%, 0%, 13%) in females at 0.3, 1, and 3 mg/kg, respectively. No erosions were noted upon completion of a 4-week recovery period.

Incidence and Severity of AMG 416-related Microscopic Changes - Dosing Phase (Day 183)

Sex:	Male				Female			
Dose (mg/kg):	0	0.3	1	3	0	0.3	1	3
No. Examined:	15	14	15	14	15	15	14	15
Parathyroid Gland								
Increased cytoplasmic eosinophilia								
minimal	0	0	10	9	0	0	9	10
Kidney								
Pigment accumulation, tubular								
minimal	0	4	7	6	0	6	7	5
mild	0	0	0	1	0	0	0	1
Administration site								
Inflammation, vascular/perivascular								
minimal	1	2	4	3	1	0	2	4
mild	0	0	0	0	0	0	1	0
Stomach								
Erosion								
minimal	0	0	1	2	0	-	0	2
mild	0	0	0	1	0	-	0	0

A dash (-) indicates absence of change in a group. NA = Not applicable.

Incidence and Severity of AMG 416-related Microscopic Changes - Recovery Phase (Day 211)

Sex:	Male		Female	
Dose (mg/kg):	0	3	0	3
No. Examined:	5	5	5	5
Parathyroid Gland				
Increased cytoplasmic eosinophilia				
minimal	0	2	0	2
Kidney				
Pigment accumulation, tubular				
minimal	0	2	0	0

- 6 month intravenous dog toxicity study
  - Doses: 0.2, 0.5, 0.9 mg/kg (0.06-, 0.15- and 0.25-fold human exposure)
  - **Emesis:** Emesis was seen sporadically throughout the study period, at low incidence, and without dose-dependence. For example, emesis was

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observed in 0/4, 0/4, 1/6 (0, 0, 17%) in males and 0/4, 0/4, 0/6 in females on Day 175. The sponsor evaluated clinical signs from prior to, during, and after starting dose. Additional clinical signs are described, below, which were ascribed to hypocalcemia in healthy animals administered etelcalcetide.

Clinical Signs (once daily)
At 0.5 and 0.9 mg/kg, etelcalcetide-related clinical findings included emesis, reduced appetite, and salivation. All showed full recovery during the 4-week recovery period. At 0.9 mg/kg, one high dose male animal was observed with severe tremors on Day 60; related to the marked decreases in serum calcium that occurred at this dose level.

Sensipar (NDA 021688 approved in 2004)

- The maximum recommended human dose of Sensipar is 180 mg/day, once daily via oral tablet (AUC_{human} = 648 ng·hr/mL)
- Chronic (6 month) oral rat toxicity study
  - Doses: 5, 25, 100 mg/kg (corresponding to 0.11-, 1.4-, and 7.5-fold human exposures, respectively)
  - **Stomach erosion**: 0/15, 0/15, 1/15 (0%, 0%, 7%) in males and 1/15, 0/15, and 2/15 (7%, 0%, 13%) in females at 5, 25, and 100 mg/kg, respectively. No erosions were observed after a treatment-free period.

Histopathology

		Males	Females
TREATMENT PERIOD		Incidence out of (15,15,15,15)	Incidence out of (15,15,15,15)
Eye, lenticular degeneration (clusters of swollen/vacuolated/ruptured/liquefied, i.e., abnormal fibers in anterior cortex of lens)	Slight to mild	0,0,1,9	0,1,0,9
Kidney, mineralization, pelvis diverticulum (clusters of crystalloid material, free or beneath pelvis epithelium or in lumen of distal collecting tubules, sometimes with thickening of pelvis epithelium)		0,0,0,7	6,4,10,10
Cecum, hyperplasia, mucosal (increased cellular basophilia and mitotic figures, goblet cell loss, inflammatory cell increase)		0,3,2,2	0,1,2,7
Heart, infiltration, mononuclear cell		8,9,9,10	4,3,2,10
Heart, degeneration and/or necrosis, myocardial (small collections of mononuclear cells in left ventricle and ventricular papillary muscle, sometimes associated with fibrosis and/or degenerated and/or necrotic myocardial fibers)		1,3,5,8	0,0,0,4
Lung, alveolar histiocytosis		3,0,1,5	1,0,0,6
Thymus, lymphoid atrophy		0,0,2,1	0,0,0,2
Stomach, erosion		0,0,0,1	0,1,0,2
RECOVERY		Incidence out of (5,0,0,5)	Incidence out of (5,0,0,5)
Eye, lenticular degeneration		1,-,-,3	0,-,-,2
Kidney, mineralization, pelvis diverticulum		0,-,-,5	1,-,-,4
Heart, degeneration and/or necrosis, myocardial		1,-,-,0	0,-,-,0
Lung, histiocytosis		0,-,-,0	0,-,-,1
Thymus, lymphoid atrophy		1,-,-,0	0,-,-,1

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- Chronic (12 month) oral monkey toxicity study
  - Doses: 5, 50, 100 mg/kg (corresponding to 0.15-, 1.6- and 1.8-fold human exposure, respectively)
  - **Emesis**: Vomiting was observed in high-dose males and females (transient during Weeks 1-3; fully recoverable). Additional clinical signs are shown,

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below.

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Clinical signs
Fair/poor appetite, emesis, soft/liquid feces in HD in Wks1-3
Decreased appetite in MD, HD and abnormal feces throughout study in LD, MD, HD
One HDf not dosed during 8 days (in 2nd month of study) due to thinness/poor appetite
No signs during recovery (signs reversible)

References on MOA of CsR in GI findings

Alfadda TI, Saleh AM, Houillier P, Geibel JP. Calcium-sensing receptor 20 years later. Am J Physiol Cell Physiol. 2014 Aug 1; 307(3):C221-31.

- Page C222 – CaSR is identified in stomach and it modulates acid secretion.
- Page C225 – C226 – CaSR and the gut
- http://www.ncbi.nlm.nih.gov/pubmed/24871857

Ward BK, Magno AL, Walsh JP, Ratajczak T. The role of the calcium-sensing receptor in human disease. Clin Biochem. 2012 Aug; 45(12): 943-53.

- Page 948 – GsR is expressed in mucosal epithelial cells of the stomach. The use of calcimimetic drugs could lead to complications like acid reflux and the progression of stomach ulcers.
- http://www.ncbi.nlm.nih.gov/pubmed/22503956

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/s/

MIYUN M TSAI-TURTON
07/13/2016

CALVIN L ELMORE
07/13/2016
I concur.

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

Pharmacology/Toxicology NDA Review And Evaluation

Application Number:	NDA 208325
Supporting Documents:	SD1; Original submission (eCTD 0000)
Applicant's Letter Date:	August 24, 2015
CDER Stamp Date:	August 24, 2015
Product:	Parsabiv® (Etelcalcetide injection)
Indication:	Secondary hyperparathyroidism (HPT) In patients with chronic kidney disease (CKD) on hemodialysis
Applicant:	Amgen
Review Division:	Division of Metabolism and Endocrinology Products
Reviewer:	Miyun Tsai-Turton, PhD, MS
Supervisor/Team Leader:	Lee Elmore, PhD
Division Director:	Jean-Marc Guettier, MD
Project Manager:	Meghna Jairath

Disclaimer
Except as specifically identified, all data and information discussed below and necessary for approval of NDA 208325 are owned by Amgen or are data for which Amgen has obtained a written right of reference. Any information or data necessary for approval of NDA 208325 that Amgen 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 208325.

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1 Executive Summary

1.1 Introduction

Parsabiv® (etelcalcetide for injection; KAI-4169) is a synthetic peptide that functions as an allosteric activator of the calcium-sensing receptor (CaSR) in the parathyroid gland. Etelcalcetide is designed for the treatment of secondary hyperparathyroidism (HPT) in patients with chronic kidney disease (CKD) on hemodialysis. Etelcalcetide is formulated for intravenous (IV) administration for chronic use and is administered three times per week as a bolus dose into the venous line of the dialysis circuit after the end of hemodialysis and prior to or during the rinse back procedure. The etelcalcetide formulation is mixed with blood outside the body. Dose levels of etelcalcetide range from 2.5 mg to 15 mg and will be titrated individually to achieve target parathyroid hormone (PTH) levels. The pharmacology of etelcalcetide is similar to that of cinacalcet tablets (Sensipar®), the only previously approved calcimimetic agent for the treatment of secondary HPT in patients with CKD undergoing dialysis.

1.2 Brief Discussion of Nonclinical Findings

Pharmacology studies with etelcalcetide demonstrated that etelcalcetide was a potent and selective allosteric activator of the CaSR, decreasing parathyroid hormone and calcium levels and preventing parathyroid gland hyperplasia. No clinically relevant safety signals were observed in cardiovascular, neurologic or respiratory safety pharmacology studies. All adverse effects were attributable to changes in serum calcium not expected to occur clinically with standard patient monitoring for hypercalcemia. Pharmacokinetic/ADME studies in rats and dogs provided confirmation of the human relevance of these animal models. Increases in plasma exposure for etelcalcetide were dose-proportional. Etelcalcetide was rapidly distributed to most tissues (highest in kidney and to a lesser extent the liver; low in brain and reproductive organs), metabolized mostly in whole blood via disulfide conjugation rather than cytochromes P450, and eliminated predominantly via renal clearance. In addition, etelcalcetide was detected in breast milk in rats at concentrations similar to plasma.

Repeat dose toxicity studies were conducted in rats and dogs; IV doses were administered daily to rats at doses up to 5 mg/kg every day for durations up to 6 months and to dogs at dose levels up to 1.5 mg/kg every other day for durations up to 9 months. The increased dosing frequency in the rats was to account for the shorter half-life of etelcalcetide in that species. Toxicities observed in rats and dogs resulted from the expected pharmacologic effects of PTH suppression in healthy animals, predominantly decreased serum calcium. Upon dosing cessation, adverse effects were reversed, corresponding to the normalization of PTH and calcium levels upon dosing cessation. The No Observed Adverse Effect Levels for hypocalcemia represented 0.7-fold and 0.15-fold the maximum clinical dose of 15 mg, based on AUC comparisons. However, these safety margins are not clinically relevant due to the greater sensitivity to PTH suppression on calcium reabsorption in healthy animals with intact kidneys versus symptomatic secondary HPT patients with little or no kidney function, elevated PTH and abnormally high serum calcium. No off-target toxicities were identified, consistent with high sensitivity and specificity of etelcalcetide for the CaSR.

Based on a weight of evidence approach etelcalcetide is considered non-genotoxic. Carcinogenicity studies were conducted in Tg rasH2 transgenic mice and Sprague-Dawley rats. Etelcalcetide was not carcinogenic in Tg rasH2 mice at up to 3.0 mg/kg/day or in rats at up to 1.6 mg/kg/day. The 1.6 mg/kg/day dose in rats represents 0.4-fold the maximum clinical dose of 15 mg administered three times per week based on AUC.

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Reproductive and development toxicity was evaluated in rats and rabbits. There were no etelcalcetide-related effects observed in the absence of maternal toxicity in these studies that covered the entire reproductive cycle. Low placental transfer of etelcalcetide was observed in rats, with fetal levels measuring 2.4 to 3.0% that of maternal levels. Maternal toxicity occurred in rats at 3.0 mg/kg/day etelcalcetide upon administration during the perinatal period. This dose produced etelcalcetide exposures 1.8-fold the clinical exposures at 15 mg administered 3 times per week based on AUC, which was associated with marked reductions in serum calcium, decreased body weight/body weight gain and food consumption, delayed estrous cycle, and increased follicular cysts. Lower mean fetal body weight, small delays in time to parturition, increased pup mortality, and transient decreases in pup growth rates were observed at this maternally toxic dose.

Local tolerance studies with etelcalcetide showed no adverse injection site reactions when administered by the IV route in dogs. There was no evidence that immunogenicity affected the validity of any studies, based both on the observations of the expected plasma etelcalcetide exposures and pharmacodynamic parameters (PTH and calcium) across all nonclinical studies.

Overall, adverse effects observed in animals administered etelcalcetide were considered to be directly or secondarily related to hypocalcemia, which is an expected pharmacological effect of suppressing PTH secretion in healthy animals. Effects were similar across species and sexes evaluated and were reversible upon cessation of dosing. No off-target toxicities were identified.

1.3 Recommendations

1.3.1 Approvability

Pharmacology/Toxicology recommends approval of etelcalcetide for the treatment of secondary hyperparathyroidism in patients with chronic kidney disease on hemodialysis.

1.3.2 Additional Nonclinical Recommendations

No postmarketing studies or requirements are recommended from the Pharmacology/ Toxicology point of view.

1.3.3 Labeling

The following represents this reviewer's recommended labeling for sections (Sections 8.1-8.2, 12.1 and 13.1-13.2) related to evaluation of nonclinical data.

Reviewer Recommended Labeling

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

There are no adequate and well-controlled studies of PARSABIV in pregnant women. In a pre- and postnatal study in rats, there was a slight increase in perinatal pup mortality, delay in parturition, and transient effects on pup growth at doses associated with maternal toxicity related to hypocalcemia. However, there were no effects on embryo-fetal development in rats and rabbits when etelcalcetide was administered during organogenesis and no effect on sexual maturation, behavior, or reproductive function in the rat offspring were observed. Animal

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studies are not always predictive of human response. It is not known whether PARSABIV can cause fetal harm when administered to a pregnant woman and PARSABIV should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Data

Animal Data

There were no effects on embryo-fetal development at 3.0 mg/kg in rats and at 1.5 mg/kg in rabbits when exposed during organogenesis at etelcalcetide exposures (1.8- and 4.3-times human exposures, respectively, at the clinical dose of 15 mg three times per week, based on AUC). At higher exposures in rats and rabbits (2.7- and 7-times human exposures, respectively, compared to patients, based on AUC), there was reduced fetal growth associated with maternal toxicities of hypocalcemia, tremoring, and reductions in body weight and food consumption.

In a pre- and postnatal development study in rats, there was a minimal increase in perinatal pup mortality, delay in parturition, and transient reductions in postnatal growth associated with maternal toxicities of hypocalcemia, tremoring, and reductions in body weight and food consumption. At the same etelcalcetide exposures (3 mg/kg), there were no effects on sexual maturation, behavior, or reproductive function in the offspring at exposures. The NOAEL was 1.5 mg/kg/day, representing 0.8 times human exposures at the clinical dose of 15 mg three times per week, based on AUC.

8.2 Lactation

Risk Summary

It is not known whether PARSABIV is present in human milk. Studies in rats showed [^{14}C]-etelcalcetide was excreted in the milk at concentrations similar to plasma. Many drugs are present in human milk and because of the potential for PARSABIV to cause adverse effects in nursing infants, a decision should be made to discontinue nursing or discontinue PARSABIV, taking into account the potential benefit of PARSABIV to the mother or the potential benefit of breastfeeding to the infant.

Data

Excretion in milk was assessed following a single intravenous dose of [^{14}C]-etelcalcetide in lactating rats. [^{14}C]-Etelcalcetide-derived radioactivity was excreted in milk at levels similar to plasma at maternal exposures similar to patients receiving PARSABIV at 15 mg three times per week.

8.3 Females and Males of Reproductive Potential

No data are available on the effect of PARSABIV on human fertility. In rats, there was no effect on male or female fertility at 3 mg/kg representing1.8-fold clinical exposure [*see Nonclinical Toxicology (13.1)*].

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12.1 Mechanism of Action

Etelcalcetide is a synthetic peptide calcimimetic agent. Nonclinical and in vitro studies have demonstrated that etelcalcetide specifically binds to and activates the CaSR, which reduces PTH secretion from the chief cells of the parathyroid gland. The reduction in PTH is associated with a concomitant decrease in serum calcium and phosphate levels.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Etelcalcetide did not induce tumors in a six-month Tg rasH2 mouse carcinogenicity study at up to 3 mg/kg in females and up to 1.5 mg/kg in males. No etelcalcetide-related tumors were observed in a lifetime study in Sprague-Dawley rats administered 0.2, 0.4, 0.8 and 1.6 mg/kg, at exposure levels representing 0.05-fold, 0.11-fold, 0.18-fold and 0.4-fold exposures achieved in patients receiving etelcalcetide at 15 mg three times per week, based on AUC.

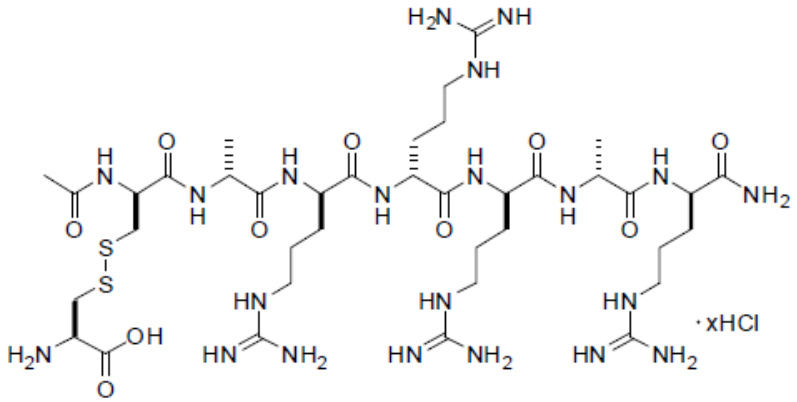
Etelcalcetide was mutagenic in some strains of bacteria (Ames), but was negative in two in vitro and two in vivo mammalian genotoxicity assays. Therefore, etelcalcetide is considered non-genotoxic in humans.

There was no effect on male or female fertility when etelcalcetide was dosed to rats at exposure levels (NOAEL = 3 mg/kg/day) 1.8-fold higher than exposures achieved in patients receiving etelcalcetide at 15 mg three times per week, based on AUC.

2 Drug Information

2.1 Drug

- CAS Registry Numbers: 1262780-97-1 (free base) and 1334237-71-6 (hydrochloride salt)
- Generic Name: Etelcalcetide
- Code Name: AMG416, KAI 4169
- Chemical Name: N-Acetyl-D-cysteinyl-S-(L-cysteine disulfide)-D- alanyl-D-arginyl-D arginyl-D-arginyl-D-alanyl- D-argininamide, hydrochloride salt
- Molecular Formula/Molecular Weight: $C_{38}H_{73}N_{21}O_{10}S_2 \bullet xHCl$, where $4 \leq x \leq 5$ and 1047.5 (monoisotopic, free base)
- Structure or Biochemical Description:



Reference ID: 3923456

- Pharmacologic Class: Calcium-sensing receptor agonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 021688 (Sensipar®, Cinacalcet hydrochloride, Amgen, Approved on March 8, 2004)

2.3 Drug Formulation

Etelcalcetide is supplied for IV administration as a sterile, single-use, preservative-free liquid solution in a 3-cc Type I glass vial with 13 mm stopper. Three product strengths are provided, (b) (4) For each product strength, the concentration of etelcalcetide as free base equivalent is 5 mg/mL, formulated with 10 mM succinic acid and 0.85% (w/v) sodium chloride, and adjusted to pH 3.3 with sodium hydroxide and/or hydrochloric acid. The deliverable volume and etelcalcetide content for each of the 3 strengths is as follows: 0.5 mL (containing 2.5 mg etelcalcetide as free base equivalent), 1.0 mL (5 mg) and 2.0 mL (10 mg).

Table 1 Composition of Etelcalcetide Drug Product

Component	Function	Standard	Concentration	Quantity per Vial for Stated Label Strength [a]		
				2.5 mg	5.0 mg	10 mg
AMG 416 Hydrochloride	Active Ingredient	In House [b]	(b) (4)	(b) (4)		
Sodium Chloride	Tonicity Agent	USP, PhEur, JP	8.5 mg/mL (0.85%)	(b) (4)		
Succinic Acid	Buffer	USP-NF, JP	(b) (4) (10 mM)	(b) (4)		
(b) (4)						
Hydrochloric Acid	pH adjustment	USP-NF, PhEur, JP	qs to pH	qs to pH	qs to pH	qs to pH
Sodium Hydroxide	pH adjustment	USP-NF, PhEur, JP[e]	qs to pH	qs to pH	qs to pH	qs to pH

qs = quantity sufficient to reach target
[a] Quantities are based on deliverable volume. Label strength is stated as AMG 416 quantity (free base equivalent).
[b] 3.2.S.4.1 (Specification)
[c] The concentration of AMG 416 as free base is determined (b) (4) (b) (4)
Weight of AMG 416 hydrochloride equivalent to the drug product label strength (as free base). (b) (4) (b) (4)
A solution of sodium hydroxide may be used for pH adjustment. The supplier tests the sodium hydroxide (b) (4) to the stated compendial standards.

2.4 Comments on Novel Excipients

There are no non-compendial excipients used in the manufacture of the etelcalcetide drug product. All excipients present in the drug product formulation are specified in the current USP, NF, PhEur, or JP monograph and are present in U.S.-approved drugs at greater concentrations, by the specified route of administration and for a comparable patient population.

2.5 Comments on Impurities/Degradants of Concern

This Pharmacology/Toxicology reviewer has no safety concerns for any impurities or degradants identified in the drug substance or product.

Drug substance

(b) (4) impurities can originate from (b) (4) (b) (4)

- In addition, one 4 week dog study (Study No 4169-NC-146) was conducted to compare a (b) (4) (b) (4) batch (b) (4) to a previous batch (Batch F4169KP1001B; used in a 9 month dog study; Study No 4169-NC-132). Another 4 week rat study (Study No 119181) was performed where a drug product (b) (4) was (b) (4) DS batch (Batch 1044951). There were no potential effects associated with drug product impurities in both studies.

(b) (4) impurities might consist of (b) (4) and their potential (b) (4) as well as leachables, (b) (4), elemental impurities, and potentially genotoxic impurities.

- The levels of (b) (4) and elemental impurities in all GMP batches met the ICH limits (ICH-Q3C for residual solvent and ICH-Q3D for elemental impurities).
- Extractables and leachables (b) (4) Based on the maximum etelcalcetide therapeutic dose of 15 mg, the estimated amount of leachables is (b) (4) µg/day, which is (b) (4) the Threshold of Toxicological Concern (TTC) of (b) (4) µg/day (b) (4)
- Potential genotoxic impurities were identified by a structural alert assessment and further assessed by (b) (4) analyses and literature review. (b) (4) was predicted to be mutagenic by (b) (4) analysis. However, further evaluation in an Ames assay showed that it was not mutagenic (Study No 118280). Based on the maximum etelcalcetide therapeutic dose of 15 mg (free base) delivered 3 times a week, other potentially genotoxic impurities (b) (4) ppm in the drug substance as free base) were therefore (b) (4) the threshold of toxicologic concern (TTC) of (b) (4) µg/day, and therefore below the level requiring qualification per (b) (4).

Drug product

- Degradants: (b) (4) (b) (4)
- Leachables: An extractable study was performed to identify potential extractables from the container system, followed by a subsequent toxicology assessment of the identified extractables. Based on the reported extractables profiles, (b) (4) (b) (4) were further evaluated by leachable testing in conjunction with stability testing. These two leachables were not observed in drug product after (b) (4) months (both reported as < (b) (4) µg/ml). These amounts are (b) (4) the permitted daily exposure, and the threshold of toxicological concern, and recommended concentration.

2.6 Proposed Clinical Population and Dosing Regimen

Etelcalcetide is intended for the treatment of secondary hyperparathyroidism (sHPT) in chronic kidney disease (CKD) patients receiving hemodialysis and is administered intravenously (IV) at

the end of hemodialysis three times a week (TIW). As an IV product given with hemodialysis, etelcalcetide administration will be controlled by the dialysis unit staff and its use will be restricted to hemodialysis subjects with sHPT. The commercial drug product will be supplied in 3 strengths of 2.5 mg/vial, 5 mg/vial and 10 mg/vial.

2.7 Regulatory Background

- **August 2010 –KAI (prior sponsor) submitted an opening IND 109773 for etelcalcetide (code name KAI-4169).**
- September 2010 – Pharmacology/Toxicology review placed in DARRTS. A Partial Clinical Hold (PCH) was instituted due to nonclinical deficiencies, based on a narrow safety margin and data did not support the proposed clinical doses. Exposure was limited to allow collection of preliminary human pharmacokinetic and plasma calcium data from End Stage Renal Disease (ESRD) patients to characterize etelcalcetide.
- January 2011 – Pharmacology/Toxicology memo was placed in DARRTS and the PCH was removed (based on sufficient exposure multiples relative to the supportive nonclinical data). However, the Pharmacology/Toxicology discipline concurred with the clinical team that a [redacted] (b) (4) was not recommended.
- October 2011 – A Full Clinical Hold (FCH) was imposed due to manufacturing facility violations. A Pharmacology/Toxicology Type C Written Response memo was placed in DARRTS. Pharmacology/Toxicology provided non-hold comments indicated that repeat-dose toxicity studies (6 month rat and 9 month dog), carcinogenicity studies, reproductive/developmental toxicity studies, and ADME studies would be expected for their nonclinical development program for etelcalcetide.
- November 2011 – The FCH was removed upon satisfactory resolution of manufacturing violations.
- January 2012 – CDER's Executive Carcinogenicity Assessment Committee (Exec CAC) did not concur with the Sponsor's lifetime rat carcinogenicity protocol.
- April 2012 – CDER's Executive Carcinogenicity Assessment Committee (Exec CAC) concurred with the Sponsor's amended lifetime rat carcinogenicity protocol.
- July 2012 – End-of-Phase 2 meeting background information was submitted by **Amgen (new Sponsor); code name was changed to AMG416)**
- May 2013 – CDER's Exec CAC concurred with the Sponsor's 6 month transgenic mouse carcinogenicity study protocol
- December 2013 – The Sponsor's lifetime rat study was terminated early with Exec-CAC concurrence (Pharmacology/Toxicology memo provided in DARRTS).
- April 2014 – Pharmacology/Toxicology Type C meeting memo placed in DARRTS. The nonclinical development program for etelcalcetide was considered adequate for NDA filing.
- December 2014 – The Agency agreed with the Sponsor's initial Pediatric Study Plan.
- April 2015 – Pharmacology/Toxicology recorded the pre-NDA memorandum in DARRTS.
- **August 2015 – Amgen submitted etelcalcetide for approval under NDA 208325.**

3 Studies Submitted

3.1 Studies Reviewed

All studies submitted were reviewed.

Reference ID: 3923456

4 Pharmacology

4.1 Primary Pharmacology

In Vitro Non-GLP Studies

Study No 119880

CaSR mRNA was expressed in thyroid tissue, likely reflective of known high expression in the parathyroid gland, kidney and pancreas.

The mRNA expression of the Calcium Sensing Receptor (CaSR) gene was evaluated in tissues from mouse, rat, cynomolgus monkey, and human. CaSR mRNA was highly expressed in the kidney and thyroid gland, and in the pancreas of human and cynomolgus monkey. Levels in other tissues were undetectable, were found in only one species, or data in other species were not available for comparison.

Study No r2002084

CaSR mRNA was expressed in following tissues: parathyroid gland (most abundant) > kidney and pancreas > stomach and small intestine > thyroid (very low).

The distribution of CaSR mRNA was characterized in tissues obtained from adult cynomolgus monkey, mouse and rat by in situ hybridization. In monkey, CaSR mRNA was abundantly expressed in the parathyroid gland (in chief cells but not in ductal or stromal cells), and to a lesser extent in kidney tubules (segments of distal tubule adjacent to glomeruli), pancreatic islets of Langerhans (but not detectable in the pancreatic acinar and ductal cells), glandular cells of the stomach and small intestine, and to the least extent in thyroid parafollicular cells. In mouse, CaSR mRNA was found to be abundantly expressed in parathyroid gland and to a lesser extent in the kidney. RT-PCR (most sensitive method) detected low levels of CaSR mRNA in mouse and rat bone.

Study No r20130053

A specific structure activity relationship (a calcimimetic cationic portion and a thiol-containing moiety) was required to activate the CaSR.

The specific structural requirements of peptide calcimimetics for CaSR activity were determined using ten peptides (3 L-amino acid polymers; and 7 D-amino acid polymers) in HEK293T cells transiently transfected with human CaSR. CaSR activity was quantified by measuring accumulation of IP-1. Peptide EC_{50} values for each were calculated and compared.

In this system, EC_{50} values were ~4 to 5-fold lower than in a stable cell line. Maximal CaSR activation was similar for each peptide across the tested series. These results suggest that the type of charged residues (arginine vs. lysine) and/or the spacing between the thiol-containing cysteine and the cationic residues are critical factors for CaSR activity.

Reference ID: 3923456

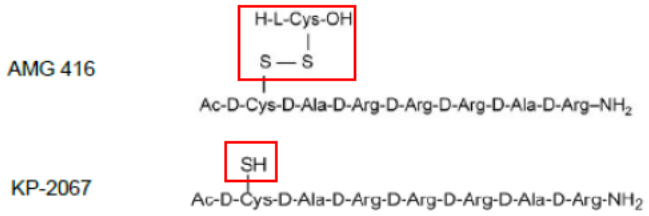
EC_{50} Values for Cationic Peptides in an In Vitro CaSR Assay

Peptide	Sequence Structure	EC_{50} (µM)*
KAI-1455	CHDAPIGYD | Ac-CYGRKKRRQRRR-NH_2	56.9
KP-1524	CPDYHDAGI | Ac-CYGRKKRRQRRR-NH_2	56.6
KP-1896	Ac-CYGRKKRRQRRR-NH_2	10.5
KP-1899	Ac-cygrkkrrqrrr-NH_2	4.1
KP-2014	Ac-crrrrr-NH_2	5.9
KP-1998	Ac-crrrrrr-NH_2	2.7
KP-2013	Ac-crrrrrrr-NH_2	1.5
KP-1956	Ac-crrrrrrrr-NH_2	0.7
KP-2033	Ac-cararrr-NH_2	35.8
KP-2067	Ac-carrrar-NH_2	5.9

Lower and upper-case letters indicate D- and L-amino acids, respectively.
*In this system EC_{50} are ~4 to 5-fold lower than what is measured in a stable cell line (e.g. KP-2067 has EC_{50} of 25 µM in stable cell line).

Etelcalcetide is a synthetic peptide comprised of 7 D-amino acids linked to L-cysteine by a disulfide bond. KP-2067 is a synthetic peptide without L-cysteine whereby both compounds share the same D-amino acid peptide backbone. The chemical structure of KP-2067 is identical to M11, a biotransformation product present in small amounts in plasma following etelcalcetide dosing. Since KP-2067 activity was similar to etelcalcetide in both in vitro and in vivo studies, KP-2067 was used in the molecular pharmacology characterization of etelcalcetide.

Sequence Structure of AMG 416 and KP-2067



Study No 4169-NC-120 (I)

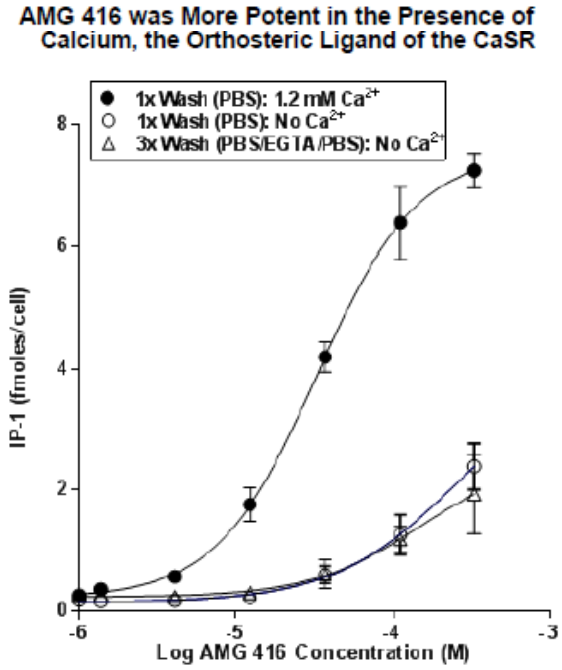
KAI-4169 (etelcalcetide) activated the CaSR signaling pathway.

To evaluate the activity of etelcalcetide on the CaSR, HEK293T cells were stably transfected with the human CaSR (HEK293T/CaSR) then treated with etelcalcetide or calcium. Untransfected HEK293T cells were included as CaSR specificity controls. Response to calcium alone was included as a positive control. CaSR activity was quantified by measuring accumulation of inositol-1-phosphate (IP-1): a stable intracellular metabolite of inositol (1,4,5) tri-phosphate (IP-3) synthesis and a surrogate of Gq (G protein, q polypeptide) activation that followed the activation of the CaSR.

The study showed that etelcalcetide was a calcimimetic that activated the CaSR signaling pathway, with partial activity in the absence of calcium, maximal activity and potency in the presence of calcium. In the presence of calcium (1.2 mM), dose-dependent increases in CaSR activation were seen in HEK293T/CaSR cells treated with (EC_{50} = 25 µM [26 µg/mL]). In the absence of calcium (through removal of calcium from the cell culture medium) the potency (EC_{50}) of etelcalcetide was approximately 20-fold lower than that observed in the presence of

1.2 mM calcium. Etelcalcetide activity was dependent on the presence of the CaSR and full activity was dependent on the presence of calcium.

Figure 1 Pharmacodynamic Study No 4169-nc-120: Full AMG416 (Etelcalcetide) Activity was Dependent on The Presence of Calcium In Vitro



HEK293T/hCaSR cells were washed either once with PBS (circles) or three times (triangles) [once with PBS, once with 2 mM EGTA and final wash with PBS] before incubation with AMG 416. As cell washing resulted in a loss of cell number from the plate, all data in this experiment were normalized to actual cell number rather than absolute IP-1 concentrations. Data are mean ± SD.

Source: Study 4169-NC-120

Study No 4169-NC-160

Cysteine in AMG416 (etelcalcetide) backbone was required for CaSR activation.

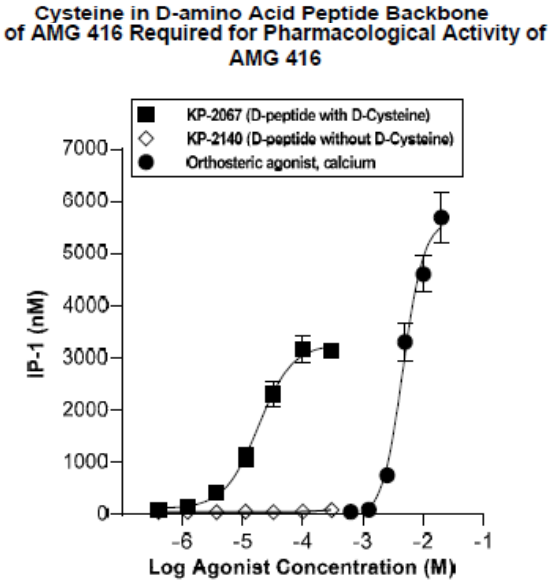
The importance of the D-cysteine in the D-amino acid peptide backbone for CaSR activation was assessed in HEK293T/CaSR cells using a peptide with a D-cysteine in the backbone (KP-2067) and a peptide (structure = Ac-arrrar-NH2) without a D-cysteine (KP-2140). In vitro studies demonstrated that the activity of KP-2067 was 29-fold higher than KP-2140, suggesting the importance of the amino-terminal cysteine for activity of the peptides to CaSR.

To determine the potential binding site of etelcalcetide to the CaSR, HEK293T cells were transiently transfected with a series of constructed and natural mutations of human CaSR then incubated with etelcalcetide or calcium. Mutated human CaSR, with either one of two substitutions at position 482 (tyrosine or serine), was not stimulated by etelcalcetide, but was fully activated by the orthosteric ligand calcium. This finding supported that an allosteric binding site for etelcalcetide on the CaSR was different from the orthosteric site for calcium.

To further determine whether CaSR cysteine 482 was directly binding to or indirectly involved in agonist peptide activation of the CaSR, the physical nature of the agonist peptide/CaSR interaction was studied. A disulfide bond formed between the D-cysteine within the peptide and

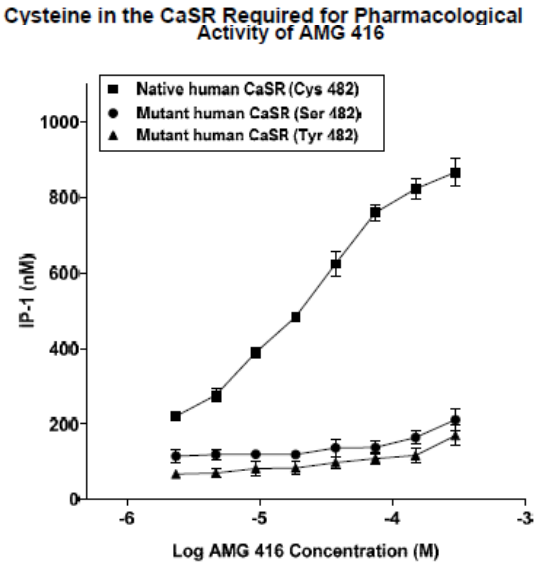
cysteine 482 in the CaSR was identified by LC-MS/MS. HEK293T/hCaSR cells were incubated with KP-2067 at concentrations approximating the EC_{20} (8 μM), EC_{50} (25 μM) and EC_{80} (80 μM). Binding of KP-2067 to cysteine 482 was monitored following LC-MS/MS. Fractional occupancy (% bound) was determined. Fractional occupancy of cysteine 482 was not detected when cells were incubated with the vehicle or 8 μM KP-2067, but increased to 5.3 ± 3.2% in the presence of 25 μM KP-2067, and further increased to 12.1 ± 4.4% in the presence of 80 μM KP-2067. These results confirmed that the D-peptide backbone of etelcalcetide was capable of forming a covalent disulfide bond with the human CaSR, and the amount of this bond correlated with the degree of CaSR activation in vitro.

Figure 2 PD Study No 4169-nc-160: Cysteine was required for the Pharmacological Activity of AMG416 (Etelcalcetide)



HEK293T/CaSR cells (stable cell line) were incubated with increasing concentrations of KP-2067, or KP-2140 (KP-2067 homologue without D-cysteine), with orthosteric CaSR agonist, calcium as a positive control. IP-1 values presented as mean ± standard error of the mean (SEM) for N = 4.

Source: Study 4169-NC-160



Transiently transfected HEK293T were stimulated with AMG 416 in the presence of 1.2 mM calcium. IP-1, a measure of Gq activation, was quantified. Data are mean ± SD.

Source: Study 4169-NC-160

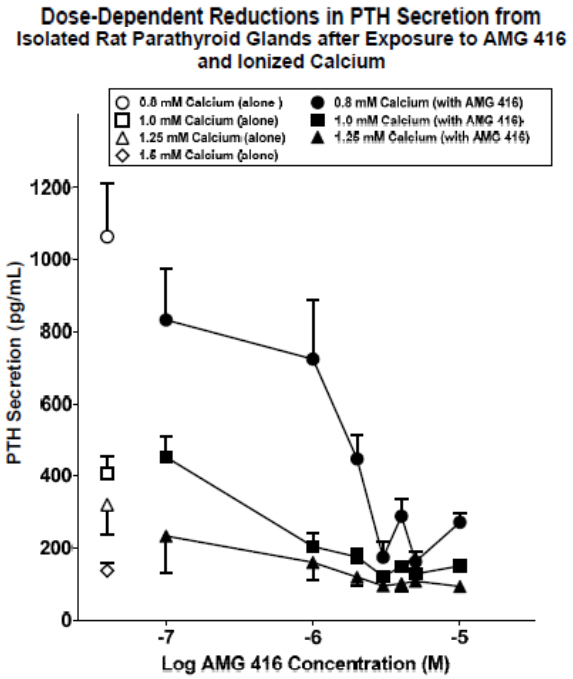
Study No r20130052

AMG416 (Etelcalcetide) inhibited PTH secretion in the presence of calcium ex vivo isolated parathyroid glands.

The potency of etelcalcetide in reducing PTH secretion was characterized ex vivo using freshly isolated parathyroid glands from female Wistar rats. Dose response curves were generated in the presence of a fixed concentration of ionized calcium (0.8 - 1.5 mM; a range of ionized calcium that is commonly observed in vivo). PTH was quantified from the conditioned media following 1 hour incubation of the glands with multiple concentrations of etelcalcetide (0.01 -10 μM).

PTH secretion was inhibited in a dose dependent manner by increasing concentrations of ionized calcium. Etelcalcetide demonstrated IC_{50} values of 0.4 - 1.2 μM (or 0.42 -1.25 μg/mL) in the presence of fixed calcium concentrations. This study showed that etelcalcetide had calcimimetic activity against isolated rat parathyroid gland by inhibiting secretion of PTH across a range of physiological relevant calcium concentrations.

Figure 3 Pharmacodynamic Study No 220130052: AMG416 (Etelcalcetide) Inhibited PTH Secretion Ex Vivo.



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Isolated rat parathyroid glands were incubated sequentially with increasing concentrations of AMG 416 in the presence of one of three fixed concentrations of ionized calcium or calcium alone. Ionized calcium concentrations were chosen to model a physiological range found in blood (0.8 mM Ca^{2+}, n = 5-11. 1.0 mM Ca^{2+}, n=6-12; 1.25 mM Ca^{2+}, n = 6-12). Maximal PTH inhibition without AMG 416 is observed with 1.5 mM ionized calcium alone (n = 10). Data presented as mean ± SEM.

Source: Study R20130052

Study No r20150023

Two biotransformation products of AMG416 (etelcalcetide), M10 or SAPC, had minimal or no activity against hCaSR when compared to AMG416.

The objective of this study was to determine the in vitro activity of etelcalcetide L-GSH, serum albumin peptide conjugate (SAPC) and glutathione disulfide (M10). A stable clone of HEK-293T overexpressing the human calcium-sensing receptor (hCaSR) was used. Activation of the hCaSR by biotransformation products were characterized by quantifying inositol monophosphate (IP-1), a stable intracellular metabolite of IP-3 synthesis and surrogate of Gq (G protein, q polypeptide) activation in vitro. Dose response effects of etelcalcetide, L-glutathione (L-GSH), M10, and SAPC were evaluated in the presence of 1.2 mM calcium.

The study results showed that there was a significant biotransformation of etelcalcetide occurred in whole blood. The major product was a serum albumin conjugate of the D-amino acid peptide back bone (SAPC). Minor amounts of low molecular weight (non-protein) biotransformation products, such as M10 (a glutathione disulfide) or M11 (same chemical structure as KP-2067) were also observed. SAPC at 100 µM was minimally active (< 14% of maximal activity observed with etelcalcetide at 100 µM) and M10 (EC_{50} 66 µM) was approximately three-fold less active than etelcalcetide.

In Vivo Non-GLP Studies

Study No 4169-NC-120 (II)

AMG 416 (Etelcalcetide) via IV administration reduced plasma PTH in a dose dependent manner regardless of the degree of renal function.

The primary pharmacodynamic response was studied in rats (normal and disease models) and normal dogs. In rats, etelcalcetide was studied in an acute model of renal insufficiency with secondary hyperparathyroidism (HPT) [1 kidney 1 clip (1K1C) or renal ischemia reperfusion injury (Renal I/R]), and a chronic model of renal insufficiency with secondary HPT [5/6 nephrectomized rats (Nx)]. In the IKIC rat model, acute renal insufficiency was created in rats by removing one kidney followed by exposure of the remaining kidney to 45 minutes of ischemia and 48 hours of reperfusion. The ensuing ischemia/reperfusion damage to the remaining kidney results in acute renal failure over the course of 2 to 3 days, which is associated with marked elevation in PTH levels. In the 5/6 nephrectomized rat model, 2/3 of one kidney was surgically removed. One week later, the other kidney was removed, leaving the rat with 1/16 of its original renal capacity. In the 2 weeks following the second nephrectomy, rats developed modest renal dysfunction resulting in increased serum creatinine (1-2 mg/dL) and plasma PTH (400-500 pg/mL).

In normal rats, etelcalcetide was administered as a single IV bolus at 0, 0.1, 0.3, 0.5 or 1 mg/kg (n=2 to 8/group). Plasma PTH was quantified every hour for four hours. Etelcalcetide caused a rapid and reversible dose-dependent decrease in plasma PTH. At 0.1 mg/kg, there was a partial PTH suppression. At 0.3 and 0.5 mg/kg, there was maximally suppressed PTH at 1 hour with recovery to 60% and 40% of pre-dose values at four hours, respectively. At 1 mg/kg, PTH levels remained fully suppressed throughout the four hour study.

In 1K1C rats, etelcalcetide was administered as a single IV bolus at 0, 0.3, 0.5, 1 or 3 mg/kg. Since 1K1C rats had much greater pre-dose PTH level than normal rats, 3 mg/kg of etelcalcetide was selected as the top dose. Unlike normal rats, baseline PTH level was markedly elevated in 1K1C rats. Nevertheless, suppression of PTH by etelcalcetide was rapid, reversible and dose-dependent. At 1 mg/kg, etelcalcetide maximally suppressed PTH for 16 hours with recovery to less than 60% of pre-dose values at 24 hour. At 3 mg/kg etelcalcetide suppressed PTH over the 24 hours of the study, even in animals with pre-dose PTH levels over 1000 pg/mL. Consistent with a reduction in PTH, a dose-dependent reduction in serum calcium was also observed.

In 5/6 nephrectomized rats, etelcalcetide was administered as an IV bolus at a dose of 1 mg/kg once daily for 28 days. Daily treatment with etelcalcetide by IV bolus over 28 days resulted in sustained PTH suppression. Consistent with the effect on PTH, a modest reduction in serum calcium was observed in treated animals.

Reference ID: 3923456

Figure 4 Pharmacodynamic Study No 4169-NC-120: Etelcalcetide Reduced PHT Levels in Normal and Acute/Chronic Renal Insufficient (1K1C and Nx) Rats Following Acute or Chronic Dosing Regimens.

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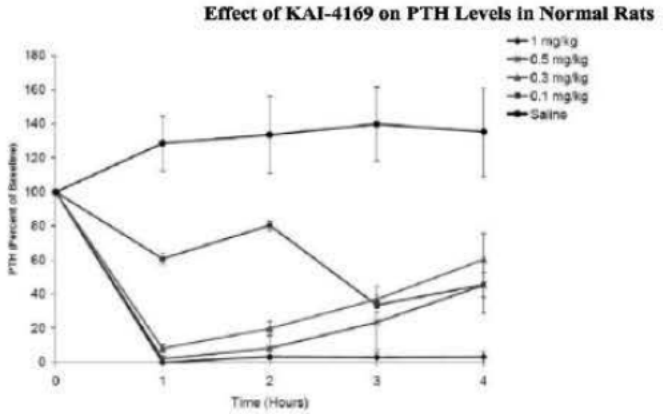
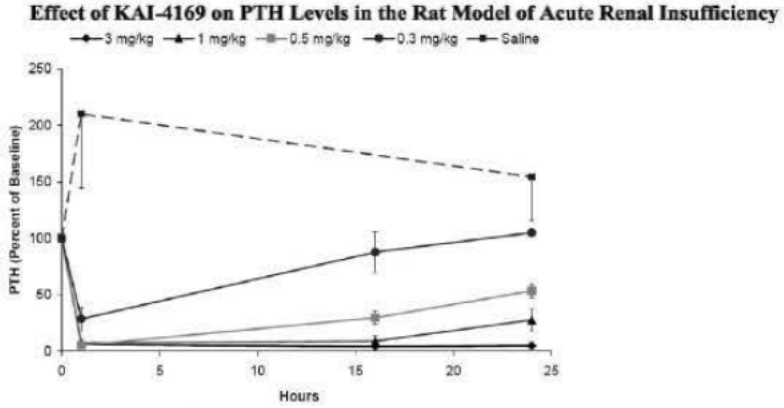
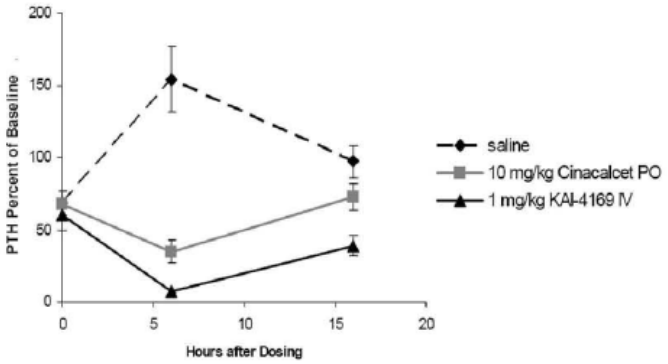


KAI-4169 was administered by IV bolus injection to normal Sprague Dawley rats at 0.1, 0.3, 0.5 or 1 mg/kg. Plasma PTH levels were measured hourly for 4 hours. Data are presented as group mean ± SEM (1 mg/kg n=2; 0.5 mg/kg n=4; 0.3 mg/kg n=7; 0.1 mg/kg n=2; saline n=4).



KAI-4169 was administered by IV bolus injection to Sprague Dawley rats with acute renal dysfunction (1K1C model) at 0.3, 0.5, 1 and 3 mg/kg. Saline vehicle was administered to a control group. Plasma PTH was determined before dosing and at 1, 16 and 24 hours after drug administration. Data are presented as group mean ± SEM (3 mg/kg n=2; 1 mg/kg n=5; 0.5 mg/kg n=6; 0.3 mg/kg n=5; saline n=4).

Change From Baseline in Plasma PTH Levels in a Rat 5/6 Nephrectomy Model Following Dosing on Day 7



Rats subjected to 5/6 nephrectomy were treated daily with KAI-4169 (1 mg/kg administered by IV bolus), saline or cinacalcet (10 mg/kg administered po) for 28 days. Plasma PTH was determined at baseline (pre-drug), pre-dose on Day 7 and 6 and 16 hours after dosing. Data are presented as group mean ± SEM (Saline n=10; cinacalcet n=11; KAI-4169 n=11).

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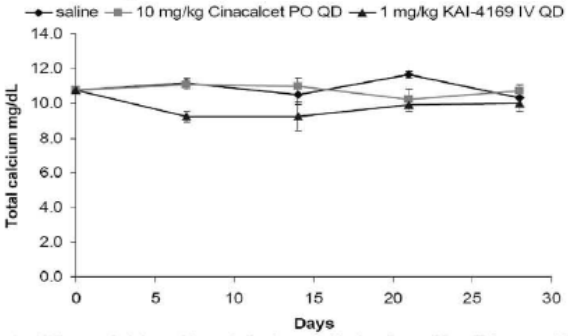
Effect of Daily IV Bolus Administration of KAI-4169 on Calcium Levels in a Rat 5/6 Nephrectomy Model of Chronic Renal Insufficiency



KAI-4169 was administered by IV bolus in rats with chronic renal insufficiency and SHPT (5/6 nephrectomy model) at 1 mg/kg daily for 28 days. Negative control animals received an identical volume of saline administered IV for 28 days. As a benchmark control, cinacalcet was administered po at 10 mg/kg daily for 28 days. Calcium was determined weekly 24 hours after drug administration. Data are presented as group mean ± SEM (n=12/group at study initiation).

Study No 4169-NC-103

Etelcalcetide Reduced PTH and Calcium in Dogs During and After 24 hr IV Infusion.

Four male beagle dogs used in the study received either vehicle (20 mM sodium succinate in normal saline) on Day 1, or 3 different doses of etelcalcetide at Days 8 (0.024 mg/kg), 17 (0.48 mg/kg) and 38 (0.192 mg/kg). Blood levels of etelcalcetide, PTH and calcium were quantified during the 24 hour period following the end of infusion. Blood samples were collected pre-dose and at 2, 4, 8, 12, 16, 20 and 24 hours after the start of infusion, and at 1, 3, 6, 12, 18 and 24 hours after the end of infusion.

Etelcalcetide plasma concentrations were dose-proportional and approached steady state for all three dose levels by the end of the 24-hour infusion and steadily declined after the end of infusion. Etelcalcetide clearance was similar (approximately 400 mL/hr/kg) across all dose-groups.

Table 2 Pharmacodynamic Study No 4169-NC-103: Pharmacokinetic Profile of AMG416 (Etelcalcetide) after Single Dose IV Administration to Dogs

Mean Non-Compartmental AMG 416 Plasma Pharmacokinetic Parameters After Single Dose Intravenous Administration to Healthy Dogs

Dose[a] (mg/kg)	C_{max} (ng/mL)	AUC (ng.hr/mL)	CL (mL/hr/kg)	$t_{1/2}$ (hr)	V_{ss} (mL/kg)
0.024	2.9	63.0	379	2.6[b]	1429[b]
0.192	23.4	457	433	5.8	4038
0.48	54.3	1207	393	8.2	4610

[a]Dose infused over 24 hr. [b]inadequate time-points to characterize the terminal portion of the PK curve; values should be interpreted with caution. AUC, area under the concentration-time curve from time 0 to the time of last observation; CL, clearance; C_{max}, maximum observed concentration; $t_{1/2}$, terminal half-life; Vss, estimated volume of distribution at steady state.

Source: Study 4169-NC-103

Mean PTH concentrations decreased at 0.192 and 0.48 mg/kg and were sustained throughout the infusion period when compared to both pre-dose and vehicle values. The magnitude and duration of PTH reduction were dose-related. Reversibility of PTH reduction was observed following the end of infusion and correlated well with drug concentrations. The length of PTH recovery to baseline (pre-dose values) was proportional to dose. Calcium levels decreased at 0.192 and 0.48 mg/kg in a dose dependent manner. Reductions in serum calcium occurred within hours after the initiation of infusion and progressively declined, subsequent to reductions in PTH. Consistent with the peak of etelcalcetide concentration, maximum reductions in calcium occurred by the end of the 24 hour infusion and mean calcium levels were 13% and 24% below pre-dose mean values for the 0.192 mg/kg and 0.48 mg/kg dose groups, respectively. Subsequently, mean calcium concentrations recovered over the remaining 24 hour post-infusion period when etelcalcetide concentration dropped. At 0.024 mg/kg, PTH and calcium appeared unaffected.

Figure 5 Pharmacodynamic Study No 4169-NC-103: AMG416 (Etelcalcetide) Reduced PHT and Serum Calcium Levels in Normal Dogs Following 24 hr IV Infusion.

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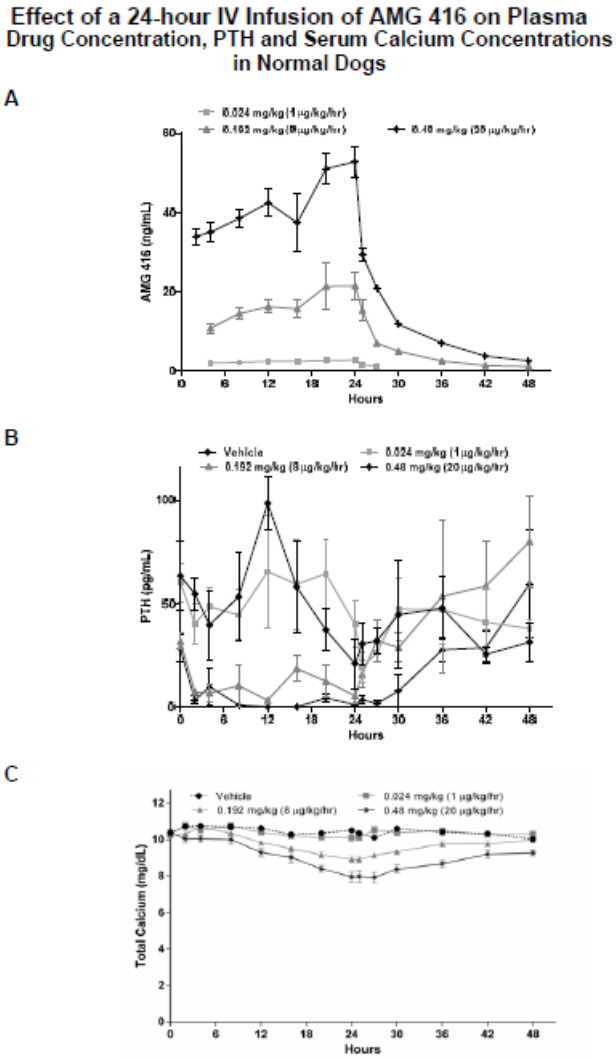


Vehicle or AMG 416 was administered by IV infusion over 24 hours to normal beagle dogs (N=4) at a total dose of 0.024, 0.192 and 0.48 mg/kg (dose rates of 1, 8 and 20 µg/kg/hr, respectively). Plasma AMG 416 concentration (A) and PTH concentration (B), and total serum calcium (C) were measured during IV infusion at 2, 4, 8, 12, 16, and 24 after the start of infusion and at 1, 3, 6, 12, 18 and 24 hours after the end of infusion. Calcium is presented as mg/dL. Data are presented as mean ± SEM.

Source: Study 4169-NC-103

Study No 4169-NC-145

AMG 416 (Etelcalcetide) treatment effectively reduced PTH, prevented soft tissue calcification and parathyroid gland hyperplasia in uremic rats following SC injections three times weekly for 6 weeks.

The efficacy of etelcalcetide was evaluated in a rat model of chronic renal insufficiency (5/6 nephrectomy, 5/6 Nx). 5/6 Nx rats were fed a high phosphate diet throughout the study to further induce secondary HPT. Four groups of 5/6 Nx rats were subcutaneously (SC) injected with vehicle or etelcalcetide at 0.3, 1, or 3 mg/kg three times weekly for 6 weeks. Blood samples were taken every two weeks for PTH and calcium assessment. Animals received 5-

bromo-2-deoxyuridine [BrDU] at 50 mg/kg by intraperitoneal injection approximately 2 and 24 hours prior to euthanasia.

Plasma PTH for vehicle treated rats was elevated (63 to 80%) relative to pre-dose baseline values across the 6 week treatment phase. There were dose-proportional decreases in PTH relative to vehicle treated animals at 2 weeks, with the 3 mg/kg group showing a substantial decrease in plasma PTH and the low dose (0.3 mg/kg) showing a modest increase from baseline values, but nominally lower than the vehicle control. The high dose (3 mg/kg) treatment effect was significant at 2 and 6 weeks.

Table 3 Pharmacodynamic Study No 4169-NC-145: Pharmacokinetic Profile of AMG416 (Etelcalcetide) after SC Administration Three Times Weekly for Six Weeks to Rats with Chronic Renal Insufficiency

AMG 416 Suppressed Plasma PTH Levels in a Rat Model of Chronic Renal Insufficiency with Secondary Hyperparathyroidism

Treatment	Dose (mg/kg)	n	PTH (% change relative to baseline) Week 2	Week 4	Week 6
Vehicle	-	12	80.4± 16.2	67.3± 19.6	63.5± 21.6
AMG 416	0.3	12[d]	66.2± 31.7	63.2± 42.2	44.7± 29.7[d]
AMG 416	1.0	12[d]	14.0± 14.5	30.6± 13.9	28.4± 19.7[d]
AMG 416	3.0	12	-50.6± 7.8[ab]	-4.3± 23.5	-32.3± 18.8[c]

[a]p < 0.001 *vs.* vehicle at week 2; [b]p < 0.001 *vs.* 0.3 mg/kg at week 2; [c]p < 0.05 *vs.* vehicle at week 6; [d]n =11 at week 6. Statistical analysis was done using one-way ANOVA and Bonferroni post-test.

Source: Study 4169-NC-145

Consistent with circulating levels of PTH, proliferating parathyroid chief cell number (as determined by 5-bromo-2-deoxyuridine [BrDU] staining) was significantly lower in the 3 mg/kg group relative to the vehicle treated group. There was a nominal reduction in the parathyroid gland (PTG) weight (assessed by the wet weight of gland normalized to body weight) following 3 mg/kg etelcalcetide treatment relative to vehicle control group.

Table 4 Pharmacodynamic Study No 4169-NC-145: AMG416 (Etelcalcetide) Prevented Parathyroid Hyperplasia after SC Administration Three Times Weekly for Six Weeks to Rats with Chronic Renal Insufficiency

AMG 416 Prevented Parathyroid Hyperplasia in a Rat Model of Chronic Renal Insufficiency with Secondary Hyperparathyroidism

n	Treatment	Dose (mg/kg)	# BrdU Positive (PT chief) Cells
12	Vehicle	n/a	127.5 ± 18.8
11	AMG 416	0.3	109.4 ± 13.2
11	AMG 416	1	74.5 ± 14.8
12	AMG 416	3	51.8 ± 15.9[a]

[a]p < 0.01 *vs.* vehicle.
Parathyroid glands were processed for immunohistochemical analysis of BrdU reactive cells. Images were visualized with Adobe Photoshop and BrdU-positive cells were manually counted. In most cases, both glands from each rat were quantitated and the average of the two used for calculating the value for each individual rat. Shown are group mean data ± SEM (n=11 for 0.3 and 1 mg/kg; n=12 for vehicle and 3 mg/kg). Statistical analysis was done using one-way ANOVA and Bonferroni post-test. PT = parathyroid

Source: Study 4169-NC-145

In addition, upon completion of dosing, hearts, aortic arches and half of each remaining kidney were removed, and processed for determination of calcium and phosphorus content as biochemical measures of tissue calcification. Etelcalcetide significantly ($p < 0.05$) reduced both calcium and phosphorus content in the aorta and phosphorus content in the heart at 3 mg/kg. There was no significant difference in mineral content in the remaining portion of the diseased kidney with the two lower doses of etelcalcetide (0.3 and 1 mg/kg).

Study No r20130074

Etelcalcetide reduced bone turnover and normalized mineralization in rats with renal insufficiency following SC injections daily for 6 weeks.

The effects of etelcalcetide and cinacalcet on bone turnover were evaluated in a rat model of secondary hyperparathyroidism with renal insufficiency. Uremia was first established in 5/6 nephrectomized (5/6 Nx) male SD rats for 8 weeks following the two-step surgery. Sham operated rats were used as non-uremic controls. Uremic rats were injected SC daily with vehicle (n = 12), etelcalcetide (n = 12) at 0.3 mg/kg, or 1 mg/kg (reduced to 0.6 mg/kg 14 days later due to the reduction in body weight, n = 11) for 6 weeks. Another set of 5/6 Nx rats were orally dosed (PO) with vehicle (n = 13), cinacalcet at 3 or 15 mg/kg (n = 10/group). Sham operated rats (n = 12) were injected SC with vehicle. Blood samples were collected at 4 hours post injection on days 1 and 42, and approximately 23 hours post injection on Days 14 and 28 just prior to the next dose during the treatment phase. The rats were euthanized after 6 weeks of treatment and histomorphometric analyses were performed on the trabecular region of the proximal tibia.

In uremic rats, there was a significant elevation of plasma PTH when compared to non-uremic controls on Day 1 of treatment (non-uremic control, 123 ± 14; etelcalcetide vehicle control, 209 ± 26; cinacalcet vehicle control, 234 ± 13 pg/mL) and after the 6 week treatment phase (non-uremic control, 108 ± 13; etelcalcetide vehicle control, 270 ± 29; cinacalcet vehicle control, 331 ± 36 pg/mL).

In uremic rats at 0.6 mg/kg etelcalcetide, the bone resorption (Oc.S/BS, osteoclast surface as percentage of bone surface) was 32% lower than vehicle-treated uremic controls. Static and dynamic bone formation parameters [osteoid surface, mineralizing surface as a percentage of bone surface (MS/BS) and bone formation rate (BFR/BS)] were dose dependently decreased compared with vehicle-treated uremic controls. The significant effects of etelcalcetide on bone formation were observed in the high dose etelcalcetide group but not in the low dose group (except MS/BS). These results indicated that etelcalcetide reduced bone turnover, which was consistent with PTH lowering effects of etelcalcetide. Osteoid volume (as percentage of bone volume; OV/BV, a parameter for mineralization) was dose-dependently reduced in etelcalcetide-treated uremic rats compared with vehicle-treated uremic controls. Furthermore, mineralization lag time (MLT, the time from matrix formation to mineralization) was dose-dependently accelerated in etelcalcetide-treated uremic rats compared with vehicle-treated uremic controls. No significant difference was observed in bone area (as percentage of tissue area) between -treated uremic rats and vehicle-treated uremic controls.

In cinacalcet-treated uremic rats, no significant differences were observed in bone formation and mineralization endpoints when compared to its vehicle-treated uremic controls. The lack of cinacalcet effects on bone was unexpected. Similar dose of cinacalcet has been reported to reduce bone formation rate and normalize bone mineralization in uremic rats and cinacalcet has been shown to normalize bone turnover in dialysis patients with secondary HPT.

Reference ID: 3923456

Table 5 Pharmacodynamic Study No r20130074: AMG416 (Etelcalcetide) Reduced Bone Turnover and Normalized Mineralization in Rats with Renal Insufficiency.

Effects of AMG 416 and Cinacalcet on Selective Bone Histomorphometric Endpoints in a 5/6 Nx Rat Model of Secondary Hyperparathyroidism

Surgery	Treatment	n	Oc.S/BS (%)	OS/BS (%)	MS/BS (%)	BFR/BS ($\mu m^3/\mu m^2$/day)	OV/BV (%)	MLT (day)
Sham	Vehicle (SC)	12	1.43 ± 0.21	9.45 ± 1.33	37.07 ± 1.88	0.47 ± 0.03	1.34 ± 0.22	1.05 ± 0.16
5/6 Nx	Vehicle (SC)	12	1.22 ± 0.11	10.81 ± 2.12	43.63 ± 1.64	0.56 ± 0.04	1.65 ± 0.38	0.94 ± 0.14
5/6 Nx	AMG 416, 0.3 mg/kg	12	0.95 ± 0.28	6.67 ± 1.65	36.39 ± 1.77[b]	0.46 ± 0.03	1.00 ± 0.30	0.68 ± 0.16
5/6 Nx	AMG 416, 0.6 mg/kg	11	0.83 ± 0.16	3.63 ± 0.70[b]	30.60 ± 2.43[b]	0.35 ± 0.04[b]	0.48 ± 0.08[b]	0.41 ± 0.06[a,b]
5/6 Nx	Vehicle (PO)	13	1.15 ± 0.10	12.54 ± 2.00	34.70 ± 1.88	0.45 ± 0.03	2.46 ± 0.88	1.52 ± 0.40
5/6 Nx	Cinacalcet 3 mg/kg	10	1.36 ± 0.27	10.07 ± 2.08	33.88 ± 1.71	0.41 ± 0.03	1.89 ± 0.54	1.18 ± 0.29
5/6 Nx	Cinacalcet 15 mg/kg	10	1.43 ± 0.11	12.06 ± 2.64	31.51 ± 1.99	0.42 ± 0.05	1.81 ± 0.45	1.29 ± 0.23

Results are expressed as the mean ± standard error of mean (SEM). [a]$p < 0.05$ vs. the sham group, [b]$p < 0.05$ vs. respective vehicle-treated 5/6 Nx group. One way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used to determine the differences between AMG 416- or cinacalcet-treated uremic rats and their respective uremic controls. Sham = fake nephrectomy, 5/6 Nx = 2/3 left kidney removal by nephrectomy + right kidney removal, BFR = bone formation rate, BS = bone surface, MLT = mineralization lag time, MS = mineralizing surface, OS/BS = osteoid surface per bone surface, Oc.S/BS = osteoclast surface per bone surface, OV/BV = osteoid volume normalized to bone volume.

Source: Study R20130074

Study No r20130075

AMG 416 (Etelcalcetide) reduced circulating levels of PTH and FGF23, reduced parathyroid glad hyperplasia and cortical porosity, and preserved bone strength in rats with chronic renal insufficiency following SC injections daily for 6 weeks.

The efficacy of etelcalcetide in controlling blood PTH, parathyroid gland hyperplasia and bone turnover was evaluated in a rat model of established secondary hyperparathyroidism. 5/6 nephrectomized (5/6 Nx) rats were injected SC daily with vehicle or etelcalcetide at 1 mg/kg (reduced to 0.6 mg/kg 11 days later due to loss in body weight for this group) for 6 weeks. Sham operated rats were injected SC daily with vehicle for 6 weeks. Blood samples were collected prior to first dose, and 4 hours post-dose on Days 2 and 42 and approximately 23 hours post-dose (just prior to next dose) on Days 14 and 28. After 6 weeks of treatment, isolated tissues were evaluated for normalized parathyroid gland weights (mg/kg body weight), proliferative index of parathyroid gland chief cells based on cell proliferation marker Ki-67 staining, femoral bone cortical porosity and mineral density by micro computed tomography (micro-CT), and bone strength (maximum load and energy to failure at the femur mid-shaft) by biomechanical testing.

The average serum PTH was about 4.7-fold greater in the 5/6Nx rats than in sham controls and no significant difference was found between the vehicle and etelcalcetide before the treatment.

Table 6 Pharmacodynamic Study No r20130075: AMG416 (Etelcalcetide) Reduced Serum PTH in Rats with Renal Insufficiency.

Effects of AMG 416 Treatment on Serum PTH in 5/6 Nx Rats with Established Secondary Hyperparathyroidism

Surgery	Treatment	n	Serum PTH (pg/mL) Predose	Day 2 – 4 h	Day 14 – 23 h	Day 28 – 23 h	Day 42 – 4 h
Sham	Vehicle	10	147 ± 32	151 ± 19	175 ± 18	178 ± 24	168 ± 20
5/6Nx	Vehicle	12	791 ± 129[a]	775 ± 131[a]	1000 ± 235[a]	1377 ± 357[a]	2205 ± 497[a]
5/6Nx	AMG 416	13	686 ± 65[a]	23 ± 6[b]	793 ± 96[a]	956 ± 124	90 ± 23[b]

Nx = Nephrectomized, 4h = 4 hours, 23 h = 23 hours

Sham and 5/6 Nx rats were fed a special diet containing high phosphate diet (0.9%) and low calcium (0.6%) to further induce secondary hyperparathyroidism. Eight weeks after the special diet, the 5/6 Nx rats were subcutaneously (SC) injected daily with vehicle or AMG 416 (1 mg/kg for the first 11 days, 0.6 mg/kg for 31 days) for 6 weeks. Sham rats were SC injected daily with vehicle for 6 weeks. Blood was collected 4h post-dosing on days 2 and 42, and 23 h post-dosing on days 14 and 28. Serum PTH was measured as described in Methods. Results are expressed as the mean ± standard error of mean (SEM) for 10-13 rats per group. [a]$p < 0.05$ vs. the sham group, [b]$p < 0.05$ vs. the vehicle-treated 5/6 Nx rats. One way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used to determine the differences between groups.

The average body weight normalized parathyroid gland weight was significantly greater in the vehicle-treated 5/6 Nx rats than sham controls. In the etelcalcetide-treated 5/6 Nx rats, the average body weight normalized parathyroid gland weight was significantly lower than that of the vehicle-treated 5/6 Nx rats. Consistent with increases in parathyroid gland weight, area-adjusted Ki-67 (also known as MKI67 – cellular marker for proliferation)-stained chief cell count was 2-fold greater in vehicle-treated 5/6 Nx rats than sham controls. In the etelcalcetide treated group, area-adjusted Ki-67-stained chief cell count was 30% less than vehicle-treated 5/6 Nx rats. In addition, serum FGF23 levels were reduced by etelcalcetide treatment in 5/6 Nx rats.

Table 7 Pharmacodynamic Study No r20130075: AMG416 (Etelcalcetide) Reduced Parathyroid Gland Weight, Parathyroid Chief Cell Proliferation, and Serum FGF23 in Rats with Renal Insufficiency.

Effects of AMG 416 Treatment on Terminal Parathyroid Gland Weight in 5/6 Nx Rats with Established Secondary Hyperparathyroidism

Surgery	Treatment	n	Parathyroid Gland Weight (mg)/ Body Weight (kg)
Sham	Vehicle	9	0.88 ± 0.12
5/6 Nx	Vehicle	9	5.91 ± 1.55[a]
5/6 Nx	AMG 416	13	2.76 ± 0.39[b]

Nx = Nephrectomized

Effects of AMG 416 Treatment on Parathyroid Chief Cell Proliferation in 5/6 Nx Rats with Established Secondary Hyperparathyroidism

Surgery	Treatment	n	Ki-67+ cell count	Total Area (um^2)	Ki-67+ cell count/ um^2 (x1000)
Sham	Vehicle	9	87.2 ± 12.4	588375 ± 52154	0.160 ± 0.027
5/6 Nx	Vehicle	9	579.0 ± 146.3[a]	1680000 ± 236517[a]	0.323 ± 0.050
5/6 Nx	AMG 416	13	247.4 ± 83.38[b]	952016 ± 67010[b]	0.226 ± 0.061

Nx = Nephrectomized

Effects of AMG 416 Treatment on Serum FGF23 in 5/6 Nx Rats with Established Secondary Hyperparathyroidism

			Serum FGF23 (pg/mL)				
Surgery	Treatment	n	Predose	Day 2	Day 14	Day 28	Day 42
Sham	Vehicle	10	62 ± 7	83 ± 7	80 ± 9	88 ± 4	100 ± 6
5/6 Nx	Vehicle	12	823 ± 102[a]	708 ± 127[a]	1426 ± 284[a]	1823 ± 337[a]	2430 ± 552[a]
5/6 Nx	AMG 416	13	1002 ± 177[a]	374 ± 63[b]	648 ± 119[b]	861 ± 164[b]	874 ± 250[b]

Results are expressed as the mean ± standard error of mean (SEM) for 10-13 rats per group. Sham = fake nephrectomy, 5/6 Nx = 2/3 left kidney removal by nephrectomy + right kidney removal. [a]$p < 0.05$ vs. the sham group, [b]$p < 0.05$ vs. the vehicle-treated 5/6 Nx rats. One way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used to determine the differences between groups.

Source: Study R20130075

Micro-CT - At the femur diaphysis, cortical porosity was significantly increased in the vehicle-treated 5/6 Nx rats when compared to sham controls. The increase in cortical porosity in the vehicle-treated 5/6 Nx rats was associated with a significant decrease in tissue bone mineral density (vTMD) compared to sham controls. Linear regression analyses demonstrated a strong linear relationship between cortical porosity and serum PTH collected 4 hours after the last dose in the vehicle-treated 5/6 Nx rats (R2 = 0.92). A strong inverse linear relationship was also observed between bone mineral density and serum PTH in vehicle-treated secondary HPT rats (R2 = 0.93). Cortical porosity was significantly lower in etelcalcetide-treated 5/6 Nx rats than vehicle-treated 5/6 Nx rats (sham rats, 0.072 ± 0.005%; vehicle-treated secondary HPT rats, 8.256 ± 2.693%; etelcalcetide- treated secondary HPT rats, 2.273 ± 0.734%). No other significant differences were observed at the femur diaphysis between etelcalcetide- and vehicle-treated secondary HPT rats.

Bone strength - At the femur midshaft, maximum load of the vehicle-treated 5/6 Nx rats was significantly lower than sham controls. After correction of maximum load for geometric parameters, the material property ultimate strength remained significantly decreased in vehicle-treated 5/6 Nx rats. Treatment with etelcalcetide prevented these significant decreases relative to sham controls. Other bone strength endpoints, namely energy to failure and toughness trended lower in the vehicle-treated 5/6 Nx rats than sham controls, and were significantly greater in etelcalcetide-treated 5/6 Nx rats. Maximum load and energy to failure at the femur mid-shaft had a strong inverse linear relationship with cortical porosity (R2 = 0.73 and R2 = 0.80, respectively) in the vehicle-treated 5/6 Nx rats. Serum PTH collected 4 hours after last dose also had a strong inverse linear relationship with maximum load and energy to failure (R2 = 0.70 and R2 = 0.75, respectively) in the same group. Thus the decrease in maximum load and energy in the vehicle-treated secondary HPT rats could be primarily attributed to the increased cortical porosity as a consequence of increased serum PTH.

Table 8 Pharmacodynamic Study No r20130075: AMG416 (Etelcalcetide) Preserved Cortical Bone Structure and Bone Strength in Rats with Renal Insufficiency.

MicroCT Analysis of Left Femur

Region	Endpoint	(Unit)	Sham Vehicle n=10	5/6 Nx Vehicle n=12	5/6 Nx AMG 416 n=13
Whole Femur	Femur Length	(mm)	41.4 ± 0.3	41.5 ± 0.3	41.4 ± 0.3
Mid-Shaft	vBMC	(mg/mm)	10.33 ± 0.31	10.14 ± 0.27	10.04 ± 0.25
Mid-Shaft	vTMD	(mg/cm^3)	1103.4 ± 4.3	996.6 ± 34.2[a]	1063.1 ± 8.2
Mid-Shaft	Porosity	(%)	0.072 ± 0.005	8.256 ± 2.693[a]	2.273 ± 0.734[b]
Mid-Shaft	Cross Sectional Area	(mm^2)	13.57 ± 0.56	14.22 ± 0.54	14.13 ± 0.35
Mid-Shaft	Bone Area	(mm^2)	8.18 ± 0.24	8.63 ± 0.50	7.98 ± 0.21
Mid-Shaft	CSMI	(mm^4)	9.45 ± 0.84	9.85 ± 0.70	9.84 ± 0.53
Mid-Shaft	Cortical Thickness	(mm)	0.705 ± 0.014	0.916 ± 0.097	0.733 ± 0.024
Mid-Shaft	Endocortical Perimeter	(mm)	9.21 ± 0.27	7.79 ± 0.80	9.42 ± 0.16
Mid-Shaft	Periosteal Perimeter	(mm)	13.58 ± 0.26	13.82 ± 0.25	13.85 ± 0.16
Distal Femur	vBMC	(mg/mm)	10.96 ± 0.30	12.51 ± 0.49[a]	11.43 ± 0.30
Distal Femur	vBMD	(mg/cm^3)	455.1 ± 10.8	484.1 ± 12.2	463.5 ± 11.2
Distal Femur	Cross Sectional Area	(mm^2)	24.12 ± 0.56	25.83 ± 0.70	24.71 ± 0.47
Distal Femur	Bone Area	(mm^2)	9.49 ± 0.29	12.14 ± 0.79[a]	10.39 ± 0.37
Distal Femur	Bone Volume Fraction	(%)	39.4 ± 1.1	46.8 ± 2.3[a]	42.1 ± 1.4
Femur Neck	vBMC	(mg/mm)	3.71 ± 0.11	3.60 ± 0.16	3.74 ± 0.14
Femur Neck	vBMD	(mg/cm^3)	776.5 ± 19.7	714.1 ± 30.4	709.0 ± 15.1
Femur Neck	Cross Sectional Area	(mm^2)	4.81 ± 0.18	5.11 ± 0.24	5.31 ± 0.25
Femur Neck	Bone Area	(mm^2)	3.88 ± 0.08	3.67 ± 0.20	3.93 ± 0.14
Femur Neck	Bone Volume Fraction	(%)	81.27 ± 1.82	73.22 ± 4.40	74.51 ± 1.41
Femur Neck	CSMI	(mm^4)	2.31 ± 0.21	1.95 ± 0.22	2.36 ± 0.21
Femur Neck	Periosteal Perimeter	(mm)	8.18 ± 0.20	8.29 ± 0.20	8.49 ± 0.19

Nx = Nephrectomized

Sham and 5/6 Nx rats were fed a special diet containing high phosphate diet (0.9%) and low calcium (0.6%) to further induce secondary hyperparathyroidism. Eight weeks after introduction of this diet, the 5/6 Nx rats were subcutaneously (SC) injected daily with vehicle or AMG 416 (1 mg/kg for the first 11 days, 0.6 mg/kg for 31 days) for 6 weeks. Sham rats were SC injected daily with vehicle for 6 weeks. Whole left femurs were scanned by microCT (32μm voxel size) and were analyzed as described in the Method. Results are expressed as the mean ± standard error of mean (SEM) for 10-13 rats per group. [a]$p < 0.05$ vs. the vehicle-treated sham group, [b]$p < 0.05$ vs. the vehicle-treated 5/6 Nx group. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used to determine the differences between groups.

Bone Strength Analysis

Site	Endpoint	(Unit)	Sham Vehicle n=10	5/6 Nx Vehicle n=12	5/6 Nx AMG 416 n=13
Femoral Shaft	Maximum Load	(N)	195.6 ± 4.6	156.3 ± 7.7[a]	178.1 ± 7.8
Femoral Shaft	Stiffness	(N/mm)	521.1 ± 25.0	442.6 ± 30.3	517.4 ± 27.8
Femoral Shaft	Energy to Failure	(N.mm)	101.7 ± 7.2	71.3 ± 8.1	109.6 ± 10.8[b]
Femoral Shaft	Ultimate Strength	(MPa)	197.4 ± 7.7	160.8 ± 11.1[a]	175.6 ± 7.6
Femoral Shaft	Elastic Modulus	(GPa)	9.43 ± 0.35	8.04 ± 0.78	8.84 ± 0.31
Femoral Shaft	Toughness	(MPa)	5.67 ± 0.44	4.21 ± 0.47	6.05 ± 0.42[b]
Femoral Neck	Maximum Load	(N)	173.9 ± 7.7	142.8 ± 8.9[a]	155.3 ± 8.1
Femoral Neck	Stiffness	(N/mm)	710.6 ± 41.4	622.3 ± 37.0	697.7 ± 29.3
Femoral Neck	Energy to Failure	(N.mm)	109.8 ± 16.4	76.4 ± 10.8	70.8 ± 6.7

Nx = Nephrectomized

Sham and 5/6 Nx rats were fed a special diet containing high phosphate diet (0.9%) and low calcium (0.6%) to further induce secondary hyperparathyroidism. Eight weeks after introduction of this diet, the 5/6 Nx rats were subcutaneously (SC) injected daily with vehicle or AMG 416 (1 mg/kg for the first 11 days, 0.6 mg/kg for 31 days) for 6 weeks. Sham rats were SC injected daily with vehicle for 6 weeks. Bone strength testing was performed at femoral shaft and neck as described in the Method. The material properties at the femur diaphysis were calculated using micro-CT data, including CSMI and the distance from the center of mass to the posterior edge (c).

Results are expressed as the mean ± standard error of mean (SEM) for 10-13 rats per group. [a]$p < 0.05$ vs. the vehicle-treated sham group, [b]$p < 0.05$ vs. the vehicle-treated 5/6 Nx group. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used to determine the differences between groups.

Study No r20130076

AMG 416 (etelcalcetide) prevented the development of secondary HPT and vascular calcification in uremic rats following daily SC administration for 4 weeks.

The effects of etelcalcetide on vascular calcification were examined in a rat model where uremia was induced by inclusion of 0.75% (w/w) adenine in a base diet (diet supplemented with 0.75% adenine; $DSA_{0.75}$). Adenine fed Wistar rats were dosed SC daily with vehicle or 0.3 mg/kg etelcalcetide for 4 weeks. Non-adenine fed (adenine-free diet; NAF) Wistar rats served as non-uremic controls. Blood samples were collected pre-dose and just prior to next dose at week 2 and 4 of the study. Rats were euthanized after 4 weeks of treatment and parathyroid gland (PTG) hyperplasia and aortic mineral content were quantified.

Plasma PTH levels were relatively variable among all rats prior to the treatment phase. Therefore the fold-change was calculated and normalized to pre-dose PTH level for each individual animal. Mean normalized PTH increased by 6.5-fold in vehicle-treated $DSA_{0.75}$ rats at week 4 which was significantly higher than in vehicle-treated NAF rats. In etelcalcetide-treated $DSA_{0.75}$ rats, mean normalized PTH (2.69 ± 0.25 fold) was significantly lower ($p< 0.05$) at week 4 than vehicle-treated DSA0.75 rats (6.51 ± 0.58 fold) and was similar to vehicle-treated NAF rats (2.17 ± 0.15 fold).

Consistent with increased PTH levels, the average body weight normalized parathyroid gland weight was significantly greater ($p< 0.05$) in vehicle-treated $DSA_{0.75}$ rats (2.49 ± 0.24 mg/kg) than in NAF rats (1.25 ± 0.11 mg/kg). In etelcalcetide-treated $DSA_{0.75}$ rats, the average normalized PTG weight (1.61 ± 0.15 mg/kg) was significantly lower ($p < 0.05$) than that of vehicle-treated $DSA_{0.75}$ rats and was not significantly different from vehicle-treated NAF rats.

Consistent with increased parathyroid gland weight in vehicle-treated $DSA_{0.75}$ rats, the average Ki-67-stained chief cell count and area-adjusted Ki-67-stained chief cell count were significantly greater than the respective values in NAF rats. In etelcalcetide-treated $DSA_{0.75}$ rats, the average Ki-67-stained chief cell count and area-adjusted Ki-67-stained chief cells were significantly lower than the respective values in vehicle-treated $DSA_{0.75}$ rats and were similar to those in NAF rats.

In vehicle-treated $DSA_{0.75}$ rats, the aortic calcium content was 10-fold greater than the value in NAF rats. In etelcalcetide-treated $DSA_{0.75}$ rats, the aortic calcium content was significantly lower than the value in vehicle-treated $DSA_{0.75}$ rats and was similar to those in NAF rats. Aortic phosphorus content was below the detection limit in all NAF and etelcalcetide-treated $DSA_{0.75}$ rats, but was measurable in 6 of 24 vehicle-treated $DSA_{0.75}$ rats.

Reference ID: 3923456

Table 9 Pharmacodynamic Study No r20130076: AMG416 (Etelcalcetide) Prevents Vascular Calcification in Uremic Rats.

Effects of AMG 416 on Vascular Calcification in a Rat Model of Secondary Hyperparathyroidism Induced by Adenine Diet

			Extracts of Aorta Segment	
Adenine	Treatment	n	Calcium (µg/g of tissue)	Phosphorus (µg/g of tissue)
_	Vehicle	23	65.2± 4.5	BLQ
+	Vehicle	24	631.3 ± 326.4[a]	1725 ± 941
+	AMG 416	24	47.5 ± 3.3[b]	BLQ

Results are expressed as the mean ± standard error of mean (SEM). [a]$p < 0.05$ vs. vehicle-treated NAF rats, [b]$p < 0.05$ vs. vehicle-treated rats fed with adenine diet. Nonparametric Kruskal-Wallis test was used to determine the difference between groups. BLQ=below the lowest limit of quantification.

Source: Study R20130076

4.2 Secondary Pharmacology

Study No 4169-NC-128

AMG416 (Etelcalcetide) did not bind to a panel of off-target receptors, ion channels, and norepinephrine transporter.

The objective of the study was to evaluate the specificity of etelcalcetide by testing the ability of etelcalcetide to stimulate or inhibit radioligand binding to a panel of 34 off-target receptors, ion channels and the norepinephrine transporter. Of those, 22 targets were human, 10 were rat, and one each was from mouse and hamster. The targets were either from recombinant cells overexpressing the protein of interest, cell lines with native expression, or in some cases tissue sources. Binding of a high-affinity radioligand specific to each target was quantified in the presence of 10 µM etelcalcetide. Assays were independently validated with a reference standard for each target. A significant response was defined as an effect of etelcalcetide to either stimulate or inhibit maximal binding of the radioligand by > 50%.

There were no significant changes in radioligand binding to any target in the presence of 10 µM etelcalcetide, indicating that etelcalcetide did not bind to a panel of related receptors, ion channels or to the norepinephrine transporter. The uniqueness of cysteine 482 to the CaSR and other biochemical studies (showing no significant binding to a panel of receptors, ion channels and a transporter) supported that the high selectivity of etelcalcetide for the CaSR.

4.3 Safety Pharmacology

Study No 4169-NC-151 (GLP)

AMG 416 (Etelcalcetide) has no effect on hERG ion channel current at concentrations tested.

The effects of etelcalcetide on the human ether-a-go-go (hERG) potassium channel current were evaluated in HEK-293 cell line stably transfected with hERG RNA using the whole cell patch clamp assay. Etelcalcetide was tested at concentrations of 0, 0.1, 0.3, 1.0, 3.0 and 10 µg/mL (9.54 µM). E-4031 (0.1 µM), a known hERG type potassium channel blocker, was included as a positive control.

There was no significant change in hERG current up to the highest concentration tested (10 µg/mL), which is at least 42-fold greater than the estimated human C_{max} (unbound) at the maximum clinical dose of 15 mg. The positive control inhibited hERG current by 89%.

Study No 4169-NC-102 (GLP)

Administration of a single IV dose of 1.5 mg/kg AMG 416 (etelcalcetide) caused a decrease in plasma PTH, an associated decrease in serum calcium and magnesium and an increase in serum phosphorus (a consequence of the expected pharmacology in animals with normal renal function). Maximal hypocalcemia occurred approximately 24 hours after dosing and was temporally associated with clinical signs of tremoring, slight QTc prolongation and increased body temperature, heart rate and blood pressure. These changes are considered secondary to hypocalcemia . There were no effects on neurological or respiratory parameters at doses of AMG 416 up to 1.5 mg/kg.

Male beagle dogs were administered a single IV dose of vehicle (Day 1) or etelcalcetide at 0.3 mg/kg (Day 9) and then 1.5 mg/kg (Day 16). Cardiovascular parameters (ECG, heart rate and mean arterial pressure) and body temperature data were recorded via telemetry prior to and through 48 hours following dose administration. Respiration rate and blood gases were evaluated prior to dosing and 6, 24 and 48 hours following dose administration. Neurological examinations were conducted prior to Day 1 and approximately 72 hours following the last dose of test article. Following a washout period, the same dogs (n = 4) were administered 1.5 mg/kg of etelcalcetide (42 days following the last dose on day 16) to evaluate the temporal relationship between plasma drug levels, serum chemistry and plasma PTH and the cardiovascular changes.

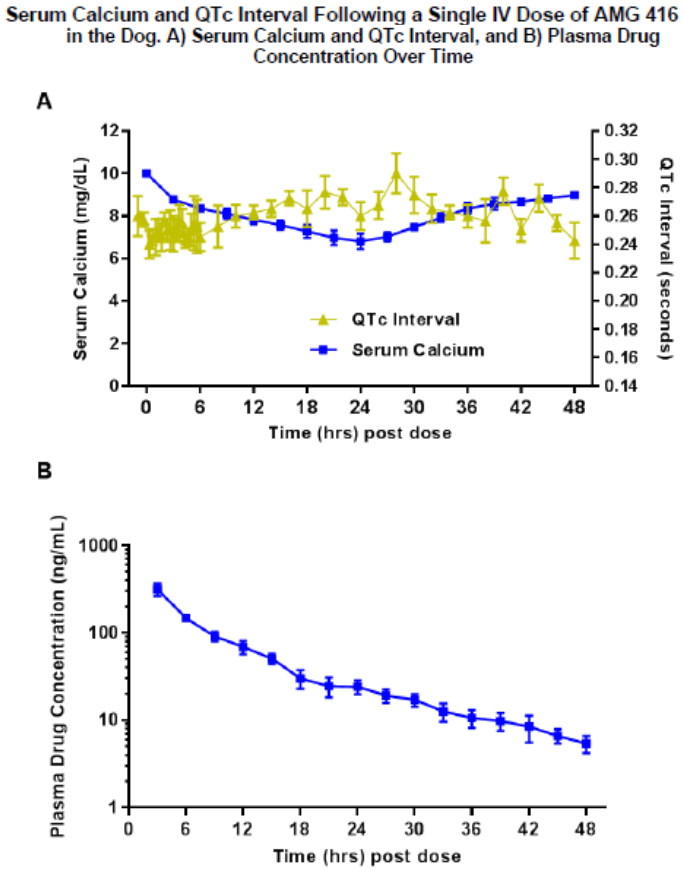
At 0.3 mg/kg, no treatment-related clinical signs or changes in body weight, food consumption, blood gases, respiration rates, neurological parameters, ECG intervals, heart rate, mean arterial pressure and body temperature were observed in male dogs. The only treatment-related changes were a significant decrease in PTH (below the lower limit of quantitation) observed at the 6-hour post-dose collection, which was correlated with a moderate decrease in serum calcium and a minor increase in serum phosphorus. These changes were reversible, not adverse and are an expected consequence of the pharmacology.
At 1.5 mg/kg, no treatment-related changes in body weight, food consumption, blood gases, respiration rates, and neurological parameters were observed in male dogs. Animals showed clinical signs of tremoring, most notably 24 hours after dosing coincident with maximal (22% to 36%) decreases in serum calcium. These effects were consistent with the significant decrease in PTH (below the lower limit of quantitation) observed at the 6-hour post-dose collection. Decreases in serum magnesium (23% to 29%) and increases in serum phosphorus (up to 47%) were also observed and were most pronounced from 21 to 24 hours post-dose. These serum ion concentrations had substantially or completely reversed to baseline values by 48 hours post dose.

At 1.5 mg/kg, etelcalcetide increased mean QTc interval 12% to 16% relative to controls, beginning from approximately 16 through 22 hours post-dose, with intermittently higher mean values thereafter at 28 to 32 hours and at 40 hours post-dose. Changes in QTc interval were temporally associated with maximum decreases in serum calcium levels, and normalized following recovery of serum calcium levels. No significant change in QTc interval was observed after dose administration when plasma drug levels were maximal during the first 6 hours. Prolongation of QTc interval is a known consequence of hypocalcemia in the dog and in humans. As a result, the increase in QTc interval was considered a secondary effect of the pharmacologic action of the drug in reducing PTH secretion and subsequent hypocalcemia. Transient increases in heart rate (20% to 31% relative to vehicle controls) were observed approximately 20 to 27 hours post-dose. Changes in heart rate were temporally associated with maximum decreases in serum calcium levels, rather than maximum plasma drug concentrations, and recovered following recovery of serum calcium levels. Increased blood

Reference ID: 3923456

pressure and temperature were also temporally associated with decreased serum calcium levels and likely reflect a response to hypocalcemia and/or stress. The increases in plasma concentrations were proportional to dose and were maximal when measured 2 min after dosing (Phase 1). Plasma drug levels for the 1.5 mg/kg dose after 24 hours, where changes in QTc interval were observed, were low (< 1% of C_{max}).

Figure 6 Safety Pharm Study No 4169-NC-102: AMG 416 (Etelcalcetide) Increased QTc Interval at Single IV dose of 1.5 mg/kg in Dogs.



Changes in serum calcium, QTc interval and plasma drug concentrations were evaluated over a 48 hour period following a single dose of 1.5 mg/kg of AMG 416 to male dogs (n = 4). A) Serum calcium (blue squares) was measured every 3 hours after dosing, up to 48 hours postdose. QTc interval (green triangles) was measured at 20 minute intervals for 30 seconds for the first 6 hours postdose, and then at 2 hour intervals for 30 seconds from 6 to 48 hours postdose. B) Plasma drug concentrations were measured every 3 hours after dosing up to 48 hours postdose.

Source: Study 4169-NC-102

Table 10 Safety Pharm Study No 4169-NC-102: TK Profile of etelcalcetide in Dogs after a Single IV Dose of etelcalcetide.

Mean AMG 416 Plasma Concentration and Toxicokinetic Parameters
After a Single IV dose of AMG 416 in Dogs

Dose (Study Day)	Time	Mean Plasma Concentration (ng/mL)	C_{max} (ng/mL)	AUC (ng•h/mL)
0.3 mg/kg (Day 9)	2 min	627.8	627.8	NC
	6 hour	29.3		
	24 hour	5.2		
1.5 mg/kg (Day 16)	2 min	3412.5	3412.5	NC
	6 hour	179.8		
	24 hour	29.6		
1.5 mg/kg[a] (Day 38)	3 hour	315.3	315.3	3549
	6 hour	147.8		
	9 hour	91.7		
	12 hour	68.9		
	24 hour	24.2		
	48 hour	5.4		

AUC = area under the concentration-time curve from time zero until last acquired timepoint; C_{max} = maximum observed concentration; NC = not calculated.

[a] Clearance (CL), terminal half-life ($t_{1/2}$) and volume of distribution (V_{ss}) were 422 mL/hr/kg, 10.2 hr, and 3719 mL/kg, respectively.

Source: Study 4169-NC-102

4.4 Drug Interaction

Study No 20130030

Coadministration of cinacalcet and AMG 416 (etelcalcetide) was additive with respect to CaSR activation in vitro.

The potential for additive or synergistic effects on CaSR signaling with co-administration of cinacalcet (AMG 073) and etelcalcetide was evaluated in two HEK293T cell lines stably transfected with the human CaSR. Response to cinacalcet and etelcalcetide was measured by quantifying the accumulation of IP-1. The co-administration study was designed in two parts.

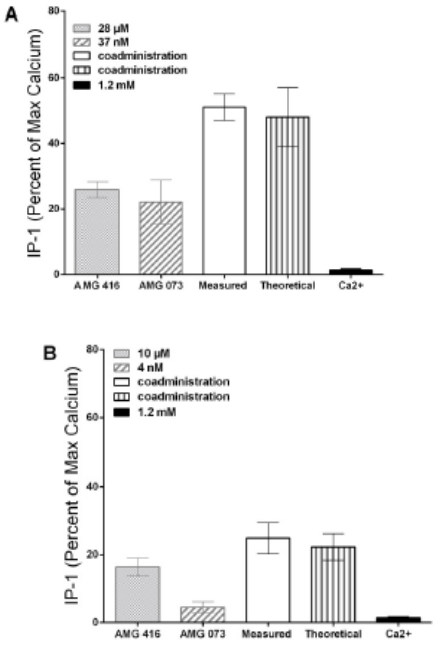
In the first part, the individual dose response curves were established for calcium and both the calcimimetics, etelcalcetide and cinacalcet. Calcimimetic activity was always measured in the presence of 1.2 mM calcium. Concentrations of both cinacalcet and etelcalcetide which provided either moderate or low degrees of CaSR activation were determined from respective individual dose response curves. In the second part, moderate or low stimulatory concentrations of the calcimimetics were tested either as fixed individual concentrations (cinacalcet or etelcalcetide alone) or following coadministration of the two calcimimetics (cinacalcet and etelcalcetide combined together). Coadministration studies were limited to combined concentrations of the calcimimetics either at each respective moderate or each respective low stimulatory concentration. Simple additivity was defined by in silico addition of CaSR activation that was observed for each calcimimetic alone and this sum of receptor activation was compared to observed receptor activity in the coadministration study. Superadditivity (or synergism) was defined as a measured activity during coadministration which is consistently and statistically greater than the in silico (simple) additivity.

In three of the four cases where additivity was observed, the theoretical additivity seen by summing the individual receptor activation values were no different than the receptor activation actually measured with a combination of cinacalcet and etelcalcetide. This was true for both cases with cell line 1 using either moderate or low stimulatory concentrations of calcimimetics. It was also true when using moderate stimulatory concentrations of calcimimetics with cell line 2.

In one case, when using low stimulatory concentrations of calcimimetics with cell line 2, combined treatment of both cinacalcet and etelcalcetide provided a degree of CaSR stimulation that was marginally greater than the theoretical value that represented simple additivity. Due to some expected variation in assay performance, this later example appears to be an exception and does not affect the conclusion of simple additivity for the combined effects of cinacalcet and etelcalcetide.

Figure 7 Drug Interaction Study No 20130030: Co-administration of AMG416 (etelcalcetide) and cinacalcet had additive effect on CaSR Activation.

CaSR Activation Following Individual or Combined Exposures with AMG 416 and/or Cinacalcet in KAI's Cell Line



Relative maximal responses of the CaSR to AMG 416 and/or cinacalcet in KAI's cell line. Cells were reseeded in 384 well plates approximately 17 hours prior to incubating with the following: (A) MODERATE: 28 µM AMG 416 alone, 37 nM cinacalcet alone, or 28 µM AMG 416 and 37 nM cinacalcet combined. (B) LOW: 10 µM AMG 416 alone, 4 nM cinacalcet alone, or 10 µM AMG 416 and 4 nM cinacalcet combined. "Measured" is the CaSR activation found with combined treatment of calcimimetics and "Theoretical" is the in silico addition of CaSR activation found with each calcimimetic alone. "Ca2+" is the basal activity found in the absence of calcimimetic, cinacalcet and AMG 416. All CaSR activation occurred at 37°C for 1.5 hours in buffer containing 1.2 mM calcium, 10 mM HEPES, 130 mM NaCl, 4.2 mM KCl, 0.5 mM $MgCl_2$, 5 mM glucose, 50 mM LiCl, 0.8% DMSO, and 0.01% BSA. IP-1 was quantified from cell lysates using the IP-One Tb kit. See Attachment 4 for data.

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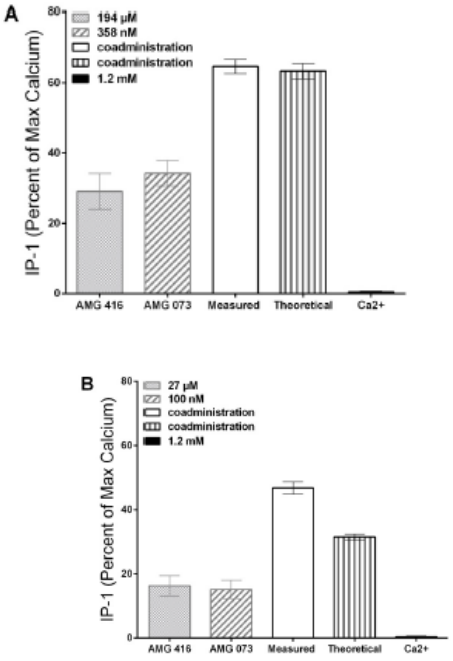
CaSR Activation Following Individual or Combined Exposures with AMG 416 and/or Cinacalcet in Amgen's Cell Line



Relative maximal responses of the CaSR to AMG 416 and/or AMG 473 in Amgen's cell line. Cells were reseeded in 384 well plates approximately 17 hours prior to incubating with the following: (A) MODERATE: 194 µM AMG 416 alone, 358 nM cinacalcet alone, or 194 µM AMG 416 and 358 nM cinacalcet combined. (B) LOW: 27 µM AMG 416 alone, 100 nM cinacalcet alone, or 27 µM AMG 416 and 100 nM cinacalcet combined. "Measured" is the CaSR activation found with combined treatment of calcimimetics and "Theoretical" is the in silico addition of CaSR activation found with each calcimimetic alone. "Ca2+" is the basal activity found in the absence of calcimimetics, cinacalcet and AMG 416. All CaSR activation occurred at 37°C for 1.5 hours in buffer containing 1.2 mM calcium, 10 mM HEPES, 130 mM NaCl, 4.2 mM KCl, 0.5 mM $MgCl_2$, 5 mM glucose, 50 mM LiCl, 0.8% DMSO, and 0.01% BSA. IP-1 was quantified from cell lysates using the IP-One Tb kit. See Attachment 5 for data.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption Non-GLP Studies

Study No 4169-NC-114

KAI-4169 (etelcalcetide) pharmacokinetics was altered in rats with compromised renal function. The extent of this impact was dependent upon the degree of renal impairment. The data indicated that the kidneys were involved in the clearance of KAI-4169 in rats following an IV bolus administration.

A set of non-GLP studies characterized the pharmacokinetics of etelcalcetide in male rats with differing levels of renal function, when delivered intravenously by rapid bolus. Pharmacokinetic parameters were calculated using WinNonlin. All animals were precannulated in the jugular vein for blood sampling and in the femoral vein for drug administration. Etelcalcetide was administered as an IV bolus in a volume of 1.7 mL/kg. Blood samples were withdrawn over the subsequent 24 hours. After each blood draw, an equal volume of saline was administered to the animal. All animals were dosed while anesthetized; samples were taken in most cases while the animals were conscious. Following the final blood draw, the animals were euthanized. Etelcalcetide was administered to Sprague-Dawley rats by intravenous bolus injection (0.3 and

1 mg/kg) in order to characterize the pharmacokinetics of this route of administration and under different degrees of renal function.

Etelcalcetide exposure (as assessed by group mean C_{max} and AUC values) increased with increasing dose in a dose-proportional manner. A comparison of data in the different models clearly indicated that both C_{max} and AUC parameters were affected by the loss of kidney function. At both the 0.3 and 1 mg/kg dose, an approximate 3-fold increase in AUC was seen in the double-nephrectomized animals when compared with the normal rats, with a corresponding 3-fold decrease in clearance. In contrast, in the less severe model of kidney dysfunction (5/6 nephrectomized rats) there was only a 1.2- and 1.4-fold increase in AUC relative to rats with normal renal function (0.3 and 1 mg/kg dose, respectively).

Table 11 Pharmacokinetic Absorption Study No 4169-NC-114: Pharmacokinetic Profile of AMG416 (Etelcalcetide) in Rats after a Single IV Dose of AMG416.

Group mean KAI-4169 pharmacokinetic summary data; sorted by dose and model

Model	Dose (mg/kg)	C_0[1] (ng/mL)	C_{max}[2] (ng/mL)		AUC_{all}[3] (hr*ng/mL)		AUC_{last} (hr*ng/mL)		CL_{all}[4] (mL/hr/kg)	$T_{1/2}$[5] (hr)
			C_{max}	SEM	AUC	SEM	AUC	SEM		
Normal	0.3	572	552	20	1258	62	1239	55	238	1.28
5/6 Neph	0.3	545	536	53	1473	170	1435	168	204	1.54
2xNeph	0.3	554	537	53	3895	380	3895	380	77	6.70
Normal	1	1726	1662	94	3738	165	3707	164	268	1.33
5/6 Neph	1	1587	1560	85	5121	370	4955	299	195	2.16
2xNeph	1	1464	1428	99	11412	578	11412	578	88	6.73

[1] Concentration at time = 0
[2] Maximum concentration
[3] Area under the time concentration curve
[4] Clearance
[5] Terminal half-life

Study No 4169-NC-157

Renal clearance was the elimination route for KAI-4169 (etelcalcetide) in rats. Similar PK profile was obtained from previous studies, Study No 4169-NC-114.

The study evaluated the plasma pharmacokinetics (PK) and urinary/fecal excretion of etelcalcetide when administered as a single intravenous (IV) injection to male Sprague-Dawley rats.

The dose of etelcalcetide administered 1.0 mg/kg, was well tolerated by all 4 rats, based on clinical signs. The plasma pharmacokinetics of intact etelcalcetide was consistent with data obtained from prior studies conducted in rats. Mean values for clearance, volume of distribution, and half-life were 351 mL/h/kg, 429 mL/kg, and 1.38 hours, respectively.

There were measurable concentrations of intact etelcalcetide in all urine samples collected over the first 48 hours post-dose in all rats, and the highest concentrations were found in the samples collected during the first 4 hours, with a progressive decline in the subsequent collection periods. The percent of the etelcalcetide dose recovered in the urine over the 48-hour collection period ranged from 0.7 to 5.4% for the 4 rats, with a mean of 3.2%.
Urinary clearance of intact etelcalcetide was calculated over the first 48 hours following administration of etelcalcetide. The mean renal clearance (CLR) value for etelcalcetide was 2.51 mL/h. Only one fecal sample had quantifiable levels of etelcalcetide, with the amount excreted being less than 0.01% of the administered dose.

Reference ID: 3923456

Table 12 Pharmacokinetic Absorption Study No 4169-NC-157: Pharmacokinetic Profile of AMG416 (Etelcalcetide) in Rats after a Single IV Dose of AMG416.

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Individual Animal and Group Mean KAI-4169 Pharmacokinetic Summary Data; Sorted by Group and Dose

Group	Dose (mg/kg)	Animal	C_{max} (ng/mL)	AUC_{all} (h*ng/mL)	AUC_{INFobs} (h*ng/mL)	Vss_{obs} (mL/kg)	Cl_{obs} (mL/h/kg)	$HL_{λz}$ (h)
1	1	1M001	1700	2470	2500	522	400	1.41
		1M002	2650	2850	2880	441	347	1.36
		1M003	3020	3020	3060	371	327	1.40
		1M004	2860	2990	3020	381	331	1.34
		N	4	4	4	4	4	4
		Mean	2560	2830	2870	429	351	1.38
		SD	591	255	255	69.4	33.5	0.0346
		CV%	23.1	9.0	8.9	16.2	9.6	2.5

Urine Volume, Concentration, Output, and Pharmacokinetic Summary Data; Sorted by Animal ID

Subject		1M001	1M002	1M003	1M004	Mean	SD	N
Body Weight (g)		278.2	283.6	292.3	278.2	283.1	6.7	4
Urinary Volume (mL)	0-4h	1.10	0.282	0	0	0.346	0.520	4
	4-8h	1.60	0.800	1.75	0.750	1.23	0.52	4
	8-12h	2.90	2.25	0.850	2.65	2.16	0.92	4
	12-24h	8.40	6.85	9.85	9.60	8.68	1.37	4
	24-48h	15.7	16.2	17.2	18.4	16.9	1.2	4
Urine Concentration (ng/mL)	0-4h	4480	23600	0	0	7020	11250	4
	4-8h	2370	6600	3660	820	3360	2450	4
	8-12h	369	870	727	227	548	300	4
	12-24h	66.9	137	94.2	51.9	87.5	37.4	4
	24-48h	22.7	28.7	25.9	15.2	23.1	5.8	4
Urinary Output (µg)	0-4h	4.93	6.66	0	0	2.90	3.42	4
	4-8h	3.79	5.28	6.41	0.62	4.03	2.51	4
	8-12h	1.07	1.96	0.62	0.60	1.06	0.64	4
	12-24h	0.56	0.94	0.93	0.50	0.73	0.24	4
	24-48h	0.36	0.46	0.44	0.28	0.39	0.08	4
	Sum	10.71	15.30	8.40	2.0	9.10	5.54	4
Urinary Output (0-8 h) (µg)		8.72	11.9	6.41	0.620	6.92	4.77	4
Plasma AUC_{all} (ng*h/mL)		2470	2850	3020	2990	2830	255	4
CL_R (mL/h)		3.53	4.19	2.12	0.21	2.51	1.76	4
Amount of Dose Excreted in Urine (%)		3.85%	5.39%	2.87%	0.72%	3.21%	1.96%	4

Feces Weight, Concentration, and Output Summary Data; Sorted by Animal ID

Subject		1M001	1M002	1M003	1M004	Mean	SD	N
Body Weight (g)		278.2	283.6	292.3	278.2	283.1	6.7	4
Feces Weight (g)	0-4h	0	0.451	0.172	0	0.156	0.213	4
	4-8h	0	0	0.425	0.922	0.337	0.439	4
	8-12h	0.647	0	0.863	0	0.378	0.445	4
	12-24h	7.74	5.31	7.59	3.22	5.96	2.14	4
	24-48h	11.3	16.1	15.2	10.8	13.3	2.7	4
Feces Concentration (ng/g)	0-4h	0	38.3	0	0	9.6	19.2	4
	4-8h	0	0	0	0	0	0	4
	8-12h	0	0	0	0	0	0	4
	12-24h	0	0	0	0	0	0	4
	24-48h	0	0	0	0	0	0.0	4
Feces Output (µg)	0-4h	0	0.017	0	0	0.004	0.009	4
	4-8h	0	0	0	0	0	0	4
	8-12h	0	0	0	0	0	0	4
	12-24h	0	0	0	0	0	0	4
	24-48h	0	0	0	0	0	0	4
	Sum	0	0.017	0.00	0.0	0.004	0.009	4
Amount of Dose Excreted in Feces (%)		0%	0.006%	0%	0%	0.002%	0.003%	4

Distribution Non-GLP Studies

Study No 4169-NC-117

AMG416 (Etelcalcetide) levels were detected in tissues: liver, kidney (highest levels) > lung, spleen, thyroid, thymus, heart > brain (very low levels).

This study characterized the major organ distribution of etelcalcetide in SD rats following IV bolus administration dose of 0.3 mg/kg. 6 hours later, animals were anesthetized, blood was removed for plasma analysis and the animals were sacrificed. Entire organs or pieces of organs were collected for analyses. Following homogenization, the samples were extracted and subjected to LC/MS for quantification of etelcalcetide levels. Because of the nature of the extraction process, etelcalcetide was chemically reduced (i.e., removal of the di-sulfide-linked L-cysteine residue) and so levels of the reduced form of etelcalcetide (KP-2067) were quantitated and reported.

Evidence of etelcalcetide exposure was consistently seen in all tissues examined with the exception of the brain where it was only detected in one out of four animals and at very low levels, suggesting minimal penetration of the blood-brain barrier. Highest levels were seen in the liver and kidney and the lowest levels in the brain. The remaining organs examined (lung, spleen, thyroid, thymus and heart) all showed detectable amounts of material, lower than what was observed in the liver and kidney.

Table 13 Pharmacokinetic Distribution Study No 4169-NC-117: AMG416 (Etelcalcetide) had the highest levels detected in liver and kidney in Rats after a Single IV Dose of AMG416.

Tissue Data for KAI-4169

Tissue	Concentration of reduced KAI-4169 (KP-2067) (ng/mL)					ng KP-2067/g Tissue
	Rat 1	Rat 2	Rat 3	Rat 4	Average	
Plasma[1]	26.0	34.0	42.0	12.0	28.5	28.5[1]
Lung	5.6	5.7	2.8	1.5	3.9	78.0
Spleen	9.3	6.6	11.4	6.8	8.5	170.6
Brain	0.0	0.0	0.0	1.4	0.4	7.0
Heart	7.8	7.2	7.1	3.3	6.4	127.0
Kidney	18.5	21.4	24.1	38.0	25.5	510.1
Liver	65.0	44.0	144.3	75.0	82.1	1641.5
Thymus	8.0	2.1	7.3	6.4	6.0	119.2
Thyroid	1.0	2.5	33.5	1.8	9.7	194.0

[1] Plasma was not boiled; value represents amount of KAI-4169 in ng/mL

Study No 4169-NC-118

AMG416 (Etelcalcetide) was 90% protein bound and binding to plasma proteins was reversible.

This study characterized the protein binding of etelcalcetide in plasma from multiple species. Equilibrium dialysis was used to determine the extent to which etelcalcetide is bound to plasma proteins. In Experiment #1, etelcalcetide was spiked into plasma from human, rat, dog and horse and subjected to rapid-equilibrium dialysis against PBS or plasma from the different species. In Experiment #2, the kinetics of protein binding was investigated. In this experiment, plasma or PBS was incubated with etelcalcetide for one hour prior to starting dialysis. Three dialysis conditions were tested: plasma dialyzed against PBS (plasma:PBS), plasma dialyzed against plasma (plasma:plasma), and PBS dialyzed against PBS (PBS:PBS). Samples of dialysate were removed periodically over the course of the study (approximately 7.5 hours) for the determination of KAI- 4169 concentrations by LC/MS analysis.

When etelcalcetide was spiked into plasma and allowed to dialyze against PBS, approximately 90% of the spiked material was retained in the plasma (starting chamber) after 6 hours of incubation. This result was consistent across all species tested. The degree of plasma protein binding determined by this method was relatively consistent across species, averaging 88, 95, 84 and 89% in human, rat, dog and horse plasma, respectively. The plasma protein binding was reversible and exchangeable, as demonstrated by the ability to readily equilibrate when

dialyzed against plasma. In addition, based on the etelcalcetide concentration recovered from the dialysis chambers, it showed there was very little loss of material as the result of etelcalcetide interaction with the dialysis apparatus.

Table 14 Pharmacokinetic Distribution Study No 4169-NC-118: KAI-4169 (etelcalcetide) was 90% Protein Bound across Species. The binding was reversible.

Protein Binding of KAI-4169

% of KAI-4169 retained in the starting chamber after 6 hours of rapid equilibrium dialysis at room temperature (n=3 ±SD).

Starting Matrix	Dialysate				
	PBS	Human	Rat	Dog	Horse
PBS	45 ± 8.3	5 ± 3.5	2 ± 1.8	8 ± 1.0	5 ± 2.4
Human	88 ± 7.8	53 ± 13.2	44 ± 5.6	52 ± 3.3	47 ± 6.9
Rat	95 ± 2.2	60 ± 7.2	54 ± 10.4	55 ± 8.7	51 ± 3.1
Dog	84 ± 7.6	44 ± 4.9	40 ± 8.8	46 ± 7.6	56 ± 9.3
Horse	89 ± 8.8	52 ± 1.5	47 ± 4.2	57 ± 9.3	59 ± 10.5

Dialysis of KAI-4169 spiked into plasma and dialyzed against phosphate buffered saline (n=1)

Time (minutes)	KAI-4169 (ng/mL)	
	Plasma	Dialysate
0	508	0
5	532	0
15	511	0
45	491	15
135	472	48
405	402	69

Dialysis of KAI-4169 spiked into plasma and dialyzed against plasma (n=1)

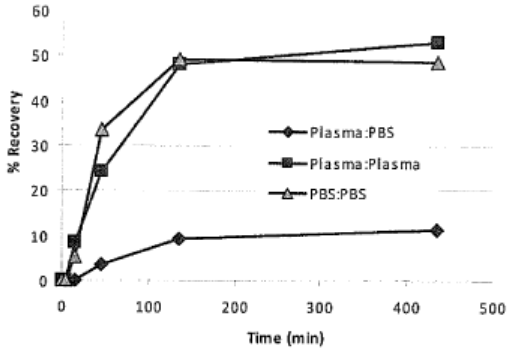
Time (minutes)	KAI-4169 (ng/mL)	
	Plasma	Dialysate
0	544	0
5	498	0
15	576	42
45	415	121
135	224	248
405	256	265

Dialysis of KAI-4169 spiked into PBS and dialyzed against PBS (n=1)

Time (minutes)	KAI-4169 (ng/mL)	
	PBS	Dialysate
0	491	0
5	512	0
15	491	25
45	388	165
135	222	236
405	278	241

Under these conditions, the amount of material remaining in the starting dialysis chamber was close to 50% regardless of which species of plasma was used. The presence of protein did not affect the rate of exchange as the rate of exchange across the dialysis membrane was equivalent in PBS and plasma. In contrast, when etelcalcetide-plasma was dialyzed against PBS, only 10% of the etelcalcetide was recovered from the PBS dialysate, indicating the majority was retained in the plasma. These data concluded that etelcalcetide was approximately 90% protein bound and that the binding to plasma proteins was reversible.

Figure 8 Pharmacokinetic Distribution Study No 4169-NC-118: The Majority of KAI-4169 (etelcalcetide) was Retained in the Plasma.



KAI-4169 was spiked into human plasma (Plasma:PBS, Plasma:Plasma) or PBS (PBS:PBS) and incubated at room temperature for 1 hour prior to initiating rapid-equilibrium dialysis against human plasma (Plasma:Plasma) or PBS (Plasma:PBS, PBS:PBS). Data are presented as % of recovered KAI-4169 in the dialysate.

Study No 117581

AMG416 (etelcalcetide) was rapidly and extensively distributed to most tissues after a single IV dose to rats with C_{max} = 1 hour post-dose. Highest exposure was kidney, to a lesser extent liver, which were organs of elimination.

The pharmacokinetics and distribution of ^{14}C-etelcalcetide were determined in male and female Sprague-Dawley rats and in male Long Evans rats after a single 2.5-mg/kg intravenous dose of ^{14}C-etelcalcetide. Radiation dosimetry parameters were also calculated for male rats in both rat strains. Blood and carcasses were collected at specified times through 168 hours post-dose in Group 1 Sprague-Dawley rats and through 2016 hours (12 weeks) post-dose in Group 2 male Long Evans rats. Blood and plasma samples were analyzed for total radioactivity content by liquid scintillation counting, and carcasses were analyzed by quantitative whole-body autoradiography.

Maximum concentrations (C_{max}) of total radioactivity in blood and plasma from male and female Sprague-Dawley rats were observed at 0.083 hours post-dose (T_{max}), with C_{max} values of 5,670 and 9,710 ng-eg/g ^{14}C-etelcalcetide/g (ng-eq/g), respectively, in male rats and 6,430 and 11,500 ng-eq/g, respectively, in female rats.

Concentrations of radioactivity in blood and plasma declined readily through 24 hours post-dose in both sexes followed by a slower elimination phase. Elimination half-lives ($t_{1/2}$) of total radioactivity in plasma were 86.2 and 143 hours for male and female Sprague-Dawley rats, respectively. Blood to plasma concentration ratios for radioactivity in male and female Sprague-Dawley rats were <0.8 at all time-points through 168 hours post-dose. Blood and plasma

Reference ID: 3923456

exposures ($AUC_{0-\infty}$) of radioactivity were 6,940 and 11,400 ng equivalents ^{14}C-etelcalcetide hours/g (ng eq·hours/g), respectively, in males and 8,010 and 13,300 ng eq·hours/g, respectively, in females.

Table 15 Pharmacokinetic Distribution Study No 117581: Pharmacokinetic Profile of etelcalcetide in Male/Female SD Rats or Male Long Evans Rats.

Pharmacokinetic parameters for radioactivity in blood and plasma after a single intravenous dose of ^{14}C-AMG 416 to male and female Sprague Dawley rats (Group 1, 2.5 mg/kg)

Sample	C_0 (ng eq/g)	T_{max} (hours)	C_{max} (ng eq/g)	$t_{½}$ (hours)	$AUC_{(0-t)}$ (ng eq·hours/g)	$AUC_{(0-\infty)}$ (ng eq·hours/g)	Cl (g/hr/kg)	Vss (g/kg)
				Males				
Blood	8023	0.083	5670	99.3[a]	6545	6942	360	9833
Plasma	13572	0.083	9710	86.2[a]	10972	11404	219	4215
				Females				
Blood	8865	0.083	6430	161[a]	7242	8005	312	14845
Plasma	15877	0.083	11500	143[a]	12386	13325	188	6494

eq Equivalents ^{14}C-AMG 416.
AUC Area under the concentration time curve.
Cl Clearance
Vss Volume of distribution at steady state.
a The number of sampling time points in this study does not support the longer half-life calculated. Therefore, calculated half-life should be interpreted with caution.

Pharmacokinetic parameters for radioactivity in blood and plasma after a single intravenous dose of ^{14}C-AMG 416 to male Long Evans rats (Group 2, 2.5 mg/kg)

Sample	C_0 (ng eq/g)	T_{max} (hours)	C_{max} (ng eq/g)	$t_{½}$ (hours)	$AUC_{(0-t)}$ (ng eq·hours/g)	$AUC_{(0-\infty)}$ (ng eq·hours/g)	Cl (g/hr/kg)	V_{ss} (g/kg)
Blood	5382	0.25	3480	181	7616	8221	304	21053
Plasma	9711	0.25	6170	142	12373	12805	195	7234

eq Equivalents ^{14}C-AMG 416.
AUC Area under the concentration time curve.
Cl Clearance
Vss Volume of distribution at steady state.
a The number of sampling time points in this study does not support the longer half-life calculated. Therefore, calculated half-life should be interpreted with caution.

TISSUE DISTRUBTION

^{14}C-etelcalcetide-related radioactivity rapidly and extensively distributed to most tissues evaluated after a single intravenous dose to male or female Sprague-Dawley rats and to male Long Evans rats. Radioactivity exposures were highest in organs of elimination, primarily kidney and to a lesser extent liver, independent of strain. Consistent with tissue exposures, concentrations of radioactivity were highest in urine from Sprague-Dawley and Long Evans rats.

High radioactivity exposures were also observed in cartilage (intervertebral, hyaline, and articular), epiphyseal line, spleen (red pulp), bone marrow, and lymph nodes in both rat strains. Distribution of radioactivity to the brain, spinal cord, and melanin containing tissues was low. After reaching C_{max}, radioactivity declined slowly and remained quantifiable in multiple tissues at the last collected time point for both groups.

Substantive concentrations of radioactivity were evident in kidney, cartilages, liver (Sprague-Dawley only), and spleen at 168 and 2,016 (Long Evans only) hours post-dose, indicating retention of or slow elimination of radioactivity from these tissues. Similar to tissue exposures, kidney cortex, kidney, kidney medulla, and liver showed the highest calculated radiation absorbed doses (mRad), independent of rat strain.

Based on the pharmacokinetic profiles of radioactivity in male Sprague-Dawley and Long Evans rats and on projected dosimetry calculations, a single 100-µCi (3.7-MBq) intravenous dose of ^{14}C-etelcalcetide would not be expected to represent a significant radiation exposure risk to healthy human male volunteers. No sex-dependent or strain-dependent differences in tissue distribution of ^{14}C-etelcalcetide-related radioactivity were evident.

Study No 118052

Etelcalcetide was not a substrate or inhibitor of transporters tested and was poorly permeable into red blood cells.

This study evaluated in vitro permeability for AMG 416 and determined whether AMG 416 is a substrate or inhibitor of the following human transporters: P-glycoprotein (Pgp) and Breast Cancer Resistance Protein (BCRP), Organic Anion Transporters 1 and 3 (OAT1, OAT3), Organic Cation Transporter 2 (OCT2), Organic Anion Polypeptide Transporters 1B1 and 1B3 (OATP1B1, OATP1B3), and Peptide Transporters 1 and 2 (PEPT1, PEPT2). The study also evaluated the direct uptake of etelcalcetide into red blood cells (RBC). The transcellular transport assay measured vectorial transport of compounds across polarized kidney epithelial cells (LLC-PK1 or MDCKII) transfected with human MDR1 (Pgp) or BCRP transporter genes. The uptake transporter assays measured the accumulation of radiolabeled test compound or probe in empty vector-, human OAT1, OAT3, OAT1B1, OATP1B3, OCT2, PEPT1, or PEPT2-transfected HEK-293 cell line monolayers. The RBC uptake assay measured accumulation of ^{14}C-etelcalcetide into erythrocytes.

The passive permeability of etelcalcetide in LLC-PK1 cell monolayers was low (0.7 x 10^{-6} cm/s). Efflux, uptake, and inhibition studies indicate that etelcalcetide was not a substrate or inhibitor of any of the tested transporters. Direct uptake studies indicate that ^{14}C-etelcalcetide was poorly permeable into red blood cells.

Table 16 Pharmacokinetic Distribution Study No 118052: AMG416 (Etelcalcetide) was not a substrate or inhibitor of transporters tested and was poorly permeable into RBCs.

Permeability and Efflux Ratio (ER) Measurements of ^{14}C-AMG 416 (5 µM) in LLC-PK1 Parental and Human MDR1-transfected Cell Lines

Transwell Measurements	LLC-PK1 Control							Human MDR1-LLC-PK1						
Compound(s)	A>B	SD	B>A	SD	Papp Avg	ER	SD	A>B	SD	B>A	SD	Papp Avg	ER	SD
^{14}C-AMG 416	0.6	0.0	0.7	0.0	0.7	1.1	0.1	0.2	0.0	0.2	0.0	0.2	1.1	0.1
^{14}C-AMG 416 + CsA	0.8	0.1	0.3	0.1	0.5	0.4	0.1	0.2	0.1	0.2	0.1	0.2	0.9	0.4
^{3}H-DIG	6.4	0.7	6.3	0.6	6.3	1.0	0.1	0.6	0.1	13.3	1.2	6.9	22.0	3.2
^{3}H-ATN	1.2	0.1	1.4	0.0	1.3	1.2	0.1	0.4	0.0	0.6	0.0	0.5	1.4	0.1

A>B = apical-to-basolateral transport permeability x 10^{-6} cm/s; B>A = basolateral-to-apical transport permeability x 10^{-6} cm/s; ER = efflux ratio; SD = standard deviation (n=3); Papp Avg = average apparent permeability ((A>B + B>A) / 2); DIG = digoxin 0.5 µM positive Pgp control; CsA = Cyclosporin A 5 µM control inhibitor; ATN = Atenolol 10 µM monolayer integrity marker control.

Direct Uptake Measurements of ^{14}C-AMG 416 Following Incubation in Washed Red Blood Cells

	% Total ^{14}C-AMG 416		
Time (hr)	Incubation Buffer Supernatants	RBC Lysate	Total Recovery
0	86.1	9.5	95.6
2	91.0	9.2	100.1

Study No 119313

The non-covalent plasma protein bindings of AMG416 (etelcalcetide) were similar between healthy and CKD human blood.

This study determined the unbound concentration of etelcalcetide (at 50, 1000 and 10,000 ng/mL in whole blood) in plasma using multiple species (SD rat, beagle dog, healthy human, humans with CKD receiving hemodialysis).

Non-covalent plasma protein binding of etelcalcetide was low in all species tested. Non-covalent binding of AMG-416 was similar in healthy and CKD human donors. Plasma protein binding was independent of concentration between 50 and 10,000 ng/mL in all species.

Table 17 Pharmacokinetic Distribution Study No 119313: AMG416 (etelcalcetide) was not a substrate or inhibitor of transporters tested and was poorly permeable into RBC.

	Mean AMG 416 Fraction Unbound in Plasma			
AMG 416 (ng/mL)	Rat	Dog	Human	CKD Human
50 – 10,000	0.77	0.67	0.53	0.59

Metabolism Non-GLP Studies

Study No 4169-NC-119

KAI-4169 (etelcalcetide) was stable in the presence of active liver fractions over the course of 1-hour incubation at 37 °C, suggesting that KAI-4169 was not subjected to hepatic metabolism.

This study characterized the metabolic stability of etelcalcetide in liver fractions from multiple species (human, dog and rat). In Experiment #1, etelcalcetide was incubated for 60 min at 37 °C with activated liver fractions (cytosolic, S9, and microsomal) from rat, dog and human to determine its metabolic stability to Phase I and Phase II hepatic enzyme systems. In Experiment #2, in order to confirm the metabolic activity of the human liver S9 fraction, the study was repeated with the inclusion of a positive control (phenacetin) to demonstrate the metabolic activity of the liver fraction.

Experiment #1: The greatest loss of parent compound (26%) was observed in the rat S9 fraction. A minimal amount of degradation of etelcalcetide was observed under the same conditions with human liver fractions (96, 85 and 104% for cytosol, S9 and microsomal fractions, respectively). Although low recovery of etelcalcetide was noted from rat cytosol and S9, as well as with human cytosol, recovery was consistent between activated and heat-inactivated control samples. The results indicated that etelcalcetide was relatively stable to enzymatic transformation in liver fractions from rat, dog and human.

Experiment #2: While the activated liver S9 fraction readily degraded phenacetin (85% degraded in 60 minutes), etelcalcetide was relatively stable, with approximately 95% remaining under the same conditions. Furthermore, neither phenacetin nor etelcalcetide was degraded in the metabolically inactive samples from which NADP+ was omitted.

Table 18 Pharmacokinetic Metabolism Study No 4169-NC-119: AMG416 (Etelcalcetide) was not Metabolized by Hepatic CYP450.

Metabolic stability of KAI-4169 in rat, dog and human activated liver fractions after 60 minutes incubation at 37°C.

KAI-4169 concentrations were determined by LC-MS. Liver fractions in control samples were inactivated by heat. Data presented are the average of two independent samples).

Test Matrix	Species	Concentration KAI-4169 (ng/mL) (average)		% KAI-4169 Remaining (active relative to control)
		Control	Activated	
Cytosol	Rat	59, 79 (69)	54, 45 (49)	84
	Dog	214, 200 (207)	206, 184 (195)	94
	Human	67, 77 (72)	58, 82 (70)	96
S9	Rat	63, 75 (69)	42, 60 (51)	74
	Dog	120, 134 (127)	103, 123 (113)	88
	Human	140, 164 (152)	128, 132 (130)	85
Microsomes	Rat	130, 148 (139)	134, 144 (139)	100
	Dog	131, 155 (143)	130, 144 (137)	95
	Human	166, 176 (171)	163, 195 (179)	104

Average recovery of phenacetin after incubation with activated human liver S9 fraction at 37°C

	Phenacetin (ng/mL)				
Time (min)	Replicate 1	Replicate 2	Replicate 3	Average (n=3)	Standard Deviation*
0	2.08	1.02	0.51	1.2	0.80
30	0.433	0.341	0.502	0.41	0.08
60	0.25	0.19	0.11	0.18	0.07

Average recovery of KAI-4169 after incubation with activated human liver S9 fraction at 37°C

	KAI-4169 (ng/mL)				
Time (min)	Replicate 1	Replicate 2	Replicate 3	Average (n=3)	Standard Deviation
0	184.9	176.8	180.7	183.7	1.51
30	182.0	163.1	175.0	168.1	7.53
60	184.2	164.5	168.7	174.8	6.00

Average recovery of phenacetin after incubation with inactivated human liver S9 fraction at 37°C.

	Phenacetin (ng/mL)				
Time (min)	Replicate 1	Replicate 2	Replicate 3	Average (n=3)	Standard Deviation*
0	2.3	3.6	1.1	2.3	1.25
30	2	3.6	1	2.2	1.31
60	2.11	3.15	1.01	2.0	1.07

Average recovery of KAI-4169 after incubation with inactivated human liver S9 fraction at 37°C

	KAI-4169 (ng/mL)				
Time (min)	Replicate 1	Replicate 2	Replicate 3	Average (n=3)	Standard Deviation
0	180.5	178.5	171.6	175.5	4.5
30	175.7	188.4	181.4	182.5	4.92
60	171.9	181.4	172.2	171.6	1.52

Study No 118358

Significant in vitro biotransformation of AMG 416 (etelcalcetide) occurred in the blood. M10 (the GSH conjugate) was major biotransformation product formed in rats, whereas M11 (reduced form) was the major biotransformation product formed in humans. All products formed were the result of redox and/or disulfide exchange.

This study determined the biotransformation of [^{14}C] etelcalcetide in rat and human hepatocytes, liver S9 fractions and kidney S9 fractions and in whole blood from rat, healthy humans and chronic kidney disease patients. The study also determined the whole blood to plasma ratio in rat, healthy human and chronic kidney disease patient. The two ^{14}C-labeled etelcalcetide molecules (10 μM) were incubated in vitro with hepatocytes, liver S9 and kidney S9 fraction from human and rat. [^{14}C]Ac-etelcalcetide (0.1 – 10 μM) was also incubated in whole blood obtained from rat, healthy human and chronic kidney disease (CKD) patient on hemodialysis. ^{14}C in blood and plasma was analyzed by liquid scintillation counting. All kidney and liver incubations and the plasma from whole blood incubations were subjected to chemical reduction with TCEP. The supernatants from the in vitro incubations were analyzed by LC-^{14}C -HRMS. [^{14}C]Ac-etelcalcetide (200 μM) was incubated in rat and healthy human blood and the protein adduct(s) in plasma were analyzed by LC-HRMS directly or isolated by affinity gel chromatography followed by SDS-PAGE separation and trypsin digestion. The PAGE gel was stained with Coomassie blue and subjected to ^{14}C imaging. The trypsin digest was analyzed by LC-^{14}C -HRMS.

Blood to plasma ratios in rat, healthy humans, and patients with CKD indicate etelcalcetide-related components did not significantly partition into red blood cells and were preferentially retained in the plasma compartment. The biotransformation products observed in human hepatocytes, S9 fractions, and whole blood were also observed in rat. Biotransformation of etelcalcetide appears to be the result of redox and/or chemical exchange rather than via metabolism by conventional metabolizing enzymes such as cytochromes P450. The biotransformation profiles of the two single ^{14}C labels on either the acetyl or the alanine (next to D-Cys) were similar, indicating that there were no changes to the D-amino acid peptide backbone of etelcalcetide. In plasma obtained after in vitro incubations in whole blood, 53 to 69% of the ^{14}C was not extractable and found to be covalently bound to plasma protein(s). The covalent protein adducts were formed by disulfide conjugation of the etelcalcetide D-amino acid peptide to serum albumin. The serum albumin amino acid residue most readily modified was Cys34 but modification of five other cysteines was also detected. Overall, significant biotransformation of etelcalcetide occurred in vitro in the blood.

Reference ID: 3923456

Table 19 Pharmacokinetic Metabolism Study No 118358: AMG416 (Etelcalcetide) was Retained in the Plasma. The Biotransformation Products were Detected in Both Human and Rat via Redox and/or Disulfide Exchange (i.e. M10 and M11).

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Blood to plasma ratios following [^{14}C]Ac-AMG 416 incubation in rat, healthy human and CKD human whole blood for 4 hr at 37 °C.

Species	[^{14}C]Ac-AMG 416 (µM)	Mean Blood Concentration (B; ^{14}C ng-eq/g)	Mean Plasma Concentration (P; ^{14}C ng-eq/g)	B/P Ratio
Rat	0.1	6.5	12.6	0.51
	1.0	76.3	133.9	0.54
	10.0	575.1	1063.6	0.52
Human (Healthy)	0.1	7.1	14.3	0.50
	1.0	57.9	110.1	0.54
	10.0	584.1	1027.0	0.56
Human (CKD patient on Hemodialysis)	0.1	7.3	10.3	0.69
	1.0	69.9	102.8	0.66
	10.0	668.0	1003.8	0.64

Biotransformation Products of [^{14}C]AMG 416 (10 µM) in primary hepatocytes, liver S9 and Kidney S9 fractions.

Component	% Radioactivity*											
	[^{14}C]Ac-AMG 416						[^{14}C]Ala-AMG 416					
	Hepatocytes		Liver S9		Kidney S9		Hepatocytes		Liver S9		Kidney S9	
	Rat	Human	Rat	Human	Rat	Human	Rat	Human	Rat	Human	Rat	Human
AMG 416	12.9	35.1	16.3	72.7	75.4	45.3	11.1	28.9	16.4	56.1	72.5	52.3
M5	ND	1.44	ND	3.5	2.2	1.7	0.5	0.9	ND	2.5	2.0	2.1
M7	ND	ND	ND	ND	ND	ND	ND	ND	0.5	1.2	ND	ND
M9	ND	ND	ND	9.4	ND	3.8	ND	ND	1.2	5.0	ND	2.2
M10	68.9	30.3	74.0	ND	3.8	ND	76.8	46.3	71.0	ND	ND	ND
M11	ND	ND	ND	3.6	ND	20.7	ND	ND	ND	3.0	3.4	25.5
M12	15.8	26.1	1.2	ND	3.8	3.8	8.0	16.8	1.3	6.8	4.5	4.1
M13	ND	2.6	ND	ND	ND	ND	ND	2.7	ND	ND	ND	ND
M14	ND	0.5	3.2	6.6	ND	3.1	1.2	1.3	2.5	2.8	4.1	1.1
M15	ND	2.9	ND	ND	ND	ND	ND	1.9	ND	ND	ND	ND
M16	2.4	1.1	ND	ND	ND	ND	2.4	1.3	ND	ND	ND	ND
M22	ND	ND	ND	ND	ND	1.4	ND	ND	ND	1.8	ND	1.2
M23	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.1	ND	0.5
Other**	ND	ND	5.3	4.1	14.8	20.3	ND	ND	7.1	17.0	13.5	11.1

*%Radioactivity in the HPLC profile equals 100*CPM counts in the area under the region of interest (ROI)/ sum of CPM counts in all ROI.
**Other, the sum of all other peaks. In liver and kidney S9 fractions, highest component was the late eluting putative protein adducts (not characterized further). Up to 9 additional metabolites detected with each at <1% administered radioactivity
ND = Not Detected

Summary of [^{14}C]AMG 416 related components in plasma obtained following [^{14}C]Ac-AMG 416 (10 µM) incubation in whole blood for 4 hr at 37 °C.

A.	Rat	Healthy Human	CKD Patient
% ^{14}C-Extractable (in supernatants)	47.5	30.8	21.9
% ^{14}C- Non-Extractable (in protein pellet)	52.5	68.6	53.0

B.	Components in Extracted Radioactivity		
	Rat	Healthy Human	CKD Patient*
Components	% ^{14}C-profile**		
AMG 416	29.3	44.6	38.2
M1	6.3^	6.9^	8.3^
M5	0.9	1.6	2.8
M10	26.2	19.2	20.1
M12	2.0	4.7	2.5
M13	2.4^	13.0^	16.6^
M14	2.9	4.9	3.4
Protein Adduct(s)	30.2	5.2	7.9

* CKD patient, chronic kidney disease patient on hemodialysis
** % ^{14}C-profile = 100*CPM counts in the area under the region of interest (ROI)/ sum of CPM counts in all ROI. CPM, ^{14}C-counts per minute.
^Radiochromatogram peaks overlap, %radioactivity determined from MS signal ratio

Percent extractable radioactivity is determined from the radioactivity present in the injection vial as compared to the starting plasma radioactivity. Percent non-extractable is the total radioactivity from the extraction pellet and the pellet formed after samples are reconstituted as compared to the starting plasma.

Study No 118436

Renal elimination was the main clearance pathway for etelcalcetide in rats with intact kidney functions. Biotransformation of etelcalcetide was predominately via replacement of the L-cysteine moiety while the D-amino acid backbone of the molecule remained intact. Circulating etelcalcetide and its biotransformation products were retained in plasma (plasma protein bound via disulfide link to the D-amino acid peptide portion of etelcalcetide).

This study determined the mass balance and excretion pathways of etelcalcetide in male and female bile-duct cannulated (BDC) Sprague-Dawley (SD) rats and in bilaterally nephrectomized (BN) male SD rats, and determined the blood and plasma pharmacokinetics in normal and bilaterally nephrectomized rat. The study also characterized the biotransformation profiles in plasma and excreta; and determined the suitability of placing the radiolabel at either of two different sites in etelcalcetide for biotransformation and other ADME studies. Total radioactivity in sample matrices was determined by liquid scintillation counting either directly or following combustion. Radioactivity in plasma and urine were profiled with and without tris(2-carboxyethyl)phosphine) (TCEP) reduction by LC-MS-^{14}C-chromatography. Structural assignments for biotransformation products were based on high resolution mass spectrometric analysis. Plasma concentrations of etelcalcetide and total M11 (a measure of all etelcalcetide related components) were determined by LC-MS/MS assay.

Purpose and ^{14}C-AMG 416 dosing information in rat ADME studies.

Group ID	Experiment ID	Kidney status	^{14}C- Site	Lot	Gender (No. of animals)	Dose mg/kg (µCi)	Purpose
1	117266	Normal	Ac	098017-01	M (4)	1.84 (~25)	Mass balance, excreta met ID
2				098017-01	F (4)	1.84 (~25)	Mass balance, excreta met ID
3				098017-01	F (4)	1.84 (~25)	B/P, plasma met ID
4	117841			120358-48	M (10)	2.5 (~50)	B/P, plasma AMG 416 and M11 bioanalysis, and plasma met ID
5	117350	Normal	Ala	098022-03	M (4)	2.5 (~38)	Mass balance, excreta met ID
6				098022-03	M (4)	2.5 (~38)	plasma met ID
7				098022-03	M (2)	2.5 (~38)	B/P
8	117752	BN	Ac	120358-48	M (6)	2.5 (~50)	Mass balance, B/P, plasma AMG 416 and Total M11 bioanalysis, and plasma met ID
9	118479	Normal	Ac	120358-48	M (1)	2.5 (~50)	^{14}C in expired air
10			Ala	098022-03	M (1)	2.5 (~38)	^{14}C in expired air

Notations: M, male; F, female; B/P, blood-to-plasma concentration ratio; Normal, rat with intact kidneys; BN, bilaterally nephrectomized rat; Ac, [^{14}C]Ac-AMG 416; Ala, [^{14}C]Ala-AMG 416.

The profiles of etelcalcetide pharmacokinetics, excretion, and biotransformation were similar in male and female rats. Profiles were also similar when the radiolabel was located either in the acyl group of the N-terminal D-cysteine or in the D-alanine adjacent to the N-terminal D-cysteine, indicating that a radiolabel incorporated at either site would be suitable for characterization of etelcalcetide disposition. Renal clearance of intact drug (approximately 50%) or the metabolites was the primary route of elimination in rats with normal kidney function; overall elimination was minimal in bilaterally nephrectomized rats. Biotransformation was predominately via replacement of the L-cysteine moiety; the D-amino acid backbone of the molecule remained intact. Circulating radioactivity (parent compound and circulating biotransformation products) was preferentially retained in plasma. A substantial proportion of

the plasma radioactivity was covalently bound to plasma proteins via an apparent disulfide link to the D-amino acid peptide portion of etelcalcetide.

Table 20 Pharmacokinetic Metabolism Study No 118436: AMG416 (Etelcalcetide) was Eliminated via Renal Clearance in Rats with Intact Kidney Functions.

Excretion of Radioactivity After a Single IV Dose (1.84 mg/kg) Administration of [^{14}C]Ac-AMG 416 to Bile-Duct Cannulated Male Rats (Group 1).

Matrix	Time point (hr)	Recovery of Radioactivity (% of Administered Dose)					
		Subject G1001*	Subject G1002	Subject G1003	Subject G1004	Mean	Standard Deviation
Urine	0-8	35.7	76.3	61.8	65.5	67.9	7.54
	8-24	9.60	7.91	14.2	11.0	11.0	3.13
	24-48	4.12	2.97	4.61	2.98	3.52	0.94
	48-72	2.31	1.34	0.64	2.08	1.35	0.72
	Subtotal	51.8	88.5	81.2	81.6	83.8	4.13
Bile	0-4	0.11	0.14	0.80	0.27	0.40	0.35
	4-8	0.02	0.02	0.07	0.03	0.04	0.03
	12-24	0.02	0.02	0.04	0.04	0.03	0.01
	24-48	0.05	0.03	0.00	0.05	0.03	0.03
	48-72	0.06	0.03	0.00	0.03	0.02	0.02
	Subtotal	0.26	0.24	0.90	0.42	0.52	0.34
Feces	0-24	27.2	1.04	1.02	0.53	0.86	0.29
	24-48	1.81	0.87	0.10	0.17	0.38	0.43
	48-72	0.30	0.21	0.00	0.09	0.10	0.11
	Subtotal	29.3	2.12	1.12	0.79	1.34	0.69
Cage wash	0-72	2.14	2.03	2.13	2.24	2.13	0.11
All	Total	83.5	92.9	85.3	85.0	87.7	4.47

* Excluded from further analysis because feces were cross-contaminated with urine.

Excretion of Radioactivity After a Single IV Dose (1.84 mg/kg) Administration of [^{14}C]Ac-AMG 416 to Bile-Duct Cannulated Female Rats (Group 2).

Matrix	Time point (hr)	Recovery of Radioactivity (% of Administered Dose)					
		Subject G2005	Subject G2006	Subject G2007	Subject G2008	Mean	Standard Deviation
Urine	0-8	59.6	55.8	50.7	68.0	58.5	7.30
	8-24	7.11	14.8	18.4	13.9	13.6	4.71
	24-48	5.55	4.26	1.83	1.98	3.41	1.81
	48-72	0.99	1.48	0.97	1.20	1.16	0.24
	Subtotal	73.3	76.3	71.9	85.1	76.6	5.94
Bile	0-4	0.13	0.11	0.33	0.12	0.17	0.11
	4-8	0.02	0.04	0.01	0.02	0.02	0.01
	12-24	0.03	0.03	0.01	0.02	0.02	0.01
	24-48	0.04	0.02	0.01	0.02	0.02	0.01
	48-72	0.07	0.04	0.05	0.01	0.04	0.03
	Subtotal	0.29	0.24	0.41	0.19	0.28	0.09
Feces	0-24	5.98	1.63	0.25	0.00	1.97	2.77
	24-48	0.54	2.45	1.68	0.58	1.31	0.92
	48-72	0.54	1.14	0.47	0.32	0.62	0.36
	Subtotal	7.06	5.22	2.40	0.90	3.90	2.77
Cage wash	0-72	1.65	3.47	2.19	1.89	2.30	0.81
	Subtotal	1.65	3.47	2.19	1.89	2.30	0.81
All	Total	82.3	85.3	76.9	88.1	83.1	4.79

Excretion of Radioactivity After a Single IV Dose (2.5 mg/kg) Administration of [^{14}C]Ala-AMG 416 to Bile-Duct Cannulated Male Rats (Group 5).

Matrix	Time point (hr)	Recovery of Radioactivity (% of Administered Dose)					
		Subject G5005	Subject G5006	Subject G5007	Subject G5008	Mean	Standard Deviation
Urine	0-8	60.3	68.1	70.5	54.4	63.3	7.34
	8-24	11.8	8.97	8.70	7.76	9.30	1.74
	24-48	4.27	2.32	2.86	2.58	3.01	0.87
	48-72	1.77	1.75	2.01	1.24	1.69	0.32
	Subtotal	78.1	81.1	84.0	66.0	77.3	7.91
Bile	0-4	1.40	0.20	0.18	0.15	0.48	0.61
	4-8	0.08	0.03	0.02	0.02	0.04	0.03
	12-24	0.05	0.04	0.03	0.03	0.04	0.01
	24-48	0.02	0.05	0.05	0.04	0.04	0.01
	48-72	0.02	0.04	0.05	0.05	0.04	0.01
	Subtotal	1.57	0.36	0.33	0.29	0.64	0.62
Feces	0-24	3.90	3.10	1.32	0.47	2.20	1.58
	24-48	0.94	2.01	0.57	1.01	1.13	0.62
	48-72	0.58	0.47	0.33	0.47	0.46	0.10
	Subtotal	5.43	5.58	2.22	1.95	3.79	1.98
Cage wash	0-72	3.92	1.90	1.54	1.83	2.30	1.09
All	Total	89.0	89.0	88.1	70.1	84.1	9.32

Excretion of Radioactivity After a Single IV Dose (2.5 mg/kg) Administration of [^{14}C]Ac-AMG 416 to Bilaterally Nephrectomized Male Rats (Group 8).

Matrix	Time point (hr)	Recovery of Radioactivity (% of Administered Dose)							
		Subject G8001	Subject G8002	Subject G8003	Subject G8004	Subject G8005	Subject G8006	Mean	Standard Deviation
G.I. Tract	0-72	12.7	11.7	9.87	9.40	6.86	14.7	10.9	2.76
Feces	0-24	0.54	0.19	0.10	0.29	0.50	0.08	0.28	0.20
	24-48	1.21	0.73	1.44	0.73	1.21	0.21	0.92	0.45
	Subtotal	1.75	0.91	1.54	1.03	1.71	0.28	1.20	0.57
Cage wash	0-72	0.07	0.03	0.02	0.02	0.04	0.02	0.03	0.02
All	Total	14.6	12.7	11.4	10.5	8.61	15.0	12.1	2.45

Excretion Profile of Expired $^{14}CO_2$ After a Single IV Dose (2.5 mg/kg) Administration of [^{14}C]-AMG 416 to Male Rats (Groups 9 and 10).

Group	Label	Time point (hr)	Recovery of Radioactivity in Expired Air (% of Administered Dose)
9	[^{14}C]Ac-AMG 416	0-24	0.19
10	[^{14}C]Ala-AMG 416	0-24	0.03

Study No 119917

The in vitro biotransformation of AMG 416 (etelcalcetide) was not mediated by cytochrome P450 enzymes. Acidification to pH 3 resulted in increased AMG 416 stability in these liver subcellular fraction incubations because thiolate anion formation was substantially reduced.

This study examined the involvement of cytochrome P450 enzymes in the in vitro biotransformation of etelcalcetide. Etelcalcetide (5 µM) was incubated in human liver microsomes (HLMs) and human liver S9 subcellular fractions (HLS9s) with or without reduced β-nicotinamide adenine dinucleotide phosphate (NADPH) and with or without 0.5% or 1% formic acid at 37 °C. Additionally, HLMs and HLS9s were pre-incubated with 1-aminobenzotriazole (ABT; 5 mM) and NADPH cofactor to inactive cytochrome P450 enzymes prior to etelcalcetide addition. Midazolam (5 µM) was used as a positive control cytochrome P450 substrate and the midazolam hydroxylase activity was used to confirm successful P450 inactivation by ABT.

Etelcalcetide stability was similar in the presence and absence of NADPH in HLMs and HLS9 fractions. There was no effect of pre-incubation of HLMs or HLS9s with ABT and NADPH on

etelcalcetide stability. Acidification of HLM and HLS9 incubations with 0.5% formic acid completely inhibited etelcalcetide disappearance.

Table 21 Pharmacokinetic Metabolism Study No 119917: The Stability of AMG416 (Etelcalcetide) was Explained by the pH of the Incubations (i.e. Formic Acid). NADPH-Dependent Enzymes (i.e. CYP450s) Did Not Play a Role in AMG416 Metabolism.

% AMG 416 remaining at 60 min in pooled human liver microsomes and S9 fractions with and without NADPH, preincubation with ABT, or acidification with formic acid

Incubation Conditions	15 min preincubation	% AMG 416 remaining at 60 min[a]	
		HLM	HLS9
	Y/N	Mean (SDp)[b]	Mean (SDp)[b]
+NADPH (1 mM)	N	44.1 (4.2)	24.2 (1.2)
-NADPH	N	40.8 (0.4)	32.0 (1.8)
+ABT (5 mM) +NADPH	Y	46.1 (1.4)	18.4 (0.1)
-ABT+NADPH	Y	49.9 (5.3)	19.0 (1.1)
Formic acid (0.5%)	N	92.9 (0.9)	94.9 (0.2)
Formic acid (1%)	N	96.0 (0.1)	90.5 (0.4)

HLM, human liver microsomes; HS9, human liver S9 fraction

[a] $\% \, AMG \, 416 \, remaining \, at \, 60 \, min = \frac{PAR_{time=60}}{PAR_{time=0}} * 100$, where peak area ratio (PAR) = peak area of AMG 416/ peak area of AMG 416 IS

[b] SDp = standard deviation of the population, n = 2 measurements at each 0 and 60 min time point

4-Hydroxy midazolam (4-OH-MDZ) formation in pooled human liver microsomes and S9 fractions with and without NADPH or preincubation with ABT.

Incubation Conditions	15 min preincubation	Peak area ratio of 4-OH-MDZ/OH-MDZ IS at 60 min	
		HLM	HLS9
	Y/N	Mean (SDp)[a]	Mean (SDp)[a]
+NADPH(1 mM)	N	22.5 (0.7)	13 (0.05)
-NADPH	N	0.15 (0.14)	0 (0)
+ABT(5 mM)+NADPH	Y	0.13 (0.02)	0.12 (0.01)
-ABT+NADPH	Y	25.4 (2.0)	12.1 (ND)[b]

[a] SDp = standard deviation of the population, n = 2 measurements at each 0 and 60 min time point
[b] ND = not determined (n = 1)

HLM, human liver microsomes; HS9, human liver S9 fraction

Excretion Non-GLP Studies

Study No 4169-NC-159

Reduced forms of KAI-4169 (etelcalcetide) and KP-212 were not detected in urine or feces.

This study determined concentration of reduced forms of etelcalcetide and KP-212 in rat urine and fecal extracts. Urine samples were acidified and then chemically reduced with trichiorethyiphosphine (TCEP) for 30 minutes at room temperature. Fecal samples were reduced without further acidification. Upon reduction, all samples were centrifuged at 4 C to remove solids. Peptide compounds were recovered from the resulting supernatant. The internal standard, KP-2129, was added after extraction to avoid reduction of the compound. Peptide related compounds were then eluted from the resin and analyzed using LC-MS/MS.

Samples were analyzed by LC/MSMS for etelcalcetide, KP-2067 (reduced), KP-212 (deacetylated), and the reduced form of KP-212.

The only compound which was present in urine within the limit of detection was KP-2067 which was the chemically reduced form of etelcalcetide. The values of etelcalcetide were comparable to the concentration of KP-2067 in urine. Likewise, etelcalcetide, KP-2067, KP-212 and reduced KP-212 were not detected in the chemically reduced fecal homogenates. Together, these observations showed that etelcalcetide was not modified through disulfide conjugation or deacetylation during urinary or fecal excretion.

Study No y15ag004

AMG416 (Etelcalcetide) was detected in milk.

This study evaluated the excretion of [^{14}C]ONO-5163 ([^{14}C]etelcalcetide) into milk, upon single IV dose administration of 2.5 mg/kg [^{14}C]ONO-5163 was given to non-fasted lactating rats at 11 days post-delivery. Milk and plasma were collected at 1, 8, 24, 48 and 72 h post-dose, and the radioactivity concentrations were measured. The radioactivity concentration in milk was 275 ng eq./mL at 1 h post-dose and 450 ng eq./mL at 8 h post-dose, which were 0.2- and 3.3-fold that in plasma, respectively. The milk/plasma ratios of radioactivity concentration were almost constant after 24 h post-dose with values ranging from 1.1 to 1.4. The radioactivity concentration in milk decreased with time in a parallel manner with that in plasma.

Table 22 Pharmacokinetic Excretion Study No y15ag004: AMG416 (Etelcalcetide) did Transfer into Milk but was Labile.

Radioactivity concentrations in milk and plasma after single intravenous administration of 2.5 mg/kg [^{14}C]ONO-5163 to lactating rats

Time after administration (h)	Radioactivity concentration (ng eq./mL)		Milk/Plasma
	Plasma	Milk	
1	1330 ± 240	275 ± 311	0.20 ± 0.21
8	176 ± 99	450 ± 39	3.31 ± 2.20
24	20.7 ± 7.4	21.3 ± 8.3	1.06 ± 0.36
48	6.37 ± 1.92	8.67 ± 0.84	1.44 ± 0.41
72	4.19 ± 3.65[a]	7.80 ± 3.00	1.43[b]

Data are expressed as the mean values ± SD of three animals.
[a] The radioactivity concentration in plasma in one animal was not detected and taken to be zero.
[b] Mean of two animals since the radioactivity concentration in plasma was not detected in one animal

Drug Interaction GLP/Non-GLP Studies

Study No 4169-NC-124 (GLP)

AMG416 (Etelcalcetide) did not inhibit CYP450 activities in human liver microsomes.

The study was to determine the inhibitory potential of the, etelcalcetide, towards human cytochrome P450 (CYP) isoforms CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in human liver microsomes. The Test Item, etelcalcetide, was evaluated for its inhibitory potential towards the activities of cytochrome P450 (CYP) isoforms CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in human liver microsomes at concentrations of 0.2, 1 and 5 pg/mL. No apparent inhibition of the CYP isoforms was observed.

Study No 4169-NC-125 (GLP)

AMG416 (Etelcalcetide) did not induce CYP450 activities in human hepatocytes.

This study was performed to determine the induction potential of etelcalcetide towards human cytochrome P450 (CYP) isoforms CYP1A2, CYP2B6, and CYP3A4 using a well characterized cryopreserved primary human hepatocyte *in vitro* assay. Human hepatocytes from three donors were used for the study. Human hepatocytes from three donors were treated with etelcalcetide at concentrations of 0.2, 1 and 5 µg/mL (Day 1), and 0.4, 2 and 10 µg/mL (Day 2 and Day 3). Under the conditions of this study, etelcalcetide was not an inducer of P450 isoforms 1A2, 2B6, and 3A4 in cryopreserved primary human hepatocytes. There was also no evidence of an inhibitory or suppressive effect of KAI- 4169 on the P450 isoforms examined in this study. In addition, etelcalcetide did not demonstrate any hepatotoxic effects.

Study No 119314 (Non-GLP)

AMG416 (Etelcalcetide) was not CYP450 inhibitor.

This study evaluated etelcalcetide as an in vitro inhibitor of six major human hepatic cytochromes P450 isozymes. Etelcalcetide (5 µM) or solvent was incubated with human liver microsomes with and without 1 mM NADPH for 30 minutes prior to transfer of an aliquot for separate incubation with six cytochrome P450 (CYP) selective substrates. The metabolism of each probe substrate was measured by LC-MSMS and the effect of pre-incubation with etelcalcetide was compared to determine its time-dependent inhibition of the relevant CYP. Etelcalcetide was not a time-dependent inhibitor of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A in human liver microsomes.

Study No 119513 (Non-GLP)

AMG416 (Etelcalcetide) had no effect on human BSEP transport activity.

This study evaluated the effect of etelcalcetide on human BSEP transport activity. Etelcalcetide had no effect on human BSEP transport of a radiolabeled bile salt (^{3}H-taurocholate).

Other Non-GLP Studies

Study No 4169-NC-121

AMG416 (Etelcalcetide) was rapidly cleared through dialysis membranes and did not bind to the membranes.

This study characterized the potential for elimination of etelcalcetide during renal demodialysis. The clearance of etelcalcetide through typical dialyzers (polyarylethersulfone and polysulfone) was determined ex vivo and hemodialysis clearance benchmarked against urea and creatinine. The experiment was conducted with a closed loop system in which heparinized equine blood was circulated through a dialysis membrane using clinical dialysis machines at standard dialysis settings. Etelcalcetide was rapidly and effectively cleared through representative polysulfone and polyarylethersulfone dialysis membranes. The etelcalcetide clearance rate was similar to the clearance of urea and creatinine markers and was equivalent for both classes of dialysis membranes.

Reference ID: 3923456

Table 23 Pharmacokinetic Study No 4169-NC-121: KAI-4169 (Etelcalcetide) did not bind to dialysis membranes and was rapidly cleared through the membranes.

Clearance of KAI-4169 Through Representative Dialyzers (analyte concentration)

Fresenius Opiflux® F160, polysulfone				
Minutes after start of dialysis	**KAI-4169 ng/mL**	**Urea mg/dL**	**Creatinine mg/dL**	**Albumin g/dL**
0	605	308	28	2276
4	193	ND	ND	2479
4*	23	3	0.3	3.4
15	52	133	14	2601
30	18	86	9	2394
60	9	43	4	2084
120	0	4	1	1800
Gambro Polyflux® Revaclear, polyarylethersulfone				
Minutes after start of dialysis	**KAI-4169 ng/mL**	**Urea mg/dL**	**Creatinine mg/dL**	**Albumin g/dL**
0	718	320	35	2478
4	258	ND	ND	2366
4*	52	<2	0.2	2.8
15	87	99	10	2084
30	28	57	6	2121
60	11	36	4	2391
120	0	3	1	2538

ND - Value not determined. * Blood taken from the venous port of the dialyzer.

All analytes were quantified in blood sampled from the arterial port of the dialyzer. In order to calculate extraction efficiency and rate of clearance, KAI-4169 was also quantified in blood taken from the venous port of the dialyzer at 4 minutes.

Extraction Efficiency and Clearance Rate of KAI-4169 using Representative Dialysis Membranes

Fresenius Opiflux® F160, polysulfone		
	Exrtaction Efficiency (%)	Clearance (mL/minute)
KAI-4169	88	44
Urea	99	50
Creatinine	99	49
Gambro Polyflux® Revaclear, polyarylethersulfone		
	Exrtaction Efficiency (%)	Clearance (mL/minute)
KAI-4169	80	40
Urea	99	50
Creatinine	99	50

Extraction efficiencies (EE) were calculated using the following equation: $EE = (C_A - C_V)/C_A * 100$ where C_A is the concentration at the arterial side of the dialyzer at 4 minutes and C_v is the concentration at the venous side of the dialyzer at 4 minutes (Table 2). Clearance rate was calculated by multiplying the EE/100 by the blood flow rate (50 mL/min).

KAI-4169 Does Not Bind to Representative Dialysis Membranes

Fresenius Opiflux® F160, polysulfone				
Minutes before start of dialysis	**KAI-4169 ng/mL**	**Urea mg/dL**	**Creatinine mg/dL**	**Albumin g/dL**
18	560	319	30	3
15	665	308	28	3
0	605	308	25	3
Gambro Polyflux® Revaclear, polyarylethersulfone				
Minutes before start of dialysis	**KAI-4169**	**Urea**	**Creatinine**	**Albumin**
18	622	330	36	3
15	744	330	36	3
0	718	330	35	3

Dialysis session was initiated in bypass mode without ultrafiltration for 35 minutes (T = -35) to achieve equilibrium. KAI-4169, urea, and creatinine was added to the extracorporeal circuit at T = −20 minutes and samples were collected at T = −18 minutes, T = −15 minutes and just before starting dialysis (T = 0 minutes).

Study No 119413

Etelcalcetide was rapidly cleared by dialyzer with very little binding to dialysis apparatus.

This study evaluated the intrinsic dialysis clearance of [^{14}C]etelcalcetide in the absence of blood , dialysis clearance of [^{14}C]etelcalcetide related products immediately after spiking [^{14}C]etelcalcetide into whole blood; and dialysis clearance of [^{14}C]etelcalcetide related materials once the biotransformation in whole blood reached a steady state. Three experiments were conducted using an in vitro dialysis setup consisting of two peristaltic pumps connected via dialysis tubing to two reservoirs and a dialysis cartridge commonly used in clinical practice. Each pump separately moved blood or fluid being dialyzed from a blood side (reservoir A), and a dialysate side (reservoir B) in a counter current fashion through the cartridge. In Experiment 1, [^{14}C]etelcalcetide was dosed to dialysate solution in reservoir A and then immediately dialyzed. In Experiment 2, [^{14}C]etelcalcetide was dosed to bovine whole blood in reservoir A and then immediately dialyzed. Creatinine, urea, and vitamin B12 were also spiked as positive control analytes in reservoir A in experiments 1 and 2. In Experiment 3, [^{14}C]etelcalcetide was dosed to bovine whole blood and incubated at 37 °C for 3 hr prior to starting dialysis. Fluids from reservoirs A and B were sampled for analysis at various time points over 2 hr.

Intact etelcalcetide was readily dialyzed by a clinical dialyzer, and there was negligible non-specific binding to the dialysis apparatus. Etelcalcetide related components were readily removed by dialysis when the dialysis procedure was initiated immediately after etelcalcetide was dosed in bovine whole blood. Dialysis of etelcalcetide related materials was significantly attenuated when etelcalcetide was pre-incubated for 3 hours in bovine whole blood.

Study No 119414

AMG416 (Etelcalcetide) and serum albumin peptide conjugate (SAPC) underwent disulfide exchange in healthy human whole blood to form other disulfides.

This study determined the biotransformation kinetics following incubation of either etelcalcetide or serum albumin peptide conjugate (SAPC) in human whole blood. Etelcalcetide (5 μM) or SAPC (2.5 μM) was incubated in pooled whole blood from healthy human volunteers (N=3) at 37 °C for up to 6 hour. At various time points, aliquots of blood were sampled and then plasma was isolated, acidified with 1% formic acid, and stored at -80 °C. For each time point an aliquot of plasma was processed for bioanalysis of etelcalcetide, biotransformation products M10-M13, total M11, and SA21-41PC. The data were fit in two kinetic models to estimate the rate constants of conversion. One model estimated the rate constants for conjugation and deconjugation between etelcalcetide and SAPC, and the conjugation and deconjugation between etelcalcetide and all biotransformation products minus SAPC. The other model estimated the rate constants for conjugation and deconjugation between etelcalcetide to all biotransformation products including SAPC.

Etelcalcetide and SAPC readily underwent disulfide exchange with endogenous thiols in healthy human whole blood to form other disulfides. In incubations with etelcalcetide, most of the disulfide exchange had occurred by 3 hours at which time SAPC was the predominant product at approximately 60% of all etelcalcetide related products. In incubations with SAPC, etelcalcetide was the most abundant product. The kinetic model-derived estimated rate constant for the disulfide exchange that resulted in formation of SAPC from etelcalcetide was 18-fold faster than the reverse reaction. Similarly, the disulfide exchanges that resulted in the formation of the aggregate of all biotransformed products (excluding SAPC) from etelcalcetide were 2-fold faster than the reverse reactions. The conversion of etelcalcetide to form an aggregate of all biotransformed products including SAPC was 10-fold faster than the reverse conversion.

Reference ID: 3923456

Analyte blood concentrations following AMG 416 (5 µM) incubation in whole blood.

	Average Blood Concentration µM (SDp)								
Time (hr)	Total M11	AMG 416	M10	M11	M12	M13	(M10-M13[1])	SAPC	Total Sum[2]
0	4.901	4.513	0.178	0.148	0.053	0.025	0.404	0.015	0.418
	(0.092)	(0.219)	(0.032)	(0.004)	(0.003)	(0.001)	(0.033)	(0.031)	(0.064)
0.5	4.965	3.400	0.212	0.140	0.164	0.221	0.737	0.597	1.334
	(0.021)	(0.031)	(0.030)	(0.027)	(0.033)	(0.000)	(0.090)	(0.046)	(0.136)
1	4.722	2.300	0.190	0.157	0.161	0.354	0.861	1.277	2.138
	(0.142)	(0.232)	(0.006)	(0.021)	(0.040)	(0.017)	(0.007)	(0.100)	(0.092)
3	5.254	0.722	0.115	0.076	0.100	0.333	0.624	3.054	3.678
	(0.313)	(0.039)	(0.001)	(0.000)	(0.002)	(0.000)	(0.004)	(0.070)	(0.066)
3.5	5.090	0.710	0.132	0.097	0.109	0.349	0.687	2.924	3.612
	(0.319)	(0.084)	(0.006)	(0.000)	(0.006)	(0.007)	(0.007)	(0.148)	(0.141)
4	4.906	0.586	0.094	0.069	0.089	0.280	0.532	3.150	3.682
	(0.000)	(0.037)	(0.028)	(0.004)	(0.013)	(0.027)	(0.064)	(0.019)	(0.045)
5	4.925	0.507	0.086	0.074	0.084	0.259	0.503	3.009	3.513
	(0.166)	(0.011)	(0.001)	(0.000)	(0.003)	(0.012)	(0.010)	(0.181)	(0.171)
6	5.153	0.444	0.066	0.080	0.068	0.227	0.442	3.057	3.499
	(0.021)	(0.006)	(0.004)	(0.000)	(0.002)	(0.014)	(0.015)	(0.118)	(0.103)

SDp = standard deviation of the population
[1]sum of M10, M11, M12, and M13 concentrations
[2]sum of AMG 416, M10, M11, M12, M13, and SAPC concentrations

Analyte blood concentrations following SAPC (2.5 µM) incubation in whole blood.

	Average Blood Concentration µM (SDp)								
Time (hr)	Total M11	AMG 416	M10	M11	M12	M13	(M10-M13[1])	SAPC	Total Sum[2]
0	1.807	0.000[b]	0.000[b]	0.040[a]	0.040[a]	0.000[b]	0.069	2.132	2.201
	(0.181)	(0.000)	(0.000)	(0.002)	(0.000)	(0.000)	(0.002)	(0.030)	(0.028)
0.5	2.074	0.012[a]	0.024	0.042[a]	.041[a]	0.006[a]	0.125	2.302	2.427
	(0.086)	(0.003)	(0.004)	(0.001)	(0.001)	(0.001)	(0.010)	(0.133)	(0.143)
1	2.082	0.024[a]	0.010[a]	0.043[a]	0.041[a]	0.009[a]	0.126	2.395	2.521
	(0.031)	(0.002)	(0.003)	(0.000)	(0.000)	(0.001)	(0.005)	(0.070)	(0.065)
3	1.965	0.080	0.011[a]	0.046[a]	0.041[a]	0.038	0.219	2.202	2.421
	(0.168)	(0.003)	(0.001)	(0.000)	(0.001)	(0.003)	(0.007)	(0.093)	(0.086)
3.5	1.938	0.083	0.013[a]	0.050[a]	0.041[a]	0.046	0.232	1.899	2.130
	(0.040)	(0.002)	(0.001)	(0.001)	(0.000)	(0.004)	(0.005)	(0.181)	(0.177)
4	1.992	0.090	0.010[a]	0.048[a]	0.041[a]	0.045	0.235	1.936	2.171
	(0.019)	(0.002)	(0.002)	(0.003)	(0.001)	(0.008)	(0.016)	(0.070)	(0.054)
5	2.046	0.093	0.010[a]	0.044[a]	0.041[a]	0.049	0.237	1.973	2.210
	(0.041)	(0.002)	(0.002)	(0.003)	(0.001)	(0.006)	(0.010)	(0.056)	(0.046)
6	1.997	0.090	0.009[a]	0.050[a]	0.041[a]	0.050	0.241	2.002	2.243
	(0.054)	(0.004)	(0.000)	(0.001)	(0.000)	(0.002)	(0.000)	(0.167)	(0.166)

[a] estimated concentration, peak area ratio was below the lowest point on standard curve (explained in section 5.7.1)
[b] below limit of detection
NC = not calculated (n=1); SDp = standard deviation of the population
[1]sum of M10, M11, M12, and M13 concentrations
[2]sum of AMG 416, M10, M11, M12, M13, and SAPC concentrations

5.2 Toxicokinetics

TK was included in toxicity studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Single acute toxicity studies were not conducted with etelcalcetide.

6.2 Repeat-Dose Toxicity

Study No 119037: 6 month IV rat study (pivotal, Amgen)

Study title: AMG 416 (Etelcalcetide): Six Month Intravenous Toxicology Study in Sprague-Dawley rats with 28 Day Recovery

Study no.:	119037
Study report location:	Amgen (Thousand Oaks, CA)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 14, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lot 1044951 with 98.9% purity

Key Study Findings

- Etelcalcetide administered intravenously daily to rats for 6 months was tolerated at levels up to 3 mg/kg.
- Dose-dependent hypocalcemia associated with tremors and reduced general activities were considered dose-limiting.
- Etelcalcetide-related changes included salivation, increased vocalization, abnormal sounds at breathing, and/or uncoordinated gait at 3 mg/kg, reduced body weight at ≥ 1 mg/kg, and reduced food consumption at 3 mg/kg.
- Etelcalcetide-related microscopic changes were observed in the parathyroid gland, kidney and spleen.
- The no-observed-adverse-effect level (NOAEL) was considered to be 1 mg/kg based on adverse hypocalcemia at 3 mg/kg.

Methods

Doses:	0.3, 1, and 3 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Intravenous injection
Dose volume:	2 mL/kg
Formulation/Vehicle:	0.27% sodium succinate dibasic hexahydrate (2.7 g/L), 2% D-mannitol (20 g/L), 1% glycine (10 g/L), 1% trehalose dihydrate (10 g/L) and 0.9% (9 g/L) benzyl alcohol in sterile water for injection, pH 4.5 ± 0.1 (pH adjusted with 1N HCl and 1N NaOH, when needed)
Species/Strain:	SD rats
Number/Sex/Group:	15/sex/group
Age:	7.5 weeks old
Weight:	191-262 g for males and 159-206 g for females
Satellite groups:	TK (6/sex/group)
Deviation from study protocol:	No significant deviations were reported

Table 24 TOX Study No 119037: 6 Month Rat Study Design.

Experimental Design

Group No.	Test Material	Dose Level (mg/kg/dose)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals			
					Dosing Phase[a]		Recovery Study[a]	
					Males	Females	Males	Females
Dosing and Recovery Animals								
1	Control	0	2	0	15	15	5	5
2	AMG 416	0.3	2	0.15	15	15	0	0
3		1	2	0.5	15	15	0	0
4		3	2	1.5	15	15	5	5
Toxicokinetics Animals								
5	Control	0	2	0	6	6	0	0
6	AMG 416	0.3	2	0.15	6	6	0	0
7		1	2	0.5	6	6	0	0
8		3	2	1.5	6	6	0	0

[a] Dosing Phase animals were euthanized on Day 183. Recovery animals were euthanized on Day 211.

Observations and Results

Mortality (twice daily)

There was no etelcalcetide-related mortality.

Clinical Signs (once daily)

There were no etelcalcetide-related clinical signs noted at 0.3 mg/kg.

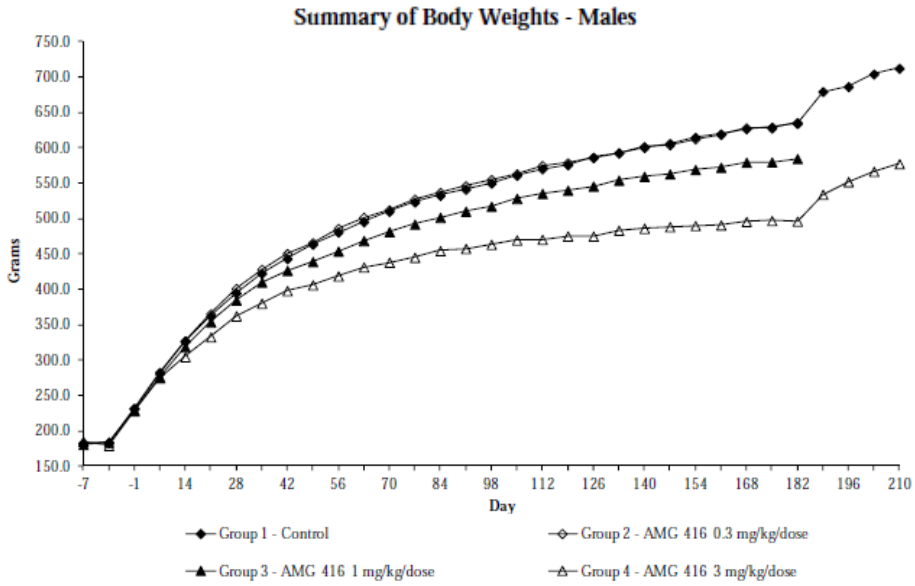
At 3 mg/kg, hypocalcemia correlated with transient slight to severe tremors post administration starting from Day 16 in males and Day 29 in females. In addition, salivation was observed from Day 32 onwards on almost every dosing occasion. From Day 62, clinical signs of decreased general activity were noted at 3 mg/kg, coupled with increased vocalization, abnormal sounds at breathing, and/or uncoordinated gait. At 1 mg/kg, decreased activity, tremors, increased vocalization, and/or salivation were occasionally noted for a few individuals. These clinical signs were observed within 1 to 2 hours post etelcalcetide administration then subsided by the following morning.

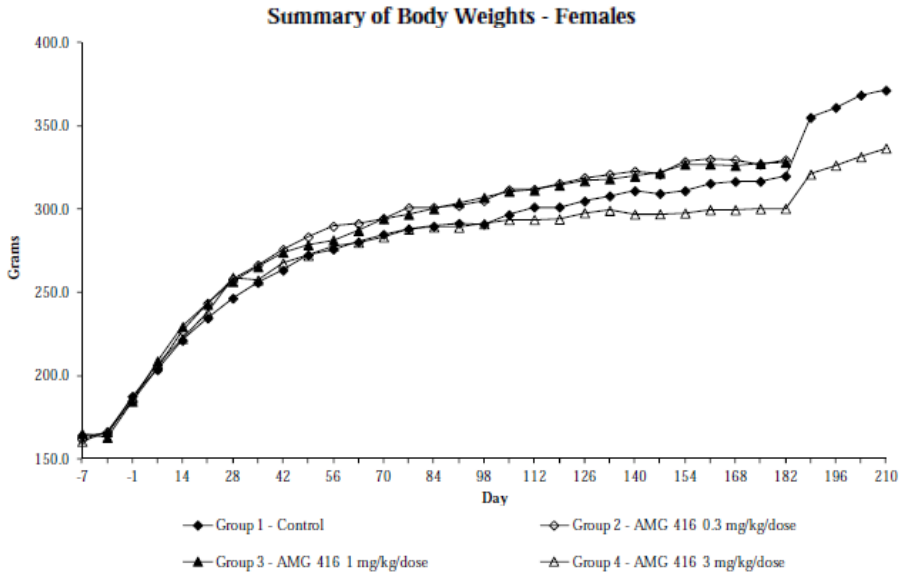
Body Weights (weekly)

Etelcalcetide at ≥ 1 mg/kg in males resulted in dose-dependent decreases in body weight (-8 and -22%, respectively), and body weight gain (-11 and -33%, respectively), compared to

controls. For high dose females, etelcalcetide caused a decrease in body weight at the end of the dosing phase (-6% compared to controls).

Figure 9 TOX Study No 119037: AMG416 (Etelcalcetide) Caused Decreases in Body Weight and Body Weight Gain in Males and Females.





Feed Consumption (weekly)
Food consumption was reduced in 3 mg/kg males correlating with the etelcalcetide-related reduced body weight.

Ophthalmoscopy (prestudy and the last week of dosing)
There were no etelcalcetide-related changes in ophthalmology at any dose level tested.

ECG

n/a

Clinical Pathology (Hematology – Days 183 and 211, Clinical Chemistry – Day 1, Week 25 – Ca and P only and Days 183 and 211, Urinalysis – Days 183 and 211)

There were no etelcalcetide-related changes in coagulation and urinalysis parameters at any dose level tested.

Dose-dependent decreases in serum calcium and increased phosphorus levels at ≥ 0.3 mg/kg were consistent with the expected pharmacology of etelcalcetide. By the end of the recovery period calcium and phosphorus changes were similar to those in the control group, except that phosphorus was minimally decreased in females.

Other etelcalcetide-related clinical pathology changes included increased alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, and/or albumin and decreased globulins, potassium and/or chloride. These changes were mostly present at the highest dose, affected males more than females, and had no histologic correlates. These changes were also reversible by the end of the recovery phase. Mild increases in red blood cell parameters and/or neutrophils, and minimal decrease in lymphocytes were also noted.

Table 25 TOX Study No 119037: AMG416 (Etelcalcetide) Caused Changes in Hematology and Clinical Chemistry Parameters in Rats.

AMG 416-Hematology Changes [a]

Sex:	Male			Female		
Dose (mg/kg):	0.3	1	3	0.3	1	3
RBC Day 183	-	-	1.08*	-	-	-
HGB Day 183	-	-	1.08*	-	-	-
HCT Day 183	-	-	1.08*	-	-	-
NEUT Day 183	-	-	1.63*	-	1.34	1.43
LYMPH Day 183	-	-	0.77	-	-	-

A dash (-) indicates absence of change in group; * = P ≤ 0.05.
[a] Change of mean hematology in groups administered AMG 416 compared to control group means.
RBC = red blood cell count, HGB = hemoglobin, HCT = hematocrit, NEUT = neutrophil count, LYMPH = lymphocyte count.

AMG 416-Clinical Chemistry Changes [a]

Sex:	Male			Female		
Dose (mg/kg):	**0.3**	**1**	**3**	**0.3**	**1**	**3**
CA Day1	0.95*	0.87*	0.74*	-	0.92*	0.75*
CA Day 170	0.93	0.90*	0.75*	0.95	0.91*	0.81*
CA Day 183	-	-	0.89*	-	0.95*	0.94*
PHOS Day1	-	1.19*	1.40*	-	1.21*	1.37*
PHOS Day 170	1.32*	1.28*	1.41*	1.24*	1.37*	1.65*
PHOS Day 183	1.21*	1.10[b]	1.36*	-	-	1.30*
AST Day 183	-	1.17	1.40	-	-	-
ALT Day 183	-	1.40*	1.55*	-	-	-
ALP Day 183	-	-	1.27	-	-	1.47*
UREAN Day 183	-	1.22*	1.25*	-	1.21*	1.20*
CREAT Day 183	-	1.12	1.14*	1.15*	1.15*	1.17*
ALB Day 183	-	-	1.06*	-	-	-
GLOB Day 183	-	-	0.93*	-	-	-
A/G Day 183	-	-	1.14*	-	-	-
K Day 183	-	-	0.90*	-	-	-
CL Day 183	-	-	0.97*	-	-	0.96*

A dash (-) indicates absence of change in group; * = P ≤ 0.05.
[a] Change of mean clinical chemistry in groups administered AMG 416 compared to control group means.
[b] Value calculated after exclusion of Nos. 3003,3006, 3010 considered outliers.
CA = calcium, PHOS = phosphorus, ALT = alanine aminotransferase, AST = aspartate aminotransferase, ALP = alkaline phosphatase, UREAN = blood urea nitrogen, CREAT = creatinine, ALB = albumin, GLOB = globulin, A/G = albumin/globulin, K = potassium, CL = chloride.

Gross Pathology (scheduled termination)
There were no etelcalcetide related macroscopic findings at 0.3 mg/kg. At ≥ 1 mg/kg, etelcalcetide-related macroscopic observations were observed in the stomach of males. In the stomach, there was dark focus in 1 of 15 males at 1 mg/kg and in 4 of 14 males at 3 mg/kg and depressed focus in 3 of 15 males at 1 mg/kg and in 2 of 14 males at 3 mg/kg.

Organ Weights (scheduled termination)
At 3 mg/kg, there was a decrease in spleen weight, which was considered at least in part secondary to decreased terminal body weight.

Table 26 TOX Study No 119037: AMG416 (Etelcalcetide) Decreased Spleen Weight in Rats.

AMG 416-related Organ Weight Changes - Percent Difference from Controls - Dosing Phase (Day 183)

Sex:	Male			Female		
Dose (mg/kg):	0.3	1	3	0.3	1	3
No. Examined:	14	15	14	15	14	15
Spleen						
Absolute	-2	-13*	-34*	-1	2	-16*
Relative (% body wt)	-4	-7	-15*	-8	-5	-14*
Relative (% brain wt)	-1	-12	-31*	-3	-1	-15*

* = P ≤ 0.05.
Note: The numerical values in the table represent the respective percent increase [+] or percent decrease [-] from control mean value [(treated group mean - control group mean) ÷ control group mean] × 100.

Histopathology

Adequate Battery
Yes

Peer Review
Yes

Histological Findings
etelcalcetide-related microscopic changes included non-dose related increases in renal tubular epithelial cell pigment at all dose levels, increased cytoplasmic eosinophilia of the parathyroid gland at ≥ 1 mg/kg, secondary microscopic changes were noted in the stomach (erosion) at ≥ 1 mg/kg (attributed to secondary to stress related to decreased body weight/body weight gain and hypocalcemia), vascular/perivascular inflammation at the administration site (attributed to tail vein injection procedure). All etelcalcetide-related changes were partially (parathyroid gland and kidney) or completely (spleen and stomach) reversible after a 28-day recovery period.

Table 27 TOX Study No 119037: Microscopic Findings.

Incidence and Severity of AMG 416-related Microscopic Changes - Dosing Phase (Day 183)

Sex:	Male				Female			
Dose (mg/kg):	0	0.3	1	3	0	0.3	1	3
No. Examined:	15	14	15	14	15	15	14	15
Parathyroid Gland								
Increased cytoplasmic eosinophilia								
minimal	0	0	10	9	0	0	9	10
Kidney								
Pigment accumulation, tubular								
minimal	0	4	7	6	0	6	7	5
mild	0	0	0	1	0	0	0	1
Administration site								
Inflammation, vascular/perivascular								
minimal	1	2	4	3	1	0	2	4
mild	0	0	0	0	0	0	1	0
Stomach								
Erosion								
minimal	0	0	1	2	0	-	0	2
mild	0	0	0	1	0	-	0	0

A dash (-) indicates absence of change in a group. NA = Not applicable.

Incidence and Severity of AMG 416-related Microscopic Changes - Recovery Phase (Day 211)

Sex:	Male		Female	
Dose (mg/kg):	0	3	0	3
No. Examined:	5	5	5	5
Parathyroid Gland				
Increased cytoplasmic eosinophilia				
minimal	0	2	0	2
Kidney				
Pigment accumulation, tubular				
minimal	0	2	0	0

Toxicokinetics

(Days 183 and 211)

Exposures were similar (within 2-fold) between sexes at each dose level. On Study Day 182, the exposure to etelcalcetide (AUC_{all}) increased approximately 3.4 fold for a 3.3-fold increase in dose (0.3 to 1 mg/kg) and approximately 2.7-fold increase for a further 3-fold increase in dose (1 to 3 mg/kg).

Table 28 TOX Study No 119037: TK Parameters.

Toxicokinetic Parameter Estimate of AMG 416 - Combined Sexes

	Study Day 1		Study Day 182	
Dose (mg/kg)	C_{max} (ng/mL)	AUC_{all} (ng·hr/mL)	C_{max} (ng/mL)	AUC_{all} (ng·hr/mL)
0.3	405	510	769	992
1	1330	1760	2540	3440
3	3760	5030	6840	9300

Dosing Solution Analysis

The dose formulations were within specification. Homogeneity testing showed that the formulation technique used produced homogeneous preparations.

The safety profile of etelcalcetide demonstrated in rats in this pivotal (6 month) chronic study was comparable to that of the original 3/6-month chronic study (Study No 4169-NC-133 conducted when KAI was the Sponsor). Both studies showed hypocalcemia-associated adverse effects (clinical signs, changes in body weight and food consumption) and the plasma exposures and the NOAEL (1 mg/kg) were similar between these two studies. In this study, intracytoplasmic golden-brown pigment in renal proximal tubules, and an increase in cytoplasmic eosinophilia of the parathyroid gland was noted.

Study No 4169-NC-133: 3/6 month IV rat study (conducted by KAI)

Study title: A Combination 3- and 6-Month Intravenous Toxicity and Toxicokinetic Study of KAI-4169 (Etelcalcetide) in Sprague-Dawley Rats, with Respective 2- and 4-Week Recovery Periods (GLP; testing facility: (b) (4)

Study description:
This study evaluated the potential toxicity and toxicokinetics of etelcalcetide when administered daily by intravenous (IV) bolus injection to Sprague-Dawley rats for 91 or 182 consecutive days in 2.0 mL/kg/day. The reversibility and potential delayed effects during a 2- or 4-week recovery period, respectively, were assessed. Etelcalcetide or the vehicle/control article (consisting of 0.27% sodium succinate dibasic hexahydrate, 2% D-mannitol, 1% glycine, 0.9% benzyl alcohol, and 1% trehalose in sterile water for injection). Toxicity parameters: cageside, unscheduled post-dose and detailed observations; body weights and body weight changes; food consumption; clinical pathology (clinical chemistry, hematology, coagulation, and urinalysis) parameters; ophthalmology; gross pathology; absolute and relative organ weights; and microscopic pathology. Blood (plasma) samples were collected for TK evaluation at 5 time points each on Days 1, 91 and 182 from TK animals. Blood samples were also collected for measurement of serum calcium and phosphorus evaluation prior to dosing and at 8 hours post-dosing on Days 1, 45, 91 and 182. All animals assigned the interim, terminal, and interim recovery and terminal recovery subgroups were euthanized and necropsied following final blood collection on Day 92, Day 106, Day 183 and Day 211, respectively.

Table 29 TOX Study No 4169-NC-133: 3/6 Month Rat Study Design.

Group	Treatment	Dose Level (mg/kg/day)	Concentration (mg/mL)	Number of animals/sex				
				IS	IR	TS	TR	TK
1	Buffer Solution	0	0	10	5	10	5	0
2	KAI-4169	0.3	0.15	10	0	10	0	9
3	KAI-4169	1.0	0.5	10	0	10	0	9
4	KAI-4169	3.0	1.5	10	5	10	5	9

IS – Interim-sacrifice animals (sacrificed on Day 92); IR – Interim recovery-sacrifice animals (sacrificed on Day 106); TS – Terminal-sacrifice animals (sacrificed on Day 183); TR – Terminal recovery-sacrifice animals (sacrificed on Day 211); TK – Toxicokinetic animals

Study findings:
- Repeated intravenous bolus injection of etelcalcetide daily at doses of 0.3 or 1.0 mg/kg/day for 182 consecutive days was well tolerated by male and female Sprague-Dawley rats.
- There was no mortality and no test article-related effects on ocular condition or coagulation parameters in main study rats. One male TK animal given 3.0 mg/kg/day

Reference ID: 3923456

was found dead on Day 167, most likely due to hypocalcemia resulting from an exaggerated pharmacologic effect of the test article.

- At 0.3 mg/kg/day, the only etelcalcetide related finding was expected pharmacology related changes in serum calcium (decreased) and phosphorus (increased).
- At 1.0 mg/kg/day, etelcalcetide related findings included changes in serum calcium and phosphorus, mild lymphoid necrosis in the thymus of a few animals, and a minimal decrease in the size of the splenic red pulp (correlating with decreased spleen weight). The latter two changes were likely secondary to stress associated with hypocalcemia.
- At 3.0 mg/kg/day, etelcalcetide related findings included mild and transient clinical sign (tremors), lower body weights and body weight gains (correlating with reduced food consumption in males), expected pharmacology-related changes in serum calcium and phosphorus, minor changes in a few other clinical chemistry parameters, decreased thymus weights (correlating with atrophy and lymphoid necrosis), decreased spleen weight (correlating with decreased size of the splenic red pulp), dark red discoloration of the gastric mucosa (correlating with minimal or mild erosions), and an increase in incidence and severity of follicular cysts in the ovary. All the above changes were completely or partially recovered after a 2-week or 4-week recovery period.
- The degree of the hypocalcemia in the group treated with 3.0 mg/kg/day etelcalcetide was considered moderately adverse, based on the magnitude of the effect and its associated transient clinical signs and other evidence of stress in the animals. The erosion in the stomach of individual animals given 3.0 mg/kg/day was also considered moderately adverse.
- The TK analysis revealed generally dose-related systemic exposure to etelcalcetide, with slightly greater exposure in females vs. males (≤ 2.0-fold differences) on Day 1, but no clear evidence of a gender difference on Days 91 and 182, and with a similar moderate accumulation of etelcalcetide in the blood compartment on Days 91 and 182, relative to Day 1.
- The NOAEL was 1.0 mg/kg/day, which was associated with the AUC_{all} values of 2,100 and 1,460 h ng/mL for males and females on Day 1, respectively, and 3550 h ng/mL for the gender-combined average from Days 91 to 182.

Table 30 TOX Study No 4169-NC-133: Microscopic Findings.

Incidence[a] of Selected Microscopic Findings

Interim Sacrifice (Day 92)

Sex:	Males				Females			
Group:	1	2	3	4	1	2	3	4
Treatment:	Vehicle[b]	KAI-4169			Vehicle[b]	KAI-4169		
Treatment (mg/kg/day):	0	0.3	1.0	3.0	0	0.3	1.0	3.0
Thymus								
Lymphoid necrosis								
minimal	-	-	-	-	-	1	-	1
mild	-	1	3	2	1	2	1 2	
moderate	-	-	-	2	1	-	3	1
Atrophy								
minimal	-	-	-	-	-	-	-	2
moderate	1	-	-	-	-	-	-	1
Spleen								
Decreased size (red pulp)								
minimal	-	1	1	4	1	NE	NE	1
mild	-	-	-	1	1	NE	NE	1
moderate	-	-	-	2	-	NE	NE	-
Stomach (glandular)								
Erosion (glandular stomach)								
minimal	-	NE	NE	-	-	-	-	1
mild	-	NE	NE	-	-	-	1	1
Hemorrhage (glandular stomach)								
minimal	-	NE	NE	-	1	-	1	2
Edema (glandular stomach)								
mild	-	NE	NE	-	-	-	1	1
Neutrophil infiltrate (glandular and/or non-glandular stomach)								
minimal	-	NE	NE	-	1	-	-	2
mild	1	NE	NE	-	1	-	2	-
Ovary								
Follicular cysts								
minimal	NA	NA	NA	NA	2	2	1 5	
mild	NA	NA	NA	NA	-	-	-	1
moderate	NA	NA	NA	NA	-	-	-	1

[a] Expressed as the number of animals with findings in each group (10 animals/sex/group)
[b] Buffer solution
"-" = not present; "NE" = not examined; "NA" = not applicable

Table 31 TOX Study No 4169-NC-133: TK Parameters.

Day	Group	Dose (mg/kg)	Gender	C_{max} (ng/mL)	SE C_{max} (ng/mL)	AUC_{all} (h*ng/mL)	SE AUC_{all} (h*ng/mL)	AUC_{INFobs} (h*ng/mL)	Cl_{obs} (mL/h/kg)	V_{SSobs} (mL/kg)	$HL_{\lambda z}$ (h)
1	2	0.3	F	468	50.6	598	54.4	604	497	324	1.57
			M	354	27.9	433	32.8	436	689	361	1.55
			F + M	411	36.3	515	45.8	520	577	346	1.57
	3	1.0	F	1660	180	2100	190	2120	472	304	1.66
			M	1200	89.9	1460	106	1470	681	346	1.62
			F + M	1430	137	1780	169	1790	557	328	1.64
	4	3.0	F	5490	382	6830	172	6920	434	287	1.84
			M	3560	237	4510	293	4570	657	453	1.78
			F + M	4520	476	5670	521	5740	522	351	1.81
91	2	0.3	F	810	32.2	999	28.8	1010	298	166	1.58
			M	753	77.0	933	68.2	941	319	183	1.58
			F + M	781	39.5	966	36.0	974	308	174	1.58
	3	1.0	F	2910	174	3520	158	3550	282	149	1.69
			M	3040	202	3670	180	3690	271	138	1.62
			F + M	2980	122	3590	112	3620	276	144	1.66
	4	3.0	F	8250	439	10200	346	10400	289	191	1.84
			M	8600	215	10700	84.2	10800	279	164	1.61
			F + M	8420	232	10500	185	10600	284	177	1.72
182	2	0.3	F	739	48.6	915	56.3	926	324	207	1.80
			M	925	80.1	1150	111	1160	258	160	1.74
			F + M	832	59.2	1030	74.9	1040	288	181	1.76
	3	1.0	F	2670	351	3360	333	3400	294	203	1.82
			M	3200	175	3950	164	3990	251	148	1.69
			F + M	2940	213	3650	209	3700	271	172	1.76
	4	3.0	F	7140	407	8710	568	8820	340	213	1.92
			M	9040	145	11200	71.7	11400	264	170	1.78
			F + M	7900	516	9730	677	9850	305	193	1.85

Study No 4169-NC-107: 4 week IV rat study

Study title: A 28-Day Repeat-Dose Intravenous Toxicity and Toxicokinetic Study of KAI-4169 (Etelcalcetide) in Sprague-Dawley Rats with a 28-Day Recovery Period (GLP; testing facility: (b) (4)

Study description:
Etelcalcetide or the vehicle (0.27% sodium succinate dibasic hexahydrate, 2% D-mannitol, 1% glycine and 1% D-(+)-trehalose dihydrate in sterile water for injection) was administered once daily via IV bolus injection at a dose volume of 2.0 mL/kg/day. Toxicity parameters: cageside, unscheduled post-dose and detailed observations; body weights and body weight changes; food consumption; clinical pathology (clinical chemistry, hematology, coagulation, and urinalysis) parameters; ophthalmology; gross pathology; absolute and relative organ weights; and microscopic pathology. Blood plasma samples were collected for parathyroid hormone (PTH) and TK evaluations at 5 and 4 time points, respectively, on Days 1 and 28 for test article-treated groups. Blood samples were also collected for calcium and phosphorus evaluation at 8 hours post-dosing on Days 1 and 28. All terminal and recovery animals were sacrificed and necropsied following the final blood collection on Day 29 and Day 57, respectively.

Group	Treatment	Dose Level (mg/kg/day)	Concentration (mg/mL)	Number of animals		
				TS	RS	TK
1	Buffer Solution	0	0	10	5	0
2	KAI-4169	0.3	0.15	10	0	6
3	KAI-4169	1.0	0.5	10	0	6
4	KAI-4169	3.0	1.5	10	5	6

TS – Terminal-sacrifice animals (Day 29); RS – Terminal recovery-sacrifice animals (Day 57); TK – Toxicokinetic animals

Study findings:

- Repeated intravenous bolus injection of etelcalcetide daily at doses up to 1.0 mg/kg/day for 28 consecutive days in male and female Sprague-Dawley rats was well tolerated.
- A comparable dose-related PTH reduction was observed in rats that were dosed with etelcalcetide once or for 28 days.
- Liver had the highest concentration of etelcalcetide (KP-2067), followed by the kidney. The concentrations of etelcalcetide (KP-2067) in liver and kidney were dose-dependent. Brain contained very low or non-quantifiable levels of etelcalcetide. There was minimal accumulation of etelcalcetide in liver, kidney or brain with daily IV administration for 28 days at doses of 0.3 and 3.0 mg/kg/day.
- The TK parameters showed generally dose-related systemic exposure to etelcalcetide (based on plasma concentrations), with slightly greater exposure in females vs. males (≤ 2.0-fold differences), and slight accumulation of etelcalcetide in the blood compartment with daily dosing.
- Bolus injection of etelcalcetide at 3.0 mg/kg/day had a marked decrease in serum calcium and increase in serum phosphorous and associated clinical signs (tremors and prostration). There were no microscopic changes that were considered clearly test article-related. However, the magnitude of the hypocalcemia in the group treated with 3.0 mg/kg/day was considered adverse.
- The NOAEL (no-observed adverse effect level) is considered to be 1.0 mg/kg/day.

Study No 2082-NC-100: 7 day IV rat study

Study title: KP-2082 (Etelcalcetide): An Exploratory 7-Day Intravenous Repeat-Dose Study of KP-2082 in Sprague-Dawley Rats (non-GLP; testing facility: (b) (4)

Study description:
This study evaluated the toxicity and toxicokinetic profile of KP-2082 (etelcalcetide) when administered by intravenous bolus injection to rats for 7 consecutive days. Toxicity parameters: mortality, clinical observations, body weight and food consumption, hematology and clinical chemistry at multiple intervals. Blood samples were taken for analysis of toxicokinetics (plasma concentration of etelcalcetide), plasma histamine concentration (sponsor did not give an explanation why this was included but perhaps this was to evaluate general health check on immune system and stress level), and plasma parathyroid hormone (PTH) concentration. Tissues samples were also collected from TK/histamine animals on Day 7, after their last blood sampling, for possible test article concentration analyses. On Day 8, the Main Study animals were euthanized and were subjected to a macroscopic examination, and selected tissues were examined by light microscopy. Vehicle control was 0.9% sodium chloride for injection.

Treatment Group	KP-2082 Dose Level (mg/kg/day)	Dose Conc. (mg/mL)	Dose Volume (mL/kg)	Number of Animals			
				Main Study		TK/Histamine	
				M	F	M	F
1. Control*	0	0	2.0	4	4	3	3
2. Low Dose	0.5	0.25		4	4	3	3
3. Mid Dose	2	1.0		4	4	3	3
4. High Dose	5	2.5		4	4	3	3

*Group 1 animals received the control/vehicle article alone.
M: male; F: female; TK: toxicokinetics

Study findings:
- Intravenous bolus injection of etelcalcetide to rats for 7 consecutive days at dose levels of 0.5 and 2 mg/kg/day was well tolerated.

- The expected dose-related decreases in plasma PTH and serum calcium were pronounced and cumulative in high -dose (5 mg/kg/day) animals. This pharmacologic effect was also accompanied by increases in serum phosphorus (also expected with calcimimetic agents).
- Apart from the expected changes in serum calcium and phosphorus, the only effect of etelcalcetide on the mid-dose animals (2 mg/kg/day) was a minor reduction in body weight gain.
- In high-dose animals, the magnitude of the hypocalcemia (approximately 40% reduction, relative to concurrent control values) was sufficient to cause substantial adverse sequelae. This might be responsible for many of the changes observed in high-dose animals during the study, including the death of one animal on Day 7.
- Changes were limited to high-dose animals. Reductions in spleen and thymus weight were observed in these animals. Microscopic changes were noted in the thymus and stomach (i.e., mild to moderate lymphoid atrophy/depletion in the thymus and minimal focal/multifocal erosion with neutrophils or mixed cell infiltration in the stomach). The nature of the lesion in the stomach was uncertain, but the thymic lymphoid atrophy and reduction in spleen weight (without a histologic correlate) were secondary to stress in the high-dose animals.

Study No 119036: 6 month IV dog study (pivotal)

Study title: AMG 416 (Etelcalcetide): Six Month Intravenous Toxicology Study in a Beagle Dog with 28 Day Recovery

Study no.:	119036
Study report location:	Amgen (Thousand Oaks, CA)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 14, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lot 1044951 with 98.9% purity

Key Study Findings

- Beagle dogs were administrated etelcalcetide by IV bolus injection at dose levels of 0.2, 0.5 and 0.9 mg/kg every other day for 6 months followed by a 1-month recovery period.
- Etelcalcetide-related findings were:
  - Clinical findings (i.e. emesis, reduced appetite, and salivation) at ≥0.5 mg/kg.
  - Reduced body weight gains and associated food consumption at ≥0.2 mg/kg.
  - An increase in heart rate corrected QT (QTc) interval at ≥ 0.2 mg/kg in females and at ≥0.5 mg/kg in males.
  - Mildly decreased reticulocytes for females at ≥ 0.5 mg/kg.
  - Minimally to markedly decreased calcium for males and females at ≥ 0.2 mg/kg, and minimally to mildly increased phosphorus for females at ≥ 0.5 mg/kg and males at ≥ 0.2 mg/kg.
  - Minimal to mild intracytoplasmic golden-brown pigment in the kidney proximal tubules at ≥ 0.2 mg/kg
  - All of which showed recovery during the 4-week recovery period, except for the microscopic changes in the kidney.
- Based on marked decreases in serum calcium and associated tremors that occurred at 0.9 mg/kg, the NOAEL was 0.5 mg/kg, corresponding to steady state C_{max} and AUC_{all} values of 1,230 ng/mL and 1,490 ng hr/mL, respectively.

Reference ID: 3923456

Methods

Doses:	0, 0.2, 0.5 and 0.9 mg/kg
Frequency of dosing:	Every other day
Route of administration:	Intravenous injection
Dose volume:	0.5 mL/kg
Formulation/Vehicle:	0.27% Sodium succinate dibasic hexahydrate (2.7 g/L), 2% D-mannitol (20 g/L), 1% glycine (10 g/L), 1% trehalose dihydrate (10 g/L) and 0.9% (9 g/L) benzyl alcohol in sterile water for injection, pH 4.5 ± 0.1 (pH adjusted with 1N NaOH and/or 1N HCL as needed)
Species/Strain:	Beagle dogs
Number/Sex/Group:	4-6/sex/group
Age:	6 months old
Weight:	7.8-9.2 kg for the males and 6.4-7.6 kg for the females
Deviation from study protocol:	No significant deviations were reported.

Table 32 TOX Study No 119036: 6 Month Dog Study Design.

Experimental Design

Group No.	Test Material	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals[a]	
					Males	Females
1	Control Article	0	0.5	0	6	6
2	AMG 416	0.2	0.5	0.4	4	4
3	AMG 416	0.5	0.5	1.0	4	4
4	AMG 416	0.9	0.5	1.8	6	6

[a] There was a total of 4 animals per sex in Groups 1 and 4 main study cohort and 2 animals per sex in the recovery cohort. For Groups 2 and 3, there were 4 animals per sex in the main study cohort only.

Observations and Results

Mortality (twice daily)

All animals survived to their scheduled study termination.

Clinical Signs (once daily)

At 0.5 and 0.9 mg/kg, etelcalcetide-related clinical findings included emesis, reduced appetite, and salivation. All showed full recovery during the 4-week recovery period. At 0.9 mg/kg, one high dose male animal was observed with severe tremors on Day 60; related to the marked decreases in serum calcium that occurred at this dose level.

Body Weights (weekly)

Group average body weights were reduced slightly within the first week of dosing at ≥ 0.5 mg/kg. These decreases persisted in a generally dose-dependent manner for the first four weeks of dosing, followed by similar body weight changes across all etelcalcetide groups compared to controls throughout the remainder of the dosing period.

Body weight gain was decreased overall during the dosing period at ≥0.2 mg/kg, in both males and females and was also generally dose dependent. Body weights increased during the 4-week recovery period (0.9 mg/kg males and females), indicating recovery. The decrease in body weight gain at ≥ 0.2 mg/kg was not considered to be adverse because body weights were

sustained or increased compared to prestudy values and treatment for decreased body weights was limited to food supplementation provided with the daily food ration.

Table 33 TOX Study No 119036: AMG416 (Etelcalcetide) Caused Decreases in Body Weight Gain in Male and Female Dogs.

Mean Body Weight Gain (kg) at End of Dose (Day 175) Compared to Prestudy / % Weight Gain Increase Compared to Control

Dose Group	Male	Female
Group 1 (Control dose)	2.4/NA	1.7/NA
Group 2 (0.2 mg/kg/day)	2.0/75%	1.2/76%
Group 3 (0.5 mg/kg/day)	1.4/58%	0.83/52%
Group 4 (0.9 mg/kg/day)	1.3/51%	0.9/56%

NA = not applicable

Feed Consumption (once daily)
At 0.5 and 0.9 mg/kg, etelcalcetide-related reduced appetite accompanied by reductions in body weights required that some of the animals be given food supplementation (i.e., broth/canned food) along with their daily food ration, which began by Week 2.
At 0.2 mg/kg, two animals required food supplementation on Week 17. Food supplementation for a majority of the animals was discontinued by Week 13/14 of the 26 week dose regimen, indicating recovery to reduced appetite.

Ophthalmoscopy (pretreatment and during Week 26)
There were no etelcalcetide-related ophthalmic changes at any dose level.

ECG (prestudy, on Day 2, during Week 26, and in Week 30 of the recovery period)
There was an etelcalcetide-related minor increase in heart rate corrected QT (QTc) interval at ≥ 0.2 mg/kg in females at Days 2 and 177 and at 0.5 and 0.9 mg/kg in males at Day 177, with recovery by the end of the 4-week dose-free period. These changes in QTc were anticipated based on the decrease in serum calcium.

Table 34 TOX Study No 119036: AMG416 (Etelcalcetide) Caused QTc Prolongation.

QTc Interval Increase Compared to Concurrent Controls

Group No.	Day 2 M/F	Day 177 M/F	Week 30 (Recovery) M/F
Group 2	- / 6%	- / 8.7%	NA
Group 3	- / 7%	5.7% / 5.8%	NA
Group 4	- / 12%	4.2% / 11.8%	- / 5%

M = males; F = females; NA = not applicable; - = similar/comparable to control dose values

Clinical Pathology (Hematology (Days 181 and 209), Clinical chemistry (Days 1, 2, 169, 170 – Ca and P only, 181, and 209), Urinalysis (Days 181 and 209))

Etelcalcetide-related changes in clinical pathology parameters were limited to mildly decreased reticulocytes for females at ≥ 0.5 mg/kg, minimally to markedly decreased calcium for males and females at ≥ 0.2 mg/kg, and minimally to mildly increased phosphorus for females at ≥ 0.5 mg/kg and males at ≥ 0.2 mg/kg. Changes in calcium and phosphorus were an expected pharmacological effect of etelcalcetide. Recovery was observed during the 4-week recovery period.

Reference ID: 3923456

Table 35 TOX Study No 119036: AMG416 (Etelcalcetide) Decreased Calcium and Increased Phosphorus Levels.

AMG 416-related Calcium Fold-changes†

Dose mg/kg	Day 1, Pre	Day 1 4 hr PD	Day 1 8 hr PD	Day 1 12 hr PD	Day 2 24 hr PD	Day 169 Pre	Day 169 4 hr PD	Day 169 8 hr PD	Day 169 12 hr PD	Day 170 24 hr PD	Day 181 24 hr PD	Day 209
Males												
0.2	-	0.95*	0.91*	0.90*	0.91*	0.95	0.91*	0.88*	0.88*	0.91*	-	NA
0.5	-	0.89*	0.82*	0.76*	0.86*	0.95	0.84*	0.80*	0.77*	0.85*	0.94*	NA
0.9	-	0.88*	0.78*	0.73*	0.79*	0.91*	0.81*	0.74*	0.70*	0.79*	0.90*	-
Females												
0.2	-	0.91*	0.87*	0.86*	0.91*	-	0.91*	0.84*	0.86*	0.92*	-	NA
0.5	-	0.91*	0.83*	0.79*	0.84*	0.94*	0.87*	0.81*	0.80*	0.88*	0.93*	NA
0.9	-	0.83*	0.75*	0.71*	0.75*	0.93*	0.80*	0.72*	0.72*	0.81*	0.88*	-

hr = hour; NA = not applicable; pre = predose; PD = postdose

† Data presented as fold-change in mean values for groups administered AMG 416 compared to control group mean values

A dash (-) indicates no change; (*) indicates statistical significance at $P \leq 0.05$.

There were 4 animals per sex in the control, 0.2, 0.5, and 0.9 mg/kg AMG 416 groups, which comprised the main study cohort and 2 animals per sex in the control and 0.9 mg/kg AMG 416 groups, which comprised the recovery cohort.

AMG 416-related Phosphorus Fold-changes†

Dose mg/kg	Day 1 Pre	Day 1 4 hr PD	Day 1 8 hr PD	Day 1 12 hr PD	Day 2 24 hr PD	Day 169 Pre	Day 169 4 hr PD	Day 169 8 hr PD	Day 169 12 hr PD	Day 170 24 hr PD	Day 181 24 hr PD	Day 209
Males												
0.2	-	-	-	-	-	-	1.16	1.14	-	1.10	-	NA
0.5	-	-	-	1.13*	-	1.15*	1.28*	1.13	1.14	1.15*	-	NA
0.9	-	-	-	1.11	-	1.27*	1.36*	1.25*	1.29*	1.29*	1.14	-
Females												
0.2	-	-	-	-	-	-	-	-	-	-	-	NA
0.5	-	-	-	1.15*	-	-	-	1.10	-	-	-	NA
0.9	-	-	-	1.14*	-	-	1.20*	1.22*	1.15*	1.15*	-	-

hr = hour; NA = not applicable; pre = predose; PD = postdose

† Data presented as fold-change in mean values for groups administered AMG 416 compared to control group mean values

A dash (-) indicates no change; (*) indicates statistical significance at $P \leq 0.05$.

There were 4 animals per sex in the control, 0.2, 0.5, and 0.9 mg/kg AMG 416 groups, which comprised the main study cohort and 2 animals per sex in the control and 0.9 mg/kg AMG 416 groups, which comprised the recovery cohort.

Gross Pathology (scheduled termination – Day 182 or Day 210)
There were no macroscopic findings.

Organ Weights (scheduled termination – Day 182 or Day 210)
There were no AMG-416 related organ weight changes.

Histopathology

(Scheduled termination – Day 182 or Day 210)
Adequate Battery
Yes

Peer Review
Yes

Histological Findings
Etelcalcetide-related microscopic changes were limited to the kidney at ≥0.2 mg/kg.
There was a minimal to mild intracytoplasmic golden-brown pigment in the kidney proximal tubules. Increased pigment was considered etelcalcetide-related due to increased incidence in females and increased incidence and severity in males that was dose-related. The change was

not considered adverse because there were no associated degenerative microscopic findings in the kidney and no changes in renal clinical pathology parameters. In the 0.9 mg/kg group, the microscopic changes in the kidney were similar at the end of recovery to those seen at the end of dosing.

Table 36 TOX Study No 119036: Microscopic Findings.

Incidence of AMG 416-related Microscopic Observations - Dosing Phase (Day 182)

	Male				Female			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg)	0	0.2	0.5	0.9	0	0.2	0.5	0.9
No. animals examined	4	4	4	4	4	4	4	4
KIDNEY								
Pigment accumulation, tubular, cytoplasmic, multifocal								
Minimal	0[a]	2	1	1	1	3	4	3
Mild	0	1	3	3	0	0	0	0

[a] Number of animals with the finding

Incidence of AMG 416-related Microscopic Observations - Recovery Phase (Day 210)

	Male		Female	
Group	1	4	1	4
Dose (mg/kg)	0	0.9	0	0.9
No. animals examined	4	4	4	4
KIDNEY				
Pigment accumulation, tubular, cytoplasmic, multifocal				
Minimal	0[a]	0	0	1
Mild	0	2	0	0

[a] Number of animals with the finding

Toxicokinetics (Days 1, 2, 181 and 182)

After the dose administered on study day 181, the mean etelcalcetide exposures (AUC_{all}) were 572, 1,490, and 2,410 ng·hr/mL with mean maximum observed drug concentration (C_{max}) values of 480, 1,230, and 1,960 ng/mL at the 0.2, 0.5, and 0.9 mg/kg/dose, respectively, for combined sexes.

Exposures were similar (within 2-fold) between the male and female dogs at each dose level. On study day 181, the mean exposure to etelcalcetide (AUC_{all}) increased approximately 2.6 fold for a 2.5-fold increase in dose (0.2 to 0.5 mg/kg/dose) and approximately 1.6-fold increase for a further 1.8-fold increase in dose (0.5 to 0.9 mg/kg/dose).

After intravenous injection administration every other day for 181 days, the exposure of etelcalcetide appeared to be similar upon repeated dosing.

Table 37 TOX Study No 119036: TK Parameters.

Mean Toxicokinetic Parameter Estimate of AMG 416 after Intravenous Administration in Dogs

Gender	Dose (mg/kg)	Study Day 1			Study Day 181		
		C_{max} (ng/mL)	AUC_{all} (ng·hr/mL)	t1/2, z (hr)	C_{max} (ng/mL)	AUC_{all} (ng·hr/mL)	t1/2, z (hr)
Male/Female	0.2	467	551	6.23	480	572	8.02
	0.5	1210	1440	6.38	1230	1490	8.73
	0.9	2150	2530	6.91	1960	2410	8.02

Dosing Solution Analysis

The dose formulations analyzed were within specifications. Homogeneity testing showed homogeneous preparations.

The safety profile of etelcalcetide demonstrated in dogs in this pivotal (6 month) chronic study was comparable to that of the original 3/9-month chronic study (Study No 4169-NC-132 by KAI). Both studies showed hypocalcemia-associated adverse effects (clinical signs, changes in body weight and food consumption, intracytoplasmic golden-brown pigment in renal proximal tubules) and the plasma exposures and the NOAEL (0.5 mg/kg) were similar between these two studies. In this study, a mild decrease in reticulocytes in females was noted.

Study No 4169-NC-132: 3/9 month IV dog study (KAI)

Study title: A Combination 13- and 39-Week Intravenous Toxicity, Toxicokinetic and Pharmacodynamic Study of KAI-4169 (Etelcalcetide) in Beagle Dogs, with Respective 2- and 4-WeekRecovery Periods (GLP; testing facility: (b) (4)

Study description:
This study evaluated the toxicity and toxicokinetics of etelcalcetide when administered every other day by intravenous (IV) bolus injection to beagle dogs for 91 or 273 days. The reversibility and potential delayed effects during a 2- or 4-week recovery period, respectively, were assessed. Etelcalcetide or the vehicle/control article (consisting of 0.27% sodium succinate dibasic hexahydrate, 2% D-mannitol, 1% glycine, 0.9% benzyl alcohol, and 1% trehalose in sterile water for injection) was administered every other day via IV bolus injection at a dose volume of 0.5 mL/kg/day. Toxicity parameters: cageside, unscheduled post-dose and detailed observations; body weights and body weight changes; food consumption; clinical pathology (clinical chemistry, hematology, coagulation, and urinalysis) parameters; ophthalmology; gross pathology; absolute and relative organ weights; and microscopic pathology. Blood (plasma) samples were collected for TK evaluation at 7 time points each on Days 1 and at 8 time points on Days 91, 183, and 273 from TK animals. Blood samples were also collected for measurement of serum calcium and phosphorus evaluation at 4-5 time points on Days 1, 45, 91, 183 and 273. An additional serum chemistry sample was also collected for the high-dose group on Day 24 in order to document serum Ca and P levels prior to reducing the dose level on Day 25/27. Blood (serum) samples for parathyroid hormone (PTH) analysis were collected from all available study animals at several time points on Days 1, 91, 183, and 273. The data from the Day 1 samples were not interpretable because the lower limit of quantification of the assay was higher than the PTH levels in most of the samples. The decision was made not to analyze the samples on Days 91, 183, and 273. Therefore, the PTH data were archived with the study records, but were not reported. All animals assigned to the interim, interim recovery, terminal, and terminal recovery subgroups were euthanized following the final blood collection on Days 92, 105, 274, or 301, respectively.

Reference ID: 3923456

Table 38 TOX Study No 4169-NC-132: 3/9 Month Dog Study Design.

Group No.	Treatment	Dose Level (mg/kg/dose)[a]	Conc. (mg/mL)	No. of Animals/Sex			
				IT	IR	TS	TR
1	Buffer Solution (Vehicle)	0	0	3	2	3	2
2	KAI-4169	0.2	0.4	3	–	3	–
3	KAI-4169	0.5	1.0	3	–	3	–
4	KAI-4169	1.2/0.9[b]	2.4/1.8[b]	3	2	3	2

IT – Interim sacrifice animals (sacrificed on Day 92)
IR – Interim recovery sacrifice animals (sacrificed on Day 105)
TS – Terminal sacrifice animals (sacrificed on Day 274)
RS – Terminal recovery sacrifice animals (sacrificed on Day 301)
[a] Based primarily on loss of body weight and the severity of hypocalcemia, doses were skipped for four high-dose (Group 4) animals on one to five occasions over the course of the study.
[b] The dose level for the high-dose group was reduced from 1.2 to 0.9 mg/kg/dose beginning on Day 25 and Day 27 for males and females, respectively. The formulation concentration was reduced from 2.4 to 1.8 mg/mL accordingly.

Study findings:

- Repeated (every other day) administration of etelcalcetide via intravenous bolus injection at dose levels up to 1.2/0.9 mg/kg/dose (highest dose level) for 273 days to male and female beagle dogs was well tolerated.
- No mortality or moribundity occurred throughout the study.
- The TK parameters confirmed dose-related exposure of all etelcalcetide-treated dogs. There was no evidence of a gender difference in exposure. The primary exposure parameters (C_{max} and AUC_{INFobs}) were generally proportional to dose. There was also a moderate accumulation on Day 273, relative to Day 1.
- Etelcalcetide related findings included:
  - Clinical signs such as lethargy, prostration, and reduced activity in high-dose animals, which were most pronounced during the early part of the interim treatment period prior to the dose level reduction (1.2 to 0.9 mg/kg/dose).
  - Significantly lower body weight gain or weight loss in most high-dose animals during most of the interim study period that was apparently related to reduced food intake (but no effects on these parameters during the remainder of the study, owing to the dose level reduction).
  - A slight increase in the heart rate-corrected QT interval (QTcF) in high dose males at the end of the interim and terminal treatment periods.
  - Dose-related decreases in serum Ca and increases in serum P at all dose levels throughout the study.
  - Slight decreases in mean serum CK, LDH, Cl, K, and/or slight increases in mean serum Na at 0.5 and/or 1.2/0.9 mg/kg/dose.
  - Decreases in the indicators of circulating RBC mass and/or reticulocyte counts, primarily in the high-dose group on Day 45 and, to a much lesser extent, on Day 92, with no effect thereafter.
  - An increased severity of microscopically observable brown pigment (presumably lipofuscin) in the kidneys.
- With minor exceptions, all of these changes, including serum Ca and P, were completely or partially reversed after the 2-week (interim) or 4-week (terminal) non-treatment recovery periods. Effects observed in this study, such as the clinical signs and changes in body weight, food consumption, QTcF prolongation, serum Ca and P, and red blood cell parameters, were either direct or indirect effects of the intended pharmacologic action of etelcalcetide in reducing PTH secretion.
- The reduction in serum calcium at the high-dose level (1.2/0.9 mg/kg/dose) was considered moderately adverse, particularly during the interim treatment period and

most notably prior to the dose level reduction, based on the percent decrease in serum calcium and the associated clinical signs and body weight loss in the high-dose animals, mainly during the interim treatment period. Other changes were not considered adverse.

- The NOAEL was 0.5 mg/kg/dose, based on marked hypoglycemia at 1.2/0.9 mg/kg/dose which was associated with the gender-combined average AUC_{all} value of 2,430 h*ng/mL on Day 273.

Table 39 TOX Study No 4169-NC-132: Microscopic Findings.

Incidence[a] of Test Article-Related Microscopic Findings
Terminal Sacrifice (Day 274)

Sex:	Males				Females			
Group:	1	2	3	4	1	2	3	4
Treatment:	Vehicle[b]	KAI-4169			Vehicle[b]	KAI-4169		
Treatment (mg/kg/dose):	0	0.2	0.5	1.2/0.9	0	0.2	0.5	1.2/0.9
Kidney								
Golden-brown pigment, proximal tubules								
minimal	3	-	-	-	1	2	2	-
mild	-	3	2	3	-	1	1	2
moderate	-	-	1	-	-	-	-	1

[a] Expressed as the number of animals with findings in each group (3 animals/sex/group)
[b] Buffer solution
"-" = not present

Incidence[a] of Test Article-Related Microscopic Findings
Terminal Recovery Sacrifice (Day 301)

Sex:	Males		Females	
Group:	1	4	1	4
Treatment:	Vehicle[b]	KAI-4169	Vehicle[b]	KAI-4169
Treatment (mg/kg/dose):	0	1.2/0.9	0	1.2/0.9
Golden-brown pigment, proximal tubules				
minimal	1	-	1	-
mild	-	2	-	2

[a] Expressed as the number of animals with findings in each group (2 animals/sex/group)
[b] Buffer solution
"-" = not present

Table 40 TOX Study No 4169-NC-132: TK Parameters.

Day	Group	Dose (mg/kg)	Gender	C_{max} (ng/mL)	AUC_{all} (h*ng/mL)	AUC_{INFobs} (h*ng/mL)	Cl_{obs} (mL/h/kg)	V_{SSobs} (mL/kg)	$HL_{\lambda z}$ (h)
1	2	0.2	F	484	729	757	266	1310	6.31
			M	366	595	621	325	1700	6.68
			F + M	425	662	689	295	1510	6.50
	3	0.5	F	1270	1920	1990	254	1250	6.43
			M	1040	1480	1540	329	1570	6.68
			F + M	1150	1700	1760	292	1410	6.56
	4	1.2/0.9	F	3550	4820	5030	241	1200	6.82
			M	2790	4020	4220	286	1500	7.34
			F + M	3170	4420	4620	264	1350	7.08
91	2	0.2	F	255	402	448	456	3560	9.53
			M	345	469	514	400	2920	8.69
			F + M	300	436	481	428	3240	9.11
	3	0.5	F	544	895	967	523	3540	7.99
			M	751	977	1070	475	3390	8.86
			F + M	648	936	1020	499	3460	8.43
	4	1.2/0.9	F	1440	2400	2650	362	2570	8.48
			M	1680	2340	2580	362	2630	9.13
			F + M	1560	2370	2610	362	2600	8.82
183	2	0.2	F	402	731	768	261	1440	7.10
			M	496	722	782	264	1640	8.40
			F + M	449	726	775	262	1540	7.75
	3	0.5	F	1100	1910	2020	251	1520	7.19
			M	1330	1880	2020	250	1440	8.83
			F + M	1220	1900	2020	250	1480	8.01
	4	1.2/0.9	F	2480	3600	3800	238	1310	7.31
			M	2850	4100	4420	207	1270	8.83
			F + M	2660	3850	4110	223	1290	8.07
273	2	0.2	F	718	951	1010	201	1160	7.64
			M	720	908	965	210	1200	7.61
			F + M	719	930	987	205	1180	7.63
	3	0.5	F	1990	2320	2410	208	962	6.87
			M	2230	2550	2720	185	1040	8.59
			F + M	2110	2430	2570	197	1000	7.73
	4	1.2/0.9	F	3800	4330	4560	199	1040	7.54
			M	4000	4720	5020	183	1010	8.18
			F + M	3900	4520	4790	191	1020	7.86

Study No 4169-NC-106: 4 week IV dog study

Study title: A 4-Week Intravenous Toxicity, Toxicokinetic and Pharmacodynamic Study of KAI-4169 (Etelcalcetide) in Beagle Dogs (GLP; testing facility: (b) (4)

Study description:
This study characterized the potential toxicity, toxicokinetics and pharmacodynamics of etelcalcetide, which was administered every other day by intravenous bolus injection to beagle dogs over a 27-day period. The reversibility and potential delayed effects during a 4-week recovery period were also evaluated. Toxicity parameters: mortality/morbidity, cageside and detailed clinical observations, body weights and body weight changes, food consumption, ophthalmology, electrocardiogram, clinical pathology (clinical chemistry, hematology, coagulation and urinalysis), gross necropsy, absolute and relative organ weights, and microscopic examination (on all tissues from the terminal (Day 28)-sacrificed dogs in the vehicle control and 1.5 mg/kg/dose groups, and on target organs for all lower-dose terminal-sacrifice and all recovery-sacrifice dogs). In addition, formulation, toxicokinetic and PTH analyses were also performed. The vehicle control was a buffer solution containing 0.27% sodium succinate dibasic hexahydrate, 2% mannitol, 1% glycine and 1% trehalose.

Group No.	Treatment	Dose Level (mg/kg/dose)	Concentration (mg/mL)	No. of Animals/Sex	
				TS	RS
1	Buffer Solution (Vehicle)	0	0	3	2
2	KAI-4169	0.1	0.2	3	–
3	KAI-4169	0.3	0.6	3	–
4	KAI-4169	1.5	3.0	3	2

TS – Terminal sacrifice on Day 28
RS – Recovery sacrifice on Day 56

Study findings:

- Treatment with etelcalcetide via once every-other-day intravenous injection over a 27-day period at dose levels up to 0.3 mg/kg/dose was well tolerated.
- The highest dose level of 1.5 mg/kg/day was associated with marked hypocalcemia and hyperphosphatemia. This was an exaggerated pharmacological response to the dramatic reduction in PTH secretion. No other etelcalcetide-related changes in clinical pathology parameters were observed.
- The pronounced hypocalcemia in the high-dose group was responsible for various clinical signs observed during the 27-day treatment period, as well as inappetence, which led to a modest reduction in weight gain.
- The slight prolongation of the QTc interval in the 1.5 mg/kg/dose animals was expected sequelae of hypocalcemia. The only etelcalcetide-related anatomic pathology finding was thymic involution in the high-dose animals, associated with a reduction in thymus size (at necropsy) and thymus weight, which likely reflected increased stress in these animals.
- The NOAEL was 0.3 mg/kg/dose, based on marked hypocalcemia and QTc prolongation observed at 1.5 mg/kg/day.
- TK analyses: On Days 1 and 27 (the first and last dosing days), systemic exposure to etelcalcetide was generally dose-proportional. Mean observed clearance (Cl_{obs}), apparent steady-state volume of distribution (VSS_{obs}), and terminal half-life (t½ ; $HL_{\lambda z}$ values were generally dose-independent. There was a slight accumulation of etelcalcetide in the blood compartment with every-other-day dosing.
- TK analyses (based on KP-2067, the process of homogenization and extraction results in the chemical reduction of etelcalcetide [removal of the disulfide-linked cysteine], yielding KP-2067): On Day 28 (one day after the last dose), the mean concentrations of etelcalcetide in liver, heart and kidney were dose-related between the 0.1 and 1.5 mg/kg/dose levels. For both dose levels, the mean concentrations in liver and kidney were similar and were much higher than in heart. The levels of etelcalcetide in brain were extremely low.

Study No 4169-NC-101: 7 day (2 phased) IV dog study

Study title: A Two-phase Intravenous Dose Range-Finding Study of KAI-4169 (Etelcalcetide)in Beagle Dogs (non-GLP; testing facility: (b) (4)

Study description:
This study was to evaluate the potential toxicity, toxicokinetics and pharmacodynamics of etelcalcetide given intravenously to beagle dogs once daily for 7 consecutive days. Phase I - The treatment was discontinued after Day 2 for the high-dose group (5.0 mg/kg/day), and after Day 3 for other groups due to pronounced clinical signs. Phase II - Following a 10-11 day washout period, a modified treatment regimen was initiated on Day 13, with dosing once every other day over a 7-day period. Vehicle control was buffer solution.

Phase I (Day 1 to Day 3)

Group	Treatment	Dose Level (mg/kg/day)	Conc. (mg/mL)	No. Animals	
				Male	Female
1	Buffer Solution (Vehicle)	0	0	2	2
2	KAI-4169	0.5	0.25	2	2
3	KAI-4169	2.0	1.00	2	2
4	KAI-4169	5.0*	2.50	2	2

* No dose was given to Group 4 animals on Day 3.

Phase II (Day 13 to Day 19):

Group	Treatment	Dose Level (mg/kg/dose)	Conc. (mg/mL)	No. Animals	
				Male	Female
1	Buffer Solution (Vehicle)	0	0	2	2
2	KAI-4169	0.5	1.00	2	2
3	KAI-4169	0.2	0.40	2	2
4	KAI-4169	0.08	0.16	2	2

Study findings:

- Phase I: Etelcalcetide given intravenously to beagle dogs once daily at 2.0 mg/kg for 3 consecutive days and 5.0 mg/kg for 2 consecutive days was not tolerated by the dogs, due to the development of marked hypocalcemia (an exaggerated pharmacologic effect), which resulted in lethargy, tremors, convulsions, prostration, dyspnea, salivation, emesis, reduced or no food and water intake. Consequently, dosing was stopped in Phase I after 2 or 3 doses.
- Phase II: After a 10-11 day washout period following Phase I, etelcalcetide was given intravenously to beagle dogs once every other day over a 7-day period at up to 0.5 mg/kg/dose, and this was well tolerated.
- A dose-related decrease in serum calcium was observed that was substantially less than in Phase I, attributed to the lower dose levels and lesser dosing frequency.
- Marked decreases in plasma PTH were observed at all dose levels in both study phases, and the duration of the PTH suppression was generally dose-related.
- There was no clear effect on plasma histamine concentration at 5 minutes after dosing in Phase I, when a histamine response was observed to be maximal (based on previous studies conducted by the Sponsor with other peptides). Therefore, other plasma samples collected in Phase I were not analyzed, and no samples were collected in Phase II.
- The maximum tolerated dose (MTD) in dogs for etelcalcetide was 0.5 mg/kg given every other day.

7 Genetic Toxicology

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

Study No 4169-NC-104 (pivotal)

Study title: Bacterial Reverse Mutation Assay

Study no.:	4169-NC-104
Study report location:	KAI Pharmaceuticals (South San Francisco, CA)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 14, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	F4169KP0901B with 95.3% purity

Key Study Findings

- The plate incorporation method was used for both phases – initial toxicity-mutation assay (Phase I) and confirmatory mutagenicity assay (Phase II). Test stains: *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537; *Escherichia coli* WP2 uvrA.
- In the initial toxicity-mutation assay (dosing range from 1.5-5000 μg per plate), positive mutagenic response were seen in TA100 without S9 (2.3X increase) and in TA 1535 without S9 (4.3X increase) and with S9 (5.4X increase). No positive mutagenic responses were seen with other tester strains.
- In the confirmatory mutagenicity assay (dosing range from 50-5000 μg per plate), positive mutagenic response were seen in TA100 without S9 (2.5X increase) and in TA 1535 without S9 (10.9X increase) and with S9 (16.4X increase). The greater response observed in TA1535 was due to low revertant count for the control plates.
- Etelcalcetide was considered to be positive in the Ames assay. The positive response was observed in 2 out of 5 bacterial strains tested.

Methods

Strains:	*Salmonella typhimurium*: TA98, TA100, TA1535, and TA1537; *Escherichia coli*: WP2 uvrA (with or without S9)
Concentrations in definitive study:	50, 150, 500, 1500 and 5000 μg
Basis of concentration selection:	Maximum of 5000 μg per guidance, in the absence of cytotoxicity or precipitation
Negative control:	Succinate buffer (10 mM at pH 4.5) was used as the solvent for dose solution preparation and as the vehicle control.
Positive control:	See tables below.
Formulation/Vehicle:	10 mM sodium succinate, 1% glycine *(w/v)*, 2% mannitol *(w/v)*, 1% trehalose *(w/v)*, pH 4.5
Incubation & sampling time:	48-72 hrs

Reference ID: 3923456

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98, TA1535 and TA1537	Rat	2-aminoanthracene (Sigma Aldrich Chemical Co., Inc.) Lot No. 03403ED Exp. Date 22-Jan-2012 CAS No. 613-13-8 Purity 99.8%	1.0
TA100			2.0
WP2 *uvr*A			10

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98	None	2-nitrofluorene (Sigma Aldrich Chemical Co., Inc.) Lot No. 03319JD Exp. Date 28-Feb-2011 CAS No. 607-57-8 Purity 98.1%	1.0
TA100, TA1535		sodium azide (Alfa Aesar) Lot No. G24R025 Exp. Date 10-Feb-2010 CAS No. 26628-22-8 Purity 99.8%	1.0
TA1537		9-aminoacridine (Sigma Aldrich Chemical Co.) Lot No. 106F06682 Exp. Date 30-Oct-2010 CAS No. 90-45-9 Purity >97%	75
WP2 *uvr*A		methyl methanesulfonate (Sigma Aldrich Chemical Co., Inc.) Lot No. 76296KJ Exp. Date 02-Jun-2012 CAS No. 66-27-3 Purity 99.8%	1,000

Study Validity

- Tester strains: All salmonella tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the uvrB gene. Cultures of TA100 must demonstrate the presence of the pKM101 plasmid R-factor.
- Negative controls values: All cultures must demonstrate the characteristic mean number of spontaneous revertants in the untreated and vehicle controls – TA100: 80-240, TA1535: 5-45.
- Tester strain titers: Tester strain culture titers must be $\geq 0.3 \times 10^9$ cells/ml.
- Positive control values: The mean of each positive control must exhibit $\geq$ 3X increase in the number of revertants over the mean value of the respective vehicle control.
- Toxicity: A minimum of 3 non-toxic dose levels is required to evaluate assay data. A dose toxic level is considered if 1) >50% reduction in the mean number of revertants/plate (accompanied by an abrupt dose-dependent drop) as compared to the mean vehicle control value and/or if 2) at least a moderate reduction in the background lawn (toxicity and degree of precipitation code: 3, 4 or 5).

Code	Description	Characteristics
1 or no code	Normal	Distinguished by a healthy microcolony lawn.
2	Slightly Reduced	Distinguished by a noticeable thinning of the microcolony lawn and possibly a slight increase in the size of the microcolonies, compared to the vehicle control plate.
3	Moderately Reduced	Distinguished by a marked thinning of the microcolony lawn, resulting in a pronounced increase in the size of the microcolonies compared to the vehicle control plate.
4	Extremely Reduced	Distinguished by an extreme thinning of the microcolony lawn, resulting in an increase in the size of the microcolonies compared to the vehicle control plate, such that the microcolony lawn is visible to the unaided eye as isolated colonies.
5	Absent	Distinguished by a complete lack of any microcolony lawn greater than or equal to 90% of the plate.
6	Obscured by Particulate	The background bacterial lawn cannot be accurately evaluated due to microscopic test article particulate.
NP	Non-Interfering Precipitate	Distinguished by precipitate on the plate that is visible to the naked eye, but any precipitate particles detected by the automated colony counter total less than or equal to 10% of the revertant colony count (e.g., less than or equal to 3 particles on a plate with 30 revertants).
IP	Interfering Precipitate	Distinguished by precipitate on the plate that is visible to the naked eye, and any precipitate particles detected by the automated colony counter exceed 10% of the revertant colony count (e.g., greater than 3 particles on a plate with 30 revertants). These plates are counted manually.

Study Results

Initial toxicity-mutation assay

The maximum dose tested was 5000 μg per plate (a concentration of 50 mg/mL and a plating aliquot of 100μL). Positive mutagenic responses were observed with TA100 without S9 (2.3x increase) and with TA1535 with or without S9 (4.3x and 5.4x increases respectively). No positive mutagenic responses were observed with other strains with or without S9. Due to unacceptable vehicle control value (for TA1537) and untreated control values (for WP2 uvrA), these two strains were retested. Neither precipitate nor appreciable toxicity was seen.

Confirmatory mutagenicity assay

The doses tested were 50, 150, 500, 1500 and 5000μg per plate. Neither precipitate nor appreciable toxicity was seen. Positive mutagenic responses were observed with TA100 without S9 (2.5x increase) and with TA1535 with or without S9 (10.9x and 16.4x increases respectively). No positive mutagenic responses were seen with the remaining test conditions.

Table 41 Genetox Study No 4169-NC-104: KAI-4169 (Etelcalcetide) was Mutagenic in Two Strains, TA100 and TA1535.

Confirmatory Mutagenicity Assay without S9 activation

Study Number: AC33FX.503.BTL
Experiment: B3
Exposure Method: Plate incorporation assay

Study Code: AC33FX
Date Plated: 12/31/2009
Evaluation Period: 1/6/2010 to 1/7/2010

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	**KAI-4169**	5000 µg	*27*	*10*	*0.7*	24[A], 38[A], 19[A]
		1500 µg	*25*	*8*	*0.7*	20[A], 22[A], 34[A]
		500 µg	*23*	*8*	*0.6*	27[A], 29[A], 14[A]
		150 µg	*42*	*9*	*1.1*	33[A], 43[A], 51[A]
		50 µg	*32*	*3*	*0.9*	32[A], 34[A], 29[A]
	10 mM succinate buffer at pH 4.5 ± 0.1	100 µL	*37*	*5*		43[A], 33[A], 36[A]
	Untreated Control		*29*	*3*		32[A], 26[A], 29[A]
TA100	**KAI-4169**	5000 µg	*233*	*27*	*2.5*	258[A], 235[A], 205[A]
		1500 µg	*189*	*11*	*2.0*	201[A], 186[A], 180[A]
		500 µg	*141*	*20*	*1.5*	119[A], 158[A], 145[A]
		150 µg	*130*	*22*	*1.4*	154[A], 126[A], 110[A]
		50 µg	*118*	*12*	*1.2*	105[A], 125[A], 125[A]
	10 mM succinate buffer at pH 4.5 ±0.1		*95*	*7*		97[A], 101[A], 88[A]
	Untreated Control		*121*	*5*		116[A], 126[A], 120[A]
TA1535	**KAI-4169**	5000 µg	*197*	*25*	*16.4*	226[A], 185[A], 181[A]
		1500 µg	*85*	*20*	*7.1*	108[A], 73[A], 75[A]
		500 µg	*31*	*10*	*2.6*	42[A], 22[A], 29[A]
		150 µg	*21*	*4*	*1.8*	19[A], 19[A], 26[A]
		50 µg	*14*	*4*	*1.2*	18[A], 10[A], 13[A]
	10 mM succinate buffer at pH 4.5 ± 0.1	100 µL	*12*	*7*		14[A], 5[A], 18[A]
	Untreated Control		*14*	*6*		13[A], 9[A], 20[A]

Key to Automatic & Manual Count Flags

[M]: Manual count [A]: Automatic count

Confirmatory Mutagenicity Assay without S9 activation

Study Number: AC33FX.503.BTL
Experiment: B3
Exposure Method: Plate incorporation assay

Study Code: AC33FX
Date Plated: 12/31/2009
Evaluation Period: 1/6/2010 to 1/7/2010

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA1537	**KAI-4169**	5000 µg	*7*	*3*	*1.0*	4[A], 9[A], 9[A]
		1500 µg	*5*	*1*	*0.7*	5[A], 6[A], 4[A]
		500 µg	*6*	*3*	*0.9*	9[A], 4[A], 5[A]
		150 µg	*4*	*1*	*0.6*	5[A], 5[A], 3[A]
		50 µg	*7*	*2*	*1.0*	8[A], 5[A], 9[A]
	10 mM succinate buffer at pH 4.5 ± 0.1	100 µL	*7*	*2*		6[A], 6[A], 10[A]
	Untreated Control		*7*	*2*		9[A], 6[A], 6[A]
WP2*uvrA*	**KAI-4169**	5000 µg	*47*	*6*	*0.8*	48[A], 52[A], 41[A]
		1500 µg	*47*	*4*	*0.8*	50[A], 48[A], 42[A]
		500 µg	*62*	*6*	*1.1*	68[A], 57[A], 62[A]
		150 µg	*61*	*9*	*1.0*	69[A], 64[A], 51[A]
		50 µg	*57*	*5*	*1.0*	59[A], 61[A], 52[A]
	10 mM succinate buffer at pH 4.5 ± 0.1	100 µL	*59*	*2*		59[A], 57[A], 60[A]
	Untreated Control		*49*	*5*		46[A], 54[A], 46[A]

TA98	**2NF**	1.0 µg	*175*	*21*	*4.7*	195[A], 175[A], 154[A]
TA100	**SA**	1.0 µg	*481*	*22*	*5.1*	469[A], 506[A], 467[A]
TA1535	**SA**	1.0 µg	*386*	*47*	*32.2*	380[A], 436[A], 343[A]
TA1537	**9AAD**	75 µg	*286*	*38*	*40.9*	259[A], 329[A], 269[A]
WP2*uvrA*	**MMS**	1000 µg	*431*	*15*	*7.3*	426[A], 448[A], 420[A]

Key to Positive Controls

2NF	2-nitrofluorene
SA	sodium azide
9AAD	9-Aminoacridine
MMS	methyl methanesulfonate

Key to Automatic & Manual Count Flags

[M]: Manual count [A]: Automatic count

Confirmatory Mutagenicity Assay with S9 activation

Study Number: AC33FX.503.BTL
Experiment: B3
Exposure Method: Plate incorporation assay

Study Code: AC33FX
Date Plated: 12/31/2009
Evaluation Period: 1/6/2010 to 1/7/2010

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	**KAI-4169**	5000 µg	*35*	*4*	*0.7*	34[A], 32[A], 40[A]
		1500 µg	*28*	*4*	*0.6*	31[A], 24[A], 28[A]
		500 µg	*17*	*2*	*0.3*	15[A], 18[A], 18[A]
		150 µg	*36*	*5*	*0.7*	31[A], 40[A], 38[A]
		50 µg	*38*	*6*	*0.8*	45[A], 36[A], 33[A]
	10 mM succinate buffer at pH 4.5 ± 0.1	100 µL	*49*	*6*		47[A], 56[A], 45[A]
	Untreated Control		*37*	*9*		27[A], 43[A], 42[A]
TA100	**KAI-4169**	5000 µg	*153*	*12*	*1.2*	157[A], 140[A], 162[A]
		1500 µg	*136*	*15*	*1.1*	153[A], 131[A], 125[A]
		500 µg	*130*	*9*	*1.0*	130[A], 121[A], 138[A]
		150 µg	*132*	*18*	*1.1*	142[A], 143[A], 111[A]
		50 µg	*119*	*13*	*1.0*	134[A], 112[A], 111[A]
	10 mM succinate buffer at pH 4.5 ± 0.1	100 µL	*125*	*13*		139[A], 124[A], 113[A]
	Untreated Control		*116*	*11*		119[A], 103[A], 125[A]
TA1535	**KAI-4169**	5000 µg	*142*	*6*	*9.5*	147[A], 136[A], 144[A]
		1500 µg	*82*	*11*	*5.5*	92[A], 84[A], 70[A]
		500 µg	*37*	*9*	*2.5*	27[A], 40[A], 43[A]
		150 µg	*22*	*6*	*1.5*	29[A], 19[A], 18[A]
		50 µg	*21*	*7*	*1.4*	28[A], 15[A], 19[A]
	10 mM succinate buffer at pH 4.5 ± 0.1	100 µL	*15*	*4*		15[A], 11[A], 18[A]
	Untreated Control		*14*	*4*		10[A], 15[A], 18[A]

Key to Automatic & Manual Count Flags

[M]: Manual count [A]: Automatic count

Confirmatory Mutagenicity Assay with S9 activation

Study Number: AC33FX.503.BTL
Experiment: B3
Exposure Method: Plate incorporation assay

Study Code: AC33FX
Date Plated: 12/31/2009
Evaluation Period: 1/6/2010 to 1/7/2010

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA1537	**KAI-4169**	5000 µg	*8*	*2*	*1.3*	9^A, 5^A, 9^A
		1500 µg	*8*	*3*	*1.3*	8^A, 5^A, 10^A
		500 µg	*8*	*5*	*1.3*	6^A, 4^A, 13^A
		150 µg	*7*	*2*	*1.2*	8^A, 8^A, 5^A
		50 µg	*7*	*1*	*1.2*	6^A, 8^A, 8^A
	10 mM succinate buffer at pH 4.5 ± 0.1	100 µL	*6*	*2*		6^A, 8^A, 4^A
	Untreated Control		*7*	*3*		4^A, 10^A, 6^A
WP2*uvr*A	**KAI-4169**	5000 µg	*51*	*5*	*0.9*	50^A, 56^A, 47^A
		1500 µg	*59*	*5*	*1.0*	64^A, 54^A, 60^A
		500 µg	*48*	*5*	*0.8*	51^A, 43^A, 51^A
		150 µg	*64*	*8*	*1.1*	71^A, 66^A, 56^A
		50 µg	*70*	*5*	*1.2*	74^A, 64^A, 71^A
	10 mM succinate buffer at pH 4.5 ± 0.1	100 µL	*58*	*7*		65^A, 52^A, 56^A
	Untreated Control		*57*	*8*		48^A, 62^A, 61^A
TA98	**2AA**	1.0 µg	*485*	*72*	*9.9*	408^A, 550^A, 496^A
TA100	**2AA**	2.0 µg	*981*	*44*	*7.8*	1007^A, 930^A, 1006^A
TA1535	**2AA**	1.0 µg	*52*	*6*	*3.5*	57^A, 54^A, 46^A
TA1537	**2AA**	1.0 µg	*29*	*7*	*4.8*	26^A, 23^A, 37^A
WP2*uvr*A	**2AA**	10 µg	*189*	*43*	*3.3*	172^A, 238^A, 157^A

Key to Positive Controls

2AA 2-aminoanthracene

Key to Automatic & Manual Count Flags

^{M}: Manual count ^{A}: Automatic count

Study No 4169-NC-113 (pivotal)

Study title: Bacterial Reverse Mutation Assay with KAI-4169 (Etelcalcetide) (Treat and Plate Method)

Study no.:	4169-NC-113
Study report location:	KAI Pharmaceuticals (South San Francisco, CA)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 14, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	F4169KP0901B with 95.3% purity

Key Study Findings

- The modification of the plate incorporation method ("treat and plate" method) was used. Test stains: *Salmonella typhimurium* TA100 and TA1535.
- In the assay (dosing range from 15-5000 µg per plate), positive mutagenic response were seen in TA 1535 without S9 (6.9X increase at 5000µg) and with S9 (7.6X increase at 5000µg). On the other hand, equivocal result was concluded with TA 100 since slight increases (1.5X increase at 5000µg) in revertant counts were observed with or without S9.
- Etelcalcetide was considered to be positive in the Ames assay.

Methods

Strains: *Salmonella typhimurium*: TA100 and TA1535

	(with or without S9)
Concentrations in definitive study:	15. 50, 150, 500, 1500, 5000 μg
Basis of concentration selection:	a follow-up to a pervious bacterial mutagenicity study (Study No. 4160-NC-104)
Negative control:	Succinate buffer (10 mM at pH 4.5) was used as the solvent for dose solution preparation and as the vehicle control.
Positive control:	See tables below
Formulation/Vehicle:	10 mM succinate and 2% mannitol, 1% glycine and 1% trehalose (weight/volume), pH 4.5 ± 0.1
Incubation & sampling time:	48-72 hrs

This study was a follow-up to a previous bacterial mutagenicity assay (Study No. 4169-NC-104) in which etelcalcetide was found to be positive in tester strains TA100 and TA1535, using the standard plate incorporation method. In the present study, a specialized "treat and plate" procedure (also referred to as "treat and wash") was employed, which is commonly used for assessing the mutagenicity of peptide test articles. The assay was performed in one phase, using the treat and plate modification of the pre-incubation method, to evaluate the mutagenic potential of the test article. This modification essentially entails a shorter period of exposure of the test system to the test article, which minimizes the possibility of obtaining false-positive results due to metabolism of the peptide and release of histidine and/or tryptophan.

Strain	S9 Activation	Positive Control	Concentration (μg/plate)
TA1535	Rat	2-aminoanthracene (Sigma Aldrich Chemical Co., Inc.) Lot No. 03403ED Exp. Date 22-Jan-2012 CAS No. 613-13-8 Purity 99.8%	1.0
TA100			2.0
TA100, TA1535	None	sodium azide (Sigma Aldrich Chemical Co.) Lot No. 71980 Exp. Date 28-Dec-2010 CAS No. 26628-22-8 Purity 99.8%	1.0

Study Validity

- Tester strains: All salmonella tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the uvrB gene. Cultures of TA100 must demonstrate the presence of the pKM101 plasmid R-factor.
- Negative controls values: All cultures must demonstrate the characteristic mean number of spontaneous revertants in the untreated and vehicle controls – TA100: 80-240, TA1535: 5-45.
- Tester strain titers: Tester strain culture titers must be $\geq 0.3 \times 10^9$ cells/ml.
- Positive control values: The mean of each positive control must exhibit ≥ 3X increase in the number of revertants over the mean value of the respective vehicle control.
- Toxicity: A minimum of 3 non-toxic dose levels is required to evaluate assay data. A dose toxic level is considered if 1) >50% reduction in the mean number of revertants/plate (accompanied by an abrupt dose-dependent drop) as compared to the mean vehicle control value and/or if 2) at least a moderate reduction in the background lawn (toxicity and degree of precipitation code: 3, 4, or 5).

Reference ID: 3923456

Study Results

Mutagenicity assay

The doses tested were 15, 50, 150, 500, 1500, and 5000 μg per plate. Slight increases in revertant counts were seen with TA100 with or without S9 (1.4x or 1.5x max increases at 1500 or 5000 μg per plate). However, these increases did not meet the criteria required for evaluation as positive (i.e. dose-related increases with a max response of ≥ 2x the mean vehicle control value. Therefore, the responses with TA100 were equivocal. On the other hand, positive mutagenic responses were observed with TA1535 with or without S9 (6.9x and 7.6x increases at 5000 μg per plate respectively). No positive mutagenic responses were seen with the remaining test conditions.

Table 42 Genetox Study No 4169-NC-113: KAI-4169 (Etelcalcetide) was Mutagenic in TA1535 Strain

Mutagenicity Assay without S9 activation

Study Number: AC33FX.507.BTL
Experiment: B1
Exposure Method: Pre-incubation assay

Study Code: AC33FX
Date Plated: 3/12/2010
Evaluation Period: 3/18/2010 to 3/22/2010

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA100	**KAI-4169**	5000 μg	*133*	*15*	*1.4*	118^{A} 3, 147^{A} 3, 134^{A} 3
		1500 μg	*151*	*21*	*1.5*	174^{A} 2, 134^{A} 2, 146^{A} 2
		500 μg	*85*	*6*	*0.9*	78^{A}, 89^{A}, 89^{A}
		150 μg	*128*	*12*	*1.3*	142^{A}, 118^{A}, 125^{A}
		50 μg	*122*	*61*	*1.2*	102^{A}, 74^{A}, 191^{A}
		15 μg	*99*	*31*	*1.0*	74^{A}, 133^{A}, 89^{A}
	10 mM succinate buffer at pH 4.5 ±0.1	350 μL	*98*	*5*		93^{A}, 102^{A}, 99^{A}
	Untreated Control		*88*	*6*		93^{A}, 89^{A}, 82^{A}
TA1535	**KAI-4169**	5000 μg	*68*	*11*	*7.6*	62^{A} 3, 80^{A} 3, 61^{A} 3
		1500 μg	*62*	*0*	*6.9*	62^{A} 2, 62^{A} 2, 62^{A} 2
		500 μg	*39*	*16*	*4.3*	57^{A}, 32^{A}, 28^{A}
		150 μg	*21*	*3*	*2.3*	20^{A}, 24^{A}, 18^{A}
		50 μg	*14*	*1*	*1.6*	14^{A}, 15^{A}, 14^{A}
		15 μg	*9*	*3*	*1.0*	10^{A}, 11^{A}, 5^{A}
	10 mM succinate buffer at pH 4.5 ±0.1	350 μL	*9*	*2*		9^{A}, 11^{A}, 8^{A}
	Untreated Control		*9*	*1*		9^{A}, 9^{A}, 8^{A}
TA100	**SA**	48 μg/mL	*749*	*23*	*7.6*	739^{A}, 733^{A}, 776^{A}
TA1535	**SA**	48 μg/mL	*298*	*29*	*33.1*	332^{A}, 284^{A}, 279^{A}

Key to Positive Controls

SA	sodium azide

Key to Plate Postfix Codes

3	Moderately reduced background
2	Slightly reduced background

Key to Automatic & Manual Count Flags

^{M}: Manual count ^{A}: Automatic count

Mutagenicity Assay with S9 activation

Study Number: AC33FX.507.BTL
Experiment: B1
Exposure Method: Pre-incubation assay

Study Code: AC33FX
Date Plated: 3/12/2010
Evaluation Period: 3/18/2010 to 3/22/2010

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA100	**KAI-4169**	5000 µg	*132*	*7*	*1.5*	126[A], 140[A], 130[A]
		1500 µg	*123*	*21*	*1.4*	147[A], 106[A], 116[A]
		500 µg	*88*	*9*	*1.0*	93[A], 93[A], 78[A]
		150 µg	*73*	*10*	*0.8*	62[A], 75[A], 82[A]
		50 µg	*78*	*8*	*0.9*	82[A], 69[A], 84[A]
		15 µg	*65*	*4*	*0.7*	69[A], 64[A], 61[A]
	10 mM succinate buffer at pH 4.5 ±0.1	350 µL	*88*	*25*		116[A], 69[A], 80[A]
	Untreated Control		*83*	*11*		96[A], 78[A], 75[A]
TA1535	**KAI-4169**	5000 µg	*48*	*9*	*6.9*	47[A], 40[A], 57[A]
		1500 µg	*29*	*6*	*4.1*	31[A], 33[A], 22[A]
		500 µg	*20*	*5*	*2.9*	24[A], 14[A], 22[A]
		150 µg	*12*	*2*	*1.7*	14[A], 10[A], 11[A]
		50 µg	*10*	*1*	*1.4*	11[A], 10[A], 10[A]
		15 µg	*7*	*2*	*1.0*	5[A], 9[A], 8[A]
	10 mM succinate buffer at pH 4.5 ±0.1	350 µL	*7*	*3*		6[A], 11[A], 5[A]
	Untreated Control		*6*	*2*		8[A], 4[A], 6[A]
TA100	**2AA**	15 µg/mL	*404*	*17*	*4.6*	389[A], 400[A], 422[A]
TA1535	**2AA**	50 µg/mL	*31*	*2*	*4.4*	31[A], 29[A], 32[A]

Key to Positive Controls

2AA 2-aminoanthracene

Key to Automatic & Manual Count Flags

[M]: Manual count [A]: Automatic count

Study No 4169-NC-123 (clinical batch)

Study title: A Bacterial Reverse Mutation Assay of KAI-4169 (Etelcalcetide) Lot # F4169KP0901A Performed with Tester Strains TA100 and TA1535 (GLP, testing facility: (b) (4)

Study description:
One clinical batch of etelcalcetide (Lot #F4169KP0901A, 98.3% purity) was tested in the Bacterial Reverse Mutation Assay using *Salmonella typhimurium* tester strains TA100 and TA1535 in the presence and absence of a metabolic activation system derived from Aroclor-induced rat liver (S9). Etelcalcetide has previously been tested in the standard plate incorporation method (Study No. 4169-NC-104) and a modified treat and plate method (Study No. 4169-NC-113) and was shown to be positive in tester strains TA1535 and TA100. Both of those studies were conducted with a nonclinical batch of etelcalcetide (Lot F4169KP0901B, 95.2% purity). This present study was conducted with the clinical batch of etelcalcetide to determine whether this material produces similar or different responses. The assay was performed in two phases (initial toxicity-mutation and confirmatory mutagenicity) using the plate incorporation method.

Study findings:

- In the initial toxicity-mutation assay, the maximum dose tested was 5,000 µg per plate. The test article formed soluble and clear solutions in the vehicle over the concentration range of 0.015 to 50 mg/mL. Positive mutagenic responses were observed with tester strain TA100 in the absence of S9 activation (3.4-fold maximum increase) and with tester strain TA1535 in the presence and absence of S9 activation (15.9- and 12.5-fold

maximum increases, respectively). No positive mutagenic response was observed with tester strain TA100 in the presence of S9 activation. Neither precipitate nor appreciable toxicity was observed.

- In the confirmatory mutagenicity assay, the dose levels tested were 50, 150, 500, 1500 and 5000 µg per plate. As observed in the initial assay, positive mutagenic responses were observed with tester strain TA100 in the absence of S9 activation (3.1-fold maximum increase) and with tester strain TA1535 in the presence and absence of S9 activation (11.5- and 13.5-fold maximum increases, respectively). No positive mutagenic response was observed with tester strain TA100 in the presence of S9 activation. Neither precipitate nor appreciable toxicity was observed.
- Etelcalcetide (Lot # F4169KP0901A) caused positive mutagenic responses (ranging from 3.1- to 15.9-fold maximum increases) with tester strain TA100 in the absence of S9 and with tester strain TA1535 in the presence and absence of S9.
- This study suggested that the mutagenicity induced by etelcalcetide was not likely due to an impurity.

Study No 4169-NC-126 (impurity)

Study title: A Bacterial Reverse Mutation Assay of KAI-4169 (Etelcalcetide) Lot 2501978 Performed with Tester Strains TA100 and TA1535 (GLP, testing facility: (b) (4)

Study description:
Etelcalcetide (Lot 2501978, 98.9% purity) was tested in the Bacterial Reverse Mutation Assay using *Salmonella typhimurium* tester strains TA100 and TA1535 in the presence and absence of S9. This study was conducted with a batch that has a higher purity than those used in the previous studies (Study Nos. 4169-NC-I04 and 4169-NC-123, Lot F4169KP0901B, 95.2% purity), to address whether the mutagenicity is caused by an impurity. The assay was performed in one phase, using the plate incorporation method, to evaluate the mutagenic potential of the test article.

Study findings:

- In the mutagenicity assay, the maximum dose tested was 5,000 µg per plate. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1,500 and 5,000 µg per plate. The test article formed soluble and clear solutions in 10 mM succinate buffer (PH 4.5 ± 0.1) from 0.015 to 50 mg/mL. Positive mutagenic responses were observed with tester strain TA100 in the absence of S9 activation (2.4-fold maximum increase) and with tester strain TA1535 in the presence and absence of S9 activation (8.1- and 9.7-fold maximum increases, respectively). No positive mutagenic response was observed with tester strain TA100 in the presence of S9 activation. No precipitate was observed. Toxicity, reflected by a reduction in revertant counts, was observed at 5,000 µg per plate with tester strain TA100 in the presence of S9 activation only.
- Etelcalcetide (Lot 2501978) was considered to be positive with tester strains TA100 and TA1535. The results are similar to those obtained from previous bacterial mutagenicity studies conducted with other batches of etelcalcetide that have lower purity; hence, the similar results obtained with the high-purity lot tested in this study strongly suggest that the bacterial mutagenicity is unrelated to impurities in KAI -4169.

7.2 *In Vitro* Assays in Mammalian Cells

Study No 4169-NC-105 (pivotal)

Study title: *In Vitro* Mammalian Chromosome Aberration Test

Study no.:	4169-NC-105
Study report location:	KAI Pharmaceuticals (South San Francisco, CA)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 14, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	F4169KP0901B with 95.3% purity

Key Study Findings

- Human peripheral blood lymphocytes (HPBL), with or without S9, were used in the in vitro mammalian chromosome aberration assay.
- Etelcalcetide was concluded to be negative for the induction of structural and numerical chromosome aberrations in both non-activated and S9-activated test systems in HPBL.

Methods

Cell line:	human peripheral blood lymphocytes (HPBL) from a healthy non-smoking 23 yr old adult male
Concentrations in definitive study:	up to 5,000 μg
Basis of concentration selection:	MTD
Negative control:	Succinate buffer (10 mM at pH 4.5) was used as the solvent for dose solution preparation and as the vehicle control.
Positive control:	In the S9-non activated system, mitomycin C (Sigma-Aldrich Lot No. 098K1681) in sterile distilled water (Gibco Lot No. 633348) to stock concentrations of 3 and 6 μg/ml was used. In the S9-activated system, cyclophosphamide (Sigma-Aldrich Lot No. 068K1131) in sterile distilled water to stock concentrations of 0.1 and 0.15 mg/ml was used.
Formulation/Vehicle:	10 ruM succinate buffer, pH 4.5 ±l
Incubation & sampling time:	For preliminary toxicity assay: 4 hrs (with and without S9) and 20 hrs continuously without S9. For the chromosome aberration assay (see table below): 4 or 20 hrs (in the non-activated system) and 4 hr followed by 16 hrs (in the activated system).

Treatment Condition	Treatment Time	Recovery Time	Dose levels (μg/mL)
Non-activated	4 hr	16 hr	313, 625, 1250, 2500, 5000
	20 hr	0 hr	313, 625, 1000, 1250, 4000, 5000
S9-activated	4 hr	16 hr	156, 313, 625, 1000, 1250, 2000, 3000, 4000

Reference ID: 3923456

Study Validity

- The frequency of cells with structural chromosome aberrations in the vehicle controls must be within the historical range for vehicle controls.
- The percentage of cells with chromosome aberrations in the positive control must be statistically increased relative to the solvent control.

Study Results

Preliminary toxicity assay

HPBL were treated for 4 hours up to 5,000 μg/ml with/without S9 and continuously for 20 hours without S9. In S9 (4hr) exposure group, visible precipitate was seen in treatment medium at concentration ≥ 1,500 μg/ml, whereas concentrations ≤ 500 were soluble in treatment medium. In non-S9 (4hr and 20 hr) exposure groups, no precipitate was observed at the end of the treatment period. No substantial toxicity was seen at any dose level in the no non-S9 (4 hr) exposure group. On the other hand, substantial toxicity was observed at ≥ 1,500 μg/ml in the S9 (4 hr) exposure group and at 5,000 μg/ml in the non-S9 (20 hr) exposure group.
The doses for the chromosome aberration assay: 313-5,000 μg/ml for both non-S9 (4 hr and 20 hr) groups, and 156-4,000 μg/ml for S9 (4 hr) group.

Chromosome aberration assay

With the S9 (4hr) exposure group, visible precipitate was seen in treatment medium at concentration ≥ 625 μg/ml, whereas concentrations ≤ 313 were soluble in treatment medium. With non-S9 (4hr and 20 hr) exposure groups, no precipitate was observed at the end of the treatment period. The percentage of cells with structural or numerical aberrations in the etelcalcetide group was not significantly increased relative to solvent control at any dose level. This study concluded that etelcalcetide was not genotoxic in human peripheral blood lymphocytes.

Reference ID: 3923456

Table 43 Genetox Study No 4169-NC-105: KAI-4169 (Etelcalcetide) did not Induce Chromosome Aberrations In Vitro.

SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
Succinate buffer	-S9	4	11.4	200	200	0.000	±0.000	0.0	0.0
KAI-4169									
1250	-S9	4	10.1	200	200	0.000	±0.000	0.0	0.0
2500	-S9	4	10.1	200	200	0.000	±0.000	0.0	0.0
5000	-S9	4	9.0	200	200	0.005	±0.071	0.0	0.5
MMC, 0.6	-S9	4	6.2	200	100	0.280	±0.570	0.0	22.0**
Succinate buffer	+S9	4	9.3	200	200	0.000	±0.000	0.0	0.0
KAI-4169									
156	+S9	4	9.1	200	200	0.000	±0.000	0.0	0.0
313	+S9	4	8.4	200	200	0.000	±0.000	0.0	0.0
625	+S9	4	7.0	200	200	0.000	±0.000	0.0	0.0
CP, 15	+S9	4	2.4	200	100	0.150	±0.386	0.0	14.0**
Succinate buffer	-S9	20	13.4	200	200	0.000	±0.000	0.0	0.0
KAI-4169									
1250	-S9	20	9.8	200	200	0.000	±0.000	0.0	0.0
4000	-S9	20	7.5	200	200	0.000	±0.000	0.0	0.0
5000	-S9	20	6.9	200	200	0.005	±0.071	0.0	0.5
MMC, 0.3	-S9	20	5.4	200	50	0.380	±0.635	0.0	32.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using the Fisher's Exact test.

Study No 4169-NC-112 (gene mutation test, CHO)

Study title: *In Vitro* Mammalian Cell Gene Mutation Test (CHO/HGPRT Assay)

Study no.: 4169-NC-112
Study report location: KAI Pharmaceuticals (South San Francisco, CA)
Conducting laboratory and location: (b) (4)
Date of study initiation: January 29, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: F4169KP0901A with 98.3% purity

Key Study Findings

- KAI-4160 was tested in the CHO/HGPRT mutation assay with or without S9.
- In the preliminary toxicity assay, the max concentration of etelcalcetide was 5,000 µg/ml. There was no visible precipitate in the treatment medium at any concentration. No substantial toxicity was seen at any concentration with or with S9. Based on these findings, the concentrations chosen for the mutagenesis assay ranged from 500-5,000 µg/ml.
- In the mutagenesis assay, no positive response (i.e. treated cultures with mutant frequencies >40 mutants per 10^6 clonable cells) was detected. No visible precipitate or toxicity was seen in treatment medium at any concentrations.
- Etelcalcetide was shown to be negative in the CHO/HGPRT mutation assay.

Methods

Cel line:	CHO-K_1 cells
Concentrations in definitive study:	500, 1,000, 2,000, 4,000, 5,000 μg/mL
Basis of concentration selection:	MTD
Negative control:	Succinate buffer (10 mM at pH 4.5) was used as the solvent for dose solution preparation and as the vehicle control.
Positive control:	In the S9-non activated system, ethyl methanesulfonate (Sigma Lot No. 116K0765) to a final concentration of 0.2 μl/ml was used. In the S9-activated system, benzo(a)pyrene (Sigma Lot No. 007K1025) to a final concentration of 4 μg/ml was used.
Formulation/Vehicle:	Succinate buffer (10 mM at pH 4.5)
Incubation & sampling time:	The CHO cells were exposed for 5 hrs at 37±1°C to the vehicle alone, positive controls and test articles, with or without S9.

Study Validity

- Negative controls: The cloning efficiency of the solvent control must be 50%. The spontaneous mutant frequency in the solvent control must fall within the range of 0-25 mutants per 10^6 clonable cells.
- Positive controls: The positive control must induce a mutant frequency ≥ 3x that of the solvent control and must exceed 40 mutants per 10^6 clonable cells.
- Test article-treated cultures: There must be ≥ 4 analyzable test article concentrations with mutant frequency data. The highest test article concentration must produce 80-90% toxicity, unless limited by solubility or the max required concentration.

Study Results

Preliminary toxicity assay

There was no precipitate in the treatment medium at any concentration up to 5,000 μg/ml. No substantial toxicity (i.e., cloning efficiency ≤ 50%) was seen at any dose level, with or without S9. Cloning efficiency at 5,000 μg/ml relative to the solvent controls was 91% without S9 and 157% with S9. The doses for the mutagenesis assay: 500-5,000 μg/ml for both non-activated and S9-activated cultures.

Mutagenesis assay

No positive responses (i.e. cultures with mutant frequencies > 40 mutants per 10^6 clonable cells) were seen. No visible precipitate was observed the treatment medium at any concentration. No toxicity was observed at any concentrations. Relative cloning efficiency was 88% and 83% at the highest concentration tested with or without S9 respectively. This study concluded that etelcalcetide was not mutagenic in mammalian CHO cells.

Reference ID: 3923456

Table 44 Genetox Study No 4169-NC-112: KAI-4169 (Etelcalcetide) Tested Negative in Gene Mutation Assay In Vitro.

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CHO/HGPRT MUTATION ASSAY
Non-activated (-S9) Study using KAI-4169

Treatment (µg/mL)		PRECIP	Cloning Efficiency Plates Plate Counts 1	2	3	Average Colonies	Cloning Efficiency (%)	Selection (Mutation) Plates Plate Counts 1	2	3	4	5	Average Colonies	Mutants/10^6 Clonable Cells	Relative Total Growth (%)
Solvent	A		66	61	89	66.0	66	0	0	0	0	1	0.4	3.0	100
	B		65	56	59			2	0	0	0	1			
EMS (0.2µL/mL)	A		36	39	55	43.0	43	8	4	10	5	12	7.6	88.4	47
	B		49	47	32			4	7	7	13	6			
500	A		73	70	85	72.5	73	0	1	2	3	1	0.8	5.5	59
500	B		69	69	69			1	0	0	0	0			
1000	A		57	61	60	46.8	47	1	1	2	2	0	1.0	10.7	71
1000	B		27	27	49			2	0	1	0	1			
2000	A		65	70	72	56.0	56	0	2	0	1	0	0.5	4.5	84
2000	B		53	36	40			0	1	0	0	1			
4000	A		69	103	103	88.0	88	5	1	7	4	3	2.0	11.4	68
4000	B		83	90	80			0	0	0	0	0			
5000	A		62	57	46	49.8	50	0	0	1	1	0	0.6	6.0	88
5000	B		51	45	38			0	0	2	2	0			

Solvent = succinate buffer

A and B are duplicate cultures

$$\text{Cloning efficiency} = \frac{\text{average colonies}}{\text{100 cells/dish}}$$

$$\text{Mutants/}10^6\text{ clonable cells} = \frac{\text{average mutant colonies}}{\text{cloning efficiency X 2 x }10^4\text{ cells}} \times 10^6$$

A and B are duplicate cultures

CHO/HGPRT MUTATION ASSAY
Activated (+S9) Study using KAI-4169

Treatment (µg/mL)		PRECIP	Cloning Efficiency Plates Plate Counts 1	2	3	Average Colonies	Cloning Efficiency (%)	Selection (Mutation) Plates Plate Counts 1	2	3	4	5	Average Colonies	Mutants/10^6 Clonable Cells	Relative Total Growth (%)
Solvent	A		76	95	74	71.8	72	0	1	0	0	1	1.5	10.4	100
	B		54	68	64			4	4	2	0	3			
B(a)P (4µg/mL)	A		50	54	41	48.7	49	27	37	36	30	38	33.3	342.1	91
	B		45	41	61			33	36	38	32	26			
500	A		75	72	75	62.2	62	0	1	0	0	0	0.1	0.8	96
500	B		46	63	42			0	0	0	0	0			
1000	A		52	95	59	59.8	60	0	0	0	0	0	2.1	17.5	82
1000	B		45	52	56			6	5	4	3	3			
2000	A		46	50	53	52.5	53	0	0	0	0	0	0	0	81
2000	B		60	42	64			0	0	0	0	0			
4000	A		58	43	57	54.2	54	1	1	0	0	0	1.0	9.2	90
4000	B		41	55	71			3	2	3	0	0			
5000	A		50	57	77	59.5	60	1	0	1	0	1	0.8	6.7	83
5000	B		63	42	68			1	2	1	0	1			

Solvent = succinate buffer

A and B are duplicate cultures

$$\text{Cloning efficiency} = \frac{\text{average colonies}}{\text{100 cells/dish}}$$

$$\text{Mutants/}10^6\text{ clonable cells} = \frac{\text{average mutant colonies}}{\text{cloning efficiency X 2 x }10^4\text{ cells}} \times 10^6$$

A and B are duplicate cultures

Study No g-11-063 (gene mutation test, V79)

Study title: Gene mutation test of ONO-5163HCL in V79 cells (non-GLP, testing facility: Ono Pharmaceutical, Osaka, Japan)

Study description:
This study assessed the potential of etelcalcetide (ONO-5163HCL; Lot F4169KP1001B, purity: 98.7%) to induce genetic mutations. A gene mutation test was performed in V79 cells (derived from Chinese hamster lung) using the hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene as the indicator. A cytotoxicity test was performed to determine the concentrations for use in the gene mutation test. To assess cytotoxicity, the cell density was determined after 2 days of culture. To assess mutagenicity, cells were reseeded after the 6-day expression period and were cultured for 9 days in medium containing 5 µg/mL 6-thioguanine (6-TG). The number of mutant colonies was then counted, the plating efficiency of the reseeded cells was calculated, and the mutation frequency was calculated from these parameters.

Study findings:
No cytotoxicity was noted in any of the groups treated with etelcalcetide (ONO-5163HCL) for 4 hours at concentrations up to 5,000 µg/mL with and without S9 mix. Visual inspection of the culture medium revealed no precipitation at the beginning or end of the treatment period in the cultures without S9 mix. In the cultures with S9 mix, no precipitation was seen in the culture medium at the beginning of the treatment period, while there was apparent precipitation of S9 at the end of the treatment period.

In all etelcalcetide (ONO-5163HCL) groups incubated with and without S9 mix, the mutation frequency was less than 3 times that in the negative control group and was within the range of the historical negative control data. These findings indicated a negative result. A confirmation test was performed under the same conditions and it also yielded a negative result. Etelcalcetide (ONO-5163HCL) did not induce genetic mutations in V79 cells under the conditions of the present study.

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

Study No 4169-NC-122 (Pivotal)

Study title: Rat Bone Marrow Erythrocyte Micronucleus Test Following Intravenous Infusion of KAI-4169 (Etelcalcetide)

Study no:	4169-NC-122
Study report location:	KAI Pharmaceuticals (South San Francisco, CA)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 20, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	F4169KP0901A with 98.3% purity

Key Study Findings

- The IV infusion of etelcalcetide over 30 min on 3 days at doses ≤5 mg/kg/day did not induced a significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow of male rats.
- Etelcalcetide was negative in the rat micronucleus assay.

Reference ID: 3923456

Methods

Doses in definitive study:	1.25, 2.5 and 5 mg/kg
Frequency of dosing:	Daily for three consecutive days
Route of administration:	Intravenous route (30 min infusion)
Dose volume:	5 mL/kg/day
Formulation/Vehicle:	Succinate buffer (10 mM at pH 4.5)
Species/Strain:	polychromatic erythrocytes (MPCEs) in the bone marrow of male Sprague-Dawley rats
Number/Sex/Group:	5 male rats
Satellite groups:	n/a
Basis of dose selection:	Max tolerated dose (MTD)
Negative control:	Succinate buffer (10 mM at pH 4.5) was used as the solvent for dose solution preparation and as the vehicle control.
Positive control:	Cyclophosphamide (CP) at a dose of 40 mg/kg 24 hrs prior to bone marrow collection

Group	Treatment (5 mL/kg/treatment)	Number of Rats Dosed
	Test Article: KAI-4169	
1	5 mg/kg/day	3
2	7.5 mg/kg/day	3
3	10 mg/kg/day	3
4	12.5 mg/kg/day	3

Treatment (5 mL/kg/treatment)	Number of Male Rats Dosed	Number of Male Rats Used for Bone Marrow Collection at 24 hr Post-Last Infusion
Vehicle Control: 10 mM succinate buffer, pH 4.5 ± 0.1	5	5
Test Article: KAI-4169		
Low dose (1.25 mg/kg)	5	5
Mid dose (2.5 mg/kg)	5	5
High dose (5 mg/kg)	5	5
Positive Control: CP (40 mg/kg)*	5	5*

*Animals were intravenously administered CP at ~ 24 hours prior to termination for bone marrow collection.

Study Validity

- The incidence of micronucleated polychromatic erythocytes in the vehicle control groups did not exceed the historical vehicle control range.
- The incidence of micronucleated polychromatic erythrocytes in the male positive control groups was significantly increased relative to the respective vehicle control group.

Study Results

Dose range finding phase (to identify the maximum tolerated dose of etelcalcetide), 3 cannulated male rats per group were infused with etelcalcetide at 5, 7.5, 10 or 12.5 mg/kg/day for 3 days.

- Mortality was observed at 7.5, 10 and 12.5 mg/kg/day groups, except 5 mg/kg/day group. The clinical signs, such as piloerection, lethargy, tremors, and etc) were seen in all treatment groups. These AEs were attributed to pronounced hypocalcemia.
- There were decreases in mean body weight in rats given 7.5, 10, and 12.5 mg/kg/day (by 6.0%, 11.3%, and 10.1% respectively).

- Based on the mortality and clinical signs observed, 5 mg/kg/day was determined to be the maximum tolerated dose for etelcalcetide and was used as the high dose in the definitive micronucleus study.

Definitive phase (to evaluate the potential of etelcalcetide to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow), 5 cannulated male rats per group were infused with etelcalcetide at 0, 1.25, 2.5 or 5 mg/kg/day for 3 days.

- No mortality was observed at any treatment groups.
- The clinical signs, such as piloerection, were seen in 5 mg/kg/day group on Days 2 and 3.
- No appreciable reductions in the ratio of polychromatic erythrocytes to total erythrocytes were seen in bone marrow of etelcalcetide treated groups, suggesting no hematopoietic toxicity with all doses tested.
- No statistically significant increase in the incidence of micronucleated polychromatic erythrocytes was observed at 24 hrs after the last infusion, in test article groups, relative to the vehicle control group.

Table 45 Genetox Study No 4169-NC-122: KAI-4169 (Etelcalcetide) was Tested Negative in Micronucleus Assay In Vivo.

Following Intravenous Infusion of KAI-4169 in Male Sprague Dawley Rats

		Number of Animals With Observed Signs/Number of Surviving Animals									
		Day 0			Day 1			Day 2			
Treatment **	Observation	During Dosing	Post-Dose	Pre-Dose	During Dosing	Post-Dose	Pre-Dose	During Dosing	Post-Dose	Day 3	Number of Animals Found Dead/Total Number of Animals Dosed
10 mM succinate buffer, pH 4.5 ± 0.1	Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	0/5
KAI-4169 1.25 mg/kg/day	Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	0/5
2.5 mg/kg/day	Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	0/5
5 mg/kg/day	Normal Piloerection	5/5 0/5	5/5 0/5	5/5 0/5	5/5 0/5	5/5 0/5	5/5 0/5	5/5 0/5	0/5 5/5	0/5 5/5	0/5
Cyclophosphamide 40 mg/kg	Normal	N/A*	N/A*	N/A*	N/A*	N/A*	N/A*	N/A*	5/5	5/5	0/5

*Observations are not applicable since animals were dosed only once on Study Day 2.
** The vehicle and test article were administered on three consecutive days at a volume of 5 mL/kg/day and the positive control was administered at a volume of 10 mL/kg (~24 hours prior to bone marrow collection).

Summary of Bone Marrow Micronucleus Analysis
Following Intravenous Infusion of KAI-4169 in Male Sprague Dawley Rats

Treatment**	Sex	Time (hr)	Number of Animals	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Number of MPCE/1000 PCE (Mean +/- SD)	Number of MPCE/PCE Scored
10 mM succinate buffer, pH 4.5 ± 0.1	M	24	5	0.465 ± 0.03	---	0.7 ± 0.27	7 / 10000
KAI-4169 1.25 mg/kg/day	M	24	5	0.474 ± 0.03	2	0.5 ± 0.50	5 / 10000
2.5 mg/kg/day	M	24	5	0.486 ± 0.03	5	0.4 ± 0.42	4 / 10000
5 mg/kg/day	M	24	5	0.465 ± 0.04	0	0.4 ± 0.22	4 / 10000
Cyclophosphamide 40 mg/kg	M	24	5	0.339 ± 0.03	-27	21.7 ± 3.99	*217 / 10000

*Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables)
** The vehicle and test article were administered on three consecutive days at a volume of 5 mL/kg/day and the positive control was administered at a volume of 10 mL/kg (~ 24 hour prior to bone marrow collection).

Induction of Micronucleated Polychromatic Erythrocytes in Bone Marrow Collected 24 Hours Following Intravenous Infusion of KAI-4169 in Male Sprague Dawley Rats

Treatment **	Sex	Animal Number	PCE/Total Erythrocytes	Micronucleated PCE (Number/PCE scored)
10 mM succinate buffer, pH 4.5 ± 0.1				
	M	201	0.513	2 / 2000
		202	0.438	1 / 2000
		203	0.475	1 / 2000
		204	0.431	2 / 2000
		205	0.466	1 / 2000
KAI-4169 1.25 mg/kg/day	M	206	0.436	2 / 2000
		207	0.508	0 / 2000
		208	0.444	1 / 2000
		209	0.488	2 / 2000
		210	0.494	0 / 2000
2.5 mg/kg/day	M	211	0.515	1 / 2000
		212	0.440	1 / 2000
		213	0.461	2 / 2000
		214	0.511	0 / 2000
		215	0.503	0 / 2000
5 mg/kg/day	M	216	0.498	1 / 2000
		217	0.408	0 / 2000
		218	0.447	1 / 2000
		219	0.494	1 / 2000
		220	0.478	1 / 2000
Cyclophosphamide monohydrate 40 mg/kg	M	221	0.319	42 / 2000
		222	0.390	48 / 2000
		223	0.314	33 / 2000
		224	0.334	54 / 2000
		225	0.336	40 / 2000

**The vehicle and test article were administered on three consecutive days at a volume of 5 mL/kg/day and the positive control was administered at a volume of 10 mL/kg (~24 hours prior to bone marrow collection).

7.4 Other Genetic Toxicity Studies

Study No 116904 (28 day Muta™ Mouse study)

Study title: AMG 416 (Etelcalcetide): 28-Day Subcutaneous Mutagenicity Study in the Male Muta™Mouse (GLP, testing facility: (b) (4)

Study description:
Etelcalcetide was tested for its ability to induce gene mutation in the *lacZ* transgene in liver and bone marrow from male Muta™ mice (CD2-lacZ80/HazfBR strain). In the range-finding component of the study, Muta™ mice were dosed once daily by the SC route with vehicle and at dose levels of 8 mg/kg for 7 days, or 4 or 6 mg/kg for up to 28 days. Based on mortality and clinical signs of toxicity, 4 mg/kg was determined to be the maximum tolerated dose for etelcalcetide in Muta™ mice. In the main study, Muta™ mice were dosed once daily by the SC route with etelcalcetide at 1, 2, or 4 mg/kg for 28 days. A vehicle control and untreated control group were also included. Animals were euthanized 3 days after the last dose. Satellite animals were included for exposure assessment.

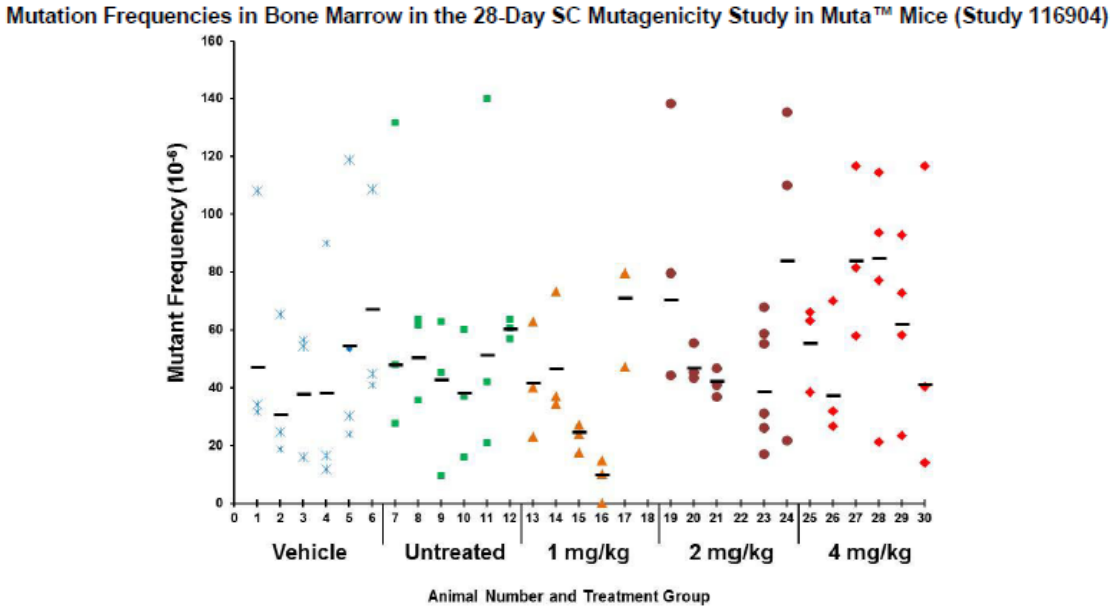
Study findings:
- Mortality or clinical signs of toxicity were not observed.
- The increases in C_{max} and AUC were proportional to dose, and AUC exposures after the last dose were comparable to exposures on day 1.
- In liver, there were no statistically significant differences in mean mutant frequency between groups treated with etelcalcetide and the concurrent vehicle control.
- In bone marrow, there were also no statistically significant differences between groups treated with etelcalcetide and the concurrent vehicle control, but a significant ($p < 0.05$) dose response test was observed. The dose response test may be influenced by an unusually low mutation frequency at 1 mg/kg. As all bone marrow mutation frequencies for all animals treated with etelcalcetide were within the bone marrow historical control range and were comparable with the concurrent vehicle control range, the significant dose response was considered to be of no biological relevance.
- There were no significant increases in mutant frequency over concurrent controls, and data for all treated animals were within the historical control range. Therefore it was concluded that etelcalcetide does not induce mutations in either liver or bone marrow following 28 days of SC dosing in the mouse.

Study Day	Dose (mg/kg)	C_{max} (ng/mL)	AUC (ng•hr/mL)
Day 1	1	534	596
	2.5	980	1040
	5.0	1890	2150
Day 28	1	373	472
	2.5	556	800
	5.0	1550	1980

AUC = area under the concentration-time curve from time zero until last acquired timepoint (24 hr); C_{max} = maximum observed concentration.

Reference ID: 3923456

Table 46 Genetox Study No 116904: AMG416 (Etelcalcetide) did not Induce Mutations in Liver or Bone Marrow Following 28 Days of SC Dosing.



8 Carcinogenicity

Study No 116848: 2 year rat study

CDER's Executive Carcinogenicity Assessment Committee (Exec CAC) agreed that the study was acceptable despite the early termination of the study in males and females due to the vehicle control. The Committee concurred that there were no drug-related neoplasms in the study.

Study title: AMG 416 (Etelcalcetide): 24 month subcutaneous carcinogenicity study in the Sprague-Dawley rat

Study no.:	116846
Study report location:	Amgen (Thousand Oaks, CA)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 21, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lot 1041722 with 99.3% purity Lot 1037979 with 98.9% purity
CAC concurrence:	Yes

Note: The dose selection of this study was based on dose range finding studies, Study No 4169-NC-147 (4 week) and Study No 4169-NC-148 (14 week).

Key Study Findings

- The carcinogenic potential of etelcalcetide was studied in SD rats. Males and females were dosed at 0 (saline), 0 (vehicle control), 0.2, 0.4, 0.8 and 1.6 mg/kg/day once daily for 104 weeks via subcutaneous injection. The protocol was reviewed and doses up to 0.8 mg/kg/day were recommended by Exec CAC on April 18, 2012.

- Two high dose female dose groups were terminated on Week 89 due to group number fell below 15. The study was terminated on Week 92 due to vehicle control group fell below 20.
- In main study groups, two high dose groups (0.8 and 1.6 mg/kg/day) had less than 15 animals and were terminated on Week 89 (vs. 25 in saline group on Week 92, where the study was terminated due to vehicle control group fell below 20. Mortalities at 1.6 mg/kg/day were observed in a 13 week DRF study, attributable to hypoglycemia. However, there was no AMG-416 related clinical observation or food consumption noted up to Week 92.
- The FDA statistical reviewer commented that the tests did show a statistically significant dose response relationship in mortality across controls and treated groups in female rats when compared to the saline control (p=0.021). The pairwise comparisons results did show statistically significant increased mortality in two highest dose groups (0.8 and 1.6 mg/kg/day) when compared to the saline control in female rats (p=0.008 and 0.0179 respectively). The tests didn't show a statistically significant dose response relationship in mortality across control and treated groups in male rats.
- Etelcalcetide-related decreases in mean body weight were noted in males at 1.6 mg/kg/day when compared to vehicle controls. This is attributed to hypocalcemia, an expected pharmacologic effect of etelcalcetide.
- No etelcalcetide-related gross findings, tumors, or non-neoplastic microscopic findings were observed.
- Increased incidence of fibrosis at the injection site (scapular area) was noted in vehicle control and etelcalcetide injected males and females, but with little effect in the saline control group. This change was considered vehicle-related and not etelcalcetide-related.
- In TK groups, at Week 26, etelcalcetide exposure (AUC_{0-t}) values of 0.228, 0.541, 0.867, and 2.02 µg*hr/mL with maximum observed drug concentration (C_{max}) values of 0.118, 0.265, 0.449, and 1.00 µg/mL at the 0.2, 0.4, 0.8, and 1.6 mg/kg/day dose levels were measured, respectively for combined sexes.
- The study showed that etelcalcetide was non tumorigenic in SD rats of either sex following once daily subcutaneous injections for 89 or 92 weeks.

Adequacy of Carcinogenicity Study
Yes, based on Exec CAC concurrence with the study doses up to 0.8 mg/kg/day.

Appropriateness of Test Models
Yes

Evaluation of Tumor Findings
Significance was based on the FDA statistical reviewer's p-values for dose response relationship or pairwise comparisons. The threshold for statistical significance - Common tumors (background rate greater than 1%) require p<0.005 for trend and p<0.01 for pairwise. Rare tumors (background rate of 1% or less) require p<0.025 for trend, p<0.05 for pairwise.

Evaluation Criteria for Common and Rare Tumors	
Test for Positive Trends	**Control-High Pair-wise Comparisons**
Common and rare tumors will be tested at 0.005 and 0.025 significance levels, respectively	Common and rare tumors will be tested at 0.01 and 0.05 significance levels, respectively

Methods
Doses: 0.2, 0.4, 0.8 and 1.6 mg/kg/day

Frequency of dosing:	Once daily
Dose volume:	1 mL/kg
Route of administration:	Subcutaneous injection
Formulation/Vehicle:	0.27% sodium succinate dibasic hexahydrate (2.7 g/L), 2% D-mannitol (20 g/L), 1% glycine (10 g/L), 1% trehalose (10 g/L), and 0.9% benzyl alcohol (9 g/L) in sterile water for injection
Basis of dose selection:	MTD; Mortality and reduction in body weight gain observed in mid-dose/high-dose animals at ≥ 1.6 mg/kg/day. Doses of 0 (saline), 0 (vehicle control), 0.2, 0.4 and 0.8 mg/kg/day are recommended by Exec CAC.
Species/Strain:	SD rats
Number/Sex/Group:	65/sex/group
Age:	4 weeks old
Animal housing:	AAALAC-accredited facility with controlled room at 68-79°F, 30-70% relative humidity, and 12 h light/12 h dark cycle
Paradigm for dietary restriction:	*Ad libitum* access to drinking water and in meal
Dual control employed:	Saline and vehicle
Interim sacrifice:	No
Satellite groups:	TK
Deviation from study protocol:	No significant deviations were reported.

Table 47 Carci Study No 116848: 2 Year Rat Study Design.

Study Design Table					
Group Number	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals	
				Male	Female
Main Study					
1	0 (saline)	1	0	65	65
2	0 (vehicle)	1	0	65	65
3	0.2	1	0.20	65	65
4	0.4	1	0.40	65	65
5	0.8	1	0.80	65	65
6	1.6	1	1.60	65	65
Toxicokinetics					
7	0 (saline)	1	0	3+2[a]	3+2[a]
8	0 (vehicle)	1	0	3+2[a]	3+2[a]
9	0.2	1	0.20	6+3[a]	6+3[a]
10	0.4	1	0.40	6+3[a]	6+3[a]
11	0.8	1	0.80	6+3[a]	6+3[a]
12	1.6	1	1.60	6+3[a]	6+3[a]
Sentinel Animals					
98	-	-	-	25[b]	25[b]

[a]Additional TK animals included as replacement animals.
[b]Pretest and at 6, 12, 18 months and termination on study, 2 to 5 surviving males and females from the sentinel group were terminated and assessed for sero-reactivity to rodent pathogens.

Observations and Results

Mortality (twice daily)

The study was halted early at Week 92 due to the number of control animals in male and female groups reaching 20/group. In males, survival over the course of the study at end of Week 92 was 38% (25/65), 32% (20/65), 38% (25/65), 25% (16/65), 35% (23/65), and 29% (19/65) for saline control, vehicle control, 0.2, 0.4, 0.8, and 1.6 mg/kg/day etelcalcetide respectively. In

females, survival over the course of the study at end of Week 89 was 38% (25/65), 32% (20/65), 32% (21/65), 28% (18/65), 23% (15/65), and 23% (15/65) for saline control, vehicle control, 0.2, 0.4, 0.8, and 1.6 mg/kg/day etelcalcetide respectively for females.

In females, two highest groups (0.8 and 1.6 mg/kg/day) were terminated earlier than other groups on Week 89 when its group number hits below 15 (vs. 25 in saline group on Week 92, where the study was terminated due to vehicle control group fell below 20). Mortalities at 1.6 mg/kg/day were observed in a 13 week DRF study, attributable to hypocalcemia.

FDA statistical reviewer commented that the tests did show a statistically significant dose response relationship in mortality across controls and treated groups in female rats when compared to the Saline control (p=0.021). The pairwise comparisons results did show statistically significant increased mortality in two highest dose groups (0.8 and 1.6 mg/kg/day) when compared to the saline control in female rats (p=0.008 and 0.0179 respectively). The tests didn't show a statistically significant dose response relationship in mortality across control and treated groups in male rats.

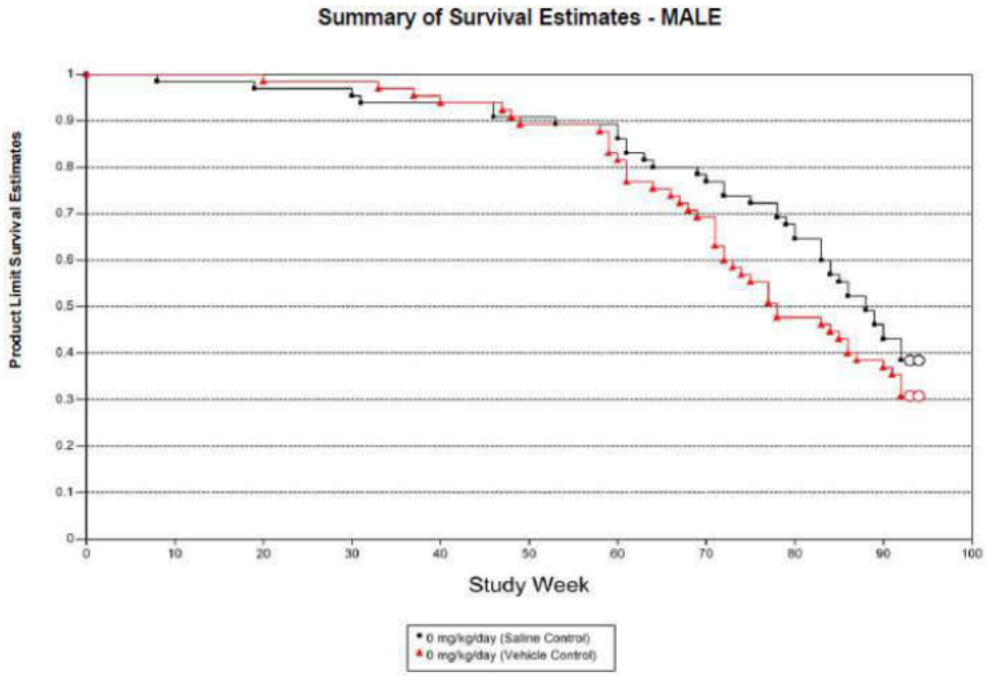
In males, the most common causes of death or moribundity across all groups were pituitary tumors or could not be determined. In females, the most common causes of death or moribundity across all groups were pituitary tumors and mammary tumors. This is an anticipated finding as spontaneous pituitary tumors and mammary tumors are well known causes of early mortality in long-term rat studies. In addition, the vehicle control group has lower survival rate when compared to saline control group. Only vehicle control was used in all toxicity studies; no saline group was included.

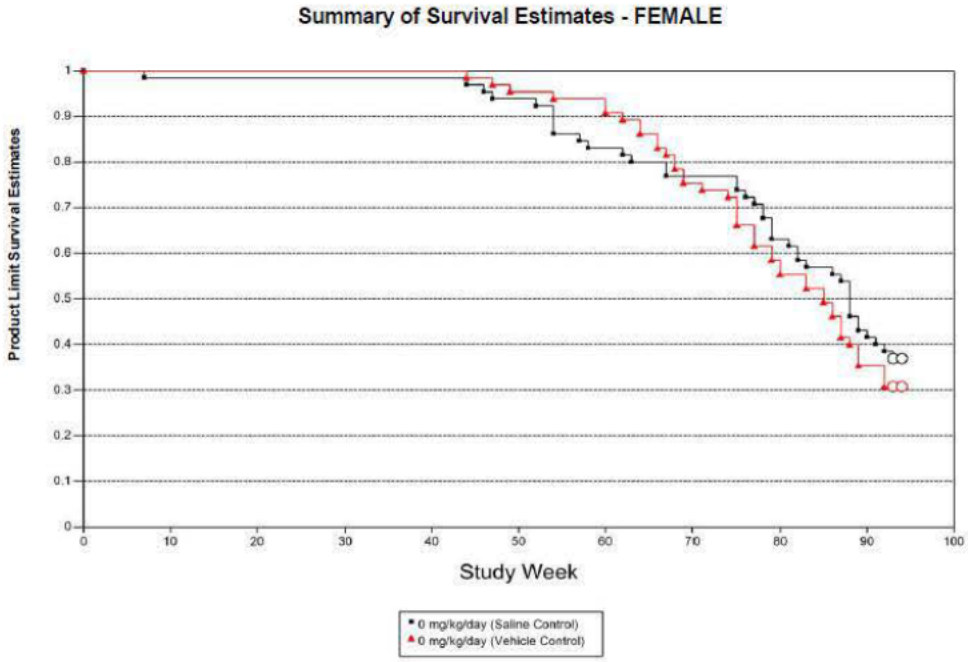
Total Survival (end of Week 92 Feb 19, 2014)		
Group/Dose Level	Male	Female
1 (Saline Control)	25/65	25/65
2 (Vehicle Control)	20/65	20/65
3 (0.2 mg/kg)	25/65	21/65
4 (0.4 mg/kg)	16/65	18/65
5 (0.8 mg/kg)	23/65	0/65*
6 (1.6 mg/kg)	21/65	0/65**

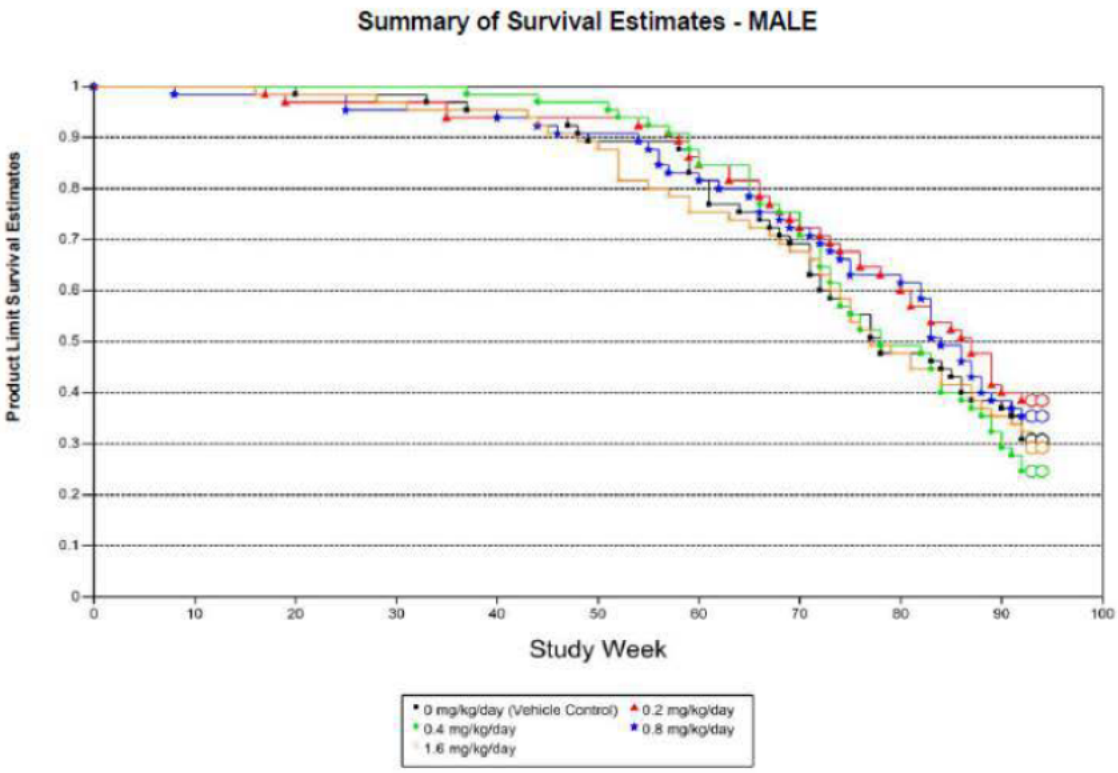
*Group 5 females terminated at n=15 on 1/30/2014

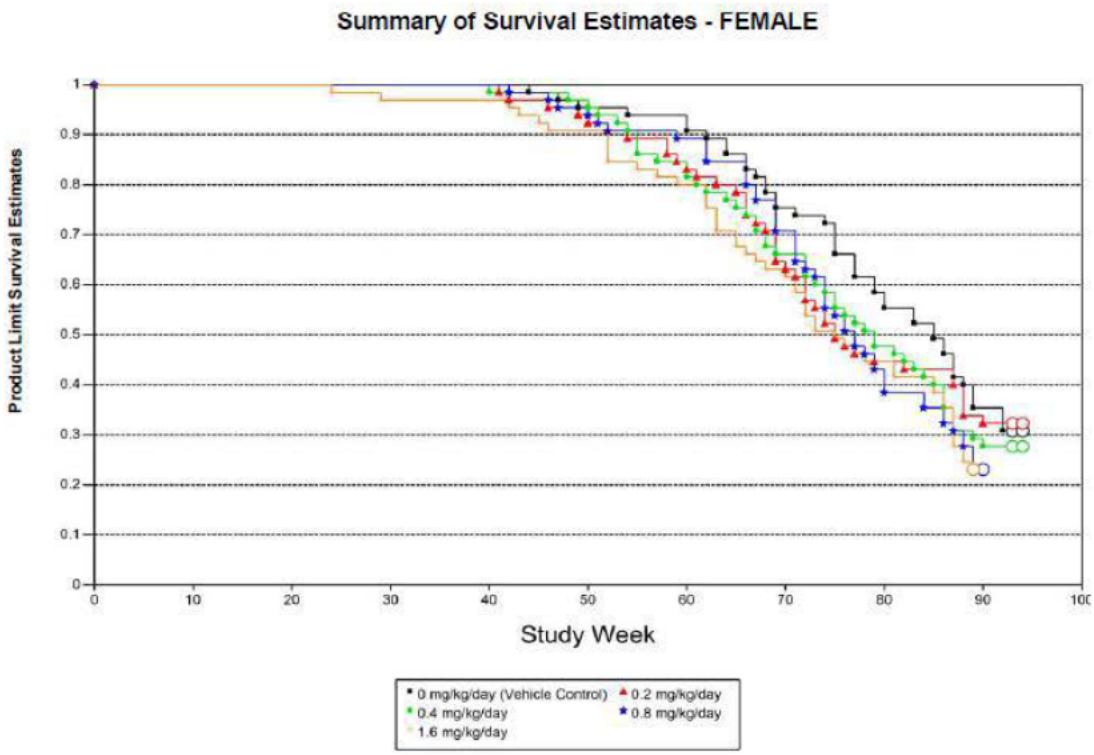
**Group 6 females terminated at n=15 on 1/29/2014

Figure 10 Carci Study No 116848: Survival.









Clinical Signs (once weekly)
No etelcalcetide related clinical signs were noted.

Some common findings in both saline and vehicle control groups and all etelcalcetide-treated groups included aggressive behavior, hypersensitivity to touch, vocalization, rigid body, hunched posture, swelling in numerous regions of the body, sparse hair, and scabbed areas. These findings were associated with the expected spontaneous reaction to repeated daily SC injections and subsequent effects from repeated injections or were typically seen in laboratory rats of this age and strain.

Body Weights (weekly through first 14 weeks, every other week through 28 weeks, monthly thereafter)
An etelcalcetide-related decrease in mean body weight was noted in males at 1.6 mg/kg/day (-11.7%, p<0.05) and females (-15.1%, p<0.01) when compared to vehicle controls (other groups were within 10% or less of vehicle control mean body weight) during the course of this study. Changes in body weights were also observed in repeat dose toxicity studies.

Table 48 Carci Study No 116848: Body Weight.

Summary of Group Mean Body Weight (g)								
	Main Study Males							
Dose Level (mg/kg/day)	Day 182	(%)	Day 364	(%)	Day 532	(%)	Day 644	(%)
Saline Control	683.6	-1.5	793.6	+0.5	840.0	+4.1	836.4	+1.5
Vehicle Control	693.9	NA	789.9	NA	807.3	NA	824.2	NA
0.2	695.8	+0.3	792.5	+0.3	844.2	+4.6	792.6	-3.8
0.4	681.2	-1.8	786.9	-0.4	831.1	+2.9	808.6	-1.9
0.8	679.1	-2.1	772.6	-2.2	808.9	+0.2	777.1	-5.7
1.6	651.5	-6.1	710.2	-10.1	740.8	-8.2	727.7	-11.7 [a,b]

(%) - Percent difference from vehicle control
NA – Not applicable
[a] Significantly different from 0 mg/kg/day (Saline Control); (p<0.01)
[b] Significantly different from 0 mg/kg/day (Vehicle Control); (p<0.05)

Summary of Group Mean Body Weight (g)								
	Main Study Females							
Dose Level (mg/kg/day)	Day 182	(%)	Day 364	(%)	Day 532	(%)	Day 644	(%)
Saline Control	341.7	-3.4	407.0	-3.2	469.6	-2.0	481.2	-7.2
Vehicle Control	353.9	NA	420.7	NA	479.1	NA	518.7	NA
0.2	349.2	-1.3	419.2	-0.4	467.9	-2.3	495.3	-4.5
0.4	351.6	-0.6	423.5	+0.7	477.7	-0.3	500.2	-3.6
0.8	365.0	+3.1	436.7	+3.8	490.4	+2.4	472.6 [a]	(-7.9) [a]
1.6	355.3	+0.4	405.1	-3.7	454.8	-5.1	435.4 [a]	(-15.1) [a,b]

(%) - Percent difference from vehicle control
NA – Not applicable
[a] Females at 0.8 and 1.6 mg/kg/day were not alive on Day 644. The mean body weights listed for 0.8 and 1.6 mg/kg/day represent values collected on Day 616 and the % difference calculated from the Day 616 vehicle control mean body weight of 513.1 grams.
[b] Significantly different from 0 mg/kg/day (Vehicle Control); (p<0.01).

Feed Consumption (weekly through first 14 weeks, every other week through 28 weeks, monthly thereafter)
No etelcalcetide-related effect on food consumption was observed. Food consumption was similar among groups. This did not correlate with reduced body weight observed in this study.

Table 49 Carci Study No 116848: Food Consumption.

Average Food Consumption; g/animal/day (Week 1 through Week 92)				
	Male		Female	
Dose Level (mg/kg/day)	Mean	(%) Difference from Vehicle Control	Mean	(%) Difference from Vehicle Control
Saline Control	29.59	-1.4	20.64	-1.4
Vehicle Control	30.02	NA	20.93	NA
0.2	29.81	-0.7	20.63	-1.4
0.4	29.98	-0.1	20.55	-1.8
0.8	30.42	+1.3	21.79	+4.1
1.6	30.06	+0.1	21.16	+1.1
NA – Not applicable				

Gross Pathology
No etelcalcetide-related macroscopic findings were noted in either sex. Macroscopic observations were of the type commonly seen in rats of this age and strain and were considered incidental and unrelated to administration of etelcalcetide.

Histopathology
Peer Review
Yes

Neoplastic
There were no etelcalcetide-related neoplastic microscopic findings noted in either sex.

Non Neoplastic
There were no etelcalcetide-related non-neoplastic microscopic findings noted in either sex.

An increased incidence of fibrosis at the injection site (scapular area) was present in vehicle control and etelcalcetide injected males and females. In males, the percent incidences of fibrosis were 3%, 66%, 55%, 62%, 86%, and 80% in saline control, vehicle control, 0.2, 0.4, 0.8, and 1.6 mg/kg/day, respectively. In females, the percent incidences of fibrosis were 2%, 54%, 45%, 43%, 38%, and 43% in saline control, vehicle control, 0.2, 0.4, 0.8, and 1.6 mg/kg/day, respectively. The incidences of fibrosis at injection sites were similar in vehicle control groups and etelcalcetide injected groups of males and females. This change was considered vehicle related and not etelcalcetide-related. The clinical formulation does not use the same vehicle and fibrosis was not seen in clinical trials and will not pose a risk in human.

Other microscopic findings in these animals were of the type typically seen in rats of this strain and age and were considered incidental to test article administration.

Toxicokinetics (Day 183)
Increases in exposure were dose-related and roughly dose-proportional. No accumulation data was available since blood samples were collected on Day 183.

Table 50 Carci Study No 116848: TK Parameters.

Toxicokinetic Parameter Estimate of AMG 416 After Daily Subcutaneous Injection Administration in Rats of 0.2, 0.4, 0.8, and 1.6 mg/kg/day

		Study Day 183		
Sex	Dose (mg/kg/day) SC	t_{max} (hr)	C_{max} (µg/mL)	AUC_t (µg•hr/mL)
Male	0.2	0.50	0.126	0.241
	0.4	0.50	0.282	0.537
	0.8	0.50	0.505	0.962
	1.6	0.50	1.12	2.15
Female	0.2	0.50	0.110	0.214
	0.4	0.50	0.241	0.542
	0.8	0.50	0.392	0.772
	1.6	0.50	0.884	1.90
All	0.2	0.50	0.118	0.228
	0.4	0.50	0.265	0.541
	0.8	0.50	0.449	0.867
	1.6	0.50	1.00	2.02

AUC_t= area under the concentration-time curve from time zero to the time of the last quantifiable concentration; C_{max} = maximum observed drug concentration during a dosing interval; t_{max} = time to reach C_{max}.

All values were rounded to 3 significant figures after calculations were performed, except t_{max}, which is rounded to 2 significant figures.

Dosing Solution Analysis

The results of the analysis of etelcalcetide samples indicated that the dose formulations were prepared accurately, with the exception of the Week 13 dose concentration at 0.2 mg/mL (accuracy of 118% vs. 85-115% of the nominal value). The low and high concentration solutions were homogeneous, there was no detectable etelcalcetide in the vehicle samples, and the test article was stable under the conditions of use. No significant effects on the study interpretation are expected due to this abnormally high dose concentration at a single time point.

Study No 116846: 6 month Tg rasH2 mouse study

The Executive Carcinogenicity Assessment Committee agreed that the study was acceptable, noting prior approval with the study protocol. The Committee concurred that there were no drug-related neoplasms in the study.

Study Title: etelcalcetide: 26 week repeated dose subcutaneous carcinogenicity study in Tg.rasH2 mice

Study no.:	116846
Study report location:	Amgen (Thousand Oaks, CA)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 21, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lot 1041722 with 99.3% purity
CAC concurrence:	Yes

Note: The dose selection of this study was based on the dose range finding study, Study No 4169-NC-155 (28 day).

Key Study Findings

- The carcinogenic potential of etelcalcetide in hemizygous Tg.rasH2 mice (main study) and the toxicokinetics profile of etelcalcetide in homozygous CByB6F1 non-transgenic littermate mice (TK cohort) were studied. Males were dosed at 0 (saline), 0 (vehicle control), 0.375, 0.75, and 1.5 mg/kg/day whereas female were dosed at 0 (saline), 0

(vehicle control), 0.3, 1, and 3 mg/kg/day once daily for 26 weeks via subcutaneous injection. The SPA was reviewed and doses up to 1.5 mg/kg/day in males and 3 mg/kg/day in females were recommended by the Exec CAC on May 8, 2013.

- In main study groups, no AMG-416 related mortality, clinical observation, body weights, body weight gains, or food consumption were noted. No AMG-416 related gross findings, tumors, or non-neoplastic microscopic findings were observed.
- There were no etelcalcetide-related tumors identified in males and females.
- In positive control group (with urethane), clinical signs and changes in body weights were expected. There was significantly increased incidence of pulmonary tumors and hemangiosarcomas in males and females when compared to the vehicle control group.
- In TK groups, during Week 26, males had mean etelcalcetide exposures (AUC_t) ranging from 0.232 to 1.04 µg•hr/mL (with mean C_{max} ranging from 0.142 to 0.466 µg/mL) and females had mean etelcalcetide exposures ranging from 0.155 to 1.70 µg•hr/mL (with mean C_{max} ranging from 0.126 to 1.07 µg/mL). Exposures during Week 26 were similar for females and slightly higher for males when compared to the first dose. Exposure was dose proportional over each dose range.
- The study showed that etelcalcetide was non tumorigenic in Tg.rasH2 mice of either sex following once daily subcutaneous injections for 26 weeks. Exposures at the highest doses evaluated in males (1.5 mg/kg/day) and females (3.0 mg/kg/day) were 1.04 µg•hr/mL and 1.70 µg•hr/mL, respectively.

Adequacy of Carcinogenicity Study
Yes, based on Exec CAC concurrence with study doses.

Appropriateness of Test Models
Yes

Evaluation of Tumor Findings
According to the sponsor, the incidence of tumors was analyzed by Peto's mortality-prevalence method, without continuity correction, incorporating the context (incidental, fatal, or mortality-independent) in which tumors were observed.

Methods

Doses:	Male: 0 (saline), 0 (vehicle control), 0.375, 0.75, and 1.5 mg/kg/day Female: 0 (saline), 0 (vehicle control), 0.3, 1, and 3 mg/kg/day
Frequency of dosing:	Once daily for 26 weeks
Dose volume:	10 ml/kg
Route of administration:	SC injection
Formulation/Vehicle:	0.27% sodium succinate dibasic hexahydrate (2.7 g/L), 2% D-mannitol (20 g/L), 1% glycine (10 g/L), 1% trehalose dihydrate (10 g/L) and 0.9% (9 g/L) benzyl alcohol in sterile water for injection (WFI) at pH 4.5±0.1
Basis of dose selection:	MTD; Mortality and clinical signs observed in males at ≥ 3 mg/kg/day and clinical signs (i.e. tremors, rapid/shallow breathing, hyperactivity, decreased motor activity) observed in females at 6 mg/kg/day in Study No 4169-NC-155 (28 day toxicity study in wild type CByB6F1 mice)

Reference ID: 3923456

Species/Strain:	Tg.rasH2 mice
Number/Sex/Group:	25/sex/group
Age:	5 weeks of age
Animal housing:	AAALAC-accredited facility with controlled room at 69-75°F, 30-70% relative humidity, and 12 h light/12 h dark cycle
Paradigm for dietary restriction:	*Ad libitum* access to drinking water and in meal
Dual control employed:	Yes (1000 mg/kg urethane intraperitoneal on Days 1, 3, and 5 and 0.9% saline)
Interim sacrifice:	No
Satellite groups:	TK cohort
Deviation from study protocol:	No significant deviations were reported.

Table 51 Carci Study No 116846: 6 Month Tg-Mouse Study Design.

Study Design

Group	Dose Levels (mg/kg/day)[E]	Main Cohort (Tg.rasH2)		TK Cohort (CByB6F1)	
		Male	Female	Male	Female
1[A]	0 (Saline Control)	25	25	-	-
2[B]	0 (Vehicle Control)	25	25	8	8
3[C]	1000 (Positive Control, Urethane)	10	10	-	-
4[D]	0.30	-	25	-	38
5	0.375	25	-	38	-
6	0.75	25	-	38	-
7	1.00	-	25	-	38
8	1.50	25	-	38	-
9	3.00	-	25	-	38
Total		**135**	**135**	**122**	**122**

[A]Group 1 animals received sterile saline for injection
[B]Group 2 animals received the vehicle (buffer solution, pH 4.5 ± 0.1: 0.27% sodium succinate dibasic hexahydrate (2.7 g/L), 2% D-mannitol (20 g/L), 1% glycine (10 g/L), 1% trehalose (dihydrate) (10 g/L) and 0.9% (9 g/L) benzyl alcohol in water for injection)
[C]Group 3 animals received three intraperitoneal (i.p.) injections once each on Days 1, 3 and 5 only, with 1000 mg/kg/day of urethane in sterile saline.
[D]Groups 4-9 animals received AMG 416 formulated in the vehicle once daily by subcutaneous injection for up to 26 weeks.
[E]All treatments were administered at a dose volume of 10 mL/kg body weight.

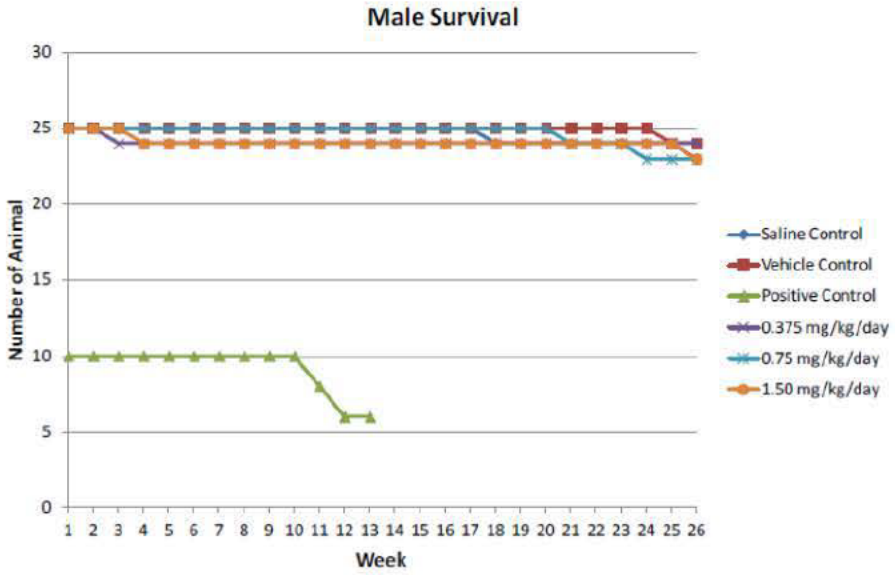
Observations and Results

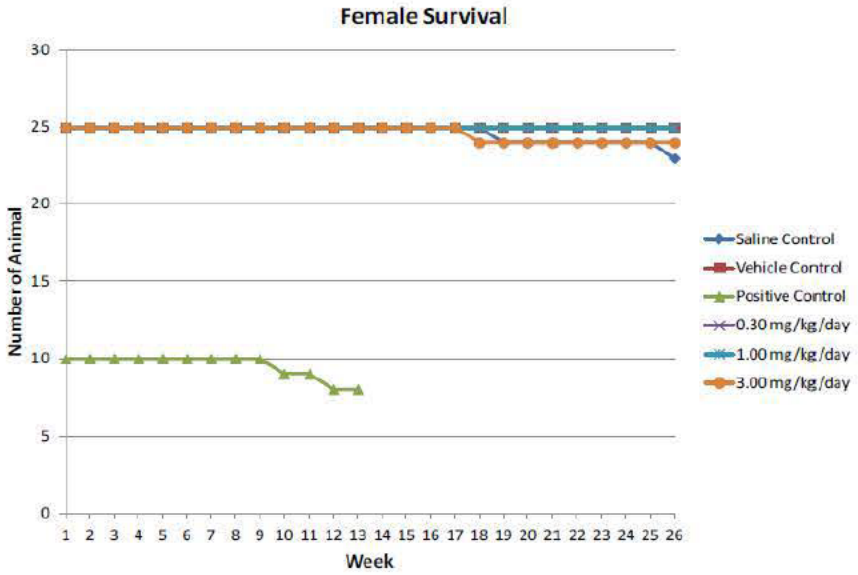
Mortality (twice daily)

There was no etelcalcetide-related mortality. Similar and low incidence of mortality was noted in saline, vehicle, and etelcalcetide-treated groups. It was also comparable to historical incidences for this species and duration of study.

In main study, for males, survival rate for the saline control, vehicle control, 0.375, 0.75 and 1.50 mg/kg/day was 92% (23/25), 96% (24/25), 96% (24/25), 96% (24/25) and 92% (23/25), respectively. For females, survival rate for the saline control, vehicle control, 0.30, 1.00 and 3.00 mg/kg/day was 92% (23/25), 100% (25/25), 100% (25/25), 100% (25/25) and 96% (24/25), respectively.

Figure 11 Carci Study No 116846: Survival.





Main Study Mortality

Group	Dose Levels (mg/kg/day)	Animal Number	Day of Removal	Mode of Death	Cause of Death
Males					
1 (Saline Control)	0	6809	183	Moribund Sacrifice	Marked pyogranulomatous inflammation in multiple organs
		6825	122	Moribund Sacrifice	Multicentric hemangiosarcoma
2 (Vehicle Control)	0	6833	173	Moribund Sacrifice	Multicentric lymphoma
5	0.375	6877	22	Natural Death	Undetermined
6	0.75	6903	146	Natural Death	Multicentric hemangiosarcoma
8	1.50	6917	177	Natural Death	Undetermined
		6926	29	Moribund Sacrifice	Undetermined
Females					
1 (Saline Control)	0	6937	128	Natural Death	Multicentric lymphoma
		6943	178	Natural Death	Mesothelial proliferation
9	3.00	7055	127	Moribund Sacrifice	Undetermined

In TK cohort, all mice survived until their scheduled TK sacrifice at the end of treatment.

Clinical Signs (once daily)
There were no etelcalcetide-related cage side and detailed (hands-on) observations. However, there were scabs at the site of injections (i.e., ear, body dorsal, and neck, with higher incidences in 1.50 mg/kg/day males compared to vehicle males), but with no histopathology correlates. These findings were vehicle- or injection procedure-related.

Body Weights (once during predose, on Day 1, and weekly thereafter)
There was no etelcalcetide-related effect on body weight.

Feed Consumption (weekly)
There was no etelcalcetide-related effect on food consumption.

Gross Pathology (scheduled sacrifice)
Gross finings noted at the sites of injection, including discoloration, focus, nodules or masses, were due to vehicle and the injection procedure and not due to etelcalcetide, e.g. gross findings.

Table 52 Carci Study No 116846: Macroscopic Findings.

Combined Incidence of Gross Findings at SOI

Males					
	Group 1	Group 2	Group 5	Group 6	Group 8
Number examined	25	25	25	25	25
Number of animals with gross findings	7	15	16	19	12
Females					
	Group 1	Group 2	Group 4	Group 7	Group 9
Number examined	25	25	25	25	25
Number of animals with gross findings	14	14	16	12	14

Nominal Dose:
Group 1 (Saline Control) Group 2 (Vehicle Control) Group 4 – 0.30 mg/kg/day
Group 5 – 0.375 mg/kg/day Group 6 – 0.75 mg/kg/day Group 7 – 1.00 mg/kg/day
Group 8 – 1.50 mg/kg/day Group 9 – 3.00 mg/kg/day

Histopathology

Peer Review

Yes.

Neoplastic

There were no etelcalcetide-related tumors identified in males or females. The positive control (urethane) group showed the expected profile and incidence of tumors for this strain of mice administered three times by the IP route. Significance was based on the FDA statistical reviewer's p-values for dose response relationship or pairwise comparisons. The threshold for statistical significance - Common tumors (background rate greater than 1%) require p<0.005 for trend and p<0.01 for pairwise. Rare tumors (background rate of 1% or less) require p<0.025 for trend, p<0.05 for pairwise.

The following is a brief summary of tumors observed in positive control animals as it relates to the study's validity.

- Lung (adenoma and carcinoma) - there was a statistically significant increase (p < 0.05) in the incidence of pulmonary tumors when compared to the vehicle control mice, proving the validity of the study. The incidence of single and multiple adenomas, carcinomas, and that of combined incidence of all pulmonary tumors in the saline, vehicle and etelcalcetide-treated mice, were comparable. It fell within the historical control range established at (b) (4)
- Hemangiosarcomas in the spleen - there was a statistically significant increase (p < 0.05) in the incidence of splenic hemangiosarcomas when compared to vehicle control mice, proving the validity of the study. The incidence of splenic hemangiosarcomas in the saline, vehicle and etelcalcetide-treated mice was comparable. It fell within the historical control range established at (b) (4)
- Hemangiosarcomas in other organs - there were no statistically significant differences for the grouping of hemangiosarcomas in different organs, including spleen, between the saline, vehicle and etelcalcetide-treated mice in both sexes. The combined incidence of all hemangiosarcomas in different organs, in the saline, vehicle control and the etelcalcetide-treated groups, fell within the historical range established at (b) (4)
- In addition, there were no other statistically significant differences in the incidence of other tumors between the saline, vehicle and etelcalcetide-treated mice of both sexes. It fell within the historical control ranges established at (b) (4) except for salivary gland lymphangioma in males and liver adenomas in females, which have not been observed previously in our historical control database. The incidence of salivary gland

lymphangioma in males was the same in the saline and the etelcalcetide-treated mice and liver adenomas in females occurred in saline and vehicle control females only.

Table 53 Carci Study No 116846: Tumor Findings.

Tumors in Lungs

Males	Group 1	Group 2	Group 3	Group 5	Group 6	Group 8	HCR (%)
Number Examined	25	25	10	25	25	25	
Lung, adenoma, single	2	1	0	1	3	1	0-24
Lung, adenoma, multiple	0	0	10*	0	0	1	0-4
Lung, carcinoma	1	0	0	0	0	0	0-8
Number of Animals with Lung tumors	3	1	10*	1	3	2	NA
Females	Group 1	Group 2	Group 3	Group 4	Group 7	Group 9	HCR (%)
Number Examined	25	25	10	25	25	25	
Lung, adenoma, single	0	1	0	2	1	4	0-24
Lung, adenoma, multiple	0	0	10*	1	0	0	0-4
Lung, carcinoma	0	0	4*	1	0	0	0-4
Number of Animals with Lung tumors	0	1	10*	4	1	4	NA

Nominal Dose:
Group 1 (Saline Control) Group 2 (Vehicle Control) Group 3 (Urethane, Positive Control)
Group 4 – 0.30 mg/kg/day Group 5 – 0.375 mg/kg/day Group 6 – 0.75 mg/kg/day
Group 7 – 1.00 mg/kg/day Group 8 – 1.50 mg/kg/day Group 9 – 3.00 mg/kg/day
HCR: (b) (4) Historical Control Range % in vehicle control mice based on 25 mice in each dose group (See Appendix D).
NA: Not applicable
* Statistically significant when compared to vehicle control (See Appendix D).

Hemangiosarcomas in the Spleen

Males	Group 1	Group 2	Group 3	Group 5	Group 6	Group 8	HCR (%)
Number Examined	25	25	10	25	25	25	
Hemangiosarcomas	2[1]	2	9*	2	1	2	0-16
Females	Group 1	Group 2	Group 3	Group 4	Group 7	Group 9	HCR (%)
Number Examined	25	25	10	25	25	25	
Hemangiosarcomas	1	1	9*	4	2	0	0-16

Nominal Dose:
Group 1 (Saline Control) Group 2 (Vehicle Control) Group 3 (Urethane, Positive Control)
Group 4 – 0.30 mg/kg/day Group 5 – 0.375 mg/kg/day Group 6 – 0.75 mg/kg/day
Group 7 – 1.00 mg/kg/day Group 8 – 1.50 mg/kg/day Group 9 – 3.00 mg/kg/day
HCR: (b) (4) Historical Control Range % in vehicle control mice based on 25 mice in each dose group (See Appendix D).
* Statistically significant when compared to vehicle control (See Appendix D).
1 = One mouse had multicentric hemangiosarcoma including spleen.

Hemangiosarcomas in Different Organs Including Spleen

Males						
	Group 1	Group 2	Group 5	Group 6	Group 8	HCR (%)
Number Examined	25	25	25	25	25	
Spleen	2[1]	2	2	1	2	0-16
Multicentric	1	0	0	1	0	0-4
Muscle	0	0	0	1	0	NPO
Total animals with hemangiosarcoma	3	2	2	3	2	NA
Females						
	Group 1	Group 2	Group 4	Group 7	Group 9	HCR (%)
Number Examined	25	25	25	25	25	
Spleen	1	1	4	2	0	0-16
Forelimb	0	0	1	0	0	0-8
Uterus	0	0	0	2	0	0-8
Total animals with hemangiosarcoma	1	1	5	4	0	NA

Nominal Dose:
Group 1 (Saline Control) Group 2 (Vehicle Control) Group 4 – 0.30 mg/kg/day
Group 5 – 0.375 mg/kg/day Group 6 – 0.75 mg/kg/day Group 7 – 1.00 mg/kg/day
Group 8 – 1.50 mg/kg/day Group 9 – 3.00 mg/kg/day
HCR: (b) (4) Historical Control Range % in vehicle control mice based on 25 mice in each dose group (See Appendix D).
NPO: Not Previously Observed in the (b) (4) Historical Control Database.
NA: Not applicable.
1 = One mouse had multicentric hemangiosarcoma including spleen and was therefore not counted under multicentric in the table above.

Other Tumors

Males						
	Group 1	Group 2	Group 5	Group 6	Group 8	HCR (%)
Number Examined	25	25	25	25	25	
Lymphoma, multicentric	0	1	1	0	0	0-4
Liver, adenoma	1	0	0	0	0	0-4
Harderian gland, adenoma	0	0	1	0	2	0-8
Harderian gland, carcinoma	0	1	1	0	0	0-4
Salivary gland, lymphangioma	1	0	0	0	1	NPO
Skin, papilloma	0	0	0	1	1	0-8
Stomach, squamous cell carcinoma	0	0	1	0	0	0-4
Females						
	Group 1	Group 2	Group 4	Group 7	Group 9	HCR (%)
Number Examined	25	25	25	25	25	
Lymphoma, multicentric	1	0	0	0	0	0-8
Liver, adenoma	2	1	0	0	0	NPO
Thymus, thymoma	0	1	0	0	1	0-4
Harderian gland, adenoma	0	1	1	1	0	0-16
Harderian gland, carcinoma	0	0	1	2	0	0-8

Nominal Dose:
Group 1 (Saline Control) Group 2 (Vehicle Control) Group 4 – 0.30 mg/kg/day
Group 5 – 0.375 mg/kg/day Group 6 – 0.75 mg/kg/day Group 7 – 1.00 mg/kg/day
Group 8 – 1.50 mg/kg/day Group 9 – 3.00 mg/kg/day
HCR: (b) (4) Historical Control Range % in vehicle control mice based on 25 mice in each dose group (See Appendix D).
NPO: Not Previously Observed in the (b) (4) Historical Control Database.

Non Neoplastic

The non-neoplastic microscopic findings noted in male or female mice at injection sites (i.e., inflammatory findings, ulceration, hemorrhages, and epidermal hyperplasia) were due to the vehicle and the subcutaneous injection procedure and were not due to etelcalcetide.

Table 54 Carci Study No 116846: Non-Tumor Findings.

Findings at SOI Skin

Males					
	Group 1	Group 2	Group 5	Group 6	Group 8
Number Examined	25	25	25	25	25
Subcutaneous Inflammation					
Minimal	7	8	3	4	10
Mild	4	9	12	14	7
Moderate	1	5	4	3	2
Hemorrhage					
Minimal	0	1	0	0	1
Mild	1	4	5	8	2
Moderate	0	6	7	6	4
Marked	0	4	4	2	3
Females					
	Group 1	Group 2	Group 4	Group 7	Group 9
Number Examined	25	25	25	25	25
Subcutaneous Inflammation					
Minimal	13	12	4	16	10
Mild	4	6	6	5	8
Moderate	0	2	7	2	2
Marked	0	1	0	0	0
Hemorrhage					
Minimal	0	0	2	1	2
Mild	0	3	5	5	5
Moderate	0	5	6	2	0
Marked	0	1	0	0	1

Nominal Dose:
Group 1 (Saline Control) Group 2 (Vehicle Control) Group 4 – 0.30 mg/kg/day
Group 5 – 0.375 mg/kg/day Group 6 – 0.75 mg/kg/day Group 7 – 1.00 mg/kg/day
Group 8 – 1.50 mg/kg/day Group 9 – 3.00 mg/kg/day

Toxicokinetics (Day 1 and Week 26)

Increases in exposure were dose-related and roughly dose proportional.

After daily subcutaneous administration for 177 days, the accumulation ratios (AR) ranged from 1.19 to 1.79 for males and 0.882 to 1.19 for females compared to Day 1.

Table 55 Carci Study No 116846: TK Parameters.

Summary of Estimated TK Results for AMG 416 on Day 1

Study Day 1				
Sex	Dose (mg/kg/day)	t_{max} (hr)	C_{max} (µg/mL)	AUC_t (µg•hr/mL)
Male	0.375	0.25	0.218	0.194
	0.75	0.25	0.475	0.429
	1.50	0.25	0.453	0.581
Female	0.30	0.25	0.154	0.176
	1.00	0.25	0.411	0.444
	3.00	0.25	2.02	1.82

AUC_t study day 177/AUC_t study day 1; AUC_t = area under the concentration-time curve from time zero to the time of the last quantifiable concentration; C_{max} = maximum observed drug concentration during a dosing interval; t_{max} = time to reach C_{max}
All values were rounded to 3 significant figures after calculations were performed, except t_{max} values, which were rounded to 2 significant figures.

Summary of Estimated TK Results for AMG 416 on Day 177

Study Day 177					
Sex	Dose (mg/kg/day)	t_{max} (hr)	C_{max} (µg/mL)	AUC_t (µg•hr/mL)	AR
Male	0.375	0.25	0.142	0.232	1.19
	0.75	0.25	0.334	0.625	1.46
	1.50	0.25	0.466	1.04	1.79
Female	0.30	0.25	0.126	0.155	0.882
	1.00	0.25	0.381	0.528	1.19
	3.00	0.25	1.07	1.70	0.931

AR = accumulation ratio = AUC_t study day 177/AUC_t study day 1; AUC_t = area under the concentration-time curve from time zero to the time of the last quantifiable concentration; C_{max} = maximum observed drug concentration during a dosing interval; t_{max} = time to reach C_{max}
All values were rounded to 3 significant figures after calculations were performed, except t_{max} values, which were rounded to 2 significant figures.

Dosing Solution Analysis

All formulations were within specifications except for the 1st, 3rd, 4th and 5th preparations for 0.30 mg/kg/day females (Group 4) and the 3rd (both primary and secondary) and 4th preparations for 0.375 mg/kg/day males (Group 5), which were found to be below the acceptable range. The cause was not identified. Only the formulations for the low-dose groups were out of range and results were not affected.

Etelcalcetide in the vehicle, at a concentration of 0.03 mg/mL, was determined to be stable for at least 24 hours when stored at room temperature and for at least 27 days at 2-8 °C. Etelcalcetide in vehicle, at a concentration of 0.30 mg/mL, was determined to be stable for at least 24 hours when stored at room temperature and for at least 15 days at 2-8 °C.

Summary of Out of Specification Analytical Results

Analysis No. (Week)	Date of Analysis (Start/End)	Date of Formulation Preparation	Group	Nominal Concentration (mg/mL)	Measured Mean Concentration (mg/mL)	% Target (repeat)
First (1)	05-Jul-13/ 07-Jul-13	05-Jul-13	4	0.03	0.0266	88.5
Third (13)	25-Sep-13/ 28-Sep-13	25-Sep-13	4	0.03	0.0256	85.5
			5	0.0375	0.0327	87.1 (85.2)
Fourth (14)	03-Oct-13/ 05-Oct-13	03-Oct-13	4	0.03	0.0246	81.9
			5	0.0375	0.0322	85.9
Fifth (16)	17-Oct-13/ 23-Oct-13	17-Oct-13	4	0.03 unfiltered	0.0266	88.6
			4	0.03 filtered	0.0254	84.8

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study No 119088: Combined Fertility and Early Embryonic Development/Embryo-fetal Development Toxicity Study in Rats (pivotal)

Study title: AMG 416 (etelcalcetide): Fertility and Embryo-Fetal Development Study in the Sprague Dawley Rat

Study no.: 119088
Study report location: Amgen (Thousand Oaks, CA)
Conducting laboratory and location: (b) (4)
Date of study initiation: February 19, 2014
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Lot 1044951 with 98.9% purity

Key Study Findings

- Male and female Sprague-Dawley rats received etelcalcetide once daily for 28 days (males) or 15 days (females) prior to mating, during cohabitation, and through Week 8 for males and through GD 17 for females at 0.75, 1.5, and 3 mg/kg.
- In males, etelcalcetide-related effects included reduced body weight gains at ≥0.75 mg/kg, an increased incidence of clinical signs and reduced body weights at ≥1.5 mg/kg, and reduced food intake at 3 mg/kg.
- In females, similar reductions in body weights, body weight gain and food intake were noted, but were limited to the 3 mg/kg dose group. Etelcalcetide-related clinical signs were noted at ≥1.5 mg/kg during the premating and gestation periods.
- These findings are consistent with the intended pharmacological effects of hypocalcemia and associated stress.
- At 3 mg/kg, etelcalcetide reduced the number of estrous stages during the 14 day premating dose period as a result of prolonged periods of diestrus (≥6 days), consistent with stress secondary to hypocalcemia.
- However, etelcalcetide did not result in any effects on mating, fertility, male reproductive assessments (organ weights or sperm parameters), embryo-fetal survival or fetal body

weights. Etelcalcetide also did not produce any fetal external, visceral, or skeletal malformations or variations at 3 mg/kg/day.
- The NOAEL for maternal toxicity was 0.75 mg/kg/day.
- The NOAEL for fertility/early embryonic development and embryo-fetal development was 3 mg/kg/day.

Methods

Doses:	0.75, 1.5, and 3 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	2 mL/kg
Route of administration:	IV injection
Formulation/Vehicle:	0.27% Sodium succinate dibasic hexahydrate (2.7 g/L), 2% D-mannitol (20 g/L), 1 % glycine (10 g/L), 1% trehalose dihydrate (10 g/L) and 0.9% (9 g/L) benzyl alcohol in sterile water for injection (WFI), pH 4.5 ± 0.1
Species/Strain:	Crl:CD(SD) Sprague-Dawley rats
Number/Sex/Group:	25/sex/group
Satellite groups:	n/a
Study design:	Females were administered etelcalcetide or the vehicle control beginning 15 days before cohabitation continuing through Gestation Day (GD) 17. Based on the dosing schedule, males were administered 56 to 59 doses and females were administered 33 to 49 doses of etelcalcetide or vehicle control.
Deviation from study protocol:	Yes but no impact on study result

Table 56 ReproTOX Study No 119088: Combined Fertility and Early Embryonic Development/Embryo-fetal Development Rat Study Design.

Experimental Design

Group No.	Test Material	Dose Level (mg/kg/day)	Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Rats (Assigned Rat Numbers)	
					Male	Female
1	Vehicle Control Article	0	0	2	25 (7401-7425)	25 (7501-7525)
2	AMG 416	0.75	0.375	2	25 (7426-7450)	25 (7526-7550)
3	AMG 416	1.5	0.75	2	25 (7451-7475)	25 (7551-7575)
4	AMG 416	3	1.5	2	25 (7476-7500)	25 (7576-7600)

All animals were monitored for viability (morbidity/moribundity), clinical signs, body weights, body weight changes, food consumption, mating and fertility, and macroscopic observations. Male rats were further evaluated for sperm motility, sperm concentration and reproductive organ weights.

Female rats were evaluated for the estrous cycle for 14 consecutive days before initiation of dose administration, 14 consecutive days prior to the initiation of cohabitation, and during the cohabitation period until evidence of mating was observed. Female rats were also evaluated for gravid uterine weights, ovarian and uterine examinations (i.e., number and distribution of corpora lutea, implantations, live and dead fetuses, and early and late resorptions), and the F_1 generation fetuses were examined for body weights, sex and external, visceral and skeletal morphology.

Observations and Results
Mortality
All males and females survived to scheduled euthanasia.

Clinical Signs
Male
At ≥1.5 mg/kg/day, etelcalcetide-related clinical signs such as whole body tremors and slight excess salivation were observed. At 3 mg/kg/day, mild dehydration, hunched posture, ataxia, low carriage, rales, prostration, decreased motor activity, tip toe walk, pale ears, tachypnea, and thin body condition were seen. These clinical signs were consistent with hypocalcemia, the expected pharmacology of etelcalcetide, and associated stress. At 0.75 mg/kg/day, there were no etelcalcetide-related clinical signs.

Female
At ≥1.5 mg/kg/day, etelcalcetide-related clinical signs such as whole body tremors, ataxia, low carriage, and prostration were observed during the premating period. At 3 mg/kg/day, mild dehydration, pale ears, decreased motor activity, cold to touch, tip toe walk, mydriasis, impaired righting reflex, and vocalization to touch, and hunched posture were seen. Most of these clinical signs persisted during the gestation period. Also at ≥1.5 mg/kg/day, slight to moderate excess salivation were observed during the gestation period. At 3 mg/kg/day, red or brown perivaginal substance, pale paws, and tachypnea were also observed. These clinical signs were consistent with hypocalcemia. At 0.75 mg/kg/day, there were no etelcalcetide-related clinical signs.

Body Weight
Male
At ≥0.75 mg/kg/day, mean body weight gain was reduced for the premating dosing interval (DS 1 to 28; 44% to 92% of controls) and overall (DS 1 to 57; 54% to 91% of controls). In addition, the mean body weight was reduced on DS 57 (95% of controls) at 1.5 mg/kg/day and between DS 8 and 57 at 3 mg/kg (85% to 91% of controls) when compared to controls.

Female
At 3 mg/kg/day, mean body weight gain was reduced for the premating dosing interval (DS 1 to 15; 64% of controls) and the mean body weight was reduced on DS 8 and DS 11 (96% of controls). Mean maternal body weight gain was reduced for the dosing intervals of GD 0 to 7 (65% of controls) and GD 15 to 18 (88% of controls) and overall for the interval of GD 0 to 21 (93% of controls) when compared to controls. In addition, the mean maternal body weight at 3 mg/kg/day was also reduced between GD 2 and GD 20 (94% to 96% of controls).

Feed Consumption
Male
At 3 mg/kg/day, etelcalcetide reduced mean food consumption (g/day and g/kg/day) during the premating dosing interval of DS 1 to 28 (81% and 89% of controls, respectively) and overall for the interval of DS 1 to 57 (84% and 95% of controls, respectively) compared to controls.

Female
At 3 mg/kg, there were corresponding reductions in mean food consumption (g/day and g/kg/day) for the premating dosing interval of DS 1 to 15 compared to controls (78% and 80% of controls, respectively). These effects of etelcalcetide on food consumption in the 3 mg/kg dose group persisted into the gestation period, resulting in a reduction in mean maternal food intake for the dosing interval of GD 0 to 7 (87% and 92% of controls, respectively).

Reference ID: 3923456

Toxicokinetics
n/a

Dosing Solution Analysis
All prepared dose formulations were acceptable.

Necropsy
There were no etelcalcetide-related macroscopic observations noted at necropsy in male and female rats.

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

Male

There were no effects on mating and fertility parameters, male reproductive organ weights or sperm parameters (vas deferens sperm motility or caudal epididymal sperm density) at any dose.

Table 57 ReproTOX Study No 119088: Rat Fertility Parameters (Male).

SUMMARY OF MATING AND FERTILITY: MALE RATS

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
RATS IN COHABITATION	N	25	25	25	25
DAYS IN COHABITATION b,c MEAN±S.D.		3.0 ± 3.3	2.7 ± 2.6	2.6 ± 2.7	2.4 ± 1.1
RATS THAT MATED c	N(%)	25(100.0)	24(96.0)	25(100.0)	25(100.0)
FERTILITY INDEX d,e	N/N	23/ 25	24/ 24	23/ 25	23/ 25
	(%)	(92.0)	(100.0)	(92.0)	(92.0)
RATS WITH CONFIRMED MATING DATES	N	25	24	25	25
MATED WITH FEMALE f					
DAYS 1-7	N(%)	23(92.0)	24(100.0)	24(96.0)	25(100.0)
DAYS 8-14	N(%)	2(8.0)	0(0.0)	1(4.0)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION e	N/N	23/ 25	24/ 25	23/ 25	23/ 25
	(%)	(92.0)	(96.0)	(92.0)	(92.0)

a. Dose administration occurred on Study Day 1 through the day before euthanasia.
b. Restricted to rats with a confirmed mating date and rats that did not mate.
c. Includes only one mating for each male rat.
d. Number of pregnancies/number of rats that mated.
e. Includes only one pregnancy for each rat that impregnated more than one female rat.
f. Restricted to rats with a confirmed mating date.

SUMMARY OF SPERM MOTILITY, COUNT AND DENSITY: MALE RATS

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
RATS TESTED	N	25	25	25	25
VAS DEFERENS SPERM MOTILITY					
MOTILE PERCENT	MEAN±S.D.	88.8 ± 5.3 [24]b	86.4 ± 12.2	88.0 ± 5.1 [24]b	87.1 ± 7.9
CAUDA EPIDIDYMAL SPERM COUNT					
SPERM DENSITY c	MEAN±S.D.	1228.59 ± 606.77	1505.46 ± 753.73	1097.72 ± 551.40	1210.68 ± 670.41

[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred on Day 1 of study through the day before euthanasia.
b. Excludes values for rats with motility data that reflected drifting debris.
c. The sperm density was calculated by dividing the sperm count by the volume in the image area (76.6 x 10^{-6} mL), multiplying by 2 (dilution factor + 1) and multiplying by 10^{-6} to obtain the sperm concentration (million sperm/mL). The calculated sperm concentration value (rounded to 1 decimal place) was multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Appendix 9 for the weight of the left cauda epididymis) to obtain the sperm density (million sperm/gram of tissue). The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

Female

The number of females (N=5) that exhibited ≥6 days of persistent diestrus during the premating dosing period was increased at 3 mg/kg, which in turn, decreased the number of estrous cycles per 14 days during the premating dose period compared to controls (2.7 cycles vs. 3.2 cycles in controls). All mating and fertility parameters were comparable for all groups.

Table 58 ReproTOX Study No 119088: Rat Fertility Parameters (Female).

SUMMARY OF ESTROUS CYCLING, MATING AND FERTILITY: FEMALE RATS

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
ESTROUS CYCLING OBSERVATIONS					
RATS EVALUATED	N	25	25	25	25
PREDOSE ESTROUS CYCLING					
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	3.6 ± 0.5	3.4 ± 0.5	3.7 ± 0.4	3.6 ± 0.5
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
PRECOHABITATION ESTROUS CYCLING					
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	3.2 ± 0.7	3.4 ± 0.6	3.2 ± 0.6	2.7 ± 0.9
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N(%)	0(0.0)	0(0.0)	1(4.0)	5(20.0)**

a. Dose administration occurred on Study Day 1 through Gestation Day 17.
** Significantly different from the vehicle control group value (p≤0.01).

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
MATING OBSERVATIONS					
RATS IN COHABITATION	N	25	25	25	25
DAYS IN COHABITATION b	MEAN±S.D.	3.0 ± 3.3	2.8 ± 3.0	2.6 ± 2.7	2.4 ± 1.1
RATS THAT MATED	N(%)	25(100.0)	25(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX c	N/N	23/ 25	25/ 25	23/ 25	23/ 25
	(%)	(92.0)	(100.0)	(92.0)	(92.0)
RATS WITH CONFIRMED MATING DATES	N	25	25	25	25
MATED BY FIRST MALE d					
DAYS 1-7	N(%)	23(92.0)	24(96.0)	24(96.0)	25(100.0)
DAYS 8-14	N(%)	2(8.0)	0(0.0)	1(4.0)	0(0.0)
MATED BY SECOND MALE d	N(%)	0(0.0)	1(4.0)	0(0.0)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION	N/N	23/ 25	25/ 25	23/ 25	23/ 25
	(%)	(92.0)	(100.0)	(92.0)	(92.0)

a. Dose administration occurred on Study Day 1 through Gestation Day 17.
b. Restricted to rats with a confirmed mating date and rats that did not mate.
c. Number of pregnancies/number of rats that mated.
d. Restricted to rats with a confirmed mating date.

Cesarean Section Data
(Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Pregnancy was confirmed in 23 or 25 rats in each dose group, including controls. There were no etelcalcetide-related effects on gravid uterine weights or any ovarian or uterine parameters (corpora lutea, implantation sites, litter size, embryo-fetal survival, fetal weights, and fetal sex).

Table 59 ReproTOX Study No 119088: Rat C-Section Data.

SUMMARY OF OVARIAN AND UTERINE CONTENTS: FEMALE RATS

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
RATS TESTED	N	25	25	25	25
PREGNANT	N(%)	23(92.0)	25(100.0)	23(92.0)	23(92.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON GESTATION DAY 21	N	23	25	23	23
CORPORA LUTEA	MEAN±S.D.	16.1 ± 2.4	16.6 ± 2.7	16.3 ± 1.5	15.3 ± 1.9
IMPLANTATIONS	MEAN±S.D.	15.7 ± 2.0	15.5 ± 3.2	15.4 ± 1.3	14.5 ± 2.4
% PREIMPLANTATION LOSS	MEAN±S.D.	2.1 ± 3.9	7.3 ± 15.1	4.8 ± 5.6	5.4 ± 8.6
LITTER SIZES	MEAN±S.D.	14.7 ± 2.0	14.5 ± 3.9	14.3 ± 2.4	13.3 ± 2.8
LIVE FETUSES	N	339	363	330	307
	MEAN±S.D.	14.7 ± 2.0	14.5 ± 3.9	14.3 ± 2.4	13.3 ± 2.8
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	1.0 ± 1.1	1.0 ± 2.6	1.1 ± 1.6	1.2 ± 1.3
EARLY RESORPTIONS	N	22	24	25	27
	MEAN±S.D.	1.0 ± 1.1	1.0 ± 2.6	1.1 ± 1.6	1.2 ± 1.3
LATE RESORPTIONS	N	1	0	0	0
	MEAN±S.D.	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
% POSTIMPLANTATION LOSS	MEAN±S.D.	6.2 ± 6.7	6.0 ± 16.6	7.4 ± 11.1	8.8 ± 11.8

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100
a. Dose administration occurred on Study Day 1 through Gestation Day 17.

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
RATS TESTED	N	25	25	25	25
PREGNANT	N(%)	23(92.0)	25(100.0)	23(92.0)	23(92.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON GESTATION DAY 21	N	23	25	23	23
DAMS WITH ANY RESORPTIONS	N(%)	14(60.9)	7(28.0)	13(56.5)	14(60.9)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	23(100.0)	25(100.0)	23(100.0)	23(100.0)
PLACENTAE APPEARED NORMAL	N(%)	23(100.0)	25(100.0)	23(100.0)	22(95.6)

a. Dose administration occurred on Study Day 1 through Gestation Day 17.

SUMMARY OF LITTER OBSERVATIONS: FEMALE RATS

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
LITTERS WITH ONE OR MORE LIVE FETUSES	N	23	25	23	23
IMPLANTATIONS	MEAN±S.D.	15.7 ± 2.0	15.5 ± 3.2	15.4 ± 1.3	14.5 ± 2.4
LIVE FETUSES	N	339	363	330	307
	MEAN±S.D.	14.7 ± 2.0	14.5 ± 3.9	14.3 ± 2.4	13.3 ± 2.8
LIVE MALE FETUSES	N	163	170	177	160
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	48.0 ± 10.6	47.3 ± 11.7	54.2 ± 12.6	51.7 ± 13.3
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.48 ± 0.23	5.64 ± 0.39	5.59 ± 0.31	5.72 ± 0.32
MALE FETUSES	MEAN±S.D.	5.63 ± 0.29	5.75 ± 0.38	5.74 ± 0.32	5.85 ± 0.36
FEMALE FETUSES	MEAN±S.D.	5.32 ± 0.21	5.53 ± 0.39	5.42 ± 0.33	5.56 ± 0.32
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	6.2 ± 6.7	6.0 ± 16.6	7.4 ± 11.1	8.8 ± 11.8

a. Dose administration occurred on Study Day 1 through Gestation Day 17.

Offspring
(Malformations, Variations, etc.)

There were no etelcalcetide-related fetal external, visceral, or skeletal malformations or variations.

Table 60 ReproTOX Study No 119088: Rat Offspring Parameters.

SUMMARY OF FETAL GROSS EXTERNAL EXAMINATIONS: FEMALE RATS

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
LITTERS EVALUATED	N	23	25	23	23
LITTERS WITH LIVE FETUS(ES)	N	23	25	23	23
FETUSES EVALUATED	N	339	363	330	307
LIVE	N	339	363	330	307
NO FETAL ALTERATIONS WERE IDENTIFIED AT GROSS EXTERNAL EXAMINATION					

a. Dose administration occurred on Study Day 1 through Gestation Day 17.

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SUMMARY OF FETAL VISCERAL EXAMINATIONS: FEMALE RATS

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
LITTERS EVALUATED	N	23	25	23	23
LITTERS WITH LIVE FETUS(ES)	N	23	25	23	23
FETUSES EVALUATED	N	165	176	157	147
LIVE	N	165	176	157	147
VESSELS: INNOMINATE ARTERY ABSENT					
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
VESSELS: SITUS INVERSUS					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)	0(0.0)

a. Dose administration occurred on Study Day 1 through Gestation Day 17.

SUMMARY OF FETAL SKELETAL EXAMINATIONS: FEMALE RATS

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
LITTERS EVALUATED	N	23	25	23	23
LITTERS WITH LIVE FETUS(ES)	N	23	25	23	23
FETUSES EVALUATED	N	174	187	173	160
LIVE	N	174	187	173	160
SKULL: ZYGOMATIC ARCH, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	1(4.3)
FETAL INCIDENCE	N(%)	1(0.6)b	0(0.0)	0(0.0)	1(0.6)h
SKULL: SQUAMOSAL, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	1(4.3)
FETAL INCIDENCE	N(%)	1(0.6)b	0(0.0)	0(0.0)	1(0.6)h
SKULL: FRONTAL, CONTAINS AN INTERFRONTAL					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)g	0(0.0)	0(0.0)
CERVICAL VERTEBRAE: ARCH, REDUCED VENTRAL PROCESS					
LITTER INCIDENCE	N(%)	2(8.7)	1(4.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	2(1.1)	1(0.5)	0(0.0)	0(0.0)
CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRA					
LITTER INCIDENCE	N(%)	0(0.0)	3(12.0)**	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	4(2.1)**e,f	0(0.0)	0(0.0)
CERVICAL VERTEBRAE: ARCH, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	1(4.3)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	1(0.6)h
THORACIC VERTEBRAE: CENTRUM, BIFID					
LITTER INCIDENCE	N(%)	8(34.8)	4(16.0)**	3(13.0)**	1(4.3)**
FETAL INCIDENCE	N(%)	10(5.7)b	5(2.7)**f	4(2.3)**	1(0.6)**

** Significantly different from the vehicle control group value (p<0.01).

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GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
LITTERS EVALUATED	N	23	25	23	23
LITTERS WITH LIVE FETUS(ES)	N	23	25	23	23
FETUSES EVALUATED	N	174	187	173	160
LIVE	N	174	187	173	160
RIBS: SHORT					
LITTER INCIDENCE	N(%)	1(4.3)	1(4.0)	0(0.0)	1(4.3)
FETAL INCIDENCE	N(%)	1(0.6)	1(0.5)g	0(0.0)	1(0.6)i
RIBS: THICKENED					
LITTER INCIDENCE	N(%)	2(8.7)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	2(1.1)c,d	0(0.0)	0(0.0)	0(0.0)
RIBS: INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	2(8.7)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	2(1.1)c,d	0(0.0)	0(0.0)	0(0.0)
RIBS: WAVY					
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)d	0(0.0)	0(0.0)	0(0.0)
RIBS: BROAD					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)g	0(0.0)	0(0.0)
MANUBRIUM: FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)g	0(0.0)	0(0.0)
PELVIS: PUBIS, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	2(8.7)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	2(1.2)	0(0.0)

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
LITTERS EVALUATED	N	23	25	23	23
LITTERS WITH LIVE FETUS(ES)	N	23	25	23	23
FETUSES EVALUATED	N	174	187	173	160
LIVE	N	174	187	173	160
STERNAL CENTRA: SUMMARIZATION (SUMMARIZATION OF ASYMMETRIC, INCOMPLETELY OSSIFIED, DUPLICATED, FUSED)					
LITTER INCIDENCE	N(%)	2(8.7)	2(8.0)	0(0.0)	1(4.3)
FETAL INCIDENCE	N(%)	2(1.1)	2(1.1)	0(0.0)	1(0.6)
STERNAL CENTRA: ASYMMETRIC					
LITTER INCIDENCE	N(%)	1(4.3)	1(4.0)	0(0.0)	1(4.3)
FETAL INCIDENCE	N(%)	1(0.6)	1(0.5)e	0(0.0)	1(0.6)i
STERNAL CENTRA: INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
STERNAL CENTRA: DUPLICATED					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)g	0(0.0)	0(0.0)
STERNAL CENTRA: FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)g	0(0.0)	0(0.0)

a. Dose administration occurred on Study Day 1 through Gestation Day 17.
b. Fetus 7508-9 had other skeletal alterations.
c. Fetus 7514-9 had other skeletal alterations.
d. Fetus 7524-13 had other skeletal alterations.
e. Fetus 7546-5 had other skeletal alterations.
f. Fetus 7546-15 had other skeletal alterations.
g. Fetus 7549-11 had other skeletal alterations.
h. Fetus 7587-16 had other skeletal alterations.
i. Fetus 7588-9 had other skeletal alterations.

Reference ID: 3923456

SUMMARY OF FETAL OSSIFICATION: FEMALE RATS

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
LITTERS EXAMINED	N	23	25	23	23
FETUSES EXAMINED	N	174	187	173	160
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN±S.D.	0.98 ± 0.10	1.00 ± 0.00	0.99 ± 0.04	1.00 ± 0.00
VERTEBRAE					
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	13.02 ± 0.06	13.06 ± 0.20	13.02 ± 0.04	13.03 ± 0.09
LUMBAR	MEAN±S.D.	5.97 ± 0.07	5.94 ± 0.20	5.98 ± 0.05	5.97 ± 0.09
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.03 ± 0.14
CAUDAL	MEAN±S.D.	7.37 ± 0.82	7.35 ± 0.65	7.33 ± 0.60	7.63 ± 0.61
RIBS (PAIRS)	MEAN±S.D.	13.01 ± 0.04	13.06 ± 0.20	13.01 ± 0.03	13.02 ± 0.06
STERNUM					
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	4.00 ± 0.00	3.99 ± 0.04	4.00 ± 0.02	3.97 ± 0.11
XIPHOID	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.02	1.00 ± 0.00	0.99 ± 0.04
FORELIMB b					
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.02	4.00 ± 0.02	3.98 ± 0.06
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	8.24 ± 0.78	8.49 ± 0.49	8.16 ± 0.59	8.05 ± 0.92
HINDLIMB b					
TARSALS	MEAN±S.D.	0.00 ± 0.00	0.03 ± 0.10	0.02 ± 0.06	0.02 ± 0.07
METATARSALS	MEAN±S.D.	4.85 ± 0.25	4.94 ± 0.09	4.91 ± 0.16	4.90 ± 0.22
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	6.60 ± 1.02	6.75 ± 1.06	6.58 ± 1.02	6.61 ± 1.09

a. Dose administration occurred on Study Day 1 through Gestation Day 17.
b. Calculated as average per limb.

The safety profile of etelcalcetide demonstrated in rats in this combined fertility and embryo-fetal development study was comparable to that of the original fertility (Study No 4169-NC-152) and embryo-fetal development (Study No 4169-NC-150) studies by KAI. Both studies showed no effects on male and female fertility and no effects on embryo-fetal development at doses up to 3 mg/kg. Similar maternal effects associated with hypocalcemia (clinical signs, changes in body weight and food consumption, consistent with changes in serum calcium) were noted in both studies.

Study No 4169-NC-152: Fertility and Early Embryonic Development

Study title: An Intravenous Study of the Potential Effects of KAI-4169 on Fertility and Early Embryonic Development through Implantation in Rats (GLP, testing facility: (b) (4)

Study description:
Etelcalcetide (at 0.75, 1.5, and 3.0 mg/kg/day, Lot No. F4169KP1001B) or vehicle (0.27% sodium succinate dibasic hexahydrate [2.7 g/L], 2% D-mannitol [20 g/L], 1% glycine [10 g/L], 1% trehalose [10 g/L], and 0.9% benzyl alcohol [9 g/L] in sterile water for injection, USP) was given to SD rats (~ 9-11 weeks of age; n = 25/sex/group) by intravenous injection once daily in a dose volume of 2 mL/kg. Male rats received 28 daily doses prior to mating and were also dosed throughout the mating period through 1 day prior to euthanasia (a total of 58-59 doses). Female rats received 14 daily doses prior to cohabitation and were dosed through Gestation Day 7 (a total of 22-36 doses). One female rat with no evidence of mating was dosed through the day prior to euthanasia (a total of 38 doses). All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. In addition, blood samples were collected from TK animals (n = 8/sex/group) approximately 8 hours after dose administration on the last day of the pre-mating

treatment period for analysis of serum calcium concentration. For female rats, a laparohysterectomy was performed on Gestation Day 15 with evidence of mating. The female with no evidence of mating was presumed to be pregnant and necropsied 10 days following completion of the mating period based on body weight gain. For male rats, they were euthanized following the necropsy of the last female. Spermatogenic evaluations were conducted on all males. Selected organs from all animals at the scheduled necropsies were weighed or retained.

Group Number	Treatment	Dosage Level (mg/kg/day)	Dosage Volume (mL/kg)	Number of Males	Number of Females
1	Vehicle Control	0	2	25	25
2	KAI-4169	0.75	2	25	25
3	KAI-4169	1.5	2	25	25
4	KAI-4169	3.0	2	25	25

Study findings:

- Administration of etelcalcetide at 0.75, 1.5, and 3.0 mg/kg/day resulted in dose-related hypocalcaemia, which was an expected effect stemming from its pharmacologic activity.
- Parental toxicity, manifested by clinical signs (tremors, red and/or yellow staining on various body surfaces, and salivation), lower mean body weights, body weight gains or body weight losses, with correspondingly lower mean food consumption, was noted in the 3.0 mg/kg/day group males and females.
- The NOAEL for systemic toxicity of etelcalcetide was 1.5 mg/kg/day in both sexes.
- There were no etelcalcetide-related effects on reproductive performance or embryonic development observed at any dosage level.
- The NOAEL for male and female reproductive toxicity and embryonic toxicity was 3 mg/kg/day.

Results of Male Reproductive Performance

Parameter	Dosage Level (mg/kg/day) 0	0.75	1.5	3.0	(b) (4) HC* Mean (Range)
Male Mating Index (%)	92.0	96.0	96.0	96.0	97.1 (88.0-100.0)
Male Fertility Index (%)	92.0	96.0	96.0	92.0	93.8 (87.5-100.0)
Male Copulation Index (%)	100.0	100.0	100.0	95.8	96.6 (88.0-100.0)

* = (b) (4) historical control data

Results of Female Reproductive Performance

Parameter	Dosage Level (mg/kg/day) 0	0.75	1.5	3.0	(b) (4) HC* Mean (Range)
Female Mating Index (%)	100.0	100.0	100.0	100.0	99.4 (95.0-100.0)
Female Fertility Index (%)	100.0	100.0	100.0	96.0	96.0 (88.0-100.0)
Female Conception Index (%)	100.0	100.0	100.0	96.0	96.7 (88.0-100.0)
Estrous Cycle Length (days)	4.1	4.0	4.2	4.4*	4.2 (3.7-5.2)
Pre-Coital Interval (days)	2.9	2.8	2.9	3.1	2.9 (2.0-3.9)

* = (b) (4) historical control data
* = Statistically significant at 0.05 compared to the control group.

9.2 Embryonic Fetal Development

See Section 9.1 for the results of the pivotal combined fertility and early embryonic development/embryo-fetal development toxicity study in rats

Study No 4169-NC-150: Embryo-fetal Development Toxicity Study in Rats

Study title: An Intravenous Dose Range-Finding Study of the Effects of KAI-4169 (Etelcalcetide) on Embryo/Fetal Development in Rats (GLP; testing facility: (b) (4)

Study description:
Etelcalcetide (at 0.8, 1.6, or 3.2 mg/kg/day) or vehicle (0.27% sodium succinate dibasic hexahydrate, 2% D-mannitol, 1% glycine, 1% trehalose, and 0.9% benzyl alcohol in sterile water for injection) was given to female Crl:CD(SD) rats (~ 14 weeks of age) once daily via intravenous injection through the lateral caudal vein in a dose volume of 2 mL/kg from Gestation Days 6 through 17. All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. In addition, blood samples for analysis of serum calcium concentrations were collected from all surviving rats at approximately 8 hours after dose administration on Gestation Days 6 and 17. On Gestation Day 20, a laparohysterectomy was performed on each surviving female. The uteri, placentae, and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed, and examined for external malformations and developmental variations.

Group Number	Treatment	Dosage Level (mg/kg/day)	Dosage Volume (mL/kg)	Number of Females
1	Vehicle Control	0	2	25
2	KAI-4169	0.8	2	25
3	KAI-4169	1.6	2	25
4	KAI-4169	3.2	2	25

Study findings:

- Administration of etelcalcetide at dosage levels of 0.8, 1.6, and 3.2 mg/kg/day resulted in dose-related hypocalcaemia, which is an expected effect stemming from its pharmacologic activity.
- Lower mean body weights and mean body weight gains, with corresponding reduced mean food consumption, were noted at 3.2 mg/kg/day.
- Therefore, a dosage level of 1.6 mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for maternal toxicity.
- The degree of hypocalcaemia in the 3.2 mg/kg/day group was potentially adverse and was likely the underlying cause of the effects on body weight gain, food consumption, and clinical condition observed in this study.
- Based on the lack of effects on intrauterine growth, survival, and fetal morphology, a dosage level of 3.2 mg/kg/day was considered to be the NOAEL for embryo/fetal development in rats.

Study No 4169-NC-140: Dose Range-Finding Embryo-fetal Development Toxicity Study in Rats

Study title: An Intravenous Dose Range-Finding Study of the Effects of KAI-4169 (Etelcalcetide) on Embryo/Fetal Development in Rats (GLP; testing facility: (b) (4)

Study description:

Reference ID: 3923456

Etelcalcetide (at 0.5, 1.5, 3.0, or 4.5 mg/kg/day) or vehicle (0.27% sodium succinate dibasic hexahydrate, 2% D-mannitol, 1% glycine, 1% trehalose, and 0.9% benzyl alcohol in sterile water for injection) was given to female Crl:CD(SD) rats (~ 14 weeks of age) once daily via intravenous injection through the lateral caudal vein in a dose volume of 2 mL/kg from Gestation Days 6 through 17. All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. In addition, blood samples for analysis of serum calcium concentrations were collected from all surviving rats at approximately 8 hours after dose administration on Gestation Days 6 and 17. On Gestation Day 20, a laparohysterectomy was performed on each surviving female. The uteri, placentae, and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed, and examined for external malformations and developmental variations.

Group Number	Treatment	Dosage Level (mg/kg/day)	Dosage Volume (mL/kg)	Number of Females
1	Vehicle Control	0	2	8
2	KAI-4169	0.5	2	8
3	KAI-4169	1.5	2	8
4	KAI-4169	3.0	2	8
5	KAI-4169	4.5	2	8

Study findings:

- Administration of etelcalcetide at dosage levels of 1.5, 3.0, and 4.5 mg/kg/day resulted in dose-related hypocalcaemia, which is an expected pharmacologic effect.
- Clinical signs, including tremors, piloerection, and yellow and/or red material findings were noted at 3.0 and 4.5 mg/kg/day. These effects have been observed in other studies conducted by the Sponsor and were attributed to the adverse sequelae of hypocalcaemia.
- In the presence of the above-noted maternal toxicity, lower mean fetal weights were also noted in the 4.5 mg/kg/day group.
- All of the adverse effects on maternal animals observed in this study may be secondary to hypocalcaemia.
- Based on the results of this study, dosage levels of 0.8, 1.6, and 3.2 mg/kg/day were selected for a definitive embryo/fetal development study of etelcalcetide administered via intravenous injection to bred Crl:CD(SD) rats.

Study No 119089: Embryo-fetal Development Toxicity Study in Rabbits (pivotal)

Study title: Etelcalcetide: Fertility and Embryo-Fetal Development Study in the New Zealand White Rabbit

Study no.:	119089
Study report location:	Amgen (Thousand Oaks, CA)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 19, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lot 1044951 with 98.9% purity

Key Study Findings

- Female rabbits received etelcalcetide once daily from GD 7 through GD 19 at 0.375, 0.75, and 1.5 mg/kg/day.

Reference ID: 3923456

- There were no etelcalcetide-related unscheduled necropsies.
- Maternal body weight gain was reduced in the 1.5 mg/kg dose group for the dosing intervals of GD 10 to 13 (20% of controls) and GD 13 to 16 (63% of controls), and during the post-dose interval of GD 20 to 29 (84% of controls). Overall, the average maternal body weight gain was 89% of controls for the interval of GD 7 to 29.
- Administration of etelcalcetide at 1.5 mg/kg also reduced absolute maternal food consumption for the interval of GD 7 to 20 (86% of controls) and GD 7 to 29 (92% of controls).
- There were no maternal macroscopic observations noted at necropsy.
- On GD 11, the mean maternal AUC_t was 4.07, 9.01, and 20.8 µg•hr/mL and the mean maternal Cmax was 0.879, 2.00, and 4.43 µg/mL in the 0.375, 0.75, and 1.5 mg/kg dose groups, respectively.
- Pregnancy was confirmed in 21 or 22 rabbits in each dose group, including controls. There were no etelcalcetide-related effects on gravid uterine weights or any ovarian or uterine parameters (corpora lutea, implantation sites, litter size, embryo-fetal survival, fetal weights, and fetal sex).
- There were no etelcalcetide-related fetal external, visceral, or skeletal malformations or variations. The NOAEL for both maternal toxicity and embryo-fetal development was 1.5 mg/kg/day.

Methods

Doses:	0.375, 0.75, and 1.5 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	2 mL/kg
Route of administration:	IV injection
Formulation/Vehicle:	0.27% Sodium succinate dibasic hexahydrate (2.7 g/L), 2% D-mannitol (20 g/L), 1 % glycine (10 g/L), 1% trehalose dihydrate (10 g/L) and 0.9% (9 g/L) benzyl alcohol in sterile water for injection (WFI), pH 4.5 ± 0.1
Species/Strain:	New Zealand White female rabbits
Number/Sex/Group:	22
Satellite groups:	n/a
Study design:	Females were administered etelcalcetide or the vehicle from Gestation Day (GD) 7-19. Based on the dosing schedule, males were administered 56 to 59 doses and females were administered 33 to 49 doses of etelcalcetide or vehicle control.
Deviation from study protocol:	Yes but no impact on study result

Table 61 ReproTOX Study No 119089: Rabbit Embryo-Fetal Development Study Design.

Experimental Design

Group No.	Test Material	Dose Level (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Rabbits (Assigned Rabbit Numbers)
1	Vehicle Control Article	0	0	2	22 (3901-3922)
2	AMG 416	0.375	0.188	2	22 (3923-3944)
3	AMG 416	0.75	0.375	2	22 (3945-3966)
4	AMG 416	1.5	0.75	2	22 (3967-3988)

All animals were monitored for maternal viability, maternal clinical signs, maternal body weights, maternal body weight changes, and maternal food consumption. The animals were also evaluated for macroscopic observations, and ovarian and uterine contents (i.e., number and distribution of corpora lutea, implantations, live and dead fetuses, and early and late resorptions), and gravid uterine weights were collected. Fetuses were evaluated for sex, body weights, and external, visceral, and skeletal abnormalities. Blood samples were collected on GD 11 to determine the toxicokinetics of etelcalcetide in pregnant rabbits.

Observations and Results

Mortality

There were no etelcalcetide-related unscheduled necropsies. There were four unscheduled necropsies (2, 1, and 1 at 0.375, 0.75, and 1.5 mg/kg, respectively) that occurred between GD 18 and GD 21. Due to the low incidence and lack of dose response these unscheduled necropsies were considered to be unrelated to etelcalcetide.

Clinical Signs

There were no etelcalcetide-related clinical signs at 0.375 or 0.75 mg/kg.
At 1.5 mg/kg, the only etelcalcetide-related clinical sign was scant feces (N=10 of 22), which was associated with reductions in food consumption.

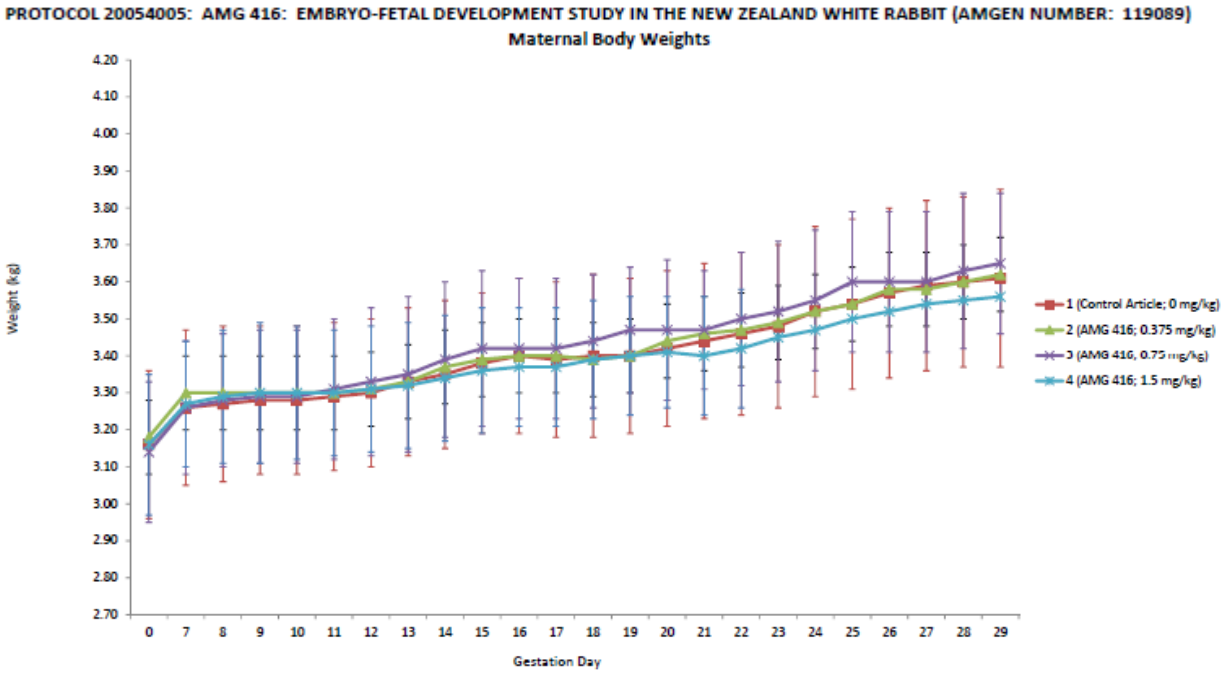
Body Weight

There were no etelcalcetide-related effects on maternal body weights or maternal body weight gain at 0.375 or 0.75 mg/kg.

At 1.5 mg/kg, maternal body weight gain was reduced for the dosing intervals of GD 10 to 13 (20% of controls) and GD 13 to 16 (63% of controls). During the post-dose period, the average maternal body weight gain at 1.5 mg/kg was reduced for the intervals of GD 20 to 24 (60% of controls) and GD 20 to 29 (84% of controls). Overall, the average maternal body weight gain was reduced for the interval of GD 7 to 29 compared to controls (89% of controls).
There were no etelcalcetide-related effects on the average gravid uterine weights, the corrected (GD 29C = corrected maternal body weight) maternal body weight, or the corrected maternal body weight gain.

Reference ID: 3923456

Figure 12 Repro TOX Study No 119089: Rabbit Body Weight.



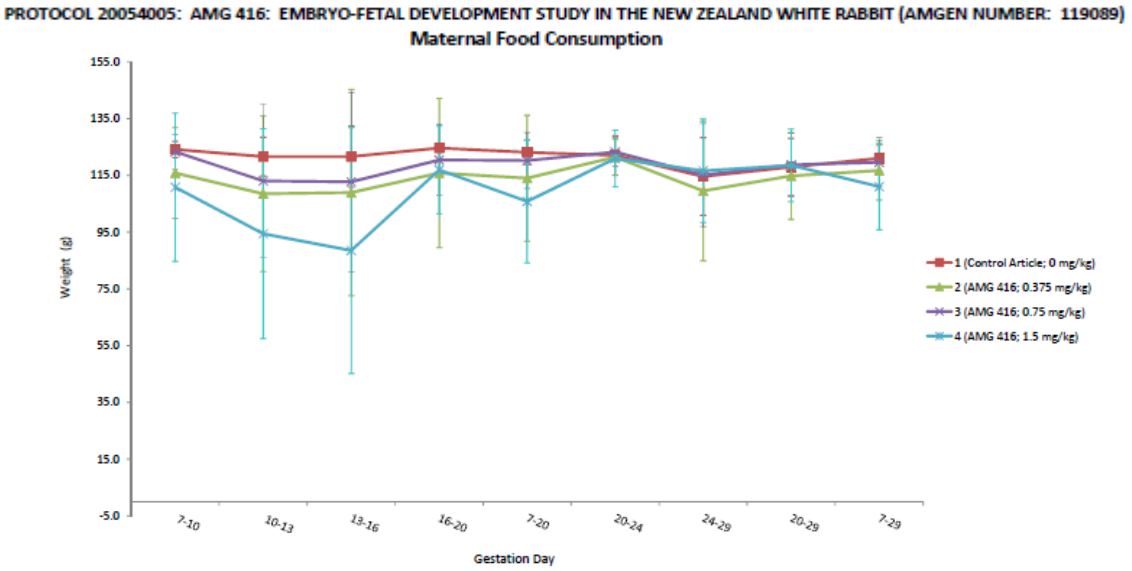
Feed Consumption

There were no etelcalcetide-related effects on the average maternal food consumption at 0.375 or 0.75 mg/kg.

At 1.5 mg/kg/day, maternal food consumption was significantly reduced ($p \leq 0.01$) for the interval of GD 7 to 20 compared to controls (86% of controls for both g/day and g/kg/day). Within the dosing period, maternal food consumption at 1.5 mg/kg was reduced at each tabulated interval between GD 7 and GD 20 (73% to 94% of controls). The most pronounced reductions in the average maternal food intake were between GD 10 and GD 16, with statistically significant reductions ($p \leq 0.01$) noted for the intervals of GD 10 to 13 and GD 13 to 16 (absolute only) compared to controls. The reductions in maternal food consumption in the 1.5 mg/kg dose group, specifically between GD 10 and GD 16, correlated with reduced maternal body weight gain.

During the post-dose period, the average maternal food intake (g/day and g/kg/day) was comparable across all remaining groups. Overall, at 1.5 mg/kg, absolute and relative food consumption was 92% of controls for the interval of GD 7 to 29.

Figure 13 Repro TOX Study No 119089: Rabbit Food Consumption.



Toxicokinetics
n/a

Dosing Solution Analysis
All study samples that were analyzed had mean concentrations that were within or equal to the acceptance criteria of ± 10% (individual values within or equal to ± 15%) of their theoretical concentrations.

Necropsy
There were no etelcalcetide-related macroscopic observations in the rabbits that survived to scheduled euthanasia.

Cesarean Section Data
(Implantation Sites, Pre- and Post-Implantation Loss, etc.)
There were no etelcalcetide-related effects on any ovarian or uterine parameter.
The litter averages for corpora lutea, implantations, the percentage of preimplantation loss, litter sizes, live and dead fetuses, early and late resorptions, the percentage of postimplantation loss, fetal body weights, the percentage of resorbed conceptuses, and the percentage of live male fetuses were comparable among the four dose groups and did not significantly differ from controls.

No mother had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. There were no observed abnormalities in any placentae.

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Table 62 ReproTOX Study No 119089: Rabbit C-Section Data.

SUMMARY OF OVARIAN AND UTERINE CONTENTS

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.375	0.75	1.5
RABBITS TESTED	N	22	22	22	22
PREGNANT	N(%)	21(95.4)	22(100.0)	21(95.4)	22(100.0)
UNSCHEDULED EUTHANASIA	N(%)	0(0.0)	2(9.1)	1(4.8)	1(4.5)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON GESTATION DAY 29	N	21	20	20	21
CORPORA LUTEA	MEAN±S.D.	10.1 ± 2.6	10.0 ± 1.5	9.9 ± 2.0	9.8 ± 1.8
IMPLANTATIONS	MEAN±S.D.	8.8 ± 1.4	9.5 ± 1.4	9.5 ± 2.0	9.1 ± 1.7
% PREIMPLANTATION LOSS	MEAN±S.D.	11.0 ± 13.0	4.1 ± 7.6	4.0 ± 6.0	6.8 ± 8.9
LITTER SIZES	MEAN±S.D.	8.5 ± 1.4	9.2 ± 1.2	9.3 ± 1.9	8.8 ± 1.9
LIVE FETUSES	N	178	184	186	186
	MEAN±S.D.	8.5 ± 1.4	9.2 ± 1.2	9.3 ± 1.9	8.8 ± 1.9
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.3 ± 0.5	0.3 ± 0.7	0.2 ± 0.4	0.3 ± 0.5
EARLY RESORPTIONS	N	3	1	2	5
	MEAN±S.D.	0.1 ± 0.4	0.0 ± 0.2	0.1 ± 0.3	0.2 ± 0.4
LATE RESORPTIONS	N	3	5	2	1
	MEAN±S.D.	0.1 ± 0.4	0.2 ± 0.7	0.1 ± 0.3	0.0 ± 0.2
% POSTIMPLANTATION LOSS	MEAN±S.D.	3.1 ± 5.0	2.8 ± 6.3	2.1 ± 4.5	3.6 ± 5.9

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100
a. Dose administration occurred on Gestation Days 7 through 19.

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.375	0.75	1.5
RABBITS TESTED	N	22	22	22	22
PREGNANT	N(%)	21(95.4)	22(100.0)	21(95.4)	22(100.0)
UNSCHEDULED EUTHANASIA	N(%)	0(0.0)	2(9.1)	1(4.8)	1(4.5)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON GESTATION DAY 29	N	21	20	20	21
DOES WITH ANY RESORPTIONS	N(%)	6(28.6)	4(20.0)	4(20.0)	6(28.6)
DOES WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	21(100.0)	20(100.0)	20(100.0)	21(100.0)
PLACENTAE APPEARED NORMAL	N(%)	21(100.0)	20(100.0)	20(100.0)	21(100.0)

a. Dose administration occurred on Gestation Days 7 through 19.

SUMMARY OF LITTER OBSERVATIONS

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.375	0.75	1.5
LITTERS WITH ONE OR MORE LIVE FETUSES	N	21	20	20	21
IMPLANTATIONS	MEAN±S.D.	8.8 ± 1.4	9.5 ± 1.4	9.5 ± 2.0	9.1 ± 1.7
LIVE FETUSES	N	178	184	186	186
	MEAN±S.D.	8.5 ± 1.4	9.2 ± 1.2	9.3 ± 1.9	8.8 ± 1.9
LIVE MALE FETUSES	N	76	89	87	79
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	42.9 ± 12.6	48.8 ± 13.4	47.1 ± 18.4	43.1 ± 15.2
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	42.67 ± 4.71	41.30 ± 3.51	42.00 ± 4.44	40.94 ± 4.21
MALE FETUSES	MEAN±S.D.	43.63 ± 4.72	42.25 ± 3.95	42.70 ± 4.46	41.04 ± 4.43
FEMALE FETUSES	MEAN±S.D.	42.11 ± 5.08	40.35 ± 3.92	41.24 ± 5.18	40.72 ± 4.50
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	3.1 ± 5.0	2.8 ± 6.3	2.1 ± 4.5	3.6 ± 5.9

a. Dose administration occurred on Gestation Days 7 through 19.

Offspring

(Malformations, Variations, etc.)

Fetal evaluations were based on 178, 184, 186, and 186 live GD 29 Caesarean-delivered fetuses in 21, 20, 20, and 21 litters at 0, 0.375, 0.75, and 1.5 mg/kg, respectively. There were no etelcalcetide-related fetal external, visceral or skeletal malformations or variations at any dose. In addition, all ossification site averages were comparable across all groups.

Table 63 ReproTOX Study No 119089: Rabbit Offspring Parameters.

SUMMARY OF FETAL GROSS EXTERNAL ALTERATIONS

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.375	0.75	1.5
LITTERS EVALUATED	N	21	20	20	21
LITTERS WITH LIVE FETUS(ES)	N	21	20	20	21
FETUSES EVALUATED	N	178	184	186	186
LIVE	N	178	184	186	186
HEAD: DOMED					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)	0(0.0)	0(0.0)

a. Dose administration occurred on Gestation Days 7 through 19.

SUMMARY OF FETAL SOFT TISSUE ALTERATIONS

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.375	0.75	1.5
LITTERS EVALUATED	N	21	20	20	21
LITTERS WITH LIVE FETUS(ES)	N	21	20	20	21
FETUSES EVALUATED	N	178	184	186	186
LIVE	N	178	184	186	186
BRAIN: MARKED VENTRICULAR DILATION					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)	0(0.0)	0(0.0)
HEART: VENTRICLE SMALL					
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)b	0(0.0)	0(0.0)	0(0.0)
HEART: INTERVENTRICULAR SEPTAL DEFECT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)d	0(0.0)
HEART: SEPTUM ABSENT					
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	0(0.0)	0(0.0)	0(0.0)
HEART: PAPILLARY MUSCLES ABSENT					
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	0(0.0)	0(0.0)	0(0.0)
VESSELS: DISTENDED AORTA					
LITTER INCIDENCE	N(%)	2(9.5)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	2(1.1)b,c	0(0.0)	0(0.0)	0(0.0)
VESSELS: CONSTRICTED PULMONARY					
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	0(0.0)	0(0.0)	0(0.0)
GALLBLADDER SMALL					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	2(1.1)d	1(0.5)
GALLBLADDER ABSENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)	0(0.0)

FOOTNOTES

a. Dose administration occurred on Gestation Days 7 through 19.
b. Fetus 3903-3 had other soft tissue alterations.
c. Fetus 3920-6 had other soft tissue alterations.
d. Fetus 3945-9 had other soft tissue alterations.

SUMMARY OF FETAL SKELETAL ALTERATIONS

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.375	0.75	1.5
LITTERS EVALUATED	N	21	20	20	21
LITTERS WITH LIVE FETUS(ES)	N	21	20	20	21
FETUSES EVALUATED	N	178	184	186	186
LIVE	N	178	184	186	186
SKULL: NASAL, CONTAINS AN INTRANASAL					
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
SKULL: NASALS, CONTAIN AN INTERNASAL					
LITTER INCIDENCE	N(%)	1(4.8)	1(5.0)	2(10.0)	0(0.0)
FETAL INCIDENCE	N(%)	2(1.1)	1(0.5)	2(1.1)	0(0.0)
SKULL: FRONTAL, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)	0(0.0)	0(0.0)
SKULL: PARIETAL, CONTAINS A HOLE					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)	0(0.0)	0(0.0)
HYOID: ALA, ANGULATED					
LITTER INCIDENCE	N(%)	0(0.0)	2(10.0)	1(5.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	4(2.2)	1(0.5)	1(0.5)
CERVICAL VERTEBRAE: CENTRUM, NOT OSSIFIED					
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
CERVICAL VERTEBRAE: ARCH, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
THORACIC VERTEBRAE: ARCH, NOT OSSIFIED					
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)

a. Dose administration occurred on Gestation Days 7 through 19.

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.375	0.75	1.5
LITTERS EVALUATED	N	21	20	20	21
LITTERS WITH LIVE FETUS(ES)	N	21	20	20	21
FETUSES EVALUATED	N	178	184	186	186
LIVE	N	178	184	186	186
THORACIC VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION					
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
THORACIC VERTEBRAE: ARCH, SMALL					
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	1(0.5)	0(0.0)
THORACIC VERTEBRAE: HEMIVERTEBRA					
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	1(0.5)	0(0.0)
THORACIC VERTEBRAE: CENTRUM, NOT OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)	0(0.0)
THORACIC VERTEBRAE: ARCHES, FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)	0(0.0)
THORACIC VERTEBRAE: ARCH, IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)	0(0.0)
LUMBAR VERTEBRAE: HEMIVERTEBRA					
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	1(0.5)	0(0.0)
LUMBAR VERTEBRAE: CENTRA, FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)	0(0.0)

a. Dose administration occurred on Gestation Days 7 through 19.

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.375	0.75	1.5
LITTERS EVALUATED	N	21	20	20	21
LITTERS WITH LIVE FETUS(ES)	N	21	20	20	21
FETUSES EVALUATED	N	178	184	186	186
LIVE	N	178	184	186	186
CAUDAL VERTEBRAE: MISALIGNED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)	0(0.0)
RIBS: SPLIT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)
RIBS: FUSED					
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	1(0.5)	0(0.0)
RIBS: PROXIMATE					
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
RIBS: IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	1(0.5)	0(0.0)
RIBS: 11 PRESENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)	0(0.0)
RIBS: THICKENED					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)	0(0.0)	1(0.5)
MANUBRIUM: IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	1(4.8)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)

a. Dose administration occurred on Gestation Days 7 through 19.

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.375	0.75	1.5
LITTERS EVALUATED	N	21	20	20	21
LITTERS WITH LIVE FETUS(ES)	N	21	20	20	21
FETUSES EVALUATED	N	178	184	186	186
LIVE	N	178	184	186	186
STERNAL CENTRA: FUSED					
LITTER INCIDENCE	N(%)	2(9.5)	2(10.0)	3(15.0)	3(14.3)
FETAL INCIDENCE	N(%)	4(2.2)	3(1.6)	4(2.2)	3(1.6)
STERNAL CENTRA: ASYMMETRIC					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)
FORE LIMB: DIGIT, ABSENT					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)	0(0.0)	0(0.0)
FORE LIMB: METACARPAL, ABSENT					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)	0(0.0)	0(0.0)
FORE LIMB: PHALANX, ABSENT					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.5)	0(0.0)	0(0.0)

a. Dose administration occurred on Gestation Days 7 through 19.

SUMMARY OF FETAL OSSIFICATION SITES

GROUP		1	2	3	4
TEST MATERIAL		VEHICLE CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.375	0.75	1.5
LITTERS EXAMINED	N	21	20	20	21
FETUSES EXAMINED	N	178	184	186	186
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
VERTEBRAE					
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	12.57 ± 0.33	12.62 ± 0.27	12.50 ± 0.30	12.49 ± 0.29
LUMBAR	MEAN±S.D.	6.43 ± 0.33	6.38 ± 0.27	6.50 ± 0.30	6.50 ± 0.28
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	16.47 ± 0.36	16.68 ± 0.26	16.48 ± 0.32	16.41 ± 0.45
RIBS (PAIRS)	MEAN±S.D.	12.46 ± 0.32	12.51 ± 0.25	12.42 ± 0.29	12.42 ± 0.28
STERNUM					
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	3.90 ± 0.14	3.93 ± 0.10	3.84 ± 0.23	3.84 ± 0.25
XIPHOID	MEAN±S.D.	0.94 ± 0.11	0.99 ± 0.03	0.94 ± 0.13	0.95 ± 0.10
FORELIMB b					
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	4.98 ± 0.04	4.94 ± 0.10	4.94 ± 0.16	4.93 ± 0.15
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	13.92 ± 0.16	13.87 ± 0.18	13.82 ± 0.29	13.78 ± 0.28
HINDLIMB b					
TARSALS	MEAN±S.D.	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
METATARSALS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
DIGITS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
PHALANGES	MEAN±S.D.	12.00 ± 0.02	12.00 ± 0.02	11.99 ± 0.04	12.00 ± 0.02

a. Dose administration occurred on Gestation Days 7 through 19.
b. Calculated as average per limb.

The safety profile of etelcalcetide demonstrated in rabbits in this embryo-fetal development study was comparable to that of the original embryo-fetal development study (Study No 4169-NC-149) by KAI. Both studies showed no effects on embryo-fetal development at doses up to 1.5 mg/kg. Similar maternal effects associated with hypocalcemia (clinical signs, changes in body weight and food consumption) were noted in both studies. Maternal plasma drug exposures measured on Day 11 in Study No 119088 were as expected based on exposures established in the rabbit (Study No 4169-NC-141).

Study No 4169-NC-149: Embryo-fetal Development Toxicity Study in Rabbits

Study title: An Intravenous Study of the Effects of KAI-4169 (Etelcalcetide) on Embryo/Fetal Development in Rabbits (GLP; testing facility: (b) (4)

Study description:
Etelcalcetide (at 0.375, 0.75, or 1.5 mg/kg/day) or vehicle (0.27% sodium succinate dibasic hexahydrate, 2% D-mannitol, 1% glycine, 1% trehalose, and 0.9% benzyl alcohol in sterile water for injection) was given to female New Zealand White rabbits (~ 6 months of age) once daily via intravenous injection through the left marginal ear vein in a dose volume of 2 mL/kg from Gestation Days 7 through 20. All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. In addition, blood samples for analysis of serum calcium concentrations were collected from all surviving rats at approximately 8 and 24 hours after dose administration on Gestation Days 7 and 20. An additional group (Group 5) was administered the test article on a comparable regimen; however, dosing for this group was initiated 7 days following the start of dosing of Groups 1-4 to allow for an initial assessment of tolerability of the test article at the 3.0 mg/kg/day dosage level. Based on the results noted at 3.0 mg/kg/day following 7 days of dosing, the dosage level for Group 5 was selected to be 2.25 mg/kg/day. On Gestation Day 29,

a laparohysterectomy was performed on each surviving female. The uteri, placentae, and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed, and examined for external malformations and developmental variations.

Group Number	Treatment	Dosage Level (mg/kg/day)	Dosage Volume (mL/kg)	Number of Females
1	Vehicle Control	0	2	22
2	KAI-4169	0.375	2	22
3	KAI-4169	0.75	2	22
4	KAI-4169	1.5	2	22

Study findings:

- Administration of etelcalcetide intravenously to time-mated New Zealand White rabbits at dosage levels of 0.375, 0.75 and 1.5 mg/kg/day resulted in hypocalcaemia, which was an expected effect stemming from its pharmacologic activity.
- Based on clinical signs of decreased defecation, lower mean body weights and mean body weight gains and/or losses with corresponding reduced mean food consumption noted at 1.5 mg/kg/day, a dosage level of 0.75 mg/kg/day was considered to be the NOAEL for maternal toxicity.
- Based on the lack of effects on intrauterine growth, survival, and fetal morphology, a dosage level of 1.5 mg/kg/day was considered to be the NOAEL for embryo/fetal development in rabbits.

Study No 4169-NC-141: Dose Range-Finding Embryo-fetal Developmental Toxicity Study in Rabbits

Study title: An Intravenous Dose Range-Finding Study of the Effects of KAI-4169 (Etelcalcetide) on Embryo/Fetal Development in Rabbits (GLP; testing facility: (b) (4)

Study description:
Etelcalcetide (at 0.5, 1.5, or. 3.0 mg/kg/day) or vehicle (0.27% sodium succinate dibasic hexahydrate, 2% D-mannitol, 1% glycine, 1% trehalose, and 0.9% benzyl alcohol in sterile water for injection) was given to female New Zealand White rabbits ((approximately 6 months of age) once daily via intravenous injection through the left marginal ear vein in a dose volume of 2 mL/kg from Gestation Days 7 through 20. All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. In addition, blood samples for analysis of serum calcium concentrations were collected from all surviving rats at approximately 8 and 24 hours after dose administration on Gestation Days 7 and 20. An additional group (Group 5) was administered the test article on a comparable regimen; however, dosing for this group was initiated 7 days following the start of dosing of Groups 1-4 to allow for an initial assessment of tolerability of the test article at the 3.0 mg/kg/day dosage level. Based on the results noted at 3.0 mg/kg/day following 7 days of dosing, the dosage level for Group 5 was selected to be 2.25 mg/kg/day. On Gestation Day 29, a laparohysterectomy was performed on each surviving female. The uteri, placentae, and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed, and examined for external malformations and developmental variations.

Group Number	Treatment	Dosage Level (mg/kg/day)	Test Article Concentration[a] (mg/mL)	pH[b]
1	Vehicle Control	0	0	4.50
2	KAI-4169	0.5	0.25	4.49
3	KAI-4169	1.5	0.75	4.49
4	KAI-4169	3.0	1.5	4.49
5	KAI-4169	2.25	1.125	4.48

[a] = A correction factor of 1.20 was used to account for the peptide content and purity of KAI-4169
[b] = pH measurement of the first dosing formulations for each group.

Study findings:

- Administration of etelcalcetide at dosage levels of 0.5, 1.5, 2.25, and 3.0 mg/kg/day resulted in dose-related hypocalcaemia.
- Two deaths and 1 abortion occurred at 3.0 mg/kg/day.
- Clinical signs including tremors, repetitive movement of the mouth and jaws, and decreased fecal output, and lower mean body weight gains with corresponding reduced mean food consumption were noted at 1.5, 2.25, and 3.0 mg/kg/day.
- The lower mean fetal weights in the 2.25 and 3.0 mg/kg/day groups could be secondary to decreased maternal body weight and, in part, could also be a consequence of unusually high mean fetal weight for the control group in this study.
- No other effects on embryo/fetal developmental parameters were observed in this study.
- All adverse effects on maternal animals observed in this study may be secondary to hypocalcaemia.
- Based on the study results, dosage levels of 0.375, 0.75, and 1.5 mg/kg/day were selected for a definitive embryo/fetal development study of etelcalcetide administered via intravenous injection to time-mated New Zealand White rabbits.

9.3 Prenatal and Postnatal Development

Study No 116847: Pre/Postnatal Development Toxicity Study in Rats (pivotal)

Study title: AMG 416 (etelcalcetide): Pre- and Postnatal Development Study in the Sprague-Dawley Rat

Study no.:	116874
Study report location:	Amgen (Thousand Oaks, CA)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 25, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lot 1041722 with 99.3% purity

Key Study Findings

- Sprague-Dawley [Crl:CD(SD)] rats received etelcalcetide once daily on GD 7 through LD 20 at 0.75, 1.5, or 3 mg/kg.
- Etelcalcetide-related maternal effects (one unscheduled necropsy, reduced body weight and/or body weight gain and reduced food intake) were observed at 3 mg/kg during the gestation and/or lactation periods. These maternal effects are consistent with hypocalcemia, the expected pharmacological effect of etelcalcetide.
- Etelcalcetide-related clinical signs were observed at 3 mg/kg late in the gestation period (slight excess salivation and tremors) and at ≥0.75 mg/kg during the lactation period

(moderate excess salivation, mild dehydration based on skin turgor, hunched posture, and urine-stained abdominal fur).

- There was a small delay in parturition and reduced pup weights at 1.5 and 3 mg/kg, in addition to increased incidences of dehydration in the F_1 generation pups and pup mortality at 3 mg/kg.
- At 3 mg/kg, the effects of etelcalcetide on pre-weaning pup weights continued into the post-weaning period in the F_1 generation males; however, the reductions were transient and did not persist throughout the growth period.
- There were no etelcalcetide-related effects on sexual maturation, neurobehavioral or reproductive function in the F_1 generation rats at any dose. The NOAEL for maternal toxicity and pre- and post-natal development was 1.5 mg/kg/day.

Methods

Doses:	0.75, 1.5, or 3 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	2 ml/Kg
Route of administration:	Intravenous bolus injection
Formulation/Vehicle:	0.27% Sodium Succinate Dibasic Hexahydrate (2.7 g/L), 2% D-mannitol (20 g/L), 1% Glycine (10 g/L), 1% Trehalose Dihydrate (10 g/L) and 0.9% (9 g/L) Benzyl Alcohol in sterile water for injection, pH 4.5 ± 0.1 (pH adjusted with 1N NaOH and/or 1N HCL as needed)
Species/Strain:	Female Sprague Dawley rats
Number/Sex/Group:	25 females
Satellite groups:	TK group (n=4)
Study design:	Females were administered etelcalcetide or the vehicle from Gestation Day (GD) 7 to Lactation Day (LD) 20 for rats delivered a litter or presumed GD 24 (for rats that did not deliver a litter). In addition, 4 rats per group were administered the control article or etelcalcetide formulations by the same route and doses for toxicokinetic evaluation beginning on GD 7 and continuing through GD 21. Toxicokinetic animals were euthanized at 1 to 2 hours post-dose on GD 21. F_0 generation females were allowed to deliver naturally. On LD 21, F_1 generation males and females were weaned and 1/sex/litter were selected for continuation on study for the assessment of any potential effects on growth, behavior, and reproductive capacity. All dams, and any pups not selected for continued evaluation, were euthanized on LD 21.
Deviation from study protocol:	Yes but no impact on final study result

Reference ID: 3923456

Table 64 ReproTOX Study No 116847: Rat Pre- and Postnatal Development Study Design.

Experimental Design

Group No.	Test Material	Dose Level (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Female Rats (Assigned Female Rat Numbers)	
					Main Study	TK Study
1	Vehicle Control Article	0	0	2	25 (701-725)	4 (801-804)
2	AMG 416	0.75	0.375	2	25 (726-750)	4 (805-808)
3	AMG 416	1.5[a]	0.75	2	25 (751-775)	4 (809-812)
4	AMG 416	3	1.5	2	25 (776-800)	4 (813-816)

TK = Toxicokinetic Study.

[a] On GD 14 (16 July 2013), F0 generation female rat nos. 751 through 756 and 758 were administered a single dose of the 3.0 mg/kg (Group 4) formulations. See Appendix 1 - Protocol, Amendments, and Deviations.

All F_0 generation females were monitored for maternal viability, maternal clinical signs, maternal body weights, maternal body weight changes, and maternal food consumption. Additional endpoints in the F_0 generation rats that were assigned to the natural delivery phase included assessments of parturition, lactation, and maternal behavior. Maternal blood samples were collected from TK study animals on GD 7 and GD 20 at 5 minutes, 2, 5, 8, and 24 hours post-dose to determine the toxicokinetics of etelcalcetide. In addition, maternal and fetal blood samples were collected 1 to 2 hours following maternal dose administration on GD 21 to determine the concentration of etelcalcetide in pooled fetal plasma and percent of fetal plasma to maternal plasma concentrations.

Prior to weaning, all F_1 generation rats were assessed for viability, clinical signs, and body weight. During the post-weaning period, all F_1 generation rats selected for continuation on study were monitored for viability, clinical signs, body weights, food consumption, sexual maturation, changes in behavior (as evaluated by passive avoidance, motor activity, and water maze testing), and macroscopic observations. Adult F_1 males and females were cohabited for the assessment of reproductive function. The ovaries and uteri of F_1 females were examined for the number and distribution of corpora lutea, implantations, and/or viable and nonviable embryos.

Observations and Results

F_0 Dams

Survival: There were no etelcalcetide-related deaths during the gestation period.

However, at 3 mg/kg, one F_0 generation female rat was euthanized on LD 17 because of maternal body weight loss and reduced food intake in the days prior to euthanasia, thin body condition, and clinical signs (tremors, salivation, dehydration) that were consistent with hypocalcemia, the expected pharmacology of etelcalcetide.

Clinical signs: During the gestation period, there were no etelcalcetide related clinical signs at 0.75 and 1.5 mg/kg. During the latter part of the gestation period, etelcalcetide-related clinical signs in the F_0 generation females at 3 mg/kg were consistent with hypocalcemia (slight excess salivation [GD 18 to 21] and tremors [GD 15 to GD 22]).

During the lactation period, these same clinical signs in F_0 generation females at 3 mg/kg persisted together with the

observation of moderate excess salivation, mild dehydration based on skin turgor, hunched posture, and urine-stained abdominal fur. Also in F_0 generation females during this period, slight excess salivation and urine-stained abdominal fur were observed at 1.5 mg/kg and tremors were observed at 0.75 and 1.5 mg/kg.

Body weight: During the gestation period, maternal body weight gain was reduced in the 3 mg/kg dose group for the dosing intervals of GD 15 to 18 (78% of controls) and GD 18 to 20 (80% of controls) and overall for the interval of GD 7 to 20 (85% of controls) compared to controls. In addition, at 3 mg/kg, the mean maternal body weight was reduced on GD 19 and 20 (97% and 96% of controls, respectively). During the lactation period, reductions in maternal body weight continued through LD 7 (96% of controls). There were no etelcalcetide-related effects on the mean maternal body weights or maternal body weight gain during the lactation period at 0.75 and 1.5 mg/kg.

Feed consumption: During the gestation period, at 3 mg/kg, there were corresponding reductions in maternal food intake (g/day and g/kg/day) observed for the dosing intervals of GD 15 to 18 (87% and 88% of controls, respectively) and GD 18 to 20 (82% and 85% of controls, respectively) and overall for the interval of GD 7 to 20 (93% of controls) compared to controls.

During the lactation period, these effects of etelcalcetide on maternal food consumption persisted in the 3 mg/kg dose group, resulting in overall reduction in food intake for the interval of LD 1 to 14 compared to controls (91% and 93% of controls, respectively).

Uterine content: There was a small increase in the average duration of gestation at 1.5 and 3 mg/kg compared to controls (22.7 and 22.8 days, respectively, vs. 22.4 in controls), which may be due to hypocalcemia owing to the role of calcium in smooth muscle (uterine) contractions.

At 3 mg/kg, fewer liveborn pups were delivered (329 liveborn pups vs. 348 in controls), and the number of stillborn pups was increased compared to controls (6 vs. 1 in controls). The percentage of pups surviving from Day 1 postpartum to Day 4 postpartum (viability index) was reduced at 3 mg/kg (95.1% compared to 98.6% in controls), which reflected an increase in pup mortality (found dead or presumed cannibalized).

Necropsy observation: There were no maternal macroscopic detected.

Toxicokinetics: On GD 20, the mean etelcalcetide exposures (AUC_{24}) ranged from 2.24 to 8.64 µg·hr/mL with mean C_{max} ranging from 1.59 to 6.84 µg/mL. Exposures on GD 20 were slightly higher than those after the first dose on GD 7.

The observed mean percent of fetal plasma to maternal plasma

Reference ID: 3923456

concentration was 2.99%, 2.86%, and 2.39% at the 0.75, 1.5, and 3 mg/kg dose levels on GD 21, indicating low placental transfer of etelcalcetide.

Dosing Solution Analysis All prepared dose formulations were acceptable for use on this study.

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Table 65 ReproTOX Study No 116847: Rat F_0 Dams.

SUMMARY OF NATURAL DELIVERY OBSERVATIONS: F0 GENERATION FEMALE RATS

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
RATS TESTED	N	25	25	25	25
PREGNANT	N(%)	25(100.0)	25(100.0)	23(92.0)	25(100.0)
DELIVERED A LITTER	N(%)	25(100.0)	25(100.0)	23(100.0)	25(100.0)
DURATION OF GESTATION b	MEAN±S.D.	22.4 ± 0.5	22.4 ± 0.5	22.7 ± 0.4	22.8 ± 0.6*
IMPLANTATION SITES	N	368	383	356	366
PER DELIVERED LITTER	MEAN±S.D.	14.7 ± 1.4	15.3 ± 1.2	15.5 ± 1.7	14.6 ± 1.3
DAMS WITH STILLBORN PUPS	N(%)	1(4.0)	1(4.0)	2(8.7)	2(8.0)
DAMS WITH NO LIVEBORN PUPS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
GESTATION INDEX c	%	100.0	100.0	100.0	100.0
	N/N	25/ 25	25/ 25	23/ 23	25/ 25
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

a. Dose administration occurred on Gestation Day 7 through Lactation Day 20 (rats that deliver a litter) or Gestation Day 24 (rats that do not deliver a litter).
b. Calculated (in days) as the time elapsed between confirmed mating (arbitrarily defined as Gestation Day 0) and the day the first pup was delivered.
c. Number of rats with live offspring/number of pregnant rats.
* Significantly different from the control group value ($p \leq 0.05$).

SUMMARY OF LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS): F1 GENERATION LITTERS

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	25	25	23	25
PUPS DELIVERED (TOTAL)	N	349	363	336	338
	MEAN±S.D.	14.0 ± 1.6	14.5 ± 1.3	14.6 ± 1.7	13.5 ± 1.4
LIVEBORN	MEAN±S.D.	13.9 ± 1.6	14.5 ± 1.3	14.5 ± 1.6	13.2 ± 2.2
	N(%)	348(99.7)	362(99.7)	334(99.4)	329(97.3)**
STILLBORN	MEAN±S.D.	0.0 ± 0.2	0.0 ± 0.2	0.1 ± 0.3	0.2 ± 0.9
	N(%)	1(0.3)	1(0.3)	2(0.6)	6(1.8)
UNKNOWN VITAL STATUS b	N	0	0	0	3
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED					
DAY 1	N/N(%)	0/348(0.0)	1/362(0.3)	0/334(0.0)	4/329(1.2)**
DAYS 2- 4	N/N(%)	5/348(1.4)	1/361(0.3)	4/334(1.2)	12/325(3.7)**
DAYS 5- 7	N/N(%)	0/343(0.0)	0/360(0.0)	3/330(0.9)	1/313(0.3)
DAYS 8-14	N/N(%)	1/343(0.3)	0/360(0.0)	1/327(0.3)	1/312(0.3)
DAYS 15-18	N/N(%)	1/342(0.3)	0/360(0.0)	0/326(0.0)	0/302(0.0)c
DAYS 19-21	N/N(%)	0/341(0.0)	0/360(0.0)	1/326(0.3)	0/302(0.0)c
VIABILITY INDEX d	%	98.6	99.4	98.8	95.1**
	N/N	343/348	360/362	330/334	313/329
LACTATION INDEX e	%	99.4	100.0	98.5	99.3c
	N/N	341/343	360/360	325/330	302/304c

DAY(S) = DAY(S) POSTPARTUM
a. Dose administration occurred on Gestation Day 7 through Lactation Day 20 (rats that deliver a litter) or Gestation Day 24 (rats that do not deliver a litter).
b. Maternal cannibalization precluded identification of vital status at birth.
c. Excludes 9 pups from litter 799 that were euthanized on Day 17 postpartum due to death of dam.
d. Number of live pups on Day 4 postpartum/number of liveborn pups on Day 1 postpartum.
e. Number of live pups on Day 21 (weaning) postpartum/number of live pups on Day 4 postpartum.
** Significantly different from the control group value (p≤0.01).

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	25	25	23	25
SURVIVING PUPS/LITTER b					
DAY 1c	MEAN±S.D.	13.9 ± 1.6	14.5 ± 1.3	14.5 ± 1.6	13.2 ± 2.2
DAY 4	MEAN±S.D.	13.7 ± 1.8	14.4 ± 1.3	14.3 ± 1.7	12.5 ± 2.4
DAY 7	MEAN±S.D.	13.7 ± 1.8	14.4 ± 1.3	14.2 ± 1.8	12.5 ± 2.4
DAY 14	MEAN±S.D.	13.7 ± 1.7	14.4 ± 1.3	14.2 ± 1.7	12.4 ± 2.4
DAY 18	MEAN±S.D.	13.6 ± 1.7	14.4 ± 1.3	14.2 ± 1.7	12.6 ± 2.3 [24]d
DAY 21	MEAN±S.D.	13.6 ± 1.7	14.4 ± 1.3	14.1 ± 1.7	12.6 ± 2.3 [24]d
PERCENT MALE PUPS PER NUMBER OF PUPS SEXED					
DAY 1c	MEAN±S.D.	50.0 ± 17.1	49.3 ± 13.6	46.9 ± 12.1	45.5 ± 13.0
DAY 4	MEAN±S.D.	50.7 ± 17.6	49.6 ± 13.8	47.1 ± 12.6	44.9 ± 12.6
DAY 7	MEAN±S.D.	50.7 ± 17.6	49.6 ± 13.8	47.4 ± 13.4	45.0 ± 12.5
DAY 14	MEAN±S.D.	50.5 ± 17.6	49.6 ± 13.8	47.5 ± 13.4	45.2 ± 12.7
DAY 18	MEAN±S.D.	50.4 ± 17.5	49.6 ± 13.8	47.5 ± 13.4	45.2 ± 13.0 [24]d
DAY 21	MEAN±S.D.	50.4 ± 17.5	49.6 ± 13.8	47.3 ± 13.5	45.2 ± 13.0 [24]d

DAY = DAY POSTPARTUM
[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred on Gestation Day 7 through Lactation Day 20 (rats that deliver a litter) or Gestation Day 24 (rats that do not deliver a litter).
b. Average number of live pups per litter, including litters with no surviving pups.
c. Includes liveborn pups and pups that died before weighing on Day 1 postpartum.
d. Excludes litter 799, which was euthanized on Day 17 postpartum due to death of dam.

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)a		0	0.75	1.5	3
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	25	25	23	25
LIVE LITTER SIZE AT WEIGHING					
DAY 1	MEAN±S.D.	13.9 ± 1.6	14.4 ± 1.3	14.5 ± 1.6	13.0 ± 2.4
DAY 4	MEAN±S.D.	13.7 ± 1.8	14.4 ± 1.3	14.3 ± 1.7	12.5 ± 2.4
DAY 7	MEAN±S.D.	13.7 ± 1.8	14.4 ± 1.3	14.2 ± 1.8	12.5 ± 2.4
DAY 14	MEAN±S.D.	13.7 ± 1.7	14.4 ± 1.3	14.2 ± 1.7	12.4 ± 2.4
DAY 18	MEAN±S.D.	13.6 ± 1.7	14.4 ± 1.3	14.2 ± 1.7	12.6 ± 2.3 [24]b
DAY 21	MEAN±S.D.	13.6 ± 1.7	14.4 ± 1.3	14.1 ± 1.7	12.6 ± 2.3 [24]b
PUP WEIGHT/LITTER (GRAMS)					
DAY 1	MEAN±S.D.	6.4 ± 0.6	6.3 ± 0.4	6.2 ± 0.5	5.9 ± 0.5**
DAY 4	MEAN±S.D.	9.1 ± 1.1	8.7 ± 0.9	8.5 ± 1.1*	7.9 ± 1.1**
DAY 7	MEAN±S.D.	13.1 ± 1.6	12.5 ± 1.5	12.0 ± 1.6*	11.2 ± 1.6**
DAY 14	MEAN±S.D.	24.9 ± 2.9	24.1 ± 2.4	23.3 ± 2.6*	21.6 ± 2.9**
DAY 18	MEAN±S.D.	31.0 ± 3.8	30.0 ± 3.0	29.1 ± 3.3	28.2 ± 4.0* [24]b
DAY 21	MEAN±S.D.	37.8 ± 5.7	36.5 ± 4.4	35.6 ± 4.6	34.5 ± 5.2 [24]b

DAY = DAY POSTPARTUM
[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred on Gestation Day 7 through Lactation Day 20 (rats that deliver a litter) or Gestation Day 24 (rats that do not deliver a litter).
b. Excludes litter 799, which was euthanized on Day 17 postpartum due to death of dam.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

Table 66 ReproTOX Study No 116847: Rat TK Parameters.

Summary of Mean (SD) TK Parameter Estimate of AMG 416 After Intravenous Administration of 0.75, 1.5, or 3 mg/kg in Rats

Dose (mg/kg)	Gestation Day 7			Gestation Day 20		
	t_{max} (hr)	C_{max} (µg/mL)	AUC_{24} (µg·hr/mL)	t_{max} (hr)	C_{max} (µg/mL)	AUC_{24} (µg·hr/mL)
0.75	0.083	0.984 (0.349)	1.33 (0.380)	0.083	1.59 (0.563)	2.24 (0.674)
1.5	0.083	2.19 (0.236)	2.71 (0.390)	0.083	2.97 (1.21)	4.05 (1.28)
3	0.083	4.28 (1.15)	5.56 (1.43)	0.083	6.84 (1.69)	8.64 (2.11)

AUC_{24} = area under the concentration-time curve from time zero to the time 24 hours postdose; C_{max} = maximum observed drug concentration during a dosing interval; N = number of animals; t_{max} = time to reach C_{max}, reported as a median (range).

All values were rounded to 3 significant figures after calculations were performed, except t_{max}, which was rounded to 2 significant figures.

Summary of Infant and Maternal Plasma AMG 416 Concentrations

Dose (mg/kg)	Gestation Day 21		
	Mean Maternal Concentration (ng/mL)	Pooled Litter Concentration (ng/mL)	Infant:Maternal (%)
0.75	234 ± 43.4	6.76 ± 2.10	2.99 ± 1.22
1.5	487 ± 141	13.5 ± 2.03	2.86 ± 0.462
3	928 ± 61.4	22.3 ± 4.02	2.39 ± 0.290

F_1 Generation

Survival: All F_1 generation rats survived to scheduled euthanasia.

Clinical signs: At 3 mg/kg, an increased number of litters (11 out of 25) had one or more pups that were mildly dehydrated (based on skin turgor) on one or more occasion between Days 6 and 20 postpartum. There were

no etelcalcetide-related clinical signs observed in the F_1 generation males or females.

Body weight: etelcalcetide-related reductions in the average pup weight were observed at 1.5 mg/kg on Days 4, 7, and 14 postpartum (92% to 94% of controls) and at 3 mg/kg at each tabulated interval between Days 1 and 21 postpartum (85% to 92% of controls). Reflecting these reductions in pre-weaning body weights, there were etelcalcetide-related reductions in the mean body weights on Days 22 and 29 postpartum (91% and 93% of controls, respectively) at 3 mg/kg in the F_1 generation males.

Feed consumption: There were no etelcalcetide-related changes in food consumption (g/day and g/kg/day) in the F_1 generation males or females at any dose. Food consumption in the males and females during the post-weaning period and in females during the gestation period was comparable among all dose groups.

Physical development: No effect on sexual maturation. The mean day of observation of male balano-preputial separation (45.0 to 46.1 days) and of female vaginal patency (32.9 to 33.4 days) was comparable across the dose groups.

Neurological assessment: No effect on behavioral endpoints (learning and memory or activity) - There were no etelcalcetide-related effects on short-term and long-term retention in the F_1 generation males and females, as evaluated during passive avoidance testing on Day 24 postpartum ± 1 day. There were no etelcalcetide-related differences in either ambulation or fine movement in the F_1 generation males and females, as evaluated during motor activity testing on Day 60 postpartum ± 2 days. Besides increased errors per trial in water maze testing (in a non-dose dependent manner when compared to control), there were no etelcalcetide-related effects on overt coordination, swimming ability, learning and memory in the F_1 generation males and females, as evaluated during M-shaped water maze testing during on Day 70 postpartum ± 2 days.

Reproduction: No effect on mating, fertility or ovarian/uterine parameters. There were no etelcalcetide-related effects on F_1 generation offspring mating or fertility. The average number of days in cohabitation ranged from 2.0 to 3.5 days in males and 2.0 to 4.1 days in females, and the mating index was 96.0% or 100%. The male and female fertility index (number of pregnancies/number of pairs that mated) was 92.0% or 100%.

<u>MALE</u>: There were no etelcalcetide-related effects on terminal body weights or paired testes weights in the F_1 generation male rats.

<u>FEMALE</u>: Pregnancy was confirmed in 23 (92.0%), 25 (100.0%), 24 (100.0%), and 23 (92.0%) F_1 generation rats in the 0, 0.75, 1.5, and 3 mg/kg dose groups, respectively. There were no etelcalcetide-related effects on any ovarian and uterine parameter in the F_1 generation female rats. The litter averages for corpora lutea, implantations, litter sizes, viable and nonviable embryos, and the percentage of postimplantation loss were comparable among the four dose groups. There were no detectable abnormalities in any

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placentae. The increase (p≤0.01) in preimplantation loss that occurred in the 1.5 mg/kg dose group was considered unrelated to etelcalcetide because the increase was not dose dependent.

Other: There were no etelcalcetide-related macroscopic observations in the F_1 generation pups at any dose level. There were no macroscopic observations detected in the F_1 generation male or female rats at necropsy examination

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Table 67 ReproTOX Study No 116847: Rat F_1 Generations (Preweaning).

SUMMARY OF CLINICAL OBSERVATIONS FROM BIRTH TO DAY 21 POSTPARTUM: F1 GENERATION PUPS

MATERNAL DOSE GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)		0	0.75	1.5	3
LITTERS EXAMINED	N	25	25	23	25
TRANSIENT CLINICAL OBSERVATIONS:a					
DEHYDRATION, TOTAL	N/N	28/4	13/1	64/3	147/11**
MILD	N/N	26/4	13/1	64/3	145/11**
MODERATE	N/N	2/1	0/0	0/0	2/ 1
UNGROOMED COAT	N/N	42/1	77/3	236/6	139/7
COLD TO TOUCH	N/N	0/0	0/0	19/6**	5/3
NO MILK BAND PRESENT	N/N	0/0	0/0	3/3	2/2
NOT NURSING	N/N	0/0	0/0	1/1	1/1
HEAD TILT TO THE LEFT	N/N	0/0	0/0	0/0	4/1
RIGHT EYE: ENOPHTHALMOS	N/N	0/0	0/0	0/0	4/1
PALE	N/N	0/0	0/0	3/3**	0/0
LOWER MIDLINE OR BACK, SCAB b	N/N	1/1	1/1	1/1	0/0
DECREASED MOTOR ACTIVITY	N/N	1/1	0/0	1/1	0/0
BRADYPNEA	N/N	0/0	0/0	1/1	0/0
WHOLE BODY, PURPLE	N/N	0/0	0/0	1/1	0/0
TAIL, THREADLIKE	N/N	0/0	4/1	0/0	0/0
LOWER MIDLINE, WHITE	N/N	0/0	2/1	0/0	0/0
LOWER MIDLINE, SWOLLEN	N/N	3/1	0/0	0/0	0/0

N/N = TOTAL FREQUENCY (DAYS X PUPS)/LITTER WITH OBSERVATIONS
a. Tabulation restricted to adverse observations; all other pups appeared normal.
b. Scab location was not recorded for 1 pup in litter 705 on Day 21 postpartum.
** Significantly different from the control group value (p≤0.01).

MATERNAL DOSE GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)		0	0.75	1.5	3
LITTERS EXAMINED	N	25	25	23	25
PERSISTENT CLINICAL OBSERVATIONS:a					
TAIL OR PORTION OF TAIL, MISSING	N/N	1/1	17/1	0/0	0/0

N/N = TOTAL FREQUENCY (DAYS X PUPS)/LITTER WITH OBSERVATIONS
a. Tabulation restricted to adverse observations; all other pups appeared normal.

SUMMARY OF NECROPSY OBSERVATIONS: F1 GENERATION PUPS

MATERNAL DOSE GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
DOSE LEVEL (MG/KG)		0	0.75	1.5	3
LITTERS EVALUATED	N	25	25	23	25
TOTAL PUPS FOUND DEAD OR STILLBORN a,b	N	2	2	6	16
FOUND DEAD	N	1	1	4	10
STILLBORN	N	1	1	2	6
NO MILK IN STOMACH c	N(%)	1(100.0)	1(100.0)	1(33.3)d	9(90.0)
APPEARED NORMAL	N(%)	1(50.0)	1(50.0)	5(83.3)	7(43.8)
PUPS EUTHANIZED AND NECROPSIED ON DAY 17 or 21 POSTPARTUM b					
LITTERS EVALUATED	N	25	25	23	25
PUPS EVALUATED	N	291	310	275	261
APPEARED NORMAL					
LITTER INCIDENCE	N(%)	25(100.0)	25(100.0)	23(100.0)	25(100.0)
PUP INCIDENCE	N(%)	291(100.0)	309(99.7)	275(100.0)	261(100.0)
KIDNEYS: RIGHT, PELVIS, SLIGHT DILATION					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.0)	0(0.0)	0(0.0)
PUP INCIDENCE	N(%)	0(0.0)	1(0.3)	0(0.0)	0(0.0)

a. Restricted to pups in which complete necropsies were performed. Complete necropsies were not performed on pups in which autolysis or cannibalization precluded full evaluation.
b. Refer to the individual pup clinical observations appendix (Appendix 13) for external clinical observations confirmed at necropsy.
c. Analysis restricted to pups found dead and necropsied.
d. Excludes one pup of litter 759, which did not have presence of milk in stomach recorded.

Table 68 ReproTOX Study No 116847: Rat F1 Generations (Post-weaning).

SUMMARY OF SEXUAL MATURATION: F1 GENERATION RATS

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
MATERNAL DOSE LEVEL (MG/KG)		0	0.75	1.5	3
MALE RATS	N	25	25	25	25
PREPUTIAL SEPARATION a	MEAN±S.D.	45.2 ± 2.1	45.0 ± 1.8	45.9 ± 1.8	46.1 ± 2.6
BODY WEIGHT AT SEPARATION (G)b	MEAN±S.D.	218.5 ± 24.9	219.4 ± 20.8	215.2 ± 17.3	216.2 ± 23.2
FEMALE RATS	N	25	25	24c	24d
VAGINAL PATENCY e	MEAN±S.D.	33.1 ± 1.4	33.4 ± 1.5	32.9 ± 1.7	33.1 ± 1.9
BODY WEIGHT AT VAGINAL PATENCY (G)f	MEAN±S.D.	100.0 ± 12.2	106.4 ± 13.8	98.1 ± 11.6	98.4 ± 9.4

a. Average day postpartum that the prepuce was observed to be separated.
b. Average body weight on day prepuce was first observed to be separated.
c. Excludes values for rat 1766, which was found to be a male on Day 24 postpartum.
d. Excludes values for rat 1797, which appeared incorrectly recorded.
e. Average day postpartum that the vagina was observed to be patent.
f. Average body weight on day vagina was first observed to be patent.

SUMMARY OF PASSIVE AVOIDANCE PERFORMANCE: F1 GENERATION RATS

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
MATERNAL DOSE LEVEL (MG/KG)		0	0.75	1.5	3
MALE RATS					
SESSION 1a	N	24	25	23	24
TRIALS TO CRITERION	MEAN±S.D.	4.0 ± 0.9	4.1 ± 0.8	5.0 ± 1.6	4.4 ± 1.5
LATENCY TRIAL 1b	MEAN±S.D.	7.2 ± 5.3	8.6 ± 7.3	9.2 ± 6.7	9.1 ± 5.7
LATENCY TRIAL 2b	MEAN±S.D.	32.8 ± 23.2	24.8 ± 21.3	28.1 ± 23.0	33.1 ± 22.7
FAILED TO LEARN c	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SESSION 2a	N	24	25	23	24
TRIALS TO CRITERION	MEAN±S.D.	2.8 ± 0.6	3.6 ± 2.7	2.8 ± 0.6	2.8 ± 1.0
LATENCY TRIAL 1b	MEAN±S.D.	31.9 ± 24.3	29.1 ± 23.0	32.6 ± 22.4	42.4 ± 22.8
FEMALE RATS					
SESSION 1a	N	25	25	22	24
TRIALS TO CRITERION	MEAN±S.D.	5.0 ± 1.6	4.5 ± 1.0	5.2 ± 1.4	4.2 ± 0.9
LATENCY TRIAL 1b	MEAN±S.D.	6.8 ± 3.5	7.2 ± 6.2	7.0 ± 3.9	9.5 ± 6.9
LATENCY TRIAL 2b	MEAN±S.D.	26.0 ± 21.2	28.5 ± 24.4	18.9 ± 19.7	29.1 ± 20.4
FAILED TO LEARN c	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SESSION 2a	N	25	25	22	24
TRIALS TO CRITERION	MEAN±S.D.	2.9 ± 0.8	2.9 ± 0.8	3.4 ± 1.0	3.1 ± 1.3
LATENCY TRIAL 1b	MEAN±S.D.	27.5 ± 24.7	30.0 ± 26.8	24.3 ± 24.2	28.9 ± 25.0

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. The latency was recorded in seconds.
c. Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from summarization and statistical analyses.

SUMMARY OF WATERMAZE PERFORMANCE: F1 GENERATION RATS

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
MATERNAL DOSE LEVEL (MG/KG)		0	0.75	1.5	3
MALE RATS					
RATS TESTED	N	24	25	23	25
SESSION 1a	N	24	25	23	25
TRIALS TO CRITERION	MEAN±S.D.	8.2 ± 2.0	8.4 ± 2.7	8.2 ± 2.2	8.4 ± 2.3
ERRORS PER TRIAL	MEAN±S.D.	0.34 ± 0.21	0.30 ± 0.11	0.39 ± 0.21	0.45 ± 0.24
LATENCY TRIAL 2b	MEAN±S.D.	12.4 ± 10.8	10.5 ± 5.7	19.3 ± 13.7*	14.0 ± 9.8
FAILED TO LEARN c	N(%)	0(0.0)	2(8.0)	0(0.0)	0(0.0)
SESSION 2a	N	24	23	23	25
TRIALS TO CRITERION	MEAN±S.D.	6.2 ± 2.2	6.5 ± 2.1	6.2 ± 1.8	6.8 ± 2.7
ERRORS PER TRIAL	MEAN±S.D.	0.09 ± 0.15	0.09 ± 0.12	0.09 ± 0.13	0.13 ± 0.14
LATENCY TRIAL 1b	MEAN±S.D.	9.4 ± 8.9	7.8 ± 3.5	9.7 ± 4.3	9.4 ± 6.4

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. The latency was recorded in seconds.
c. Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from summarization and statistical analyses.
* Significantly different from the control group value ($p \leq 0.05$).

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
MATERNAL DOSE LEVEL (MG/KG)		0	0.75	1.5	3
FEMALE RATS					
RATS TESTED	N	25	25	22	25
SESSION 1a	N	25	25	22	25
TRIALS TO CRITERION	MEAN±S.D.	8.7 ± 2.5	8.0 ± 1.9	9.2 ± 2.6	8.5 ± 2.4
ERRORS PER TRIAL	MEAN±S.D.	0.34 ± 0.18	0.50 ± 0.23*	0.49 ± 0.24*	0.49 ± 0.19*
LATENCY TRIAL 2b	MEAN±S.D.	12.3 ± 12.1	16.3 ± 10.8	16.0 ± 11.9	17.6 ± 16.8
FAILED TO LEARN c	N(%)	0(0.0)	0(0.0)	1(4.5)	1(4.0)
SESSION 2a	N	25	25	21	24
TRIALS TO CRITERION	MEAN±S.D.	7.2 ± 3.2	6.8 ± 3.1	7.4 ± 3.8	6.5 ± 2.3
ERRORS PER TRIAL	MEAN±S.D.	0.14 ± 0.21	0.14 ± 0.20	0.16 ± 0.23	0.11 ± 0.19
LATENCY TRIAL 1b	MEAN±S.D.	10.4 ± 6.4	8.9 ± 5.7	8.1 ± 5.1	9.0 ± 4.4

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. The latency was recorded in seconds.
c. Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from summarization and statistical analyses.
* Significantly different from the control group value ($p \leq 0.05$).

SUMMARY OF MATING AND FERTILITY: F1 GENERATION MALE RATS

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
MATERNAL DOSE LEVEL (MG/KG)		0	0.75	1.5	3
RATS IN COHABITATION	N	25	25	24a	25
DAYS IN COHABITATION b	MEAN±S.D.	3.5 ± 2.9	2.0 ± 1.2*	2.9 ± 1.1	2.4 ± 1.1
RATS THAT MATED	N(%)	24(96.0)	25(100.0)	24(100.0)	25(100.0)
FERTILITY INDEX c	N/N (%)	23/ 24 (95.8)	25/ 25 (100.0)	24/ 24 (100.0)	23/ 25 (92.0)
RATS WITH CONFIRMED MATING DATES	N	24	25	24	25
MATED WITH FEMALE d					
DAYS 1-7	N(%)	22(91.7)	25(100.0)	24(100.0)	25(100.0)
DAYS 8-14	N(%)	2(8.3)	0(0.0)	0(0.0)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION	N/N (%)	23/ 25 (92.0)	25/ 25 (100.0)	24/ 24 (100.0)	23/ 25 (92.0)

a. Rat 1654 was not assigned to cohabitation because there were no available female rats.
b. Restricted to females with a confirmed mating date and females that did not mate.
c. Number of pregnancies/number of females that mated.
d. Restricted to females with a confirmed mating date.
* Significantly different from the control group value ($p \leq 0.05$).

SUMMARY OF MATING AND FERTILITY: F1 GENERATION FEMALE RATS

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
MATERNAL DOSE LEVEL (MG/KG)		0	0.75	1.5	3
FEMALES IN COHABITATION	N	25	25	24a	25
DAYS IN COHABITATION b	MEAN±S.D.	4.1 ± 5.3	2.0 ± 1.2*	2.9 ± 1.1	2.4 ± 1.1
FEMALES THAT MATED	N(%)	24(96.0)	25(100.0)	24(100.0)	25(100.0)
FERTILITY INDEX c	N/N (%)	23/ 24 (95.8)	25/ 25 (100.0)	24/ 24 (100.0)	23/ 25 (92.0)
FEMALES WITH CONFIRMED MATING DATES	N	24	25	24	25
MATED BY FIRST MALE d					
DAYS 1-7	N(%)	22(91.7)	25(100.0)	24(100.0)	25(100.0)
DAYS 8-14	N(%)	2(8.3)	0(0.0)	0(0.0)	0(0.0)
MATED BY SECOND MALE d	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FEMALES PREGNANT/FEMALES IN COHABITATION	N/N (%)	23/ 25 (92.0)	25/ 25 (100.0)	24/ 24 (100.0)	23/ 25 (92.0)

a. Excludes values for rat 1766, which was found to be a male on Day 24 postpartum.
b. Restricted to females with a confirmed mating date and females that did not mate.
c. Number of pregnancies/number of females that mated.
d. Restricted to females with a confirmed mating date.
* Significantly different from the control group value (p<0.05).

SUMMARY OF TERMINAL BODY WEIGHTS, TESTES AND EPIDIDYMIDES WEIGHTS AND RATIOS (%) OF TESTES AND EPIDIDYMIDES WEIGHT TO TERMINAL BODY WEIGHT: F1 GENERATION MALE RATS

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
MATERNAL DOSE LEVEL (MG/KG)		0	0.75	1.5	3
RATS TESTED	N	25	25	25	25
TERMINAL BODY WEIGHT	MEAN±S.D.	539.7 ± 35.5	546.5 ± 45.8	541.8 ± 50.9	526.4 ± 44.5
EPIDIDYMIDES PAIRED	MEAN±S.D.	1.50 ± 0.12	1.46 ± 0.15	1.37 ± 0.11**	1.35 ± 0.10**
EPIDIDYMIDES PAIRED (%)	MEAN±S.D.	0.278 ± 0.026	0.269 ± 0.027	0.253 ± 0.022**	0.257 ± 0.023**
TESTES PAIRED	MEAN±S.D.	3.58 ± 0.31	3.59 ± 0.34	3.52 ± 0.28	3.42 ± 0.16
TESTES PAIRED (%)	MEAN±S.D.	0.664 ± 0.066	0.658 ± 0.052	0.653 ± 0.066	0.654 ± 0.057

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.
** Significantly different from the control group value (p<0.01).

SUMMARY OF OVARIAN AND UTERINE CONTENTS: F1 GENERATION FEMALE RATS

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	AMG 416	AMG 416	AMG 416
MATERNAL DOSE LEVEL (MG/KG)		0	0.75	1.5	3
RATS TESTED	N	25	25	24a	25
PREGNANT	N(%)	23(92.0)	25(100.0)	24(100.0)	23(92.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 13 OF GESTATION	N	23	25	24	23
CORPORA LUTEA	MEAN±S.D.	15.6 ± 1.6	16.5 ± 1.8	16.4 ± 2.0	16.4 ± 1.5
IMPLANTATIONS	MEAN±S.D.	15.5 ± 1.7	15.9 ± 1.6	15.3 ± 2.0	15.7 ± 1.8
% PREIMPLANTATION LOSS	MEAN±S.D.	1.1 ± 2.6	3.2 ± 4.3	6.7 ± 5.9**	4.2 ± 7.6
VIABLE EMBRYOS	N	341	374	351	340
	MEAN±S.D.	14.8 ± 1.7	15.0 ± 2.0	14.6 ± 2.0	14.8 ± 1.8
NONVIABLE EMBRYOS	N	15	24	16	21
	MEAN±S.D.	0.6 ± 1.2	1.0 ± 1.4	0.7 ± 0.9	0.9 ± 1.1
% POSTIMPLANTATION LOSS	MEAN±S.D.	4.0 ± 6.9	6.1 ± 8.8	4.3 ± 5.5	5.6 ± 6.4
DAMS WITH ANY NONVIABLE EMBRYOS	N(%)	9(39.1)	12(48.0)	11(45.8)	13(56.5)
DAMS WITH ALL NONVIABLE EMBRYOS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE EMBRYOS	N(%)	23(100.0)	25(100.0)	24(100.0)	23(100.0)
PLACENTAE APPEARED NORMAL	N(%)	23(100.0)	25(100.0)	24(100.0)	23(100.0)

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF VIABLE EMBRYOS) / NUMBER OF IMPLANTATIONS] x 100
a. Excludes values for rat 1766, which was found to be a male on Day 24 postpartum.
** Significantly different from the control group value ($p \leq 0.01$).

10 Special Toxicology Studies

Local Tolerance GLP Studies

Study No 117458

Intravenous injection (cephalic vein) or paravenous injection (saphenous vein) administration of a single dose of etelcalcetide in dogs at 15 mg/injection site was well-tolerated with no macroscopic or microscopic changes to the injection sites.

Study title: AMG 416: Single-Dose Intravenous and Paravenous Local Tolerance Study in the Male Beagle Dog

This study evaluated the local tolerance of etelcalcetide, when administered as a single dose via intravenous or paravenous injection to the male beagle dog. Male beagles (n=3) were administered etelcalcetide at 0 (sterile water for injection USP) and 15 mg/dose via intravenous (cephalic vein) or paravenous (saphenous vein) routes at 3 mL/dose. Assessment of tolerance was conducted over a 24-hour period after injection, with necropsy approximately 24 hours after dosing. Assessment of local tolerance was based on mortality, body weight, clinical observations (including injection site irritation scoring), and macroscopic and microscopic evaluation of injection sites.

All animals survived to their scheduled necropsy. Adverse etelcalcetide-related clinical observations occurred in one animal immediately prior to necropsy. These clinical observations included severe and continuous tremors in the entire body, especially in the hind limbs; ataxia; and dilated pupils. These findings were observed in previous studies and were consistent with

the expected pharmacological effect of reducing serum calcium secondary to parathyroid hormone suppression.

There were no etelcalcetide-related injection site irritation scores or body weight changes. There were no AMG-416-related macroscopic or microscopic observations at the injection site.

Microscopic observations (minimal to slight subacute inflammation and/or hemorrhage) were similar to those observed at control article administration sites, and all changes were considered related to intravenous or paravenous (subcutaneous) injection procedures.

Incidence and Severity of Inflammation and/or Hemorrhage at Injection Sites

Sex	Male			
Treatment[a]	Control Article	AMG 416[b]	Control Article	AMG 416[b]
Anatomic Designation	Left Front Leg Cephalic Vein (Intravenous)	Right Front Leg Cephalic Vein (Intravenous)	Left Hind Leg[d] Saphenous Vein (Paravenous)	Right Hind Leg Saphenous Vein (Paravenous)
Number Examined[c]	12	12	12	12
Inflammation, subacute				
Number Unremarkable	2	1	0	1
Minimal	10	11	6	9
Slight	0	0	6	2
Hemorrhage				
Number Unremarkable	12	12	6	8
Minimal	0	0	4	4
Slight	0	0	2	0

a Animals dosed at a volume of 3 mL/injection.
b The AMG 416 dose level 15 mg of AMG 416/injection site.
c Four sections of each injection site were examined, for a total of 12 sites per animal.
d One animal (Animal No. H09676) was administered the control article in right hind leg and AMG 416 in left hind leg. The incidence and severity reported is based on the control article or test article administered to the animal.

Study No 4169-NC-111

Etelcalcetide did not cause any hemolysis up to 30 mg/ml.

Study title: Assessment of the Hemolytic Potential of KAI-4169 in Human Blood

This study assessed the hemolytic potential of etelcalcetide (Lot F4169KP0901B) in human blood. A buffered formulation of etelcalcetide similar to the formulations used in the toxicology studies (10 mM succinic acid, 2% mannitol, 1% glycine, 1% trehalose, pH 4.5) was evaluated for its potential to induce hemolysis in human blood (n = 3 donors) in vitro at concentrations of 0 (vehicle), 0.1, 0.3, 1, 3, 10, and 30 mg/mL. Phosphate buffered saline and 0.5% saponin were also included as negative and positive controls, respectively.

There was no evidence of hemolysis by phosphate buffered saline, the vehicle or by etelcalcetide at all concentrations tested. The positive control produced the expected lysis of red blood cells. In conclusion, etelcalcetide does not cause hemolysis at concentrations up to 30 mg/mL.

Study No 4169-NC-154

Etelcalcetide did not cause any hemolysis up to 5 mg/ml.

Study title: Assessment of the Hemolytic Potential of KAI-4169 in Human Blood

The objective of this study was to determine the hemolytic potential of etelcalcetide in human blood. A buffered formulation of etelcalcetide similar to the liquid drug product (10 mM succinic acid, 0.85% sodium chloride, pH 3.17) was evaluated for its potential to induce hemolysis in human blood (n=3 donors) in vitro at concentrations of 0 (vehicle), 0.05, 0.15, 0.5, 1.5 and 5 mg/mL. Phosphate buffered saline and 0.5% saponin were also included as negative and positive controls, respectively.

There was no evidence of hemolysis by phosphate buffered saline, the vehicle or by etelcalcetide at all concentrations tested. The positive control produced the expected lysis of red blood cells. In conclusion, etelcalcetide does not cause hemolysis at concentrations up to 5 mg/mL.

Mechanistic Non-GLP Studies

Study No 117144

Etelcalcetide and KP-2326 (but not KP-2140 and KP-2067) were mutagenic in TA1535 tester strain, suggesting that the specific sequence of alanines and arginines in the backbone was unlikely to be important for mutagenic activity.

Study title: Mutagenicity of KP-2326, KP-2140, KP-2067 and AMG 416 in TA1535 in the absence of S9 activation

Etelcalcetide and structurally related peptides were evaluated for mutagenic activity to identify the structural components of the molecule important for mutagenic activity. The peptides were tested using the plate incorporation method in TA1535 at concentrations ranging from 15 to 5,000 µg/plate in the absence of S9 metabolic activation.

No signs of toxicity related to the background lawn were observed and no precipitate was seen in any of the test article treated plates. With KP-2326 (rearranging alanine and arginine on the peptide backbone), significant increase in the number of revertant colonies was observed at the top concentration (5041 µg/plate) compared to the vehicle control and a concentration-related increase in the revertant colony number. With KP-2140 and KP-2067, no significant increases in the number of revertant colonies were observed compared to the vehicle control at any concentration. With etelcalcetide, significant increase in the number of revertant colonies was observed at the top two concentrations (1500 and 5000 µg/plate) compared to the vehicle control and a concentration-related increase in the revertant colony number. This study showed that etelcalcetide was mutagenic under the condition of this study.

Reference ID: 3923456

Summary of Mutagenicity (Ames) Results for AMG 416 and Structurally Related Peptides in TA1535 in the Absence of S9 Metabolic Activation (Study 117144)

Concentration (µg/plate)	Number of Revertant Colonies ± SD (Fold-increase Relative to Control) in TA1535			
	AMG 416	KP-2326	KP-2067	KP-2140
	Ac-c(C)arrrar-NH_2	Ac-c(C)rrarar-NH_2	Ac-carrrar-NH_2	Ac-arrrar-NH_2
Buffer	9 ± 2 (1.0)	12 ± 2 (1.0)	11 ± 3 (1.0)	12 ± 6 (1.0)
15	13 ± 6 (1.4)	10 ± 2 (0.9)	13 ± 1 (1.2)	10 ± 5 (0.8)
50	12 ± 3 (1.3)	15 ± 3 (1.3)	9 ± 2 (0.8)	9 ± 1 (0.7)
150	15 ± 3 (1.6)	14 ± 3 (1.2)	10 ± 2 (0.9)	6 ± 2 (0.5)
500	21 ± 6 (2.3)	15 ± 3 (1.3)	20 ± 5 (1.9)	12 ± 3 (1.0)
1500	27 ± 7 (3.0)*	19 ± 2 (1.7)	24 ± 9 (2.2)	9 ± 6 (0.7)
5000	48 ± 7 (5.3)*	36 ± 5 (3.1)*	18 ± 5 (1.7)	11 ± 2 (0.9)

a = D-alanine; Ac = acetatylated N-terminal amino acid; c = D-cysteine; C = L-cysteine; NH_2 = amidated C-terminal amino acid; r = D-arginine; SD = standard deviation.
* Significant increase (≥3-fold) in colony number compared to control.

Study No 117145

Etelcalcetide and KP-2326 were converted to homodimers. A small amount of KP-2067 homodimerization was seen after 60 min of incubation. No homodimers were detected with KP-2014. This study suggested that biotransformation of etelcalcetide was limited to disulfide exchange with the D-cysteine, and that the peptide backbone was stable in TA1535 tester strain.

Study title: Evaluation of the Metabolism of KP-2326, KP-2140, KP-2067, and AMG416 in *Salmonella Typhimurium* TA1535

Etelcalcetide and structurally related peptides were evaluated for metabolite formation in TA1535 to determine if the peptides were metabolized to their component amino acids or whether unique bacterial metabolites were produced. The tester strain TA1535 was exposed to the peptides by the pre-incubation method of the Ames test, whereby peptides were incubated with TA1535 tester strain in phosphate buffer at 37°C for 60 minutes at a final concentration of 10 mg/mL (7142 µg/mL when corrected for peptide content).

At 0 min of incubation with TA 1535, all of etelcalcetide, KP-2140, and KP-2067 were recovered in the parent form and approx. 90% of KP-2326 was recovered in parent form with remaining 10% recovered as homodimer.

At 60 min of incubation with TA 1535, ~ 21 % of etelcalcetide was converted to homodimer and 79% remaining as the parent compound, ~ 39 % of KP-2326 was converted to homodimer with 61 % remaining as the parent compound, ~ 4 % of KP-2067 was converted to homodimer with 96% remaining as the parent compound, and no metabolites were detected for KP-2140 and all of the parent compound was recovered in the parent form.

With etelcalcetide, KP-2326, and KP-2067, the corresponding homodimer were formed via the disulfide linkage of the D-Cys in the parent backbone peptide. No other metabolites were detected for any of the four compounds at start of the incubation and at the end 60 minutes of incubation with TA 1535.

Metabolism of AMG 416 and Related Peptides in TA1535 (Study 117145)

	Percent of Metabolites From all Parent-related Material After 60-Minute Incubation in Ames Assay			
	AMG 416	KP-2326	KP-2067	KP-2140
Compound	Ac-c(C)arrrar-NH_2	Ac-c(C)rrarar-NH_2	Ac-carrrar-NH_2	Ac-arrrar-NH_2
Parent	79	61	96	100
Homodimer	21	39	4	0

a = D-alanine; Ac = acetatylated N-terminal amino acid; c = D-cysteine; C = L-cysteine; NH_2 = amidated C-terminal amino acid; r = D-arginine.

Study No 117326

The study showed that it was unlikely that free L-cysteine was mediating the mutagenic activity of etelcalcetide in TA1535.

Study title: Bacterial Reverse Mutation Assay: AMG2508#5

The objective of this study was to determine if L-cysteine (AMG2508#5) was responsible for the mutagenic activity of etelcalcetide observed in TA1535, suggesting a reduction of the disulfide bond in etelcalcetide as the cause. The test article, AMG2508#5 (L-cysteine), was tested at six concentrations along with appropriate vehicle and positive controls in the absence and presence of S9 in TA1535. TA100 and TA102 were used as positive controls for mutagenic activity of L-cysteine.

A greater than 50% reduction in the number of revertant colonies was observed in tester strain TA 1535 at 500 and 5000 µg/plate without metabolic activation compared to the concurrent vehicle control; however, these reduced numbers were within the historical vehicle control range. The remaining tester strains/treatment conditions had no signs of toxicity up to the concentration of 5000 µg/plate. Precipitate was not observed in any of the tester strains/treatment conditions. Significant increase in the number of revertant colonies was observed at 5000 µg/plate with metabolic activation in TA100 and TA102 when compared to the vehicle control.

Study No 117418

KP-2067 was not mutagenic in TA1535 in the absence of metabolic activation, suggesting the importance of a thiol anion in etelcalcetide for mutagenicity.

Study title: Mutagenicity of KP-2067 in TA1535 in the Absence of S9 Activation

To confirm the non-monotonic dose response in revertant colony count observed with KP-2067 in Study No 117144, the experiment was repeated in TA1535 in the absence of S9 activation according to the same protocol used in Study 117144. Additional concentrations between 150 and 5000 µg/plate were evaluated to further assess the dose-response.

No signs of toxicity related to the background lawn were observed and no precipitate was seen in any of the test article treated plates. No significant increases in the number of revertant colonies were observed (except 2,500 µg/plate; 3 x increase) compared to the vehicle control at any concentration. Doses of 1,000 and 3,000 µg/plate also had 2.95–fold increases. However, there was no concentration dependent trend for mutagenicity.

Together with Study No 117144, these results confirmed the importance of a thiol or thiolate anion in etelcalcetide for mutagenic activity, and suggested that the oxidized form of the D-

cysteine in etelcalcetide was more potent at inducing mutations in TA1535 relative to the reduced form, KP-2067.

Summary of Mutagenicity (Ames) Results for KP-2067 in TA1535 in the

Concentration (µg/plate)	Number of Revertant Colonies ± SD (Fold-increase Relative to Control) in TA1535	
	KP-2067 (Study 117144)	KP-2067 (Study 117418)
Buffer	11 ± 3 (1.0)	7 ± 2 (1.0)
15	13 ± 1 (1.2)	NT
50	9 ± 2 (0.8)	NT
150	10 ± 2 (0.9)	9 ± 5 (1.2)
500	20 ± 5 (1.9)	17 ± 2 (2.3)
1500	24 ± 9 (2.2)	21 ± 6 (2.8)
2500	NT	22 ± 6 (3.0)
5000	18 ± 5 (1.7)	14 ± 8 (1.9)

NT = not tested; SD = standard deviation.

Study No 118039

This study showed that TA1535 was sensitive to known oxidative mutagens and suggested that TA1535 might be susceptible to ROS-mediated mutagenesis.

Study title: Investigation of Mutagenic Effects of Oxidants and Antioxidants in Three *Salmonella typhimurium* Strains

The objective of the study was to determine if TA1535 was sensitive to oxidant-induced mutagenesis, which would support the hypothesis that etelcalcetide induces mutations via ROS formation. TA100 and TA102 were used as positive controls as they were known to be responsive to oxidant induced mutagenesis). The compounds were tested using the preincubation method in the absence of S9 metabolic activation. Test articles included hydrogen peroxide, L-glutathione (reduced), 1,2,4 benzenetriol, hydroquinone, pyrogallol, superoxide dismutase, glyoxal, t-butyl hydroperoxide, cumene hydroperoxide, L-penicillamine, phenylhydrazine, and 2,6-dimethyl-1,4-benzoquinone.

All criteria for a valid study were met with the exception of test article, 2,6-dimethyl-1,4-benzoquinone, which did not have a minimum of three non-toxic concentrations.

Under the conditions of this study, test articles, such as hydrogen peroxide, L-glutathione (reduced), 1,2,4 benzenetriol, hydroquinone, pyrogallol and superoxide dismutase, were not mutagenic in the absence of S9 activation. On the other hand, test articles, such as glyoxal, t-butyl hydroperoxide, cumene hydroperoxide, L-penicillamine and phenylhydrazine, were mutagenic in the absence of S9 activation in the initial experiment.

In the repeat experiment, Glyoxal gave a positive response in TA1535 without activation, thus confirming the evaluation in the initial experiment. However, this positive finding with cumene hydroperoxide could not be confirmed in the repeat experiment.

Summary of Mutagenicity (Ames) Results for Oxidant Mutagens in TA1535 in the absence of S9 Metabolic Activation (Study 118039)

	Maximum Fold-increase in Revertant Colony Count Relative to Control[a]		
Compound	TA100	TA102	TA1535
Glyoxal	12	2.5	5.6
t-Butyl hydroperoxide	2.6	2.4	-
Cumene hydroperoxide	2.2	3.2	5.2/-[b]
L-penicillamine	4.8	-	-
Phenylhydrazine	2.2	2.8	-
Hydrogen peroxide	-	-	-
1,2,4-benzenetriol	-	-	-
Hydroquinone	-	-	-
Pyrogallol	-	-	-
2,6-Dimethyl-1,4-benzoquinone	-	-	-
L-glutathione	-	-	-
Superoxide dismutase	-	-	-

[a] Significant increases shown only (≥ 2-fold for TA100/TA102; ≥ 3-fold for TA1535)
[b] Positive results were not confirmed in repeat experiments
- indicates no significant change in revertant colony count

Study No 119371

This study suggested that TA1535 was sensitive to free radical-mediated mutation under certain oxidant conditions, and that etelcalcetide may be inducing mutations via ROS formation.

Study title: Investigation of the Reversibility of Glyoxal-induced Mutagenicity by Antioxidants

The objective of this study was to evaluate the role of ROS in the mutagenesis of glyoxal in TA1535. To test whether the mutations induced by glyoxal were mediated by ROS, the bacteria were pretreated with select antioxidants for 10 minutes before a 20-minute incubation with glyoxal. Glyoxal was tested at 250 µg/plate; a concentration that induced a significant increase in revertant colonies in both TA100 and TA1535 in a previous study (Study 118039). The compounds and their combinations were tested using the pre-incubation method in the absence of S9 metabolic activation. Test articles included glyoxal, catalase, deferoxamine, superoxide dismutase, EDTA.

The oxidant glyoxal was positive (mutagenic) in both TA100 and TA1535 strains without metabolic activation.

Pretreatment with superoxide dismutase lowered glyoxal-induced mutagenicity in TA100 (~66% reduction) and TA 1535 (~40% reduction). Pretreatment of with catalase lowered glyoxal-induced mutagenicity in TA100 (~60% reduction) and TA 1535 (~21% reduction). Pretreatment with deferoxamine mesylate salt lowered glyoxal-induced mutagenicity in TA100 (~62% reduction) and TA 1535 (~29% reduction). Pretreatment with EDTA lowered glyoxal-induced mutagenicity in TA100 (~63% reduction) and TA1535 (~39% reduction). The results demonstrate that the antioxidants superoxide dismutase and catalase, and the iron chelators desferroxamine and EDTA, were capable of reducing the glyoxal-induced increase in revertant colonies in both TA100 and TA1535.

Summary of Mutagenicity (Ames) Results for Glyoxal in the Presence and Absence of Anti-oxidants in TA100 and TA1535 in the absence of S9 Metabolic Activation (Study 119371)

Average Revertant Colony Count ± SD	TA100	TA1535
Water	84 ± 10	9 ± 3
Phosphate buffer	85 ± 10	11 ± 3
Glyoxal (250 µg/plate)	1534 ± 94	28 ± 2
Superoxide dismutase (6400 IU/plate)	95 ± 9	11 ± 3
Superoxide dismutase + Glyoxal	522 ± 7	17 ± 2
Catalase (24 µg/plate)	99 ± 19	13 ± 3
Catalase + Glyoxal	607 ± 34	22 ± 5
Desferroxamine (0.21 µg/plate)	89 ± 12	15 ± 4
Desferroxamine + Glyoxal	576 ± 8	20 ± 2
EDTA (4.1 µg/plate)	107 ± 15	19 ± 8
EDTA + Glyoxal	567 ± 14	17 ± 6

EDTA = ethylenediaminetetraacetic acid; SD = standard deviation

Impurity GLP Studies

Study No 4169-NC-146: 4 week IV dog study comparing two lots

Study title: A 4-Week Intravenous Bridging Toxicity and Toxicokinetic Study of Two Lots of KAI-4169 (etelcalcetide) in Beagle Dogs (GLP, testing facility: [redacted] (b) (4)

Study description:
This study compared the toxicity and toxicokinetics of a new lot [redacted] (b) (4)) of etelcalcetide to a previous lot (Lot No. F4169KP1001B). The two lots of etelcalcetide or the vehicle (buffer solution; consisting of 0.27% sodium succinate dibasic hexahydrate, 2% D-mannitol, 1% glycine, 1% trehalose, and 0.9% benzyl alcohol in sterile water for injection) were administered to separate groups of dogs every other day via intravenous slow-bolus injection at a dose volume of 0.5 mL/kg for 4 weeks. Parameters evaluated included cage-side and detailed clinical observations, body weights and body weight changes, qualitative food consumption, clinical pathology (clinical chemistry, hematology, coagulation and urinalysis), ophthalmology, electrocardiograms, gross pathology, absolute and relative organ weights, complete microscopic pathology and toxicokinetics. Blood samples were collected for TK evaluations at 6 time points on Day 1 and at 7 time points on Day 27. All animals were euthanized and necropsied after the final blood collection on Day 29.

Group	Treatment	Dose Level (mg/kg/dose)	Concentration (mg/mL)	No. of Animal/sex
1	Buffer Solution (Vehicle)	0	0	3
2	KAI-4169 (Lot No. F4169KP1001B)	0.4	0.8	3
3		1.0	2.0	3
4	KAI-4169 [redacted] (b) (4)	0.4	0.8	3
5		1.0	2.0	3

Study findings:

- Repeated (every other day) intravenous bolus injection of etelcalcetide of both lots at doses up to 1.0 mg/kg/dose for approximately 4 weeks in male and female beagle dogs

did not result in mortality or moribundity, and there were no test article-related effects on ocular condition, coagulation or urinalysis parameters.

- Test article-related microscopic findings were limited to the thymus, liver, bone marrow (sternum), and kidney, were similar to the changes observed in prior studies with etelcalcetide, and were similar between the two lots.

Incidence[a] of KAI-4169 Microscopic Findings

Terminal Sacrifice (Day 29)

Sex:	Males					Females				
Group:	1	2	3	4	5	1	2	3	4	5
Treatment[b]:	Vehicle	Lot 1	Lot 1	Lot 2	Lot 2	Vehicle	Lot 1	Lot 1	Lot 2	Lot 2
Dose level (mg/kg/dose):	0	0.4	1.0	0.4	1.0	0	0.4	1.0	0.4	1.0
Bone marrow (sternum)										
Hypocellularity										
Minimal	-	-	-	-	-	-	-	-	-	1
Mild	-	-	1	-	1	-	-	1	-	-
Moderate	-	-	-	-	1	-	-	-	-	-
Thymus										
Lymphoid depletion										
Minimal	1	2	-	1	1	1	1	-	2	2
Marked	-	-	1	-	2	-	-	1	-	1
Liver										
Hepatocellular vacuolation (glycogen)										
Minimal	-	1	1	2	1	1	2	-	-	2
Mild	-	-	-	1	1	-	-	1	-	1
Moderate	-	-	1	-	1	-	-	-	-	-
Kidney										
Golden brown pigment (proximal tubules)										
Minimal	-	-	2	-	2	-	NA	-	NA	-

[a]Expressed as the number of animals with findings in each group (3 animals/sex/group except thymus in Group 4 males where n = 2)
[b]Vehicle = Buffer Solution; Lot 1 = Lot F4169KP1001B; (b) (4)
- = not present; NA = not applicable/not examined

Study No 118280: Ames assay for sodium isopropyl sulfate

Study title: Sodium Isopropyl Sulfate: Bacterial Reverse Mutation Test in *Salmonella typhimurium* and *Escherichia coli* (GLP, testing facility: (b) (4)

Study description:
This study determined the potential genotoxicity of sodium isopropyl sulfate, using the bacterial reverse mutation (Ames) test. *Salmonella typhimurium* strains (TA1535, TA97a, TA98, TA100) and *Escherichia coli* strain WP2 *uvrA* were treated with sodium isopropyl sulfate at a range of concentrations up to 5000 µg/plate (the standard limit dose for this assay), in the presence and absence of a supplemented rat liver metabolic activation system (S9 mix), using the plate incorporation version of the bacterial mutation test. Bacteria were incubated with standard positive controls, and the response of the various bacterial strains to these agents confirmed the sensitivity of the test system and the activity of the S9 mix.

Study Design - Plate Incorporation Assay

Material	Formulation Conc. (µg/mL)	Final Dose (µg/plate)	No. of Replicates		No. of Strains
			0S9	+S9	
Water	-	-	3	3	5
1/ sodium isopropyl sulfate	15.8	1.58	3	3	5
2/ sodium isopropyl sulfate	50	5.0	3	3	5
3/ sodium isopropyl sulfate	158	15.8	3	3	5
4/ sodium isopropyl sulfate	500	50	3	3	5
5/ sodium isopropyl sulfate	1581	158	3	3	5
6/ sodium isopropyl sulfate	5000	500	3	3	5
7/ sodium isopropyl sulfate	15811	1581	3	3	5
8/ sodium isopropyl sulfate	50000	5000†	3	3	5
Positive controls	‡	‡	3	3	5

‡ Depends on the test organism, positive control and methodology used.
† Test item was tested at levels up to 5000 µg/plate, which is the standard limit dose recommended by regulatory guidelines.

Study findings:

- Incomplete, or absent, background lawns of non-revertant bacteria, or substantial reductions in revertant colony counts, were not obtained following exposure to sodium isopropyl sulfate, indicating that the test item was non-toxic to the bacteria at the levels tested.
- No precipitation was observed in the assay.
- No substantial increases in revertant colony numbers were obtained with any of the tester strains following exposure to sodium isopropyl sulfate, at any concentration level, in either the presence or absence of S9 mix.
- Sodium isopropyl sulfate was not mutagenic in *Salmonella typhimurium* (strains TA98, TA100, TA1535, and TA97a) or *Escherichia coli* (strain WP2*uvrA*), in the presence or absence of an *in vitro* metabolic activation system.

Study No 119181: 28 day IV rat study for etelcalcetide mixture and impurities

Study title: Mixture of AMG 416 (Etelcalcetide) and Impurities: A 28-Day Intravenous Injection Toxicity Study in the Sprague-Dawley Rat

Study description:
This study determined the potential toxicity of a "Mixture of etelcalcetide and Impurities," termed test item (Lot 1053127 with 81.6% purity), when administered intravenously for 28 days to the rat. The test item consisted of [redacted] (b) (4) at known amounts into etelcalcetide drug substance. The toxicokinetic characteristics of etelcalcetide were also determined. Animals received 0 (vehicle consisting of 0.27% sodium succinate dibasic hexahydrate (2.7 g/L), 2% D-mannitol (20 g/L), 1% glycine (10 g/L), 1% trehalose dihydrate (10 g/L) and 0.9% (9 g/L) benzyl alcohol in sterile water for injection, pH 4.5 ± 0.1), 0.3, 1, and 3 mg/kg etelcalcetide and impurities daily (n = 10/sex/group for main study; 6/sex/group for toxicokinetic phase). Scheduled necropsies were conducted at the end of the 28-day dosing phase. The endpoints were evaluated: clinical signs, body weights, food consumption, ophthalmology, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), toxicokinetic parameters, gross necropsy findings, organ weights, and histopathologic examinations.

Experimental Design

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals[a]	
					Males	Females
Dosing Animals						
1	Control	0	2	0	10	10
2	Test Item[b]	0.3	2	0.15	10	10
3		1	2	0.5	10	10
4		3	2	1.5	10	10
Toxicokinetic Animals						
5	Control	0	2	0	6	6
6	Test Item[b]	0.3	2	0.15	6	6
7		1	2	0.5	6	6
8		3	2	1.5	6	6

[a] Animals were euthanized on Day 29.
[b] Mixture of AMG 416 and impurities (aka test item).

Study findings:

- Etelcalcetide and impurities administered intravenously daily to rats for 28 days was tolerated at levels up to 3 mg/kg.
- Test item changes were considered reflective of the known safety profile of etelcalcetide and include tremors in all animals at 3 mg/kg, which was considered secondary to marked hypocalcemia observed at this dose level, in addition to clinical signs reflective of toxicity and decreased body weight gain in males. Microscopic changes in the parathyroid gland and kidney are consistent with effects of etelcalcetide.

11 Integrated Summary and Safety Evaluation

Pharmacology

- Etelcalcetide was a potent and selective allosteric activator of the calcium sensing receptor (CaSR). Etelcalcetide lowered the threshold of receptor activation by calcium.
- Etelcalcetide caused rapid and reversible decreases in circulating levels of PTH in both rats and dogs by direct actions on the parathyroid gland. It also caused decreases in total serum calcium levels in both rats and dogs in a dose-dependent manner.
- Repeated dosing of etelcalcetide prevented parathyroid gland hyperplasia and vascular calcification in renal impaired rats with secondary hyperparathyroidism (HPT). Etelcalcetide could also preserve cortical bone and strength in rats with secondary HPT.

Safety Pharmacology

- No direct effects on central nervous system or respiratory function were observed in dogs. QTc prolongation was observed in the dog as expected due to reductions in serum calcium.
- There were no direct effects on hERG when evaluated in vitro.

Pharmacokinetics

- Intravenous administration of etelcalcetide to rats and dogs with normal kidney function resulted in a dose-proportional plasma exposure with no evidence of significant accumulation.
- Renal elimination was the predominant clearance pathway in rats with the administered dose excreted as intact etelcalcetide or its metabolites. Metabolites observed in rats were also be detected in humans.
- Etelcalcetide was predominately metabolized in blood, by replacing L-cysteine in etelcalcetide with endogenous thiols in plasma (disulfide exchange rather than CYP450s). A significant portion of administered etelcalcetide, serum albumin peptide

conjugate (SAPC), was covalently bound through disulfide conjugation of the D-amino acid peptide backbone to serum albumin in rat and human plasma. This conjugation was reversible.

Summary of AMG 416 Biotransformation Products in Plasma

	S—R[a] S Ac-carrrar-NH$_2$	In vitro[d]		In vivo[e]	
	R moiety	Human	Rat	Normal Rat	BN Rat
AMG 416	L-Cys	14.1	11.7	34.5	24.3
SAPC	Cys34-Serum Albumin	71.4	58.9	42.3	18.7
M1	Thiosulfate	1.4	5.6	4.3	17.8
M10	L-GSH	1.1	14.2	10.8	19.9
M11	NA[b]	1.2	2.3	2.0	5.8
M13	L-Cysteinyl-L-glycine	3.9	1.4	ND	2.0
others[c]	Minor products	each <2%		each <3%	

BN = bilaterally nephrectomized SD rat; NA = not applicable; ND = not detected; normal = SD rat with intact kidneys; SAPC = serum albumin peptide conjugate
[a] lower case letters represent D-amino acids; [b] structure shown in Figure 1.
[c] comprehensive listing provided in Table 11 in Module 2.6.5
[d] Study 118358; [e] AUC pooled plasma; Study 118436

Repeated Dose Toxicity

- All adverse effects observed in toxicology studies (6 month rat and 9 month dog studies) were related, either directly or indirectly, to the pharmacologic activity of etelcalcetide and the expected sequelae associated with hypocalcemia resulting from suppression of PTH secretion.
- Adverse effects observed included hypocalcemia, tremoring and convulsions, in addition to the sequelae of stress associated with hypocalcemia (i.e. reduced body weight, body weight gain, and food consumption, and other clinical signs of poor health). These adverse effects were non-progressive with chronic dosing, and were reversible. No off-target toxicity was identified in rats or dogs.
- The NOAELs were 1 mg/kg/day in rats and 0.5 mg/kg every other day in dogs, corresponding to a safety margin of 0.7-fold and 0.15-fold relative to the maximum clinical dose of 15 mg TIW in secondary HPT patients based on plasma exposures (AUC).
- While these healthy animals were pharmacologically relevant, they were more much more sensitive to the hypocalcemic effects of etelcalcetide because of the greater effect of PTH on calcium reabsorption in intact kidneys, relative to secondary HPT patients who have little or no kidney function and elevated PTH and serum calcium. Therefore, these safety margins significantly over-predict human risk.
- One month toxicity study in rats and dogs showed no findings related to potential impurities, indicating no additional hazard at the tested levels.

Genotoxicity

- Etelcalcetide was mutagenic in 2 of 5 tester strains (TA100 and TA1535) in the presence and/or absence of metabolic activation. The cause of the positive mutagenic response in these two strains is unknown. However, results of follow-up studies indicated that mutagenic activity was not due to the presence of mutagenic impurities or release of free amino acids and compensation by histidine auxotrophy based on a modified treat and plate method. Further mechanistic studies (Study No 117144) and structure activity relationship data indicated that a thiol or thiolate anion of etelcalcetide was important for mutagenic activity, suggesting involvement of the generation of reactive oxygen species.
- Etelcalcetide was negative in an in vitro chromosomal aberration assay in human peripheral blood lymphocytes, and an in vivo bone marrow micronucleus study in rats.

- Additionally, etelcalcetide was negative in two mammalian cell mutagenicity assays (hypoxanthine-guanine phosphoribosyl transferase assay in Chinese hamster ovary cells and Chinese hamster lung cells).
- Etelcalcetide was also evaluated for mutagenicity in liver and bone marrow in a 28-day repeat-dose study in the in vivo Muta™ Mouse assay and was negative.

Carcinogenicity

- In 6 month transgenic rasH2 mouse study (at doses of 0.3, 1, and 3 mg/kg in females, and 0.375, 0.75 and 1.5 mg/kg in males once daily by the SC route for 26 weeks), there were no etelcalcetide-related effects on survival, clinical observations, body weight, body weight gain, food consumption or macroscopic observations. There were no etelcalcetide-related effects on tumor incidence or non-neoplastic findings. There was an increased incidence and severity of inflammatory findings and/or hemorrhages in the vehicle and etelcalcetide-treated mice that was attributed to the injection procedure and vehicle. There was an expected statistically significant increase in the incidence of pulmonary tumors and hemangiosarcomas in the male and female positive control urethane-treated groups relative to the vehicle control groups, thus supporting the validity of the study.
- In 2 year rat study (at doses of 0.2, 0.4, 0.8, and 1.6 mg/kg; representing exposure multiples of 0.05-fold, 0.11-fold, 0.18-fold and 0.4-fold respectively, via daily subcutaneous injection for 104 weeks), there were no etelcalcetide-related effects on survival, palpable masses, clinical observations, food consumption, gross pathology, or histopathology. An etelcalcetide-related decrease in mean body weight was observed in males at 1.6 mg/kg (88% of controls) when compared to vehicle controls. Due to decreasing survival in high dose groups and controls, groups were terminated early in accordance with termination plans approved by the Executive CAC. Females in the 0.8 and 1.6 mg/kg group were terminated on week 89 due to declining survival. The remaining groups/sexes were terminated at week 92 due to reduced survival in male and female rats in the vehicle group. There were no etelcalcetide-related effects on tumor incidence or non-neoplastic findings. The only non-neoplastic finding noted was an increased incidence of fibrosis at the injection site (scapular area) in vehicle control and etelcalcetide injected animals, but with little effect in the saline control group.
- Due to the increased sensitivity of animals with normal renal function to the calcium lowering effects due to PTH changes, the exposures at the high dose in rats were lower than the maximal exposures in humans administered 15 mg of etelcalcetide three times weekly. The plasma drug exposures were approximately 0.4-fold compared to secondary HPT patients at the maximum clinical dose of 15 mg TIW.

Reproductive/developmental toxicity

- In fertility and early embryonic toxicity studies (at doses of 0.75, 1.5, and 3 mg/kg, representing exposure multiples of 0.46-fold, 0.84-fold and 1.8-fold respectively, by the IV route), administration of etelcalcetide to pregnant rats resulted in dose-related hypocalcemia. Clinical signs, reduced body weight and body weight gains, with correspondingly lower mean food consumption, were observed, consistent with similar observations in repeat-dose toxicity studies in non-pregnant rats. There were no effects on reproductive organ weights or sperm parameters in either study. Mating and fertility parameters were comparable for all groups in both males and females at all dose levels in both studies. There were no etelcalcetide-related effects on embryo-fetal survival or fetal body weights. No etelcalcetide-related fetal external, visceral, or skeletal malformations or variations. In another similar study, decreased number of estrous

Reference ID: 3923456

cycles was noted in high-dose females, which delayed estrous cycle (increased follicular cysts) was also seen in high-dose females in a repeat dose toxicity study.

- In embryo-fetal toxicity studies in rats, intrauterine growth, survival and external fetal morphology was unaffected by etelcalcetide at dose levels up to 3.2 mg/kg in one study (at doses of 0, 0.8, 1.6, and 3.2 mg/kg from gestation days (GDs) 6-17 via the IV route) and up to 3.0 mg/kg in another study (at doses levels of 0, 0.075, 1.5 and 3 mg/kg from GDs 6-17 via the IV route). However, maternal effects associated with marked decreases in serum calcium (clinical signs, changes in body weight and food consumption, consistent with changes in serum calcium) were observed in both studies. At the highest dose tested in pregnant rats (3.0 mg/kg), plasma drug exposures were approximately 1.8-fold greater compared to secondary HPT patients at the maximum clinical dose of 15 mg TIW. In rabbits (at doses of 0, 0.375, 0.75, and 1.5 mg/kg from GDs 7-20 via IV route), ovarian parameters and embryo-fetal survival or growth were unaffected by etelcalcetide at dose levels up to 1.5 mg/kg. Etelcalcetide did not produce any fetal external, visceral, or skeletal malformations or variations. At the highest dose tested in pregnant rabbits (1.5 mg/kg), no effects on embryo-fetal development were observed and plasma drug exposures were approximately 4.3-fold greater compared to secondary HPT patients at the maximum clinical dose of 15 mg TIW.
- In a pre and postnatal toxicity study in rats (at doses of 0, 0.75, 1.5, or 3 mg/kg from GDs 7 – lactation day (LD) 20 by the IV route; representing exposure multiples of 0.46-fold, 0.84-fold and 1.8-fold respectively), there were no etelcalcetide-related effects on sexual maturation, behavioral endpoints (learning and memory or activity), mating, fertility or ovarian and uterine parameters in the F1 generation rats at all dose levels. A small delay in parturition, increased pup mortality and transient decreases in pup growth were associated with maternal signs of toxicity at doses accompanied by marked decreases in serum calcium. The delay in parturition was potentially due to hypocalcemia, owing to the established role of calcium in smooth muscle (uterine) contractions.

Local tolerance

- The etelcalcetide liquid drug product is mixed with blood in the venous line of the dialysis circuit outside the body, and therefore the potential for injection site reactions is low.
- However, due to the low pH and the potential for inadvertent and direct IV administration in patients, additional testing was performed. Etelcalcetide liquid drug product showed no evidence of adverse injection site reactions when administered by the intended (IV) or unintended (paravenous) route of administration in the dog.

Impurities

- In a 28-day repeat-dose toxicity study in the dog, a [redacted] (b) (4) lot of drug substance [redacted] (b) (4) was compared to the safety profile of the parent lot. The safety profile of [redacted] (b) (4) was considered comparable to the parent lot, and no new toxicities were identified.
- In a 28-day repeat-dose toxicity study in the rat, a mixture of etelcalcetide [redacted] (b) (4) impurities was administered once daily by the IV route to rats to evaluate the potential toxicity of the [redacted] (b) (4) impurities. The safety profile of the mixture of etelcalcetide and [redacted] (b) (4) - [redacted] (b) (4) impurities was considered comparable to that of etelcalcetide, and no new toxicities were identified.
- These studies demonstrate that the tested impurities do not present any additional risk at the tested levels.
- There were no genotoxic impurities identified in the drug product that would require qualification in accordance with [redacted] (b) (4)

Table 69 Safety Margin

Exposure (AUC)-based Safety Margin in Pivotal Studies of Etelcalcetide

Study	NOAEL (mg/kg)	Mean AUC (ng hr/ml)	Safety Margin based on AUC at NOAEL*
6 month rat (Study No 119037)	1.0	3400	**0.7x**
6 month dog (Study No 119036)	0.5	1490	**0.15x**
Fertility early embryo (Study No 119088 but AUC obtained from Study No 116847 on gestational day 20)	3.0	8640	**1.8x**
Embryo-fetal rat (Study No 119088 but AUC obtained from Study No 116847 on gestational day 20)	3.0	8640	**1.8x**
Embryo-fetal rabbit (Study No 119089)	1.5	20800	**4.3x**
Pre- and post-natal rat (Study No 116847)	1.5	4050	**0.8x**
6 month tg mouse (Study No 116846)	3.0	1700	**0.4x**
2 year rat (Study No 116848)	1.6	2025	**0.4x**

* $AUC_{0\text{-}168h}$ at max clinical dose of 15 mg three times a week = 33867 ng hr/ml (Study No 119343); to account for the differences in dosing regimen between species, the exposure data in animals at steady state at the NOAEL dose was calculated as $AUC_{0\text{-}24h}$ x 7 for rat/mice/rabbits or x 3.5 for dogs.

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/s/

MIYUN M TSAI-TURTON
04/27/2016

CALVIN L ELMORE
04/27/2016
I concur.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: NDA 208325 **Applicant: Amgen** **Stamp Date: August 24, 2015**

Drug Name: Etelcalcetide **NDA/BLA Type: 505b1**

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

AMG 416 (formerly known as KAI 4169) is a calcimimetic being developed for the treatment of secondary HPT (sHPT) in patients with Chronic Kidney Disease (CKD) who are receiving hemodialysis. AMG 416 is a synthetic peptide comprised of 7 D-amino acids linked to an L-cysteine via a disulfide bond that acts as an allosteric activator of the calcium-sensing receptor (CaSR). AMG416 is able to enhance signal transduction through the CaSR in parathyroid tissue, and suppresses secretion of parathyroid hormone (PTH) in a dose-dependent manner, with this suppression being reversible. As an IV product given with hemodialysis, AMG 416 administration will be controlled by the dialysis unit staff and its use will be restricted to hemodialysis subjects with sHPT.

The drug product will be supplied in 3 strengths of 2.5 mg/vial, 5 mg/vial, and 10 mg/vial. All strengths use the same formulation and primary container, and only differ in deliverable volume. The deliverable volume and AMG 416 freebase content for each of the 3 strengths are as follows: 0.5 mL (2.5 mg), 1.0 mL (5 mg) and 2.0 mL (10 mg). For each strength, the concentration of AMG 416 (free base equivalent) is 5 mg/mL, formulated with 10 mM succinic acid and 0.85% (w/v) sodium chloride, and adjusted to pH 3.3 with sodium hydroxide and/or hydrochloric acid.

There are 14 clinical trials in more than 1700 subjects. The AMG 416 clinical development program included two phase III, 26-week, placebo-controlled studies (Studies 20120229 and 20120230) in 500 subjects each and one phase III, active-controlled, 26-week study (Study 20120360) in 683 subjects comparing AMG 416 with cinacalcet (the only calcimimetic approved for the treatment of secondary HPT)

Nonclinical Development

- Nonclinical pharmacology studies consisted of in vitro cell signaling studies to characterize the molecular pharmacology and target specificity of AMG 416 on the CaSR. In vivo pharmacology studies utilized healthy and uremic rat models to characterize primary effects on PTH lowering and secondary effects on the complications of HPT.
- Nonclinical safety pharmacology studies were conducted. The cardiovascular, respiratory and central nervous system effects were evaluated in the dog, in addition to an in vitro human ether-a-go-go (hERG) assay. Doses were selected in order to identify dose-limiting toxicities, establish a no observed adverse effect level (NOAEL) and characterize safety at relevant therapeutic exposures to facilitate human risk assessment.
- Nonclinical pharmacokinetic studies were conducted in rat and dog, and the distribution, biotransformation and excretion of AMG 416 was characterized in the rat. In addition, in vitro studies were performed to characterize the dialysis and kinetic properties of AMG 416 and its biotransformation product(s).
- Nonclinical toxicology studies included repeat-dose toxicity studies in the Sprague Dawley (SD) rat and beagle dog. AMG 416 was administered by the IV route to mimic the therapeutic route of administration in patients. In chronic toxicology studies, the IV doses were administered daily to rats for up to 6 months, or every other day to dogs for up to 9 months. The increased dosing frequency in the rat was based on a shorter half-life in rat. In vitro and in vivo genotoxicity evaluation was conducted, in addition to carcinogenicity studies in the transgenic rasH2 (Tg.rasH2) mouse and SD rat. The

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

subcutaneous (SC) route achieved adequate exposures and was used in the carcinogenicity studies due to the technical difficulties of daily IV dosing for extended periods in these models. Embryo-fetal development was evaluated in the rat and rabbit, and fertility and pre- and postnatal development was evaluated in the rat. Lastly, impurity and local tolerance studies were also conducted to address any potential adverse effects due to impurities or drug product itself.

	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 (b)(1) and (b)(2) including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		IV
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	X		Sections 8 and 13
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	X		Studies re: impurities are found in Module 4 – Other Tox
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?			N/A 505b1 submission

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

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

N/A

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

N/A

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/s/

MIYUN M TSAI-TURTON
10/05/2015
FILEABLE

DAVID B CARLSON
10/05/2015
NDA is fileable from a nonclinical perspective